ISSN 0973-3930

Volume 41 | Issue 3 | July–September 2021

International Journal of Diabetes in Developing Countries

Official Publication of
Research Society for the
Study of Diabetes in India

Springer

International Journal of Diabetes in Developing Countries
Incorporating Diabetes Bulletin

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International Journal of Diabetes in Developing Countries is published 4 times a year. Volume 41 (4 issues) of will be published in 2021.

ISSN 0973-3930 print version
ISSN 1998-3832 electronic version

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International Journal of Diabetes in Developing Countries

Volume 41 · Number 3 · July–September 2021

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International Journal of Diabetes in Developing Countries (July-September 2021) 41(3) :365–368
https://doi.org/10.1007/s13410-021-00993-x

EDITORIAL

Youth-onset type 2 diabetes mellitus—a distinct entity?

S. V. Madhu[1]

Published online: 13 August 2021

Diabetes mellitus is a chronic and lifelong disease that causes a huge burden to the healthcare system. Its prevalence is increasing worldwide. Global estimates suggest that 463 million individuals have diabetes as of 2019 and that this number will increase to 700 million by 2045, whereas in India 77 million individuals have diabetes as of 2019 and this will increase to 134 million by 2045 [1]. Over 90% of cases of diabetes are of type 2 diabetes mellitus. The increase in the prevalence of diabetes among older adults is well recognised but the rising number of young people with type 2 diabetes is a more recent development and is of particular concern. Type 2 diabetes mellitus in adolescents and young adults results in adverse societal effects because of the presence of a chronic disease throughout patients' working life and consequently greater propensity for long-term complications. Type 2 diabetes in young people could be more rapid and disruptive than in patients who have diabetes later in life, leading to early morbidity and poor quality of life [2].

There is a 31% increase in type 2 diabetes in age group 10–19 years in the USA between 2001 and 2009, resulting prevalence of 0·48 per 1000 in this age group [3]. Data from the SEARCH study [4] shows an annual increase of about 7% in the incidence of type 2 diabetes between 2002–2003 and 2011–2012 among people aged 10–19 years in the USA, with substantial relative increases in all ethnic minority groups compared with non-Hispanic White people.

The registry of youth-onset diabetes in India (YDR) reported that over 25% of youth-onset diabetes less than 25 years were type 2 diabetes mellitus [5] with an incidence of 0.5 cases/100,000 in those under 19 years [6]. In those less than 19 years of age, type 2 diabetes was equally common in both genders with the mean duration of diabetes at the presentation of 7 years. Most of these patients belonged to the high socioeconomic group, over 57% were either overweight or obese and over 70% had HbA1C of more than 9% indicating poor control [7]. The prevalence of DKA among T2D in SEARCH and YDR was 5.5% and 6.6% respectively [8].

The development and progression of type 2 diabetes represent a complex interplay between genetic, epigenetic, lifestyle, demographic, socioeconomic, therapeutic and environmental factors. A majority of patients with type 2 diabetes have at least one parent with type 2 diabetes mellitus [9]. The mechanisms leading to the development of type 2 diabetes in young people are similar to those in older patients, but the speed of onset, severity and interplay of reduced insulin sensitivity and defective insulin secretion may be different in patients who develop type 2 diabetes at a younger age [10] and a better understanding of this would help in improvement in its management. Type 2 diabetes in young people was thought to be associated with chronic complications due to longer disease duration but now there is increasing evidence that the disease could have a faster rate of progression in young people.

As seen with late-onset type 2 diabetes, adolescents also have initial deterioration in β-cell function which is characterised by loss of the first phase of stimulated insulin secretion [4]. There is some evidence that the second phase of nutrient-induced insulin secretion might be compromised earlier in younger individuals with type 2 diabetes [10]. TODAY study showed a 20–35% annual decline in β-cell function in adolescents aged 10–19 years with type 2 as compared to a 7% decline in older individuals with type 2 diabetes [11]. The prevalence of obesity among children, adolescents and young adults with type 2 diabetes is much greater than in older adults with type 2 diabetes and there is an inverse relationship between BMI and age at diagnosis of type 2 diabetes [12]. Physical inactivity is associated with an increased risk of obesity. The CARDIA study [13] which was a multicentre cohort study in 5115 participants aged 18–30 years showed that physical inactivity was strongly associated with risk of type 2 diabetes, hypertension and metabolic syndrome. Intrauterine environment can affect the development of obesity and type 2 diabetes in adolescents

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and young adults. Maternal undernutrition and overnutrition are associated with an increased risk of obesity and type 2 diabetes in the adolescent period and early adulthood [14, 15]. Socioeconomic deprivation in affluent societies is a risk factor for obesity and type 2 diabetes because of consumption of energy dense foods and sedentary habits rather than because of absolute lack of availability or affordability of healthier options. Obesity is also common in affluent societies that are not deprived thus implicating individual lifestyle and behavioral choices as risk factors with the greatest effect on the pathogenesis of type 2 diabetes in young people [16, 17].

A family history of type 2 diabetes is inversely associated with the age of onset of type 2 diabetes [18]. Studies [19, 20] in Mexican and Asian populations have identified several mutations associated with type 2 diabetes in young people. The high prevalence of type 2 diabetes in the parents of young people diagnosed with type 2 diabetes reflects a stronger genetic predisposition. SEARCH study of US children and adolescents (aged 10–19 years) showed that incidence of type 2 diabetes in female individuals was nearly twice that of male individuals [4]. The risk of type 2 diabetes is increased in young women diagnosed with polycystic ovary syndrome [21] and is possibly due to insulin resistance in polycystic ovary syndrome [22, 23]. Non-alcoholic fatty liver disease (NAFLD) is a stronger risk factor for young-onset type 2 diabetes than for type 2 diabetes that develops in middle or later life. NAFLD is twice as more common in adolescents than in older patients with type 2 diabetes and is commonly associated with insulin resistance in adolescents and young adults with type 2 diabetes.

The clinical characteristics of type 2 diabetes in young people overlap substantially with those of type 1 diabetes, from which it needs to be differentiated, as well as with those of type 2 diabetes in older adults. The early loss of insulin secretory capacity in young patients of type 2 diabetes is similar to the demise of β-cell function in type 1 diabetes, whereas the high prevalence of obesity seen in young people with type 2 diabetes is more in keeping with type 2 diabetes in older adults. Adolescents who develop type 2 diabetes also show similar clinical features of insulin resistance, dyslipidemia, hypertension and polycystic ovary syndrome as seen in the late onset of disease, but the long-term consequences of the early-onset disease are only now becoming apparent [24, 25].

People diagnosed with type 2 diabetes at a younger age still have the potential to develop complications at an earlier stage of life, at a time when the complications are more likely to cause greater disability and loss of productivity compared with people diagnosed at an older age. To examine the effect of age of onset of disease on risks of complications, focusing on all-cause mortality, macrovascular events and microvascular events a comprehensive systematic review and meta-analysis was done which compiled the results of 26 studies investigating the effects of age at diabetes diagnosis on mortality and subsequent complications in 1,325,493 participants with type 2 diabetes from diverse populations across the Asia Pacific, Europe and North America. In this review, an inverse relationship was reported between age at diabetes diagnosis and risk of major diabetes complications after adjustment for the current age. Each 1-year increase in age was associated with a 4%, 3% and 5% decreased risk of all-cause mortality, macrovascular disease and microvascular disease, respectively. These effects were consistent across the individual components of the composite outcomes (CHD, cerebrovascular disease, peripheral vascular disease, retinopathy, nephropathy and neuropathy) and reversed when the models included diabetes duration rather than current age. In this review, it was seen that younger rather than older age at diabetes diagnosis was associated with a higher risk of mortality and vascular disease. Thus, early and sustained interventions to delay type 2 diabetes onset and improve blood glucose levels and cardiovascular risk profiles of those already diagnosed with the disease are essential to reduce morbidity and mortality [26]. An association of cardiometabolic multimorbidity and depression with cardiovascular events in early-onset adult type 2 diabetes has been seen from a multi-ethnic study from the USA [27].

The study by Ashraf et al. in the current issue compares clinical features, complication profile and achievement of guideline targets in early- and late-onset type 2 diabetes patients from North India. In a large study conducted in over 5000 patients, they report that nearly one-third of all type 2 diabetic patients had an early onset before 40 years of age. These patients were more obese, had a stronger family history of diabetes and displayed more severe dyslipidemia. Comorbid conditions and diabetes-related vascular complications were higher in late-onset patients. This observation is in contrast to earlier suggestions that youth-onset type 2 diabetes is likely to be more aggressive and as a consequence develop complications more often. However, in this study, as the groups were not matched for duration of diabetes and level of glycemic control, these findings have to be interpreted with caution. Long-term follow-up studies with incident rates of complications may be a better way to assess complications.

Recently, distinct clusters or subgroups of individuals with diabetes have been identified in a Scandinavian population in 8980 patients. Clusters were based on six variables (glutamate decarboxylase antibodies, age at diagnosis, BMI, HbA1c and homoeostatic model assessment 2 estimates of β-cell function and insulin resistance). These subgroups were (1) severely autoimmune diabetes (SAID), (2) severely insulin-deficient diabetes (SIDD), (3) severely insulin-resistant diabetes (SIRD), (4) mild obesity-related diabetes (MOD) and (5) mild age-related diabetes (MARD).

These subgroups differed not only with respect to clinical characteristics at diagnosis, but also with pathophysiological mechanisms and susceptibility to complications. Cluster 1 comprised 6·4% of patients and this cluster was characterised by early-onset disease, relatively low BMI, poor metabolic control, insulin deficiency and presence of anti-GAD antibodies. Cluster 2 comprised 17·5% of patients who were GADA negative but otherwise similar to cluster 1 and had young age at onset, relatively low BMI, low insulin secretion (low HOMA2-B index) and poor metabolic control. Cluster 3 had 15·3% patients and was characterised by insulin resistance (high HOMA2-IR index) and higher BMI. Cluster 4 had 21·6% of patients which was also characterised by obesity but not by insulin resistance. Cluster 5 had 39·1% of patients and this cluster had older patients than in other clusters but had only modest metabolic derangements [28]. This study also compared the diabetic complications in different clusters. Ketoacidosis at diagnosis was common in cluster 1 (31%) and cluster 2 (25%) both of which had patients with younger age at onset and were less than 5% in other clusters. Similarly, the risk of retinopathy was highest in cluster 2. Cluster 3 had the highest prevalence of non-alcoholic fatty liver disease and the highest risk of developing chronic kidney disease. We can clearly observe that age is one of the important variables in identifying these clusters and younger patients could belong to distinct clusters and older ones to others with distinct phenotypic characteristics. The long-term follow-up of these patients will provide greater clarity on the incidence of complications in them. A similar study from India defined four clusters of patients with type 2 diabetes mellitus differing in phenotypic characteristics as well as disease outcomes [29]. However, clear age-related differences were not described.

Identification and quantification of the increased risk of mortality and vascular disease due to younger age at type 2 diabetes diagnosis may enable risk stratification of people early in the condition and provide greater opportunities for interventions to reduce the risk of complication associated morbidity and mortality for this increasing population of young type 2 diabetes patients. Younger people also pose a significant challenge for clinicians who should be aware of these compounding pathologies of natural ageing and premature vascular ageing associated with young age type 2 diabetes patients.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):369–376
https://doi.org/10.1007/s13410-021-00923-x

REVIEW ARTICLE

The association between dairy products consumption with risk of type 1 diabetes mellitus in children: a meta-analysis of observational studies

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Received: 22 May 2020 / Accepted: 11 January 2021 / Published online: 26 January 2021

Abstract

Aim The cornerstone of diabetes management is the diagnosis of its risk factors and then applying appropriate therapy. The aim of the study was to find if dairy consumption increases the risk of T1DM in children.

Method A systematic search of PubMed and Scopus was done up to 22 February 2020. The overall estimates and their 95% confidence intervals were calculated using a random effects model.

Result Our meta-analysis was performed on 7 studies. We extracted 14 effect sizes for T1DM risk. The analysis indicated that dairy consumption increased the risk of T1DM (RR: 1.04, 95% CI: 1.01, 1.08), with high heterogeneity ($I^2 = 76.6\%$, P heterogeneity < 0.001).

Conclusion This meta-analysis showed a significant association between the consumption of dairy products and increased risk of T1DM with considerable heterogeneity. Further, longitudinal studies are needed to determine the causal relationship between dairy products and T1DM occurrence.

Keywords Dairy consumption · T1DM · Children · Meta-analysis

Introduction

Although type 1 diabetes mellitus (T1DM) can be diagnosed at any age, it is one of the most prevalent diseases in children [1]. It is reported that about 79,000 children aged 14 or under the age of 14 years are suffering from T1DM worldwide annually [2]. The peak of T1DM occurrence is between 5 and 7 years of age and at or near puberty [3]. T1DM is correlated with major chronic disease risk factors and increases morbidity and mortality [4, 5]. Death under 30 years usually results due to acute complications of diabetes, including diabetic ketoacidosis and hypoglycemia [6]; and cardiovascular disease is the major reason for death later in life [5, 6]. The basis of diabetes care is the identification of the risk factors and then using the appropriate treatment [7]. Genetics and exposure to environmental factors [8] may play a major role in the occurrence of type 1 diabetes. Among environmental risk factors, a significant one that may modify the risk of type 1 diabetes in early childhood is dietary quality. Limited breastfeeding duration and early exposure to complex dietary proteins are identified as risk factors for advanced beta-cell autoimmunity or type 1 clinical diabetes [6]. Supplementing breast milk with a highly hydrolyzed milk formula would reduce the cumulative incidence of diabetes-associated autoantibodies in such children [9]. All the above are factors that researchers will also investigate in order to evaluate the function of diet during early infancy and development of T1D, as they may intervene and serve as confounders as possible. However, the correlation of dairy products with health outcomes in children is not well-understood [10]. Also, there are inconsistencies about the impact of dairy on diabetes [11].

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Therefore, the purpose of this research was to explore this main question, Can dairy intake increase the risk of T1DM? We aim to determine the association between dairy products intake and T1DM risk using observational and cohort evidences.

Method

Search strategy

We systematically searched PubMed and Scopus up to 22 February 2020 with the following keywords: ((dairy product[tiab] OR milk[tiab] OR yogurt[tiab] OR cheese[tiab] OR kefir[tiab] OR butter[tiab] OR cream[tiab] OR "Dairy Products"[Mesh] OR Milk[Mesh] OR Yogurt[Mesh] OR Cheese[Mesh] OR Kefir[Mesh] OR Butter[Mesh] OR "Ice Cream"[Mesh]) AND ("type 1 diabetes"[tiab] OR "type 1 diabetic"[tiab] OR "type 1- diabetes mellitus"[tiab] OR "diabetes mellitus"[tiab] OR "diabetic patients"[tiab] OR DM[tiab] OR T1DM[tiab] OR TIDM[tiab] OR "insulin dependent"[tiab] OR IDDM[tiab] OR T1D[tiab] OR "Diabetes Mellitus, Type 1"[Mesh] OR "Diabetes Mellitus"[Mesh]) AND ("population-based" OR prospective OR "case control" OR longitudinal OR follow-up OR cohort OR retrospective OR nested OR "Longitudinal Studies"[Mesh] OR "Prospective Studies"[Mesh] OR "Case-Control Studies"[Mesh] OR "Cohort Studies"[Mesh] OR "Retrospective Studies"[Mesh])). Besides, a hand search of references of the published papers was done to detect other relevant articles. In Fig. 1, the details of the search strategy are illustrated. We also performed the systematic search for the second time in order to find any recent possible articles on 20 May 2020.

Eligibility criteria

Two trained reviewers (MSH and LSH) separately examined the eligibility of the studies twice. Studies were included if they met the following criteria: (a) observational study design; (b) involved subjects with <18 years old; (c) adequate information about any types of dairy products and the risk of type 1 diabetes mellitus; (d) publications that had provided estimates of relative risks (RRs) (odds ratios (ORs), hazard ratios (HRs), or rate ratios) with corresponding 95% confidence intervals (CIs); and (e) published in English.

Exclusion criteria

Totally, 1457 articles were found in our initial search. We excluded 856 articles by reading the title and abstract. The other 194 papers were excluded because of the following reasons: age > 18 ($n = 84$), type 2 mellitus ($n = 3$), review studies ($n = 4$), and the article without sufficient data for outcomes ($n = 103$).

Data extraction

Two independent reviewers (MSH and FDj) extracted the data. Any disagreements and differences were resolved by the study supervisor (SS-b), if necessary. The following information of studies was extracted: the first author's last name, date of publication, country, participants' age range, gender, sample size, number of cases, duration of follow-up, method of measurement of exposure and outcome, comparisons, and ORs or RRs for type 1 diabetes mellitus.

Quality assessment

The quality of included studies was evaluated by means of the Newcastle-Ottawa Scale [12]. For cohort and case-control studies that were included in the analysis, we used their own specific methods. The NOS allocates a maximum of nine points to each study: four for selection, two for comparability, and three for assessment of outcomes (nine represented the highest quality). Any inconsistencies were set by discussion.

Statistical analysis

Random effects models were applied in the current meta-analysis. Therefore, the overall effect size was calculated. Between-study heterogeneity which refers to the variation in study outcomes among studies was assessed using Cochran's Q test and I2 statistic. Sensitivity analysis was conducted to examine changes in pooled effect size when one study had been removed. Publication bias was assessed by visual inspection or using Egger's regression asymmetry test. All statistical analyses were done through Stata software, version 12.0 (Stata Corp LP, College Station, TX, USA). A p value of 0.05 was considered as statistical significance.

Results

Study selection

We found 1457 publications in our initial search. Of those, 400 records were duplicates. We reviewed titles and abstracts of all remaining publications, and as a result, another 856 publications were excluded, yielding 201 studies for full text assessing (Fig. 1). Of those, 7 publications provided sufficient information for the present meta-analysis. Thus, 6 case-control studies [13–18] and 1 cohort [19] with 5067 participants were included in the final analyses.

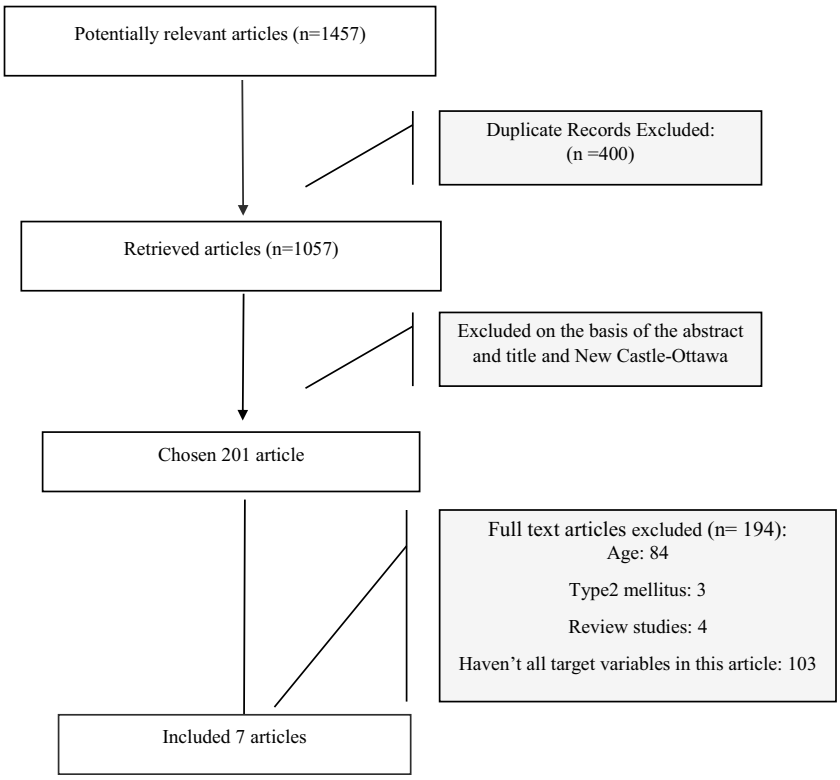

Fig. 1 Flow chart of the number of studies identified and selected into the meta-analysis

Characteristics of the included studies

Most included studies reported the association of dairy products consumption and risk of type 1 diabetes in children, and only one study assessed the risk of beta-cell autoimmune as an outcome.

General characteristics of the studies are provided in Table 1. These studies were published between 1991 and 2012 and were conducted in Finland [16–19], Germany [15], Sweden [13], and the UK [14]. All of the studies included both genders. The exposure assessment tool in six studies was the dairy products consumption questionnaire, and only one study was relied on 3-day food recall. All studies were at high quality (≥7 stars) on the basis of the Newcastle-Ottawa Scale (Table 2).

Our meta-analysis was performed on 7 studies. We extracted 14 effect sizes for type 1 diabetes risk. The overall effect of an association between dairy products consumption and risk of T1DM is illustrated in Fig. 2. The analysis indicated that dairy products consumption increased the risk of T1DM (RR: 1.04, 95% CI: 1.01, 1.08), with high heterogeneity, ($I^2 = 76.6\%$, P heterogeneity < 0.001). Based on the visual inspected funnel plot (Fig. 3) and Egger test, the studies did not have publication bias ($p = 0.161$). Sensitivity analysis results showed that excluding any of the studies was not significantly changed.

Discussion

The current meta-analysis revealed a significant linkage on the consumption of dairy products and increased risk of T1DM in children with considerable heterogeneity. In 2006, Multinational Project for Childhood Diabetes (DIAMOND) was indicated that Finland, Sweden, and the UK are the first, third, and fifth, respectively, with the very high incidence of childhood type 1 diabetes, and Germany was classified as a high-risk group [20]. Culturally, racial variation in communities of European origin has shown a higher incidence compared to non-Europeans but may cause these differences, particularly among long-term immigrants to European countries [21]. The most important risk factors for type 1 diabetes as autoimmune diabetes are genetic, family history, and environment [22]. Although the evidences proved that the diet is a potential risk factor for the induction of diabetes autoantibodies in children [17, 23], whether dairy products protect against T1DM or not is a controversial issue. Surely, kind of milk consumption pattern and specially fresh cow milk in the north European population (Finland, Sweden, the UK) [24] is an important factor in diabetes incidence and prevalence. Previous epidemiological data have shown the relationship between dairy product consumption and risk of T1DM. In line with our study, Suvi et al. found that high intakes of dairy products during childhood may be diabetogenic in children with type 1 diabetes [16]. In 2018, Canada Clinical Practice published guidelines about reducing the risk of developing

Table 1 The characteristics of included studies of dairy product intake and type 1 diabetes risk1

First author, year, country	Design	Gender	Sample size	Age range (year)	Exposure	Exposure assessment	Quantity	Effect size (95% CI)	Matching variables	Score
Virtanen et al., 2000, Finland	Nested case control	Both	35 (case), 254 (control)	1.8–16.2	Childhood milk and sour milk consumption	Questionnaire	<3 (glasses/-day)	3.24 (1.2, 8.7)	Sex, age	9
Marshall et al., 2004, UK	Case control	Both	196 (case), 381 (control)	0–16	Pre-school milk consumption	Structured interview	>1 vs. ≤1 (pint/day)	0.567 (0.395, 1)	Sex, age	9
Rosenbauer et al., 2007, Germany	Case control	Both	253 (case), 196 (control)	0–5	Current cow's milk consumption	Questionnaire or telephone interview	< 200 ml/d	0.85 (0.61, 1.18)	Sex, age	8
Virtanen et al., 1994, Finland	Case control	Both	86 (case), 86 (control)	0–14	Current milk	Questionnaire	>3 (glasses/-day)	0.95 (0.41, 2.17)	Sex, age	7
Rosenbauer et al., 2007, Germany	Case control	Both	353 (case), 328 (control)	0–5	Current cow's milk consumption	Questionnaire or telephone interview	≥ 200 ml/d	0.69 (0.51, 0.95)	Sex, age	7
Virtanen et al., 1994, Finland	Case control	Both	415 (case), 415 (control)	0–14	Current milk	Questionnaire	>3 (glasses/-day)	2.33 (0.86, 6.35)	Sex, age	7
Virtanen, 2012, Finland	Nested case control	Both	232 (case), 926 (control)	1–10.5	Fresh milk	3-day food record	750 g/d	1.05 (1, 1.1)	Birth date, sex, area, genetic risk	9
Virtanen, 2012, Finland	Nested case control	Both	232 (case), 926(control)	1–10.5	Sour milk	3-day food record	750 g/d	1.03 (0.97, 1.09)	Birth date, sex, area, genetic risk	9
Virtanen, 2012, Finland	Nested case control	Both	232(case), 926 (control)	1–10.5	Cheese	3-day food record	750 g/d	0.99 (0.87, 1.12)	Birth date, sex, area, genetic risk	9
G. Dahlquist, 1991, Sweden	Case control	Both	339 (case), 528 (control)	0–4	Frequency of intake of cow milk	Questionnaire	T1 vs T3	3 (1.07, 8.36)	Age, sex, country	7
G. Dahlquist, 1991, Sweden	Case control	Both	339 (case), 528 (control)	5–9	Frequency of intake of cow milk	Questionnaire	T1 vs T3	3.13 (1.49, 6.54)	Age, sex, country	7
G. Dahlquist, 1991, Sweden	Case control	Both	339 (case), 528 (control)	10–14	Frequency of intake of cow milk	Questionnaire	T1 vs T3	2.03 (1.24, 3.33)	Age, sex, country	7
G. Dahlquist, 1991, Sweden	Case control	Both	339 (case), 528 (control)	0–14	Frequency of intake of cow milk	Questionnaire	T1 vs T3	2.03 (1.34, 3.07)	Age, sex, country	7
Virtanen et al., 1998, Finland	Cohort	Both	725 (case)	0.4–24.9	Childhood milk and sour milk consumption	Questionnaire	<3 (glasses/-day)	2.75 (0.9, 8.4)	–	9

diabetes, and suggested nonlinear inverse associations were observed for total dairy products and yogurt, with most of the benefit being observed when increasing the intake of total dairy products from little to no dairy up to 300 to 400 g/day or yogurt up to 120 to 140 g/day, above which there was no further benefit [25].

As our knowledge, accumulating evidence-supported dairy products intake can increase the risk of type 1 diabetes [14, 15, 17, 18, 26]. Numerous mechanistic pathways suggested about the correlation between dairy products consumption and T1DM risk [27]. Most dairy products are milk based [28]; therefore, we have tried to point the mechanisms for the impact of milk on T1DM risk. Moreover, a series of studies have shown that children with newly diagnosed type 1 diabetes had elevated levels of antibodies, in particular to food antigens, lactose intolerance, and cow's milk proteins [17, 29–32]. The role of genetics, as one of other risk factors in T1DM occurrence, has been of recent interest. T1DM as a chronic immune-mediated disease with a subclinical prodromal period is characterized by selective loss of insulin-producing β-cells in the pancreatic [16] islets in genetically susceptible subjects. Auto-reactive T cells, CD4 and CD8 cells, and a series of auto-antigens like glutamic acid decarboxylase (GAD) have been implicated as active players in β-cell destruction [19, 33]. The issue of whether there is any primary autoantigen in T1D has remained controversial. Given that there are two major HLA haplotypes conferring disease susceptibility, i.e., the DR3–DQ2 haplotype and the DR4–DQ8 haplotype, one may assume that there will be at least two primary antigens in T1D [16, 22, 34]. The first signs of β-cell autoimmunity might appear already during the first year of life [33]. The enormous studies reported that a strong connection between the longitudinal consumption of cow milk in the children and the development of advanced β-cell autoimmunity is related to several previous case-control and cohort results with endpoints varied from early pre-type 1 diabetes to clinical disease [16, 19, 35]. Lamb et al., clearly explained that higher intakes of cow's milk may promote progression to type 1 diabetes in children with autoimmune islets [36], an effect that could be induced by certain fatty acids in cow's milk and meat, such as myristic [34]. Also, lipid-mediated signals can play an important role in lipotoxicity induced by fatty acids [37]. A nested case control determined that there was no relationship between the consumption of sour milk products and cheese with advanced β-cell autoimmunity [17]. In this article, the mechanism of the effect of sour milk and cheese on T1DM was mentioned kind of protein that is partly hydrolyzed to smaller peptides and amino acids. Familial history is another risk factor for type 1 diabetes [38]. Parkkola's article indicated the children with familial type 1 diabetes have an autoantibody profile, implying similar pathogenic disease mechanisms [39] Further, the duration of breastfeeding has the potential for being T1DM [40].

The recent trial published that cow's milk does not play a critical role in the development of type 1 diabetes [41]. It was reported that camel milk is safe and efficacious in improving long-term glycemic control, with a significant reduction in the doses of insulin in type 1 diabetic patients [42]. To dispose the controversy in evidence with about effect of cow milk on diabetes mellitus [16, 19, 35, 41], a systematic review published in 2017 supported that consumption of camel milk can decease blood sugar and insulin resistance [43]. Another hypothesis was discussed by Sørensen et al. that found an association between higher serum 25-hydroxyvitamin D in late pregnancy and lower risk of type 1 diabetes in

Table 2 Application of methodology criteria to case-control studies

First author, year	Study design	Selection	Comparability	Exposure	Outcome	Total
Virtanen (2000)	Nested case control	****	**	***		9
Marshall (2004)	Case control	****	**	***		9
Rosenbauer (2007)	Case control	****	**	**		8
Virtanen (1994)	Case control	***	**	**		7
Rosenbauer (2007)	Case control	***	**	**		7
Virtanen (1994)	Case control	***	**	**		7
Virtanen (2012)	Nested case control	****	**	***		9
Virtanen (2012)	Nested case control	****	**	***		9
Virtanen (2012)	Nested case control	****	**	***		9
Dahlquist (1991)	Case control	***	**	**		7
Dahlquist (1991)	Case control	***	**	**		7
Dahlquist (1991)	Case control	***	**	**		7
Dahlquist (1991)	Case control	***	**	**		7
Virtanen (1998)	Cohort	****	**		***	9

NOS Newcastle-Ottawa Scale

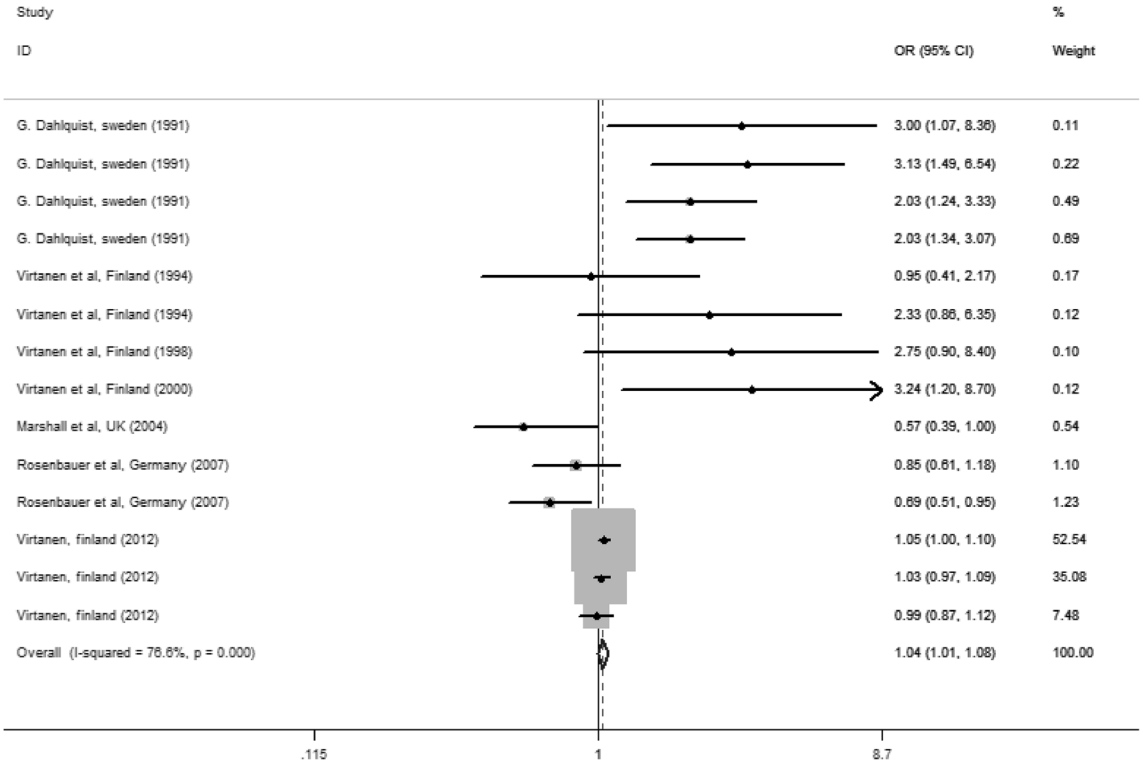

Fig. 2 Forest plot of the association between dairy product consumption with risk of type 1 diabetes in children. OR stands for odds ratio

offspring [44]. Dairy products are known to be rich in vitamin D. This has been demonstrated that vitamin D changes the balance of the T cell response in the body towards downregulation of the T-helper-1 immune response [45] and beneficial factor as it plays an important function in regulating the immune system, as well as diabetes-relevant metabolic pathways.

Our meta-analysis has several strengths; for example, our search strategy was very accurate and covered multiple databases. Further, our statistical examinations indicated no evidence of publication bias in our analyses, and, finally, to the best of knowledge, this is the first meta-analysis to be performed in this regard, although some limitations exist. At first, these studies that are selected to be included have heterogeneous risk measurement methods. The high heterogeneity is another limitation that could impact our findings powerfully. Second, owing to the small number of studies, there was no ability to better examination of the association between childhood dairy products consumption with risk of T1DM. Third, most of the studies presented some form of bias, and thus, it is hard to reach a certain conclusion. Another limitation that is necessary to mention is the quantity of milk that is different in various studies.

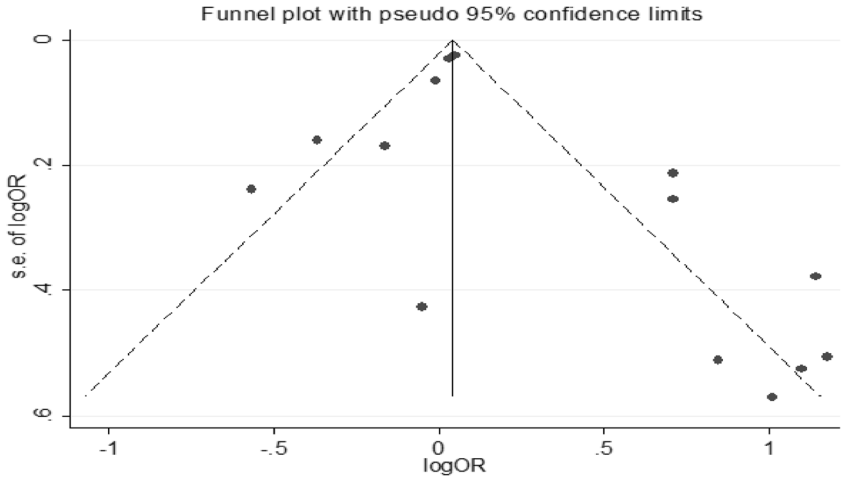

Fig. 3 Funnel plot for dairy product intake and type 1 diabetes risk

Conclusion

We found a positive association between dairy products consumption and odds of type 1 diabetes mellitus in children. Our team suggested that these findings need to be confirmed by larger trials in order to conclusively determine any relationship between dairy products intake and T1DM.

Author contribution MSH and SS-b designed the study. MRA and MSH did the literature search and screening data. FDj, MSH, and LSH performed data extraction and quality assessment, independently. FDj, LSH, FSH, and HSH analyzed and interpreted data and wrote the manuscript. SS-b finalized the manuscript and supervised the study. All authors read and approved the final manuscript.

Compliance with ethical standards

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

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):377–382
https://doi.org/10.1007/s13410-021-00924-w

SHORT ARTICLE

Is there a need to reconsider the use of metformin in COVID-19 patients with type 2 diabetes mellitus?

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Received: 2 December 2020 / Accepted: 11 January 2021 / Published online: 2 March 2021

Abstract

Introduction Diabetes has been linked with poorer outcomes in coronavirus disease (COVID-19) patients. However, the question to whether continue or withdraw metformin therapy in COVID-19 patients with type 2 diabetes mellitus remains contentious. This study aims to investigate the association between metformin and poor COVID-19 outcomes.

Methods Eligible studies published up to 21 October 2020 were included and appraised for validity, importance, and applicability. The included studies were further ranked according to the level of evidence (LOE).

Results Nine studies were included for further assessments, of which seven studies stated that metformin was not associated with poor COVID-19 outcomes (LOE II-V), while the other two with poorer designs stated otherwise (LOE V). Although metformin may increase the risk of developing acidosis and lactic acidosis (LOE IV), the observed risks were more accentuated in patients with severe COVID-19 disease or kidney impairment and in patients with > 2 daily metformin doses. Interestingly, one study revealed that metformin may even yield therapeutic role in reducing the risk of COVID-19 mortality (LOE II), although further studies are required to confirm these findings.

Conclusions Our findings indicated that metformin may be safely continued in COVID-19 patients. The benefit of metformin therapy with simultaneous continuous monitoring of COVID-19 severity and kidney function may outweigh the risks of lactic acidosis, of which incidence is relatively rare.

Keywords COVID-19 · Metformin · Type 2 diabetes mellitus · Prognosis

Clinical scenario

A 45-years-old man with a 5-year history of type 2 diabetes mellitus (T2DM) came to the hospital with mild-to-moderate coronavirus disease 2019 (COVID-19) infection. His T2DM had been well-controlled by metformin 500 mg bid, and his laboratory panel revealed a HbA_{1C} level of 43 mmol/mol and a random blood glucose level of 8.6 mmol/L, while the other tests were unremarkable.

Recent consensuses have recommended diabetic patients to temporarily cease metformin treatment during the course of COVID-19 disease [1, 2]. However, the patient expressed his concerns that he wishes to stay with the current medication. Acknowledging the patient's wishes, the general practitioner wonders whether metformin had any effect on COVID-19 outcomes.

Introduction

The disease burden of the COVID-19 pandemic has perpetually surged with millions of cases and deaths. Diabetes has been strongly associated with poor prognosis in COVID-19 patients. [3] However, the management strategy of diabetic COVID-19 patients remains contentious as previous reports showed conflicting results [1, 4]. This raises concerns about the safety of metformin, the gold standard first-line antidiabetic treatment [5], in treating COVID-19-infected T2DM patients. Hence, we studied the evidence linking metformin and poor COVID-19 outcomes in T2DM patients, thus

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providing guidance for clinicians to make real-time treatment decisions in such cases.

Clinical question

Is metformin therapy associated with poor prognosis in COVID-19-infected T2DM patients?

Methods

Search strategy and eligibility criteria

We comprehensively searched PubMed, Embase, CENTRAL, EBSCO MEDLINE, CINAHL, and gray literature databases (Google Scholar, ProQuest, Scopus, preprints) for studies investigating the effect of metformin on COVID-19 outcomes, including mortality, severity, and other prognostic surrogates, published up to 21 October 2020 using keywords listed on Supplementary Table S1. Records were screened against the eligibility criteria listed on Fig. 1, and no language restrictions were applied.

Critical appraisal

The included studies were further appraised for validity, importance, and applicability using a standardized tool [8]. Critical appraisal and literature searches were conducted by two pairs of independent adjudicators (GL and RAR, EW and IPS), with discordant judgments resolved by consensus between the authors. Lastly, the studies were ranked according to their level of evidence (LOE) [9].

Results

The initial search yielded 1094 records, of which 16 were retrieved following thorough reviews. Among them, two systematic reviews (SRs) were excluded as one had overlapping studies with smaller sample size [10], and another one pooled the unadjusted rather than the adjusted estimates [6]. In addition, five observational studies were also excluded as they were already included in the SR. Therefore, nine studies comprising of one SR of cohort studies, six retrospective cohort studies, one case series, and one case-control study qualified

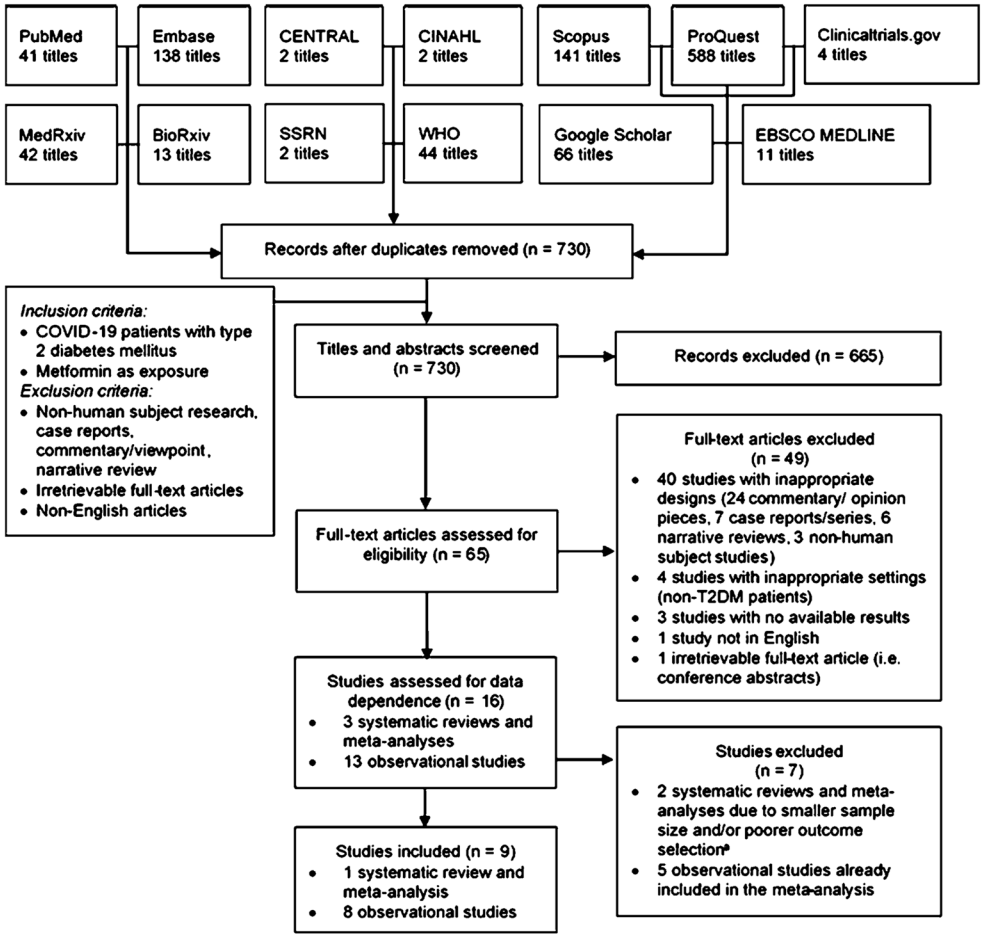

Fig. 1 Diagram flow illustrating the literature search and selection. [a]Although one of the studies included in a systematic review by Hariyanto et al. [6] was not included in the systematic review by Kow et al. [7], the study only pooled unadjusted rather than adjusted estimates; thus we decided to exclude the study by Hariyanto et al. [6] and assessed the non-duplicate study separately. CENTRAL, Cochrane Central Register of Controlled Trials; CINAHL, Cumulative Index to Nursing and Allied Health Literature. COVID-19, coronavirus disease 2019; SSRN, Social Science Research Network; T2DM, type 2 diabetes mellitus; WHO, World Health Organization COVID-19 Research Database

Table 1 Critical appraisal of the included observational studies

Author; year	Study design	Validity				Importance		Applicability		LOE[b,c,d,e]
		IR	F[a]	OB	A	Outcome [adjusted variables]	Estimates [95% CI]	Indirect.	Import.	
Cheng X; 2020 [14]	Retrospective cohort	✓	✓	✓	✓	Mortality [2–6, 8–10]	HR 1.65 [0.71–3.86] Severe: 1.56 [0.63–3.85]	✓	✓	IV[f]
						Acidosis [2–6, 8–10]	HR 2.73 [1.04–7.13] Severe: HR 3.82 [1.27–11.50]	✓	✓	IV[g,h]
						AHI [2–6, 8–10]	HR 1.02 [0.62–1.66] Mild: HR 1.62 [0.54–4.91] Severe: HR 0.81 [0.48–1.38]	✓	✓	IV[f]
						AKI [2–6, 8–10]	HR 0.65 [0.19–2.24] Severe: HR 0.47 [0.11–2.00]	✓	✗	IV[f]
						ARDS [2–6, 8–10]	HR 0.85 [0.61–1.17] Mild: HR 0.42 [0.04–4.65] Severe: HR 0.81 [0.57–1.16]	✓	✓	IV[f]
						DIC [2–6, 8–10]	HR 1.68 [0.26–10.90] Severe: HR 0.91 [0.10–7.98]	✓	✗	V[g]
						HF [2–6, 8–10]	HR 0.59 [0.41–0.83] Mild: HR 0.57 [0.27–1.19] Severe: HR 0.63 [0.42–0.96]	✓	✓	III
						Lactic acidosis [2–6, 8–10]	HR 4.46 [1.11–18.00] Severe: HR 5.65 [1.06–30.10]	✓	✓	IV[g,h]
Dashti H; 2020 [11]	Case-series	✓	?	✓	✗	Mortality	OR 1.87 [1.32–2.67][i,j]	✓	✗	V[k]
						Severity	OR 1.88 [1.54–2.28][i,j]	✓	✗	V[k]
						Hospitalization	OR 4.45 [3.84–5.17][i,j]	✓	✗	V[k]
Gao Y; 2020 [12]	Case-control	✓	✗	✓	✓	Severity [2, 3, 11, 12]	OR 3.96 [1.03–15.19]	✓	✓	V[g,h]
Goodall JW; 2020 [19]	Retrospective cohort	✓	✗	✓	✓	Mortality	HR 0.97 [0.75–1.25][i]	✓	✗	IV[l]
Izzi-Engbeaya C; 2020 [20]	Retrospective cohort	✓	✗	✓	✓	ICU or Mortality [2–4, 7, 13–18]	OR 0.94 [0.16–5.67]	✓	✓	V[g]
Mirsoleymani S; 2020 [21]	Retrospective cohort	✓	?	✗	✗	Mortality	RR 2.13 [0.95–4.77][i,j]	✗	✗	V[f,l,m]
Pérez-Belmonte LM; 2020 [13]	Retrospective cohort	✓	?	✓	✓	Mortality [1]	OR 1.16 [0.78–1.72] Metformin + DPP4i: OR 0.72 [0.39–1.27]	✓	✓	III
						Severity [1]	OR 1.05 [0.73–1.52] Metformin + DPP4i: OR 0.84 [0.49–1.41]	✓	✓	III
						In-hospital complications [1]	OR 1.17 [0.81–1.70] Metformin + DPP4i: OR 0.86 [0.50–1.47]	✓	✓	III
						Prolonged hospitalization [1]	OR 1.49 [0.96–2.33] Metformin + DPP4i: OR 1.17 [0.62–2.19]	✓	✓	III

Table 1 (continued)

Author; year	Study design	Validity				Importance		Applicability		LOE[b,c,d,e]
		IR	F[a]	OB	A	Outcome [adjusted variables]	Estimates [95% CI]	Indirect.	Import.	
Wang B; 2020 [22]	Retrospective cohort	✓	?	✓	✗	Mortality	OR 0.35 [0.01–3.08][i]	✗	✗	V[f,k,l]
						Hospitalization	OR 5.85 [0.69–278.29][i]	✗	✗	V[g,k,l]

[a] When adequacy of follow-up was not explicitly stated, limitations on the validity of follow-up domain were judged as serious when the loss to follow up rate was > 10%. [b] LOE of evidence may be upgraded by one level due to large effect size (RR > 2 or RR < 0.5) or by two levels due to very large effect size (RR > 5 or RR < 0.2). [c] LOE may be downgraded by one level each due to imprecision (wide CI: upper limit – lower limit of 95% CI > 1 [for increased risks] or > 0.8 [for decreased risks]), indirectness (study PICO not applicable to question's PICO), and poor study quality (validity score < 3). [d] LOE may be downgraded by two levels each due to very serious imprecision (very wide CI: upper limit – lower limit of 95% CI > 5 [for increased risks] or > 0.5 [for decreased risks]) or very poor study quality (validity score < 2). [e] LOE assessment starts with level III for cohort studies and level IV for case-series and case-control studies. [f] Downgraded by one level due to imprecision. [g] Downgraded by two levels due to imprecision. [h] Upgraded by one level due to large effect size. [i] Estimates were not adjusted for confounders. [j] Calculated from binary data. [k] Downgraded by one level due to poor study quality (validity score < 3). [l] Downgraded by one level due to indirectness. [m] Downgraded by two levels due to poor study quality (validity score < 2)

[1] Matched by PSM, [2] Age, [3] Sex, [4] C-reactive protein, [5] Aspartate transaminase, [6] Urea, [7] Red blood cells, [8] Creatinine, [9] Hospital site, [10] Blood glucose, [11] Lactate dehydrogenase, [12] Ethnicity, [13] Comorbidities (e.g., type 1 diabetes mellitus, active foot disease, stroke, ischemic heart disease, hypercholesterolemia, heart failure, hypertension, chronic obstructive pulmonary disease, cancer), [14] Other medications (e.g., insulin, GLP-1 mimetic, sulphonylurea, DPP4 inhibitor, number of anti-diabetic drugs, statin, ACE inhibitor, angiotensin receptor blocker), [15] Other laboratory parameters (viz., white cell count, hemoglobin, platelet count, neutrophil, lymphocytes, serum sodium) [16] On-diagnosis parameters (viz., eGFR, capillary blood glucose, respiratory rate, heart rate, NEWS score, oxygen saturation requirement), [17] Temperature, [18] Systolic and diastolic blood pressure

IR, inception and representative cohort; F, sufficient follow-up; OB, objective or blind outcome ascertainment; A, appropriate adjustment for important prognostic factors; ACE, angiotensin-converting enzyme; AHI, acute heart injury; AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; CI, confidence interval; DIC, disseminated intravascular coagulation; DPP4i, dipeptyl peptidase 4 inhibitor; eGFR, estimated glomerulus filtration rate; GLP-1, glucagon-like peptide-1; ICU, intensive care unit; Import., importance; Indirect., indirectness of population; HF, heart failure; HR, hazard ratio; LOE, level of evidence; NEWS, National Early Warning Score; OR, odds ratio; RR, relative risk

Table 2 Critical appraisal of the included systematic review

Author; year	Study design	Validity					Importance			LOE[a,b,c]
		PICO	F	A1	A2	T	Outcome	Estimates [95% CI]	Heterogeneity	
Kow CS; 2020 [7]	SR of cohort studies	✓	✗	✓	✓	✓	Mortality	OR 0.62 [0.43–0.89]	I^2 =29%; p=0.23	II[d,e]

[a] LOE of evidence may be upgraded by one level due to large effect size (RR > 2 or RR < 0.5) or by two level due to very large effect size (RR > 5 or RR < 0.2). [b] LOE may be downgraded by one level each due to imprecision (wide CI: upper limit – lower limit of 95% CI > 1 [for increased risks] or > 0.8 [for decreased risks]), indirectness (study PICO not applicable to question's PICO), inconsistency (I^2 > 50% or p < 0.10), and poor study quality (validity score < 4). [c] LOE may be downgraded by two levels each due to very serious imprecision (very wide CI: upper limit – lower limit of 95% CI > 5 [for increased risks] or > 0.5 [for decreased risks]), very serious inconsistency (I^2 > 75% or p < 0.01), or very poor study quality (validity score < 2). [d] LOE assessment starts with level III. [e] Upgraded by one level due to systematic review design

✓, no serious limitation; ✗, serious limitation; PICO, clear statement of review question; F, comprehensive strategy to find eligible studies; A1, appropriate eligibility criteria; A2, appropriate risk of bias or methodological quality assessment; T, appropriate strategy to total up the summary of findings; CI, confidence interval; I^2, I-squared value; LOE, level of evidence; OR, odds ratio; SR, systematic review

for inclusion. Details on the literature search process are illustrated by Fig. 1.

We discovered that most studies stated that metformin use was not associated with poor COVID-19 outcomes, whereas only two studies stated otherwise [11, 12]. However, both studies were underpowered to determine causalities due to poorer designs. Furthermore, one study [11] did not adjust for important confounders, and another one [12] yielded excessively wide confidence intervals (CIs), thus further diminishing the LOE (LOE V; Table 1). Among the included studies, four were deemed not clinically important as the results were not adjusted for confounders, while another one was judged inapplicable as they only included COVID-19 patients with multiple myeloma.

Interestingly, Kow et al. discovered that metformin use was associated with a lower rate of COVID-19 mortality (odds ratio [OR] 0.62, 95% CI 0.43–0.89, LOE II; Table 2) [7]. Although some study-specific estimates were inconsistent, the model only yielded non-significant low heterogeneity (I^2 = 29%, p = 0.23) [7], hence resulting in the judgment of LOE II. This was further corroborated by Pérez-Belmonte et al. who stated that either metformin monotherapy or its combination with dipeptyl peptidase-4 inhibitor remained safe for diabetic COVID-19 patients (LOE III) [13]—further ascertaining the findings. On the other hand, Cheng et al. stated that metformin was associated with increased risk of acidosis and lactic acidosis (LOE IV), although the effect was more accentuated in patients with severe COVID-19 infection (oxygen saturation < 93% or neutrophil-to-lymphocyte ratio > 3.13) or kidney impairment (estimated glomerular filtration rate < 60 mL/min/1.73 m^2), and in patients taking > 2 daily metformin doses [14] (Table 1).

Discussion

The question to whether continue or adjourn metformin therapy in COVID-19 patients remained relevant during the pandemic. Recent consensus has recommended postponing metformin therapy due to the risk of dehydration and lactic acidosis [1, 2]; however, some others argued that the impending benefits of metformin may outweigh the risks of developing adverse events as their incidence is relatively rare [15, 16]. According to our findings, it is plausible to continue metformin therapy while simultaneously increasing vigilance on the patient's COVID-19 severity and kidney function. Furthermore, our patient was not at higher risks of developing acidosis as his COVID-19 disease was not severe and his renal panel was unremarkable. In addition, his daily metformin doses were still within the safe limit [14], thus further inclining towards the use of metformin. Furthermore, it is also important to heed to patients' preference as it may substantially affect treatment adherence and outcome. [17] This is saliently important considering that poorer glycemic control has been associated with poor COVID-19 prognosis [18], implying that the benefits of continuing metformin may also outweigh the risk of noncompliance. Despite the favorable findings, further prospective studies are required to establish a firm evidence as the included studies yielded predominantly low-to-moderate LOE.

Further extrapolating our findings, recent reports have suggested the potential therapeutic role of metformin in COVID-19 [7, 10]. Several mechanisms have been hypothesized, including its ability to induce phosphorylation of angiotensin-converting enzyme-2 receptors to inhibit viral entry and replication [10]. Furthermore, metformin yields potent antioxidative, anti-inflammatory, and pulmoprotective properties, thus further suggesting the impending benefits [10]. Nonetheless, further studies are required to substantiate these premises.

Conclusion

In summary, our findings indicated that metformin may be safely continued in diabetic COVID-19 patients. However, several factors need to be considered when assessing the

benefits and risks of continuing metformin therapy, including COVID-19 severity, kidney function, and glycemic control. Rather than indifferently deferring metformin therapy, it is more imperative to continuously monitor the patient's COVID-19 disease course and renal function, deterioration of which may predict poor COVID-19 outcomes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-021-00924-w.

Authors' contribution All authors contributed to idea conception, data abstraction, and manuscript draft. GL developed the methodology and visualized the data. GL and JDB reviewed and edited the initial manuscript draft. All authors have read and approved the final manuscript for submission.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability The data that supports the findings of this study are available in the supplementary material of this article.

Declarations

Conflict of interest The authors declare no conflict of interest.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):383–388
https://doi.org/10.1007/s13410-021-00930-y

ORIGINAL ARTICLE

Epidemiological characteristics and outcomes of COVID-19 in diabetic versus non-diabetic patients

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Received: 23 November 2020 / Accepted: 20 January 2021 / Published online: 9 February 2021

Abstract

Objectives and background People with diabetes (PWD) are one of the high-risk groups for coronavirus disease 2019 (COVID-19) infection, increasing the disease mortality. This study was aimed to compare the epidemiological characteristics and outcomes of COVID-19 in diabetic versus non-diabetic individuals.

Methods In this retrospective observational study, the epidemiological characteristics of the two groups of diabetic (n=1365) and non-diabetic (n=15,026) subjects with definite diagnosis of COVID-19 in the southwestern region of Iran were compared. All clinical signs and comorbidities of the patients were evaluated. Chi-square test was used to examine the differences in qualitative variables between diabetic and non-diabetic groups.

Results Of 16,391 enrolled subjects, 8.3% had diabetes, and 28.3% of COVID-19-related deaths occurred in diabetics. Also, the mortality rate among diabetics was reported as 14.3%. The average age of diabetic patients and non-diabetic patients was 59 and 37 years, respectively. The odds of fever, cough, shortness of breath, headache, and underlying diseases, such as hypertension, cardiovascular disease, chronic lung disease, immune deficiency, and hyperlipidemia, were significantly higher in diabetic patients than in non-diabetics.

Conclusion Diabetes is associated with increased mortality rate in patients with COVID-19 and is considered as a major risk factor for COVID-19 infection, posing a major public health challenge for health policymakers in managing and controlling the disease. Therefore, development of prevention and treatment strategies aimed at reducing COVID-19 morbidity and mortality in diabetes patients is of significant importance.

Keywords COVID-19 · Diabetes · Mortality

Introduction

Diabetes is the fifth leading cause of death throughout the world (approximately 3 million deaths per year), which is mainly caused by defects in either insulin secretion or action. The prevalence of diabetes has dramatically increased over the past two decades and is projected to increase from 285 million in 2010 to 438 million in 2030 [1]. Also, according to the

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World Health Organization, the prevalence of diabetes in Iran will increase from 3 million in 2010 to 6 million in 2030 [2]. People with diabetes (PWD) are one of the high-risk groups for Coronavirus Disease 2019 (COVID-19) due to their weakened immune system [3] .

According to the World Health Organization, COVID-19 highlighted on December 30, 2019 in Wuhan, China, and quickly infected many Chinese people [4]. On January 30, 2020, WHO identified corona as a global public health concern [5]. The family of coronaviruses is the cause of a variety of well-known diseases occurring in humans, ranging from common cold to Middle Respiratory Syndrome (MERS) and Acute Severe Respiratory Syndrome (SARS), and currently COVID-19 as a new problematic member of this family [6, 7]. Symptoms of the virus range from mild to severe, including fever, cough, and difficulty breathing [8]. PWD are at higher risk of infection, especially flu and pneumonia, and with proper blood sugar control, the risk can be reduced among these individuals. In fact, it was observed that diabetes was a leading cause of death in patients with the 2009 pandemic influenza (H1N1), SARS, coronavirus, and MERS [9]. In a study conducted in Wuhan on 140 patients with COVID-19, diabetes was not a risk factor for severe COVID-19 [10]. However, another study on 150 patients (68 deaths and 82 recovered patients) in Wuhan showed diabetes as an important predictor of COVID-19 [11]. Analysis of 11 studies on patients with COVID-19 showed that hyperglycemia and diabetes were not predictors of severe COVID-19 [12]. However, among 72,314 reported cases of COVID-19 by the China Centers for Disease Control and Prevention, mortality in PWD showed an increasing trend (2.3% and 7.3% of PWD in general) [13].

Due to the fact that limited information about the complications of COVID-19 in PWD is unknown, the present study was carried out to answer the question whether COVID-19 is more severe in PWD with higher mortality rate. This study was aimed to investigate and compare the epidemiological characteristics and outcomes of COVID-19 in patients with diabetes mellitus and non-diabetic patients.

Methods

This retrospective observational study was performed on 16,391 patients with COVID-19 who were under care in the southwestern region of Iran (Khuzestan province) from March to September 2020. The total population of this region was estimated 627,970 based on the annual growth, using the databases of province health centers and the national census of 2020. Confirmation of definite COVID-19 cases by RT-PCR was performed using nasal and throat swab samples. Patients with normal vital signs (pulse, respiration rate, and blood pressure) with saturated oxygen levels above 93% and mild symptoms were sent to home quarantine and monitored daily by health professionals. Patients were hospitalized with severe symptoms such as chest pain, shortness of breath, and a saturated oxygen level of less than 90%. Patients with saturated oxygen levels between 90 and 93% were admitted to hospital or undergoing home quarantine, depending on the physicians' opinion and other clinical conditions. In the study area, there were only two hospitals that, like other medical centers in the country, performed all stages of testing and hospitalization of patients according to the protocols and national guidelines of corona management. Also, according to the patients' clinical condition, according to the doctor's prescription, a CT scan was performed for some patients to diagnose the extent of lung involvement.

All clinical and demographic information of patients, as well as the results of daily follow-up and the final outcome of each patient, were recorded by health experts. Duplicate samples have been identified and removed based on the national code of participants. Subjects were divided into two groups: diabetic group (n=1365) and non-diabetic group (15,026). First, demographic characteristics, symptoms, and other comorbid diseases were compared between diabetic and non-diabetic groups; then, the role of comorbid diseases in mortality and survival of all study subjects was compared. Finally, diabetic and non-diabetic patients were compared in terms of clinical symptoms and comorbid diseases. Variables used in this study include age, gender, final outcome (e.g., death and survival), symptoms (e.g., negative stenosis, fever, cough, lack of smell, lack of taste, fatigue, muscle pain, diarrhea, sore throat, and headache), and comorbidities, such as hypertension, diabetes, cardiovascular disease, kidney disease, liver disease, immune deficiency, high blood fats, and chronic lung disease.

Statistical analyses

Descriptive quantitative data were expressed as mean and median, and qualitative data were expressed as percentage and frequency. The distribution of continuous variables was performed using Kolmogorov-Smirnov test. Mann-Whitney test was also used to analyze abnormal data analysis. Chi-square test and Fisher's exact test were used to evaluate the differences between qualitative variables in the mortality of two study groups. Data analysis was performed using SPSS version 19.0. The significance level was set as 0.05.

Results

During the study period, a total of 16,391 cases of COVID-19 occurred in the cities affiliated to Abadan University of Medical Sciences. The average age of the patients was 38 years, with an interquartile range of 30–51 years. A total of 54.55% of the patients were male, and 62.60% (n=10,256)

had a history of contact with definite or suspected cases of COVID-19. Also, 4.21% of the patients (*n*=691) died, of whom 28.36% (*n*=196) had diabetes (Table 1). The most common symptoms displayed by subjects were fever (68.70%), cough (67.80%), and shortness of breath (49.30%). The most common comorbidities in all patients were hypertension (5.50%), cardiovascular disease (5%), chronic lung disease (1.90%), and chronic liver disease (1.70%) (Table 1).

Of all participants, 1365 (8.32%) were diabetic and 15,026 (91.68%) were non-diabetic. Also, 55.50% of diabetic patients were female, and 755 (55.30%) had a history of contact with definite or suspected cases of COVID-19. Compared with non-diabetic patients, diabetic patients were older, with an average age of 59 years (IQR: 49–67) vs. 37 years (IQR 29–49), and risk of comorbidities was higher in diabetic patients than in non-diabetic individuals, including hypertension as 418 (30.60%) vs. 478 (3.20%), heart disease as 266 (19.50%) vs. 549 (3.70%), chronic kidney disease as 72 (5.30%) vs. 213 (1.40%), chronic lung disease as 56 (4.10%) vs. 258 (1.70%), chronic liver disease as 13 (1%) vs. 27 (0.20%), immunodeficiency as 24 (1.80%) vs. 63 (0.40%), and hyperlipidemia as 18 (1.30%) vs. 34 (0.20%).

Fever, cough, and shortness of breath were significantly higher in diabetic patients compared with non-diabetic patients, and sore throat, chest pain, and reduced sense of taste and smell were significantly higher in non-diabetic patients than in diabetic patients. Diabetic patients had higher mortality (14.40% in diabetic patients vs. 3.30% in non-diabetic). The history of contact with definite or suspected cases of COVID-19 was higher in non-diabetic subjects (63.20% vs. 55.30%) (Table 1).

In addition, 15,700 patients (95.78%) survived from COVID-19. Compared with COVID-19 survivors, non-survivors were significantly older (median age of 66 years (IQR 55–76) versus 38 years (IQR 30–50)). Chronic diseases were significantly higher in non-survivors, including diabetes as 196 (28.40%) patients vs. 1169 (7.44%), hypertension as 143 (20.70%) vs. 753 (4.79%), heart disease as 142 (20.50%)

Table 1 The characteristics of patients with COVID-19 with or without diabetes

Variable	Total (*n*=16,391) Number (%)	Diabetes (*n*=1365) Number (%)	Non-diabetes (*n*=15,026) Number (%)	*p* value
Age, median (IQR)	38 (30–51)	59 (49–67)	37 (29–49)	<0.001
Gender				
Male	8940 (54.55)	607 (44.50)	8333 (55.50)	<0.001
Female	7446 (45.45)	758 (55.50)	6688 (44.50)	
Symptoms				
Fever	11258 (68.70)	1075 (78.80)	10183 (67.80)	<0.001
Cough	11106 (67.80)	1035 (75.80)	10071 (67.00)	<0.001
Dyspnea	8086 (49.30)	910 (66.70)	7176 (47.80)	<0.001
Muscular pain	1520 (9.30)	124 (9.10)	1396 (9.30)	0.801
Diarrhea	351 (2.10)	26 (1.90)	325 (2.20)	0.528
Nausea	436 (2.70)	39 (2.90)	397 (2.60)	0.636
Sore throat	1239 (7.60)	79 (5.80)	1160 (7.70)	0.010
Anorexia	78 (0.50)	6 (0.40)	72 (0.50)	0.839
Headache	618 (3.80)	57 (4.20)	561 (3.70)	0.270
Fatigue	54 (0.30)	3 (0.20)	51 (0.30)	0.460
Chest pain	121 (0.70)	4 (0.30)	117 (0.80)	0.045
Decreased sense of smell	378 (2.30)	10 (0.70)	368 (2.40)	<0.001
Decreased sense of taste	85 (0.50)	1 (0.10)	84 (0.60)	0.017
Comorbidities				
Hypertension	896 (5.50)	418 (30.60)	478 (3.20)	<0.001
Cardiovascular disease	815 (5.00)	266 (19.50)	549 (3.70)	<0.001
Immunodeficiency	87 (0.50)	24 (1.80)	63 (0.40)	<0.001
Chronic kidney disease	285 (1.70)	72 (5.30)	213 (1.40)	<0.001
Chronic pulmonary disease	314 (1.90)	56 (4.10)	258 (1.70)	<0.001
Chronic liver disease	40 (0.20)	13 (1.00)	27 (0.20)	<0.001
Hyperlipidemia	52 (0.30)	18 (1.30)	34 (0.20)	<0.001
Exposure to disease	10256 (62.60)	755 (55.30)	9501 (63.20)	<0.001
Mortality	691 (4.20)	196 (14.40)	495 (3.30)	<0.001

vs. 673 (4.28%), immunodeficiency as 14 (2.20%) vs. 72 (0.45%), chronic kidney disease as 43 (6.20%) vs. 242 (1.54%), and chronic lung disease as 31 (4.50%) vs. 283 (1.80%) (Table 2).

Of 1365 diabetic patients with COVID-19, COVID-19 non-survivors were considerably older than survivors (median age of 66 years (IQR 57–73) vs. 57 years (IQR 48–66)). The risk of heart disease was higher in non-survivors (56 patients (28.57%) vs. 193 patients (16.50%)). Gender, other comorbidities, and disease symptoms showed no significant difference with survived diabetic patients and non-survived patients (Table 3).

Discussion

This was a retrospective observational study, performed on 16,391 patients with COVID-19 in southwestern Iran. This study compared clinical features and mortality between people with type 2 diabetes and non-diabetic patients. Since the onset of COVID-19 pandemic, the disease has become widespread throughout the world, affecting a large number of people. The most vulnerable groups against COVID-19 are those with comorbid diseases, including diabetes. Various epidemiological evidences have shown that PWD are at higher risk for infectious diseases, such as measles and mumps [14]. Also, according to the results of previous studies, diabetes is one of the most common comorbidities associated with COVID-19. In our study, 8.3% of COVID-19 patients had type 2 diabetes. Other studies showed various percentages of COVID-19 patients with type 2 diabetes, as 20% [15], 52% [16], 24.9% [17], 14.5% [18], and 21.8% [19]. However, a number of studies have shown that the severity and complications of COVID-19 disease and the need for ICU stay were higher in diabetic patients than in non-diabetic individuals [9, 14, 15, 17, 20–22].

According to the results of our study, the median age of COVID-19 patients who had diabetes was higher than COVID-19 patients without diabetes (59 vs. 37), which was consistent with the results of many previous studies [17, 19, 23]. Since diabetes is a chronic disease and chronic diseases are more common at older age, which explains higher average age of COVID-19 patients with diabetes.

The results of our study showed that symptoms of COVID-19, including fever, shortness of breath, and cough, were mostly reported in diabetic patients than in non-diabetic patients, while chest pain, sore throat, and decreased sense of smell and taste were more common in non-diabetic COVID-19 patients. In a study conducted in China, fever, dry cough, and fatigue were the most common symptoms in diabetic COVID-19 patients [23], and another study showed cough and fever as the most common symptoms in these patients [24]. A systematic review study indicated that no significant differences in signs and symptoms of these differences may be due to variation in selected sample sizes in various studies, study population, and type of collected data [18]. As such, some studies have examined only patients with severe form of COVID-19, while others have examined patients with various severity of the disease. Undoubtedly, patients with different severities of the disease display different symptoms.

According to the results of our study, COVID-19-related mortality was higher in PWD than in non-diabetic individuals (14.4% vs. 3.3%). Also, a study performed on 46 cases of COVID-19 in Wuhan, China, showed that 42% of COVID-19 deaths occurred among PWD. However, small sample size of this study should be considered in assessing the validity of the results. In another study on 72,312 people in China, mortality rate was higher in diabetic patients compared with non-

Table 2 The baseline characteristics of survivors and non-survivors infected with COVID-19

Variable	Total (*n*=16,391) Number (%)	Survivors (*n*=15,700) Number (%)	Non-survivors (*n*=691) Number (%)	*p* value
Age, median (IQR)	38 (30–51)	38 (30–50)	66 (55–76)	<0.001
Gender				
Male	8940 (54.55)	8424 (54.70)	378 (54.70)	0.986
Female	7446 (45.45)	6966 (45.30)	313 (45.30)	
Comorbidities				
Diabetes mellitus	1365 (8.30)	1169 (7.44)	196 (28.40)	<0.001
Hypertension	896 (5.50)	753 (4.79)	143 (20.70)	<0.001
Cardiovascular disease	815 (5.00)	673 (4.28)	142 (20.50)	<0.001
Immunodeficiency	87 (0.50)	72 (0.45)	15 (2.20)	<0.001
Chronic kidney disease	285 (1.70)	242 (1.54)	43 (6.20)	<0.001
Chronic pulmonary disease	314 (1.90)	283 (1.80)	31 (4.50)	<0.001
Chronic liver disease	40 (0.20)	38 (0.20)	2 (0.30)	0.797
Hyperlipidemia	52 (0.30)	51 (0.32)	1 (0.10)	0.449

Table 3 The baseline characteristics of survivors and non-survivors in patients with COVID-19 with diabetes

Variable	Total (*n*=1365) Number (%)	Survivors (*n*=1169) Number (%)	Non-survivors (*n*=196) Number (%)	*p* value
Age, median (IQR)	59 (49–67)	57 (48–66)	66 (57–73)	<0.001
Gender				
Male	607 (44.50)	488 (44.30)	98 (50.00)	0.141
Female	758 (55.50)	613 (55.70)	98 (50.00)	
Symptoms				
Fever	1075 (78.80)	874 (79.40)	162 (82.70)	0.293
Cough	1035 (75.80)	861 (78.20)	156 (79.60)	0.663
Dyspnea	902 (66.08)	755 (68.60)	147 (75.00)	0.072
Muscular pain	106 (7.76)	93 (7.95)	13 (6.63)	0.393
Diarrhea	23 (1.68)	22 (1.88)	1 (0.51)	0.146
Nausea	29 (2.12)	25 (2.13)	4 (2.04)	0.841
Sore throat	68 (4.98)	63 (5.38)	5 (2.55)	0.066
Comorbidities				
Hypertension	391 (28.64)	324 (27.71)	67 (34.18)	0.181
Cardiovascular disease	249 (18.24)	193 (16.50)	56 (28.57)	<0.001
Immunodeficiency	20 (1.46)	17 (1.45)	3 (1.53)	0.989
Chronic kidney disease	71 (5.20)	57 (4.87)	14 (7.14)	0.265
Chronic pulmonary disease	53 (3.88)	45 (3.84)	8 (4.08)	0.997
Chronic liver disease	13 (0.95)	12 (1.02)	1 (0.51)	0.453
Exposure to disease	690 (50.54)	599 (51.24)	91 (46.42)	0.039D

diabetic individuals [9]. The results of our study were supported by previous studies [17–19, 25] (17-40-42-12). The higher rate of mortality in diabetic patients can be due to their weakened immune system [26], poor lung function, and reduced lung capacity along with their common respiratory problems [18]. Furthermore, COVID-19 can affect diabetes pathogenicity as well as blood sugar control [20], all of which together can lead to increased mortality rate among these people. However, further research is needed to find out if COVID-19-related mortality was actually caused by diabetes or other factors, as most of these people are elderly and may have at the same time some other chronic diseases, including hypertension and cardiovascular disease, which makes it a little difficult for us to identify the main cause of death due to COVID-19. According to the findings of this study, the incidence of hypertension and heart disease was higher in PWD who died from COVID-19 than in non-diabetics. Other studies have shown that the mortality rate of COVID-19 was significantly higher in diabetic patients with heart disease as a comorbidity compared with non-diabetic patients.

Conclusion

The findings of this study suggest that diabetes increased mortality rate in patients with COVID-19 and is a major threat to these patients. Since COVID-19 is an unknown emerging disease, disease management has posed a huge challenge to health policymakers around the world. As a result, there is a need to develop prevention and treatment strategies for high-risk people, especially those with diabetes to reduce morbidity and mortality. It is also recommended that diabetic patients should take preventive measures, such as home quarantine, avoid unnecessary visits to medical centers, control blood glucose level, and follow the prevention guidelines developed by WHO experts for diabetics.

Strengths and weaknesses

Although, in our study, laboratory results and comprehensive information from patients were not available for further analysis, our study is one of the first studies in Iran, which examined clinical features of diabetic patients with COVID-19 and also, unlike many other similar studies, our study had a large sample size and also examined all cases of the disease, while many studies have been performed on only severe form of COVID-19.

We predict that we may have a diagnostic bias in our study. Because some people with asymptomatic or mild symptoms did not come to perform the test or the result of some tests was falsely negative, of course, our high sample size solves this problem

Acknowledgments We would like to acknowledge the Department of Health of Abadan University of Medical Sciences.

Author contribution ML was responsible for the field working including data collection and management and wrote the discussion. PM was collected data and wrote the manuscript. AbH, PE, and GHH analysis data and wrote the manuscript. VA collected data and edited the final version of the manuscript.

Data Availability The data for the current study will not be shared publicly.

Declarations

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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International Journal of Diabetes in Developing Countries (July-September 2021) 41(3) :389–395
https://doi.org/10.1007/s13410-021-00968-y

ORIGINAL ARTICLE

Glycemic control among children and adolescents with type 1 diabetes during COVID-19 pandemic in Egypt: a pilot study

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Received: 24 March 2021 / Accepted: 12 June 2021 / Published online: 9 July 2021

Abstract

Background The COVID-19 pandemic and the consequences of lockdown significantly impacted glycemic control.

Aim To evaluate the impact of the pandemic and lockdown on glycemic control among Egyptian children and adolescents with type 1 diabetes.

Methods Cross-sectional study conducted through an online questionnaire. The participants were patients with type 1 diabetes and/or their caregivers

Results A total of 115 valid responses to the questionnaire were received. During the lockdown, almost 64% of patients showed worsening of their HbA1C with significant increment of HbA1c after the lockdown ($p < 0.001$). Synchronous simple telemedicine service was initiated through phone calls and social media applications, and 97% of the patients and their families were successfully able to continue follow-up. Almost 76% of the patients/caregivers showed moderate stress which was significantly correlated with HbA1C ($p < 0.05$). Fear of hospital admission and fear from shortage of medical supplies were the main COVID-19-related worries.

Conclusion The lockdown negatively impacted glycemic control and initiated a set of COVID-19 worries and stress among patients and their caregivers in Egypt. Telemedicine service, even simple tools, is effective and important for the continuity of care among patients. The limited availability and the fear of shortage of medical supply forced patients to ration glucose monitoring.

Keywords COVID-19 · Lockdown · Telemedicine · Type 1 diabetes

Introduction

On March 11, 2020, the coronavirus disease 2019 (COVID-19) was declared as a global pandemic [1]. The pandemic significantly impacted all countries, creating heavy burdens and affecting different substructures of the country. Global measures were promptly initiated to limit spread of infection, and different countries announced a series of restrictions. On March 2020, Egypt imposed a series of nationwide lockdown to control the spread of infection [1, 2].

The burden of COVID-19 has been increasing continuously, and special concerns were linked to patients with chronic disorders like diabetes [3]. The implemented restrictions and social distancing practices resulted in changes in daily routine with modifications of lifestyle and dietary habits [4]. It is expected that lockdown could initiate a series of challenges affecting continuity of medical care and availability of medical supplies, all of which may negatively impact the complex routine management of diabetes [3].

Although impact of lockdown on glycemic control among patients with type 1 diabetes has been reported in various studies [5–7], there is paucity of data addressing the impact of lockdown on management of children and adolescents with type 1 diabetes in developing countries with limited resources.

To the best of our knowledge, this is the first published study evaluating the impact of COVID-19 pandemic and lockdown on glycemic control among Egyptian children and adolescents with type 1 diabetes. The study also aimed to highlight and map factors associated with lockdown that could affect the overall glycemic control.

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Methodology

This study is a cross-sectional study approved by the local ethical committee of Ain Shams University and registered in the Clinical Trials Government (NCT04531111). The participants were patients with type 1 diabetes and/or their caregivers. Children and adolescents with type 1 diabetes, less than 18 years, were recruited from Pediatrics and Adolescents Diabetes Unit (PADU), Pediatrics Hospital, Ain Shams University.

This study was conducted through an online questionnaire; however, for patients with limited internet access, the questionnaire was carried out through phone calls (according to patients' convenience).

Initially, the questionnaire was circulated among 200 patients with type 1 diabetes, yet 50 patients were not willing to either complete the questionnaire or were not willing to share their data. The remaining 150 questionnaire forms were evaluated, 16 forms were excluded because patients had associated comorbidities, and additional 19 forms were excluded as well because forms were not completed with missing responses.

The questionnaire included four main domains as well as reporting HbA1c levels before and after lockdown. HbA1C before and after lockdown was assessed using the same methodology "cation exchange high performance Liquid chromatography (CE-HPLC)". The first domain included demographic data and disease history of the patient. The second evaluated the dietary habits, diabetes care, and lifestyle before and after lockdown. The third reviewed the insulin regimen and requirements together with frequency of hypoglycemia and hyperglycemia before and after lockdown, and finally, the last domain evaluated mainly the COVID-19-related worries among patients as well as the communications channels with health professionals.

Perceived stress scale-10 (PSS-10) (Arabic Validated version) was used (with permission) to measure patients'/caregivers' level of stress in response to the extraordinary status of the pandemic and its consequences. It is a 10-questions scale that was part of the questionnaire [8, 9]. Individual scores on the PSS can range from 0 to 40 with higher scores indicating higher perceived stress. Scores ranging from 0 to 13 were considered low stress, from 14 to 26 were considered moderate stress, and from 27 to 40 were considered high perceived stress.

Statistical methods

Analysis of data was done using Statistical Program for Social Science version 23 (SPSS Inc., Chicago, IL, USA). Continuous variables are presented either as mean ± SD or median and interquartile range (IQR). Categorical variables are presented as percentages. For comparing variables before and after lockdown, the two-tail paired Student's *t* test was performed. Categorical variables were compared using Chi square (X^2) test. Pearson correlation coefficients were used to assess the association between two normally distributed variables. Statistical significance was accepted when $p < 0.05$.

Results

A total of 115 patients responded to the questionnaire. About 60% of responders had either or both parents with a college or university degree. Almost 95% was on multiple daily injections (MDI), and only 5% was on continuous subcutaneous insulin infusion (CSII). Only 47% was on carb counting. Sixteen patients (13.9%) were using flash glucose monitoring system (IsCGMs), and the rest were on regular self-monitoring of blood sugar (SMBG) (Supplemental Table).

During lockdown, diet control and eating habits were reported as worse in almost 60% of patients. Similar to diet control, overall diabetes control was worse during the lockdown in almost 59% of patients. Only 40% tended to do SMBG at least five times daily. Fluctuation of blood sugar with attacks of hyperglycemia and hypoglycemia was reported more frequently in 66% and 59%, respectively. With lockdown and poor dietary habits, there was an increase in insulin dose (61.7%). HbA1C, a surrogate marker of diabetes control, was reported as better in only one-third of patients, while the rest had worsening of their HbA1C (Fig. 1A). HbA1C significantly increased after the lockdown ($p < 0.001$) with a percentage increment of $6.85 \pm 1.67\%$ (Fig. 1B). Subset analysis showed that HbA1c improved in patients aged less than 5 years with a percentage decrement of 4.43 ± 1.469, yet this change was not significant. Unlike younger patients, patients aged 5–10 years and adolescents showed significantly increased HbA1C after lockdown with percentage increment of 8.6 ± 1.484 and 7.43 ± 0.748, respectively (Table 1).

The questionnaire revealed that only 18% of patients were enrolled in sports and only 5% continued to perform household exercises during lockdown (Supplemental Table).

Regarding communication with healthcare professionals, most patients and families were able to continue

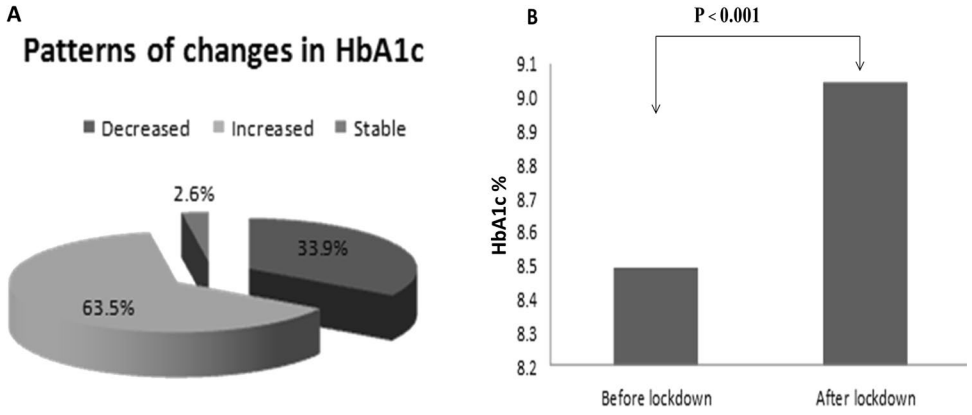

Fig. 1 Patterns of changes in HbA1C among the studied cohort during the lockdown period. During the period of lockdown, about 64% of patients showed an increase in the level of HbA1C, HbA1C was decreased in 34%, and it was unchanged in about 3% (**A**). The mean HbA1C significantly increased after lockdown (**B**). The HbA1c before lockdown ranged from 6.2 to 12% (44–108) with a mean of 8.49 ± 1.26% (69) compared to HbA1C ranging from 5.8 to 14% (40–130) after lockdown with an average of 9.04 ± 1.84% (75) ($p < 0.001$)

Table 1 Effect of lockdown on HbA1C among different age groups

	HbA1C% (IFCC)		% change	p value	Sig
	Before lockdown	After lockdown			
Age 0–5 years	8.55 ± 1.33 (70)	8.08 ± 1.22 (65)	−4.43 ± 1.47	0.280	NS
Age 5–10 years	8.26 ± 1.23 (67)	8.94 ± 1.64 (74)	8.6 ± 1.5	0.001	HS
Age 10–18 years	8.65 ± 1.27 (71)	9.28 ± 2.01(78)	7.43 ± 1.8	0.003	HS

follow-up through a synchronous simple telemedicine service (real-time telephone or social media applications). However, with this facility still around, 3% failed to communicate (Supplemental Table).

Table 2 summarizes the demographic data and diabetes management among different age groups. Data showed that almost 70% of patients less than 5 years monitored blood glucose at least 5 times/day ($p < 0.05$). There were significant differences in the pattern of diabetes control among different age groups. Patients aged 5–10 years and older patients tended to show worse control ($p < 0.01$).

When evaluating the impact of duration of diabetes, eating habits and overall diabetes control during lockdown period was the worst among patients with diabetes duration of 1–5 years with more significant attacks of hypoglycemia and hyperglycemia (Fig. 2).

The COVID-19-related worries among the studied cohort were mainly the fear to catch SARs-COV2 infection, fear from getting admitted with any diabetes-related complication, and finally from shortage of medical supplies (Supplemental Table).

On analyzing the PSS-10, almost 76% showed moderate stress. A significant positive correlation was found between HbA1C both before and after lockdown ($p < 0.05$) (Fig. 3). Severe stress was more evident among caregivers of infants and toddlers with diabetes (Table 2).

Discussion

Patients with chronic conditions including diabetes experienced challenges during the period of lockdown especially patients in developing countries with pronounced barriers in accessing healthcare services and medical supplies [3].

To achieve tight control, monitoring of blood sugar all over the day in children with type 1 diabetes is mandatory; meanwhile, those with fluctuating blood sugar or intermittent hypoglycemia, blood glucose should be checked at least four times a day with additional check in case of signs or symptoms of occurring hypoglycemia [10].

It is obvious from the current study that about 51% of the patients monitored their blood glucose less frequent during the lockdown period with 60% of patients monitoring less than three times daily. The defect in SMBG could be related to shortage of supply or lack of insurance as most supplies are covered by insurance. This negative impact of lockdown was also reported in a similar study in Middle East where a higher percentage of families (43%) had to ration or cut down the use of glucose test strips which led to more frequent hypo-/hyperglycemic excursions in glucose levels in most of their children [11].

When the lockdown was eased early in July, most patients were able to check their HbA1C, and there was

Table 2 Demographic data and diabetes management among different age groups

		Age 0–5 years	Age 5–10 years		Age 10–18 years	p value
		No. = 10	No. = 44		No. = 61	
Duration of diabetes	6 months–1 year	7 (70.0%)	9 (20.5%)		4 (6.6%)	0.000
	1–5 years	3 (30.0%)	30 (68.2%)		20 (32.8%)	
	> 5 years	0 (0.0%)	5 (11.4%)		37 (60.7%)	
Gender	Male	6 (60.0%)	21 (47.7%)		26 (42.6%)	0.571
	Female	4 (40.0%)	23 (52.3%)		35 (57.4%)	
Insulin regimen	Multiple daily injections	8 (80.0%)	43 (97.7%)	58 (95.1%)		0.074
	Insulin pump	2 (20.0%)	1 (2.3%)	3 (4.9%)		
Number of daily injection of insulin	Less than 3 times/day	0 (0.0%)	0 (0.0%)	0 (0.0%)		0.123
	3 times/day	0 (0.0%)	9 (20.5%)	6 (9.8%)		
	More than 3 times/day	10 (100.0%)	35 (79.5%)	55 (90.2%)		
Eating habits and diet control during lockdown	Same	5 (50.0%)	11 (25.0%)	13 (21.3%)		0.325
	Better	0 (0.0%)	7 (15.9%)	10 (16.4%)		
	Worse	5 (50.0%)	26 (59.1%)	38 (62.3%)		
Frequency of daily SMBG † during lockdown	Less than 3 times/day	3 (30.0%)	10 (22.7%)	21 (34.4%)		0.048
	3–4 times/day	0 (0.0%)	19 (43.2%)	16 (26.2%)		
	5–7 times/day	7 (70.0%)	15 (34.1%)	24 (39.3%)		
Diabetes control during lockdown	Same	7 (70.0%)	9 (20.5%)	11 (18.0%)		0.008
	Better	0 (0.0%)	8 (18.2%)	12 (19.7%)		
	Worse	3 (30.0%)	27 (61.4%)	38 (62.3%)		
Hyperglycemia more often after the lockdown	No	5 (50.0%)	16 (36.4%)	18 (29.5%)		0.406
	Yes	5 (50.0%)	28 (63.6%)	43 (70.5%)		
Hypoglycemia more often after the lockdown	No	4 (40.0%)	16 (36.4%)	27 (44.3%)		0.718
	Yes	6 (60.0%)	28 (63.6%)	34 (55.7%)		
Exercise before lockdown	No	9 (90.0%)	37 (84.1%)	51 (83.6%)		0.874
	Yes	1 (10.0%)	7 (15.9%)	10 (16.4%)		
Exercise during lockdown	No	10 (100.0%)	41 (93.2%)	59 (96.7%)		0.531
	Yes	0 (0.0%)	3 (6.8%)	2 (3.3%)		
Method of communication	Physical visit	0 (0.0%)	3 (6.8%)	5 (8.2%)		0.159
	Phone call	8 (80.0%)	20 (45.5%)	19 (31.1%)		
	Social media	2 (20.0%)	20 (45.5%)	35 (57.4%)		
	Couldn't communicate	0 (0.0%)	1 (2.3%)	2 (3.3%)		
Satisfaction with communication	Not satisfied	0 (0.0%)	3 (6.8%)	8 (13.1%)		0.860
	Mildly satisfied	2 (20.0%)	7 (15.9%)	14 (23.0%)		
	Satisfied	8 (80.0%)	34 (77.3%)	39 (63.9%)		
Afraid about shortage of supply	No	1 (10.0%)	4 (9.1%)	14 (23.0%)		0.142
	Yes	9 (90.0%)	40 (90.9%)	47 (77.0%)		
COVID-19-related worries	Difficulty in contacting healthcare physician	1 (10.0%)	1 (2.3%)	1 (1.6%)		0.69
	Afraid of not finding the medical care if infected with COVID-19	1 (10.0%)	5 (11.4%)	12 (19.7%)		
	Feeling more susceptible to infection	3 (30.0%)	13 (29.5%)	17 (27.9%)		
	Fear of hospital admission	5 (50.0%)	25 (56.8%)	31 (50.8%)		
PSS-10 ◆	Moderate stress	4 (40.0%)	35 (79.5%)	49 (80.3%)		0.017
	High perceived stress	6 (60.0%)	9 (20.5%)	12 (19.7%)		

†*SMBG* self-monitoring of blood glucose, ◆ *PSS-10* perceived stress scale-10

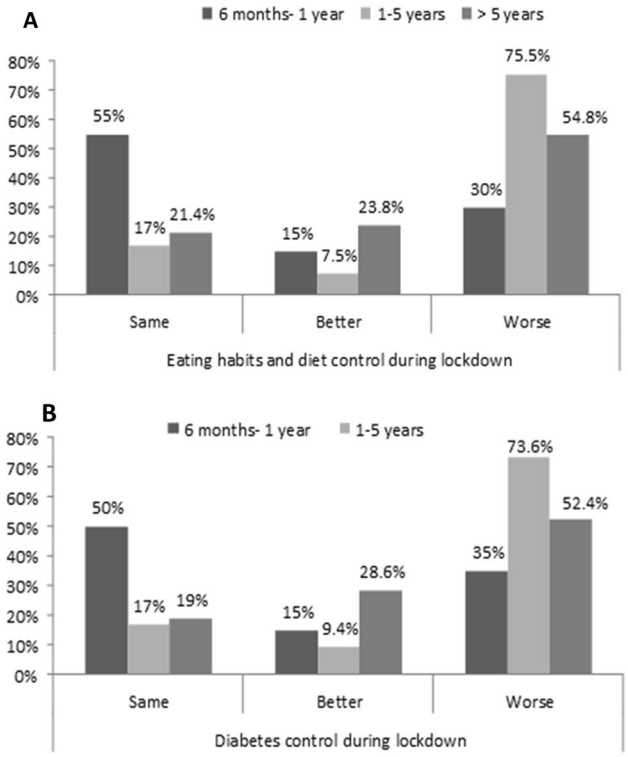

Fig. 2 Eating habits and overall diabetes control during lockdown period among patients with different duration of diabetes. Eating habits (**A**) and overall diabetes control (**B**), during lockdown period, were the worst among patients with diabetes duration of 1–5 years ($p < 0.05$)

an overall significant increase in mean HbA1C. Although being non-significant, HbA1C improved in toddlers and preschoolers; however, school children and adolescents had significant worsening. This is similar to previous studies which showed that the pandemic and lockdown negatively impacted the metabolic control of type 1 diabetes among children and adolescents [11–13]. However, data from the current study, regarding impact of lockdown on glycemic control, was in contrast to data explored by previous studies showing no worsening or even improvement of glycemic control during the period of lockdown [5–7]. Differences in study population as well as differences in standards of care together with differences in availability of medical supply could provide insight and possible explanation for such observed difference.

In concordance with a study conducted among an Indian cohort, a country with limited resources as ours, poor dietary habits and lack of physical exercise are noticeable causes of the poor glycemic control during the period of lockdown [13].

Limited outdoor activities, with increased desire for frequent snacking especially with online learning, had made it noticeable that dietary habits and control were becoming worse among almost all age groups; however, those with newly diagnosed diabetes or duration less than a year were the best in regard to maintaining diet control in comparison to those with longer duration. We speculated that parents or caregivers of patients, whose disease duration is less than a year, took over diabetes control all over the day, which may partially explain the relative improvement in dietary habits. Similarly, Shah et al. highlighted the importance of family support in maintaining a steady daily routine [14].

The pandemic impacted the practice and the standard of care offered to patients with type 1 diabetes and the implementation of telemedicine service, which became a crucial tool in managing patients [15, 16].

With the onset of lockdown, our diabetes team started to launch a telemedicine service. Synchronous simple service was used by sharing logbooks and patients' inquiries through social media applications (WhatsApp and Facebook) and phone calls. Around 97% of the patients and their families were successfully able to communicate with their medical team, and almost 70% were more or less satisfied. Similar data from a Jordanian study showed the efficacy of simple basic ways of telemedicine service in managing type 1 diabetes through sharing data from logbooks and adjusting insulin regimens [11].

This survey explored the worries associated with COVID-19 among the studied cohort and showed that the pandemic was associated with different worries among patients with diabetes. Almost half was afraid from hospital admission and contacting infection, and about 29% was feeling more

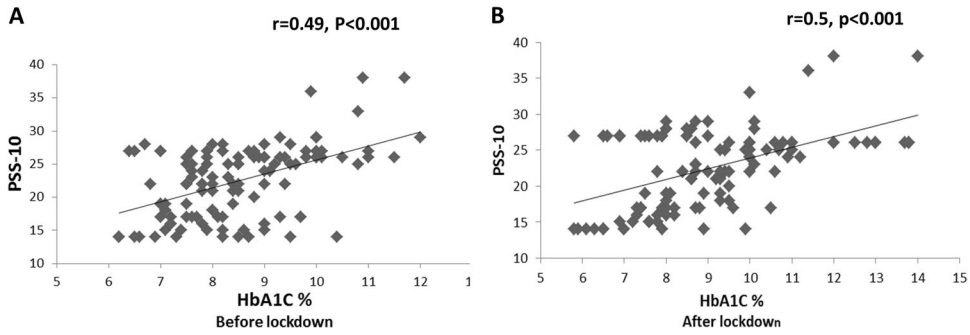

Fig. 3 Correlation between PSS-10 and HbA1C before and after lockdown. A significant positive correlation was noticed between PSS-10 and glycemic control, as reflected by HbA1C before (**A**) and after lockdown (**B**) ($p < 0.001$)

susceptible to infection. Beside the previous worries, the majority was afraid of shortage of medical supplies.

Joensen and colleagues showed that their studied cohort was most frequently worried about COVID-19, 56% was worried of being severely affected due to diabetes, 28% was afraid of being unable to manage diabetes if infected with COVID-19, and 24% was worried from lack of medical supplies [17]. In this context, different resources and diabetes fact sheets were developed aiming to support and manage patients' worries and concerns about COVID-19 [18–20].

The current study evaluated the levels of perceived stress among patients and/or caregivers during the challenging period of lockdown. Sixty percent perceived moderate stress, and 40% perceived severe stress. This was shown in a similar study where more than half of the studied cohort reported moderate stress [12].

Several studies evaluated perceived stress among patients with diabetes and correlated it with patients' glycemic control. Diabetes treatment, management and perception of complications anticipated by poorly controlled patients significantly impact patients' perception of stress [21, 22]. A significant positive correction was noticed between PSS-10 and pre-lockdown Hb A1C. Similar finding was reported by Agarwal et al., where they observed higher PSS-10 scores among adolescent patients with poor glycemic control. They attributed such observation to the fact that the pandemic could possibly augment pre-existing health-related stress [12].

Similar to the findings reported by Agarwal et al. [12], we observed a significantly positive correlation between level of perceived stress and patients' glycemic control, as reflected by Hb A1C level assessed after ease of lockdown. Stress is known to negatively impact outcome of disease, and this could be related to non-adherence to medication and disruption of healthy lifestyle as well [23].

The pandemic affected all aspects of life with special impact on patients with type 1 diabetes. The main limitation of the current study is relatively small sample size representing data from a single center. The absence of data assessing the history of COVID-19 infection in patients/caregivers as well as contact with suspected or confirmed cases is another limitation of the current study. Additionally, the disadvantages associated with the nature of online survey with its potential have limited the ability to access certain portals.

Conclusion

The pandemic had a significantly negative impact on glycemic control among Egyptian children and adolescents with type 1 diabetes. The restriction of mobility due to the lockdown created barriers in assessing health team and continuity of healthcare, all of which highlighted the importance of initiating a telemedicine service. The limited availability and the fear of shortage of medical supply forced the patients to ration glucose monitoring. The lockdown affected patients/caregivers perception of stress and initiated a set of COVID-19 worries.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-021-00968-y.

Author contribution Both Dr. Elhenawy and Dr. Eltonbary shared in designing the study, carried out the practical part of the study, drafted, reviewed, and revised the manuscript. Both authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Data availability N/A

Code availability N/A

Compliance with ethical standards

Ethical approval The study was approved by the local ethical committee of Ain Shams University and registered in the Clinical Trials Government (NCT04531111).

Consent to participate N/A

Consent for publication N/A

Conflict of interest The authors declare no competing interests.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):396–403
https://doi.org/10.1007/s13410-020-00905-5

ORIGINAL ARTICLE

Comparison of clinical features, complication profile, and achievement of guideline targets in early- and late-onset type 2 diabetes patients from North India

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Received: 15 July 2020 / Accepted: 8 December 2020 / Published online: 12 January 2021

Abstract

Introduction The prevalence of diabetes in young people is rising in India. The risk factors are high body mass index, lack of physical activity, and a positive family history. Young patients with type 2 diabetes mellitus are at a higher risk of longer disease duration and enhanced risk for chronic complications. We have studied the difference between early- and late-onset type 2 diabetes mellitus with respect to various glycemic and non-glycemic parameters.

Material and methods This is an observational cross-sectional study conducted from November 2018 to October 2019. A total of 5142 patients were included in the study. The demographic, anthropometric, clinical, and biochemical information was obtained from the record of the patients.

Results The number of patients diagnosed before 40 years of age was 1574 and 3568 patients after 40 years of age. There was female preponderance, 75.7% of the study subjects were from the urban area, and 60% had an income of < 2 lakh rupees per annum. Two-third (77%) of the patients were either overweight or obese. Significantly higher proportion of patients achieved glycemic and blood pressure targets in the early-onset type 2 diabetes mellitus group. The younger group had higher weight and body mass index, strong family history of diabetes, and more severe dyslipidemia. The comorbid conditions were significantly higher in the older age group.

Conclusion Our study has revealed that about one-third of type 2 diabetes mellitus patients are diagnosed before 40 years of age. The younger patients are more obese. Two-third (73%) of these patients have uncontrolled diabetes.

Keywords Type 2 diabetes · Early onset · Glycemic · Non-glycemic targets · Complications

Introduction

The worldwide prevalence of type 2 diabetes mellitus (T2D) in adults is around 8%, and this is expected to rise to > 10% by 2040 [1]. Though the high prevalence of T2D among older individuals is well established, the growing proportion of young individuals with T2D is a more recent phenomenon, particularly in developing countries, and it is of significant concern. The onset of T2D early in life is associated with lengthier exposure to hyperglycemia and a subsequently higher risk of chronic complications of diabetes. Moreover, the progression of T2D in young individuals might be swifter and more troublesome than in elderly patients contributing to enhanced morbidity [2]. Additionally, T2D in young adults could be associated with enhanced adverse societal effects due to the accompanying chronic disease all through the patient's working life [3].

Even though the Southeast Asian region has the largest number of people with diabetes, spending on diabetes-related disorders is just US$6 billion, which is < 1% of the global total. The adult population of India constitutes 86% of Southeast Asia's adult population of 883 million. The Southeast Asian region has the maximum number of deaths

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related to diabetes when compared to the other six International Diabetes Federation (IDF) global regions. Most of these deaths (55%) occurred in people with age < 60 years and one-quarter (27%) in individuals < 50 years of age [4].

The IDF estimated that roughly 23 million young adults aged 20–39 years had T2D worldwide in the year 2000, and by 2013, this estimate had increased to 63 million [4, 5]. A study from the UK revealed a significant increase (217/100,000 in 1996–2000 to 598/100,000 in 2006–2010) in the prevalence of T2D in individuals aged < 40 years [6].

Management of hyperglycemia in patients with T2D is rapidly changing, with emphasis on providing patient-centered care. Level of hyperglycemia and associated comorbid condition would dictate the management. Because of the availability of a wide array of anti-hyperglycemic agents, the glycemic control of T2D has become increasingly complex and, to some extent, controversial [7]. The concerns about the long-term safety and benefit of the newer agents and the effect of intensive glycemic control on macrovascular complications persist [8]. Identifying the optimal agents might be perplexing for many clinicians. There is an unmet need to recognize T2D patients' subgroups at higher risk of developing complications of diabetes and associated morbidity and mortality; this might enhance patient-centered management.

The current evidence suggests that, when compared with the elderly (≥ 65 years), younger people with diabetes may have a more aggressive disease progression, with an enhanced risk of chronic complications of diabetes and more severe hyperglycemia [9]. One study revealed that individuals with T2D diagnosed before 40 years of age had the highest risk for most of the adverse outcomes relative to controls, with an adjusted hazard ratio of 2.05 for death from any cause, 2.72 for cardiovascular death, 1.95 for non-cardiovascular death, 4.77 for heart failure, and 4.33 for coronary artery disease [10]. Another study revealed that individuals with the onset of diabetes before 40 years of age had excess hospitalizations throughout their lifespan compared with persons with late-onset T2D, with a surprisingly massive mental illness load in young adulthood [11].

These risks may be due to the differences in the degree of hyperglycemia, prolonged duration of disease, higher BMI, and poor quality of care. In this study, we have studied the difference between early-onset (≤ 40 years) and older patients' late-onset (> 40 years) T2D for various glycemic and non-glycemic parameters.

Materials and methods

Subjects

This is an observational cross-sectional study conducted at two endocrine super-specialty clinics. One center was the endocrine clinic of Rajiv Gandhi Centre for Diabetes and Endocrinology, Faculty of Medicine of J. N Medical College and Hospital, Aligarh Muslim University, Aligarh, India. The other center was the Diabetes & Endocrinology Super-Speciality Centre, Aligarh, India. We conducted the study from November 2018 to October 2019. We included all the patients diagnosed with T2D in the study as per the American Diabetes Association's criteria, 2019 [12]. To minimize the inclusion of type 1 diabetes mellitus patients, individuals diagnosed before 20 years were not included in the study. A total of 5142 patients who were attending the clinic regularly were included in the study. The study subjects were further divided into two groups depending upon the age of diagnosis: 1574 patients diagnosed before 40 years of age (group A) and 3568 diagnosed after 40 years of age (group B). The cutoff age to define early- and late-onset diabetes was taken as 40 years; this was based on previously available literature [10, 11, 13].

We obtained relevant information regarding their age, place of residence, annual income, duration of diabetes, type of diabetes, medication use (including insulin), blood sugar level (fasting and postprandial), HbA1c level, and comorbidities (hypertension, dyslipidemia, diabetic neuropathy, diabetic kidney disease, diabetic retinopathy, coronary artery disease, heart failure) from the record of these patients. Anthropometric details such as height and weight were measured as per the standard recommendations; body mass index (BMI) was calculated as weight in kg divided by height in m^2. We measured waist circumference (WC) with non-stretchable measurement tape at the level just above the iliac crest at the end of expiration, and hip circumference was measured at the widest point. Individuals were categorized according to their BMI values as "lean" (< 18.0 kg/m^2), "normal" (18.0–22.9 kg/m^2), "overweight" (23.0–24.9 kg/m^2), and "obese" (> 25 kg/m^2) [14]. A qualified ophthalmologist diagnosed diabetic retinopathy by doing a detailed fundus examination after dilation and ocular coherence tomography wherever indicated. Diagnosis of diabetic neuropathy was established based on the patient's history and examination (vibration perception and 10-g monofilament, ankle jerk, pinprick, and temperature sensation). Diabetic kidney disease was diagnosed on the basis of the presence of albuminuria and/or reduced eGFR (Cockcroft-Gault formula) in the absence of signs or symptoms of other primary causes of kidney damage [12]. Diagnosis of coronary artery disease and heart failure was based on clinical history, examination, and relevant investigations like EKG, X-ray chest, echocardiography, stress testing, coronary angiography, and CT angiography and of troponin-T levels and BNP levels.

Statistical analysis

The statistical software SPSS Version 21 (IBM SPSS Statistics for Windows, Version 20 Armonk, NY: IBM Corp) was used to analyze the data. The normality of the distribution of each variable was tested. Data were expressed as mean ± SD for normally distributed variables. The unpaired Student *t* test was used to compare mean values between different groups. The Pearson correlation coefficient was used to quantify the univariate associations between variables. All the results have been described on a 5% level of significance, i.e., p value < 0.05 considered as significant

Results

The demographic and clinical features of the study population are given in Table 1. About one-third (30.6%) of the study population was diagnosed prior to 40 years of age. There was female preponderance (57.63%) in the study subjects. Three-fourth (75.7%) of the study subjects were from the urban area. About 60% of the study subjects were having an income of less than two lakh rupees per annum. The mean fasting and postprandial glucose were 139 and 202 mg/dl respectively. The mean HbA1c was 8.69%. Only 22% of the subjects were having good glycemic control (HbA1c < 7%) as per the criteria of American Diabetes Association 2019 [10]. The subjects with HbA1c between 7 and 9% were 45.8%, and 32% had HbA1c > 9%, and 77% of the patients were either overweight or obese. Half (50%) of the patients were on single, 30.84% of patients were on two, and 17.09% were on triple drug therapy. Only 11% of the patients were on insulin therapy (Table 1).

The mean age was significantly lower in the early-onset group. Though female preponderance was observed in both the groups, it was significantly more marked in the younger group. Three-fourths of the subjects in both groups were residents of the urban area. The older age group was having more numbers from the lower income group but the difference was non-significant. The early-onset group was having a small but statistically significant higher weight and BMI. Family history was significantly more common in young onset group. History of diabetes in 1st-, 2nd-, and 3rd-degree relatives was obtained from the patients. There was no significant difference in the pattern of anti-diabetic drug use between both study groups. But the number of anti-diabetic pills was significantly high in the older age group. The early-onset group was having significantly better glycemic control as compared with the older age group. The presence of associated comorbid conditions such as hypertension, diabetic neuropathy, diabetic nephropathy, diabetic retinopathy, and coronary artery disease was significantly higher in the late-onset group (Table 2). The HbA1c level was having a significant positive correlation with

Table 1 Glycemic and non-glycemic parameters of the study population

Parameters	Values
Mean age	49.92 ± 10.88
Age at diagnosis < 40 years	1574 (30.6)
Gender	
Male	2179 (42.46)
Female	2963 (57.63)
Residence	
Rural	1250 (23.3)
Urban	3892 (75.7)
Income	
Group 1 (< 2 lakh/ annum)	3268 (60.1)
Group 2 (2–6 lakh/ annum)	1542 (31.4)
Group3 (> 6 lakh/ annum)	332 (8.5)
Family history of diabetes	1986 (38.6)
BS (F)	139 ± 58.66
BS (PP)	202 ± 97.42
HbA1c	8.69 ± 5.5
BMI	
Lean	183 (3.6)
Normal weight	943 (18.7)
Over weight	830 (16.5)
Obese	3054 (61.2)
Glycemic control	
Good (< 7%)	1143 (22.2)
Poor (7–9%)	2355 (45.8)
Very poor (> 9%)	1644 (32.0)
No of patients on insulin	572 (11.1)
Frequency of insulin	
Once daily	43 (0.8)
Twice daily	332 (6.5)
Thrice daily	43 (0.8)
Four times daily	144 (2.8)
Number of anti-diabetic rugs	
Only MNT	7 (0.14)
One	2620 (50.93)
Two	1586 (30.84)
Three	879 (17.09)
Four	48 (0.93)
Five	2 (0.09)
Drugs	
MFN	3561 (71.0)
SU	2149 (41.8)
DPP IV inhibitors	1109 (21.6)
SGLT2	295 (5.7)
Thiazolidinedione	27 (0.5)
α-Glucosidase inhibitor	126 (2.5)
Insulin	572 (11.1)
Neuropathy	779 (15.14)
Nephropathy	360 (7.01)

Table 1 (continued)

Parameters	Values
Retinopathy	835 (16.12)
HTN	1412 (27.46)
CAD	741 (14.41)

BS (F), blood sugar fasting; *BS (PP)*, blood sugar post prandial; *MNT*, medical nutrition therapy; *MFN*, metformin; *HTN*, hypertension; *CAD*, coronary artery disease

Data are mean ± SD or *n* (%) unless otherwise indicated

age, duration of diabetes, and number of drugs. In the early-onset group, 39.1%, 63.2%, 60.3%, 84.4%, and 28.4% of the study subjects achieved their target of triglyceride (< 150 mg/dl), HDL cholesterol (> 40 mg/dl for male, > 50 mg/dl for female), LDL cholesterol (< 70 mg/dl for patients with CAD, < 100 mg/dl for patients without CAD), blood pressure (< 140/90 mm of Hg), and HbA1c (< 7.0%) respectively, whereas 42.5%, 62.2%, 62.8%, 70.1%, and 19.6% of the study subjects in the late-onset group achieved their targets of triglyceride, HDL cholesterol, LDL cholesterol, blood pressure, and HbA1c respectively (Fig. 1).

Discussion

T2D is now becoming more common in the younger population. In the year 2000, individuals with age between 20 and 39 years were 13% of total 177 million adults with T2D, and this proportion increased to 16% of 382 million total adults with T2D in the year 2013 [4, 5]. Various studies from the globe have provided a similar trend of increased incidence and diabetes prevalence in the young population. Currently, India is having the second largest number of people with diabetes in the world. In 2019, there were 77 million people with diabetes in India, which is expected to rise to 101 million in 2030 [4]. There are few peculiar features of T2D in the Southeast Asian region, including India. The disease occurs in patients with lower BMI, and patients have high total body fat content, more severe insulin resistance, more intense proinflammatory cytokine milieu, and a high rate of conversion from prediabetes to diabetes. The disease's onset is almost two decades earlier in Indian patients compared with the western population. These factors are suggestive of more aggressive disease in Indian patients [15].

We have conducted this cross-sectional study to examine the various demographic, clinical, and biochemical parameters in early-onset Indian diabetic patients. We have also compared these parameters between early- and late-onset patients with T2D. A cutoff of 40 years was taken to define the young population [10, 11, 13].

The mean age of the patients was 49.92 years. About one-third (30.6%) was diagnosed to have diabetes before 40 years of age. This finding is similar to previous studies conducted in India [16]. Three-fourths of the study subjects were residing in the urban area; this finding is similar to earlier findings [17]. In our study, 57% of the study subjects were female. This finding is similar to previous studies conducted in North India [18]; this can occur because, in our society, females mostly remain indoor, their physical activity is low, and the incidence of obesity is also higher in females [19]. About 40% of our study patients were having an income of fewer than 2.0 lakhs rupee/annum, which is similar to previous studies [20]. The average blood glucose fasting, postprandial, and HbA1c in the study population was 139 mg/dl, 202 mg/dl, and 8.69%, respectively. Only 22% of the study subjects were having an HbA1c of less than 7%, suggesting good glycemic control; this could be a true reflection of diabetes management status in our country, or it can also be because it is an observational study; many newly diagnosed patients with high HbA1c were also included in the analysis. Analysis of follow-up data can provide different results. But the result of our study is not very much different from those of earlier studies conducted in India, which also denote a high proportion of patients failing to achieve their glycemic targets [21]. Only 11% of the patients were on insulin, which suggests that a very small percentage of patients were on insulin therapy despite poor glycemic status. The reason could be reluctance on the part of patients and treating physicians to start insulin therapy. Another reason could be that we do not have follow-up data or have been shifted to insulin in follow-up; this is particularly important in newly diagnosed patients. Newly detected diabetic patients are usually reluctant for insulin therapy, and they insist on oral medications. Another study from our center with follow-up data has revealed insulin's use in 38% of patients [22]. The associated comorbid conditions such as hypertension, diabetic neuropathy, diabetic retinopathy, diabetic nephropathy, and coronary artery disease were present in 27.46%, 15.14%, 16.2%, 7.01%, and 14.14% of the study subjects, respectively. These complication profiles of our patients are similar to previous studies [23–26].

The female preponderance of the subjects was significantly more in the younger population than in the older population. Similar results have been reported in many studies from different countries. The SEARCH study, which was conducted in US children and young adults, revealed that T2D in female individuals was almost twice that of young males [27]. Similar findings have been reported from developing countries such as Bangladesh and Jamaica [28, 29]. This has been ascribed to the increase in overweight and obesity in young females across the world. Young females with polycystic ovary syndrome have a significantly higher risk of developing T2D, which has been attributed to the increased insulin resistance present in individuals with polycystic ovary syndrome [30].

The older age group had more numbers from the lower income group, but the difference was non-significant. This

Table 2 Difference between early-onset and late-onset subjects

Parameters	Young subjects (age at diagnosis < 40 years)	Older subjects (age at diagnosis > 40 years)	*p* value
Number	1574	3568	
Age	38.59 ± 9.47	54.91 ± 8.02	< 0.001
Female	942 (60.0)	2007 (56.7)	< 0.001
Urban	1178 (75.6)	2713 (76.8)	NS
Duration of diabetes (years)	4.7 ± 5.9	5.4 ± 7.1	0.003
Income group			
Group 1 (< 2 lakh/ annum)	945 (60.03)	2323(65.10)	NS
Group 2 (2–6 lakh/ annum)	490 (31.13)	1052 (29.48)	NS
Group3 (> 6 lakh/ annum)	139 (8.84)	193 (5.40)	NS
Weight (kg)	66.85 ± 14.50	66.62 ± 13.35	0.005
BMI (kg/m^2)	26.72 ± 5.34	26.65 ± 4.9	0.001
BMI category			
Lean	82 (5.3)	103 (2.89)	NS
Normal weight	280 (18.1)	677 (18.98)	NS
Over weight	219 (14.15)	624 (17.49)	NS
Obese	966 (62.44)	2164 (60.64)	NS
Waist circumference (cm)	97.06 ± 12.76	98.72 ± 12.61	NS
Family history of diabetes	660 (41.9)	1326 (37.16)	0.01
Drugs			
MFN	1275 (81)	2783 (78)	NS
SU	528 (33.4)	1621 (35.2)	NS
DPP IV inhibitors	213 (13.5)	896 (25.0)	NS
SGLT2	85 (5.4)	210 (5.85)	NS
Thiazolidinedione	15 (0.9)	12 (0.3)	NS
α-Glucosidase inhibitor	25 (1.5)	101 (2.8)	NS
Insulin	174 (11.2)	398 (11.2)	
Number of drugs			
Only MNT	7 (0.4%)	0	< 0.001
1	942 (59.8)	1678 (47)	< 0.001
2	451 (28.6)	1135 (31.8)	< 0.001
3	166 (10.3)	713 (19%)	< 0.001
4	7 (0.4)	41 (1.1%)	< 0.001
Glucose control			
Good (HbA1c < 7%)	443 (28.1)	700 (19.63)	< 0.001
Poor (HbA1c 7–9%)	800 (50.8)	1555 (43.58)	< 0.001
Very poor (HbA1c > 9%)	331 (21)	1313 (36.79)	< 0.001
Lipids			
TC (mg/dl)	181.3 ± 50.7	177.1 ± 46.7	0.008
TG (mg/dl)	191.8 ± 88.7	182.2 ± 85.3	0.01
HDL (mg/dl)	46.4 ± 14.4	45.7 ± 14.4	NS
LDL (mg/dl)	96.4 ± 37.6	94.3 ± 34.7	NS
Neuropathy	125 (7.94)	654 (18.34)	< 0.001
Nephropathy	54 (3.43)	306 (8.57)	0.005
Retinopathy	179 (11.31)	656 (18.38)	< 0.001
HTN	192 (12.12)	1220 (34%)	< 0.001
CAD	106 (6.73)	635 (17.77)	< 0.001

MNT, medical nutrition therapy; *MFN*, metformin; *TC*, total cholesterol; *TG*, triglyceride; *HTN*, hypertension; *CAD*, coronary artery disease; *NS*, non-significant

Data are mean ± SD or *n* (%) unless otherwise indicated

could be because obesity is the major driver for an increased incidence of T2D in young individuals. An improvement in the standard of living and rapid urbanization has led to an increased incidence of obesity worldwide. Therefore, we can expect a surge in T2D in young people in the future. The younger group was having a small but statistically significant higher weight and BMI. Obesity in children and young adults is multifactorial in origin, but it is one of the most significant drivers for the growing prevalence of T2D in young people. The ever-increasing consumption of energy-rich foods and glucose-containing drinks and a sedentary lifestyle are the main reasons for obesity in young individuals [31].

The early-onset group was also having a significantly stronger family history of diabetes. A positive family history of T2D is associated with a reduction in the age of onset of T2D. In one study, including more than 5000 individuals from a different ethnic background, for every 10% increase in the affected family member, the age of diabetes occurrence was reduced by 1.7 years [32]. Comparable findings have been reported in studies from different countries, including India [33]. The younger patients have significantly higher levels of total cholesterol and triglyceride levels. This could be secondary to higher BMI in this subgroup of patients. Similar results have been published previously [34].

There was no significant difference in the pattern of anti-diabetic drug use between both study groups. This finding is expected as, until now, we do not have a specific strategy for managing T2D in the young population. But the number of anti-diabetic pills was significantly high in the older age group. This can be due to increased duration of disease and more severe hyperglycemia in older individuals. A similar finding has been reported in a study from our center [22]. Though the younger age group had significantly better glycemic control than the older age group, only 27% of the patients were having HBA1c less than 7%. This finding is similar to the study conducted by Yeung et al. in South Asia and Southeast Asia, in which about 27% of individuals have HbA1c of less than 7% [35]. The presence of associated co-morbid conditions such as hypertension, diabetic neuropathy, diabetic nephropathy, diabetic retinopathy, and coronary artery disease was significantly higher in the older age group. This could be because of the increased diabetes duration and poor glycemic control in the older age group. Similar findings have been reported in a study previously conducted at our center [22]. Previous studies from other parts of India have revealed retinopathy in 17.6%, diabetic nephropathy in 6.9%, and neuropathy in 19.5% [24]. Studies from the west have revealed a very high incidence of microvascular and macrovascular complications of diabetes in individuals with earlier onset of disease. But most of those studies are prospective in nature with prolonging the duration of disease and longer follow-up periods [25, 26].

The strengths of the present study include a larger number of patients of type 2 diabetes and the availability of extensive data regarding the demography, clinical, biochemical, and complication profile. Also, it is a multi-center study, including both the government setup and the private setup, which minimize inclusion bias.

The major limitation is that it is a cross-sectional study; many variables may not be present at the visit. Follow-up data is not present.

Summary

Our study has revealed that about one-third of T2D patients are very young. The younger patients are more obese and have strong family history. A large number (73%) of these

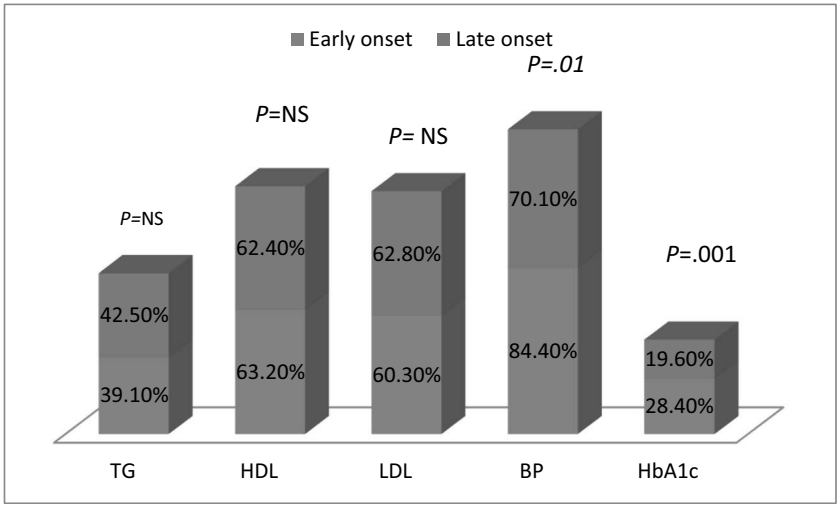

Fig. 1 Percentage of type 2 diabetes mellitus patients achieving guideline targets in the early- and late-onset group. NS, non-significant; TG, triglyceride; BP, blood pressure

populations have uncontrolled diabetes. Though the prevalence of complication was lower in young adults, but with a greater life expectancy, they are vulnerable to develop the complications in the future. With increasing prevalence of obesity and rapid urbanization, the proportion of young patients with diabetes are going to increase in the future. A specific and focused strategy for the prevention and management of diabetes in this subgroup is needed. Also, the overall control of hyperglycemia in our diabetic patient is still far from being satisfactory. We need strategies to improve the care of our patients with diabetes. Also, longitudinal studies to assess the course of the disease in the younger population are needed.

Compliance with ethical standards

Conflict of interest All the three authors declare that they do not have any conflict of interest.

Research involving human participants and/or animals Yes.

Informed consent Approval was obtained from the institutional ethics committee. The procedures used in this study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all the individuals who participated in the study.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):404–411
https://doi.org/10.1007/s13410-021-00919-7

ORIGINAL ARTICLE

Type 1 diabetes in children: a scientometric assessment of Indian research output from 1990 to 2019

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Received: 14 February 2020 / Accepted: 8 January 2021 / Published online: 25 January 2021

Abstract

Background Pediatric type 1 diabetes (T1D) is an intensively researched disease in the developed countries. However, the Indian contribution to global pediatric T1D research is limited.

Objective To provide a comprehensive analysis of Indian research on pediatric T1D over the past 3 decades.

Methods Articles on pediatric T1D published from 1990 to 2019 were retrieved from Scopus database. Specifically, the data on number of publications, top productive institutions and authors, citation analysis, highly cited articles, international collaboration, and active journals were extracted and presented using appropriate bibliometric indicators.

Results The USA leads the global ranking of 90 countries with 29.29% publications share, followed by the UK, Germany, Italy, Sweden, and Australia with 10.77 to 5.48% share. India is ranked 17th and contributed 1.98% to global output. India's publications averaged 9.74 citations per paper (CPP) compared to global average of 23.61. During 1990–2019, Indian research registered an average annual growth of 37.47% compared to global output of 12.45%. The most productive organizations were PGIMER, Chandigarh, and AIIMS, New Delhi, contributing 37 and 26 publications respectively. The most impactful publications were from MDRF, Chennai, and DRC, Chennai, with CPP of 20.36 and 19.56 and relative citation index of 2.09 and 2.01 respectively. Devi Dayal and V. Mohan were the most productive authors with 27 and 13 papers respectively.

Conclusions Indian contribution to global scientific output on pediatric T1D is lagging in quantity and quality compared to developed countries but has shown improvement in recent years. There is a need for Indian researchers to focus on collaborative research in pediatric T1D.

Keywords Type 1 diabetes · Pediatric diabetes research · Indian publications · Scientometrics · Bibliometrics

Introduction

Type 1 diabetes (T1D) is the most common endocrine disease in children with an increasing global incidence of 3–5% per year. India is witnessing a consistent increase in the incidence of T1D over the past several years along with Finland, Sweden, and Germany [1]. India now ranks first in the countries of the world for number of incident cases of T1D in children and adolescents [1]. Among the countries in the Southeast Asia region, India accounts for 93% of all children with T1D [1]. Recent data suggest that the estimated incidence of T1D in Indian youth is 4.9 cases/100000 as compared to 21.2 cases/100000 in the developed countries such as the USA [2]. However, the estimated total cases of T1D in Indian children aged 0–19 years (1,071,300) was almost equal to that of USA (1,075,900) in 2019 [1]. Furthermore, the sheer increase in the number of Indian children and adolescents with T1D from 128,500 in 2017 to the current 184,100, and the estimated number of 21,300 new T1D cases added each year, is alarming [1, 2]. Epidemiological studies also indicate an increasing prevalence of T1D in Indian children over the past decade [3, 4].

There are several reasons why conducting indigenous research in pediatric T1D is important. The characteristics of pediatric T1D differ considerably in different ethnic populations due to its heterogeneous nature [5]. The modes of

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presentation also differ and the recognition of this fact can lead to a more useful disease classification and better therapeutic decision-making. There is also a need to further understand factors that lead to high rates of diabetic ketoacidosis (DKA) at onset of diabetes or later in Indian children [6]. Several other issues of Indian children with T1D such as deaths before diagnosis, long-term complications rates and mortality, high financial burden of managing T1D, and poor follow-up rates need to be addressed through research [7–9]. In addition, research is also needed to develop low-cost insulins and delivery devices, glucose measurement devices, and use of modern gadgets and telemedicine, etc., to improve care of children with T1D in a low-resource setting like ours.

Unfortunately, however, the Indian research contribution in pediatric T1D appears limited. The global pediatric T1D research landscape is dominated by North-American and Western-European countries [10]. A recent scientometric analysis of published research in T1D shows that India lags behind several other countries with a similar disease burden, both quantitatively and qualitatively [11]. India's share in the global T1D research published during 1996–2020 was only 1.7% as compared to approximately 4 to 30% of the top 10 countries [10]. However, a precise estimation of the research output on pediatric T1D from India is unknown. The previous bibliometric analyses of India's diabetes research did not evaluate pediatric T1D separately [12–14]. Even globally, only a brief bibliometric assessment of Australian T1D research is reported by Juvenile Diabetes Research Foundation [15]. We therefore planned to undertake the evaluation of Indian research in pediatric T1D over the past 3 decades with an aim to generate some quantitative statistics which may help the policy makers to improve future research planning in this field.

Objectives

The study aims to examine the quantitative and qualitative performance of India's research on pediatric T1D based on indexed publications in international Scopus database during 1990–2019. Only publications pertaining to T1D as defined by the International Society for Pediatric and Adolescent Diabetes (ISPAD) were included for analysis [16]. In particular, the study focuses India's annual average and absolute publication growth, global share, citation impact of research output, distribution of publication output by broad sub-fields, identification of significant keywords, profile of India's top organizations and authors, the media of research communications, and characteristics of highly cited papers.

Materials and methods

Publication data for the present study was retrieved from the Scopus database (http://www.scopus.com) covering the 30-year period between 1990 and 2019. A set of keywords such as "Type 1 Diabe*" and (Child* or pediatric* or juvenile) were suffixed to "TITLE-ABS-KEY" tag and the search output further refined by period "1990–2019" using "date range tag" and country including India to identify top 10 most productive countries in pediatric T1D research. The search strings used for overall data and the India specific data are shown below:

TITLE-ABS-KEY("Type 1 Diabet*" and (Child* or pediat* or juven*)) AND PUBYEAR >1989 AND PUBYEAR <2020

TITLE-ABS-KEY("Type 1 Diabet*" and (Child* or pediat* or juven*)) AND PUBYEAR >1989 AND PUBYEAR <2020 AND (LIMIT-TO AFFILCOUNTRY, "India")

Citations were counted from the date of their publication until January 2, 2020. A complete counting method, wherein every contributing author or organization covered in multiple authorship papers is fully counted, was used. All authors or organizations of multi-authored papers received equal credit in data counting and analysis. All types of publications as listed in the Scopus were used. The quality of research was assessed in terms of citations per paper (CPP), relative citation index (RCI), and h-index (HI). The CPP was defined as the total number of citations divided by the total number of papers. The RCI, a metric of influence of a publication, was calculated by dividing the number of citations that a paper received by the average number of citations an article usually receives in that particular field. The number is then benchmarked against the median Relative Citation Ratio for all NIH-funded papers [17]. H-index, also known as Hirsch index, is defined as the maximum value of h such that the given author/journal has published h papers that have each been cited at least h times.

Results

Indian versus global publication output

The global and India's publication research output on pediatric T1D cumulated to 13,987 and 278 publications, respectively, during 1990–2019. Over the 30-year period, the global and Indian output registered an average annual growth of 12.45% and 37.47% respectively. The absolute growth in global and India's output was 325.23% and 869.23%, respectively, up from 2663 and 26 publications during 1990–2004 to 13,987 and 278 publications during 2005–2019. India's global share increased from 0.98% during 1990–2004 to 2.23% during 2005–2019, averaging 1.9% during 1990–2019. The global and India's publications averaged 23.61 and 9.74 CPP, respectively, during 1990–2019. India's citation impact, however, decreased from 14.62 CPP during 1990–2004 to 9.24 CPP during 2005–2019. Sixty (21.6%) of Indian publications

had international collaboration involving 30 countries. However, the share of international collaborative papers (ICP) decreased from 38.5% during 1990–2004 to 19.8% during 2005–2019 (Table 1). Among the collaborating countries, USA contributed the largest number of publications [27], followed by Sweden and the UK (11 each); Australia (10 publications); Canada (8 publications); Belgium, Denmark, Germany, and Malaysia (7 each); and France and Italy (6 each). Of the total Indian publications, 227 (81.65%) appeared as original articles, 22 (7.91%) as review papers, 14 (5.04%) as letters, 7 (2.52%) as conference papers, 3 (1.08%) as short notes, and 1 (0.72%) each as editorial, book chapter, or short survey. The original articles were identified through keywords as controlled studies (92), cross-sectional studies (26), case-control studies (24), cohort analysis (14), randomized clinical trials (11), practice guidelines (9), clinical trials (8), multi-center studies (7) comparative studies (7), and observational studies (7), etc.

Table 1 World and India's publication output and citations count in pediatric type 1 diabetes research, 1990–2019

Publication period	World	India					
	TP	TP	TC	CPP	TP (%)	ICP	ICP (%)
1990	49	1	10	10	2.04	0	0
1991	61	1	4	4	1.64	0	0
1992	77	0	0	0	0	0	0
1993	67	0	0	0	0	0	0
1994	61	0	0	0	0	0	0
1995	43	0	0	0	0	0	0
1996	53	1	32	32	1.89	1	100.0
1997	59	0	0	0	0	0	0
1998	110	1	0	0	0.91	0	0
1999	228	1	8	8	0.44	1	100.0
2000	242	2	34	17	0.83	0	0
2001	325	5	66	13.2	1.54	1	20.0
2002	373	3	24	8	0.80	3	100.0
2003	429	4	102	25.5	0.93	1	25.0
2004	486	7	100	14.29	1.44	3	42.9
2005	508	3	60	20	0.59	1	33.3
2006	534	5	37	7.4	0.94	2	40.0
2007	559	5	94	18.8	0.89	0	0
2008	649	9	101	11.22	1.39	1	11.1
2009	607	6	67	11.17	0.99	1	16.7
2010	694	5	120	24.00	0.72	2	40.0
2011	730	17	575	33.82	2.33	5	29.4
2012	750	9	92	10.22	1.20	1	11.1
2013	824	14	108	7.71	1.70	2	14.3
2014	832	16	319	19.94	1.92	4	25.0
2015	901	39	193	4.95	4.33	2	5.1
2016	912	29	299	10.31	3.18	4	13.8
2017	905	25	192	7.68	2.76	7	28.0
2018	971	28	55	1.96	2.88	9	32.1
2019	948	42	16	0.38	4.43	9	21.4
1990–2004	2663	26	380	14.62	0.98	10	38.5
2005–2019	11,324	252	2328	9.24	2.23	50	19.8
1990–2019	13,987	278	2708	9.74	1.99	60	21.6

TP, total papers; *TC*, total citations; *CPP*, citations per paper; *ICP*, international collaborative papers

Top 10 countries in pediatric T1D research

Although pediatric T1D research has been carried out in more than 90 countries, 82.96% of the global publication share comes from only 10 countries. The average CPP of the top 10 countries was higher as compared to the average global CPP (32.6 versus 23.6). The USA leads the ranking with almost one-third of global share followed by other developed countries (Table 2). Seven of the top 10 countries, namely the USA, Germany, Australia, Canada, Sweden, Poland, and Denmark, recorded an increase in the publication share from 0.24 to 7.47% while the other 3, namely UK, Finland, and Italy, showed a fall from 3.80 to 1.63% during the two 15-year intervals (Table 2). On correlating research contribution and prevalence in various countries, the USA was the only country showing publication share exceeding the estimated prevalence of T1D (Fig. 1). India is currently placed at 17th position in the global publications output, up from 35th position during 1990–2004.

Subject-wise distribution of research output

Medicine accounts for 82.73% of publication share in Indian research on pediatric T1D followed by biochemistry, genetics, and molecular biology with 41.01%, and immunology and microbiology with 5.76% share. Based on the activity index, it was observed that the research activities have decreased in all three subjects. Immunology and microbiology recorded the highest CPP of 23.75 followed by medicine (9.90), and biochemistry, genetics and molecular biology (8.97) (Supplementary Table 1). According to the major areas of research, 106 publications were related to clinical spectrum, 66 to epidemiology, 38 to diabetic complications, 26 to pathogenesis, 23 to genetics, and 19 to treatment outcomes.

Significant keywords

We identified 63 significant keywords from the literature on Indian pediatric T1D which throw light on the research trends, factors involved, and type of complications in research (Supplementary Table 2).

Table 2 Global publication output and share of top 10 most productive countries in pediatric type 1 diabetes research, 1990–2019

S. no.	Country	Number of papers			Share of papers (%)			TC	CPP	RCI
		1990–2004	2005–2019	1990–2019	1990–2004	2005–2019	1990–2019			
1	USA	619	3478	4097	23.24	30.71	29.29	141,857	34.62	1.47
2	UK	322	1185	1507	12.09	10.46	10.77	55,814	37.04	1.57
3	Germany	119	994	1113	4.47	8.78	7.96	35,913	32.27	1.37
4	Italy	274	735	1009	10.29	6.49	7.21	25,872	25.64	1.09
5	Sweden	158	719	877	5.93	6.35	6.27	26,566	30.29	1.28
6	Australia	92	621	713	3.45	5.48	5.10	19,792	27.76	1.18
7	Finland	173	482	655	6.50	4.26	4.68	29,984	45.78	1.94
8	Poland	115	519	634	4.32	4.58	4.53	7586	11.97	0.51
9	Canada	87	475	562	3.27	4.19	4.02	19,632	34.93	1.48
10	Denmark	78	359	437	2.93	3.17	3.12	15,349	35.12	1.49
	Total of top 10 countries	2037	9567	11,604	76.49	84.48	82.96	378,365	32.61	1.38
	Total of world	2663	11,324	13,987				330,227	23.61	1.00

TP, total papers; *TC*, total citations; *CPP*, citations per paper; *RCI*, relative citation index

Top 15 Indian organizations

During 1990–2019, 133 Indian organizations were involved in pediatric T1D research of which 54 organizations published 1 paper each, 27 organizations 2 papers each, 21 organizations 3 papers each, 16 organizations 4 papers each, 4 organizations 5 papers each, 7 organizations 6–10 papers each, and 4 organizations 14–37 papers each. The productivity of top 15 organizations varied from 4 to 37 publications per organization; together they contributed 57.91% of India's publication share and 48.56% of citation share (Table 3). Four organizations that registered their publication output above the group average of 10.73 were Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, with 37 papers; All India Institute of Medical Sciences (AIIMS), New Delhi, with 26 papers; and Madras Diabetes Research Foundation (MDRF), Chennai, and Sanjay Gandhi Postgraduate Institute of Medical Education and Research (SGPIMER), Lucknow, with 14 papers each. Five organizations recorded CPP and RCI above the group average of 8.17 and 0.84; MDRF 20.36 and 2.090; Diabetes Research Centre, Chennai, 19.56 and 2.01; Bangalore Diabetes Hospital, Bengaluru, 13.75 and 1.41; AIIMS 13.75 and 1.41; and Ramaiah Medical College, Bangalore, 9.71 and 1.0 respectively.

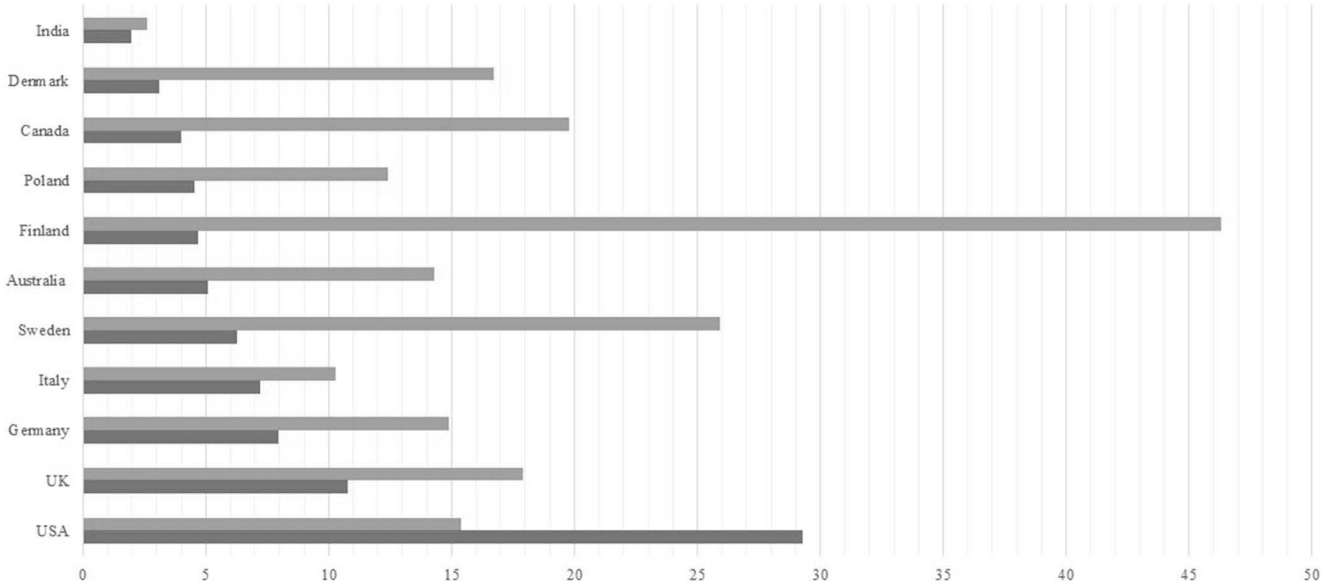

Fig. 1 Estimated prevalence of type 1 diabetes per 100000 children aged 0–14 years (orange lines) versus publication share (blue lines) of top countries

Table 3 Scientometric profile of the top 15 most productive Indian organizations in pediatric type 1 diabetes research, 1990–2019

S. no.	Name of the organization	TP	TC	CPP	HI	ICP	ICP (%)	RCI
1	Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh	37	241	6.51	7	1	2.70	0.67
2	All India Institute of Medical Research (AIIMS), New Delhi	26	280	10.77	11	6	23.08	1.11
3	Madras Diabetes Research Foundation (MDRF), Chennai	14	285	20.36	10	9	64.29	2.09
4	Sanjay Gandhi Postgraduate Institute of Medical Education & Research (SGPIMER), Lucknow	14	69	4.93	6	5	35.71	0.51
5	Diabetes Research Centre (DRC), Chennai	9	176	19.56	6	4	44.44	2.01
6	Bharti Hospital, Karnal	8	7	0.88	1	1	12.50	0.09
7	Jehangir Hospital, Pune	8	24	3.00	4	1	12.50	0.31
8	Jawaharlal Nehru Medical College (JNMC), Belgaum	7	36	5.14	3	5	71.43	0.53
9	Ramaiah Medical College (RMC), Bangalore	7	68	9.71	3	0	0.00	1.00
10	Institute of Post Graduate Medical Education & Research (IPGMER), Kolkata	7	24	3.43	3	0	0.00	0.35
11	King George's Medical University, Lucknow	6	15	2.50	2	1	16.67	0.26
12	Radhakrishan Hospital, Kurukshetra	5	1	0.20	1	1	20.00	0.02
13	Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Pondicherry	5	24	4.80	4	1	20.00	0.49
14	Bangalore Diabetes Hospital, Bengaluru	4	55	13.75	3	1	25.00	1.41
15	King Edward Memorial Hospital (KEMH)	4	10	2.50	2	1	25.00	0.26
	Total of 15 Indian organizations	161	1315	8.17	4.4	37	22.98	0.84
	Total of India	278	2708	9.74				
	Share of top 15 organizations in India's total output	57.91	48.56					

TP, total publications; *TC*, total citations; *CPP*, citations per paper; *ICP*, international collaborative papers; *RCI*, relative citation index

Top 15 most productive authors

Of the 193 Indian authors who contributed to T1D research during 1990–2019, 63 published 1 paper each, 48 published 2 papers each, 40 published 3 papers each, 17 published 4 papers each, 8 published 5 papers each, 14 published 6–10 papers each, and only 3 authors published 11–23 papers each. The research productivity of top 15 authors varied from 6 to 27 publications per author together contributing 146 (52.52%) publications and 1240 (45.79%) citations. The authors who registered their publication output above the group average of 9.73 were Devi Dayal, Viswanathan Mohan, Sanjay Kalra, and Nikhil Tandon (Table 4). Six authors registered CPP and RCI above the group average of 8.49 and 0.87: A Ramachandran, V Mohan, A Amutha, SK Bhadada, A Bhansali, and N Tandon (Table 4).

Research funding

Only 33 (11.8%) of Indian publications were funded by national or international agencies. The number of funded publications increased from only 5 during 1990–2004 to 28 during 2005–2019. Among the major Indian funding agencies, Indian Council of Medical Research (ICMR) funded 4 publications, the Department of Science and Technology (DST) funded 3 papers, while MDRF, Chennai, the Department of Biotechnology (DBT), India, DBT, West Bengal, and DST, Kerala, funded 2 publications each. The governmental support was limited to 17 (6.1%) publications through the various funding agencies. Among the foreign funding agencies, 4 publications received support from the National Institute of Health, USA, and 3 each from Medtronic and Novotronic. The funded publications received higher CPP than the average of all publications (22.33 versus 9.74).

Medium of research communication

Of the total research output, 272 (97.84%) appeared in journals, 4 (1.44%) in book series, and 1 (0.36%) each as book and undefined. Of the 115 journals which reported 274 articles, 78 published 1 paper each, 11 published 2 papers each, 12 published 3 papers each, 3 published 4 paper each, 2 published 5 and 6 papers each, and 7 published 8–28 papers each. The 15 most productive journals accounted for 51.84% share of total research output which increased from 48.15 to 52.24% between 1990 and 2004 and 2005–2019 (Supplementary Table 3). The top four most productive journals were Indian Journal of Endocrinology and Metabolism (IJEM) (28 papers), Indian Pediatrics (17 papers), Pediatric Diabetes (14 papers), and International Journal of Diabetes in Developing Countries (IJDDC) (13 papers). In terms of CPP, the top four journals were Pediatric Diabetes (22.21), Diabetes Research

Table 4 Scientometric profile of top 15 most productive Indian authors in pediatric type 1 diabetes research, 1990–2019

S. no.	Author	Affiliation	TP	TC	CPP	HI	ICP	ICP (%)	RCI
1	D. Dayal	PGIMER, Chandigarh	27	158	5.8	6	0	0.0	0.60
2	V. Mohan	DRF, Chennai	13	254	19.5	9	7	53.8	2.01
3	S. Kalra	Bharati Hospital, Karnal	11	19	1.7	3	2	18.1	0.18
4	N. Tandon	AIIMS-New Delhi	10	96	9.6	6	4	40.0	0.99
5	A. Bhansali	PGIMER, Chandigarh	9	98	10.8	4	1	11.1	1.12
6	E. Bhatia	SGPGIMER, Lucknow	9	59	6.5	5	4	44.4	0.67
7	R. Kumar	PGIMER, Chandigarh	9	22	2.4	3	0	0.0	0.25
8	S.K. Bhadada	PGIMER, Chandigarh	8	100	12.5	4	1	12.5	1.28
9	V. Bhatia	SGPIMER, Lucknow	8	51	6.3	4	5	62.5	0.65
10	S. Chowdhury	IPGMER, Kolkata	8	25	3.1	3	0	0.0	0.32
11	N. Sachdeva	PGIMER, Chandigarh	8	33	4.1	3	0	0.0	0.42
12	G. Kaur	AIIMS, New Delhi	7	52	7.4	5	3	42.8	0.76
13	A Ramachandran	DRC, Chennai	7	167	23.8	6	4	57.1	2.45
14	A. Amutha	DRC, Chennai	6	92	15.3	5	4	66.6	1.57
15	P. Dabadghao	SGPIMER, Lucknow	6	14	2.3	2	2	33.3	0.24
	Total of 15 organizations		146	1240	8.4	4.5	37	25.3	0.87
	Total of India		278	2708	9.7				
	Share of 15 authors in India's total output		52.5	45.7					

TP, total papers; *TC*, total citations; *CPP*, citations per paper; *ICP*, international collaborative papers; *HI*, Hirsch index or h-index; *RCI*, relative citation index

and Clinical Practice (19.0), Diabetic Medicine (8.40), and Indian Journal of Medical Research (5.70).

Highly cited papers

Only 14 publications received 40 to 276 CPP (average 88.71, total 1242 CPP). Of these, 7, 5, and 2 publications, respectively, were non-collaborative, international collaborative, and national collaborative. The USA and France collaborated in 4 and 3 papers each while 21 other countries collaborated with 1 paper each. The 14 highly cited papers involve 168 authors (47 Indian) and 96 organizations (20 Indian). Among 20 Indian organizations, 2 papers were contributed by PGIMER, Chandigarh, and 1 paper each by 19 other Indian organizations. The highly cited papers are published in 12 journals with 2 papers each in Pediatric Diabetes and Journal of the Indian Medical Association and 1 paper each in 10 other journals.

Discussion

Over the past 3 decades, research in the developed countries led by the USA has made significant contribution towards understanding of childhood-onset T1D [18]. The research has made dramatic improvements in the health and quality of life of children with T1D while continuing to strive for the ultimate goal of cure [18]. The contribution of developing countries including India to the global pediatric T1D research appears to be lagging in quality as well as quantity. Although India has shown a jump in global rankings and publication share over the last 15 years, its contribution is still < 2% despite the disease burden of pediatric T1D almost equal to that of the USA. Only a few research organizations such as PGIMER, AIIMS, MDRF, and SGPIMER appear to have contributed to India's publication growth of 969.23% compared to 325.23% of global output over the last 15 years.

The lower citation impact of Indian research in pediatric T1D in comparison to the global research is understandable as the funding required to conduct and publish quality research is limited due to other healthcare priorities of a developing nation [19]. The decrease in citation impact from 14.62 CPP during 1990–2004 to 9.24 CPP during 2005–2019 is also a matter of concern. The highly organized scientific research activity required to produce impactful research needs to be funded and driven by the national governments [19]. Unfortunately, however, T1D often does not find priority for governmental support due to the much highly prevalent T2D [10]. Another reason for low citations is the relatively recent increase in the pediatric T1D research activity in India, in particular of the major contributing organizations such as PGIMER and AIIMS. Citations are accumulated over time. Several original articles published in reputed journals during the last few years are likely to show their impact over time

[20–23]. In particular, recent publications emanating from the focused research by the PGIMER group on the role of T cells in pathogenesis and cellular therapy in pediatric T1D appear poised for accumulating citations over the next few years [24–29]. Other publications on the role of vitamin D in T1D by the same group are already among the well-cited papers [30–32]. Biases in citing articles from authors of developing countries may also contribute to low CPP and impact [12].

The international collaboration has shown a decrease during the last 15 years to almost half of its previous level of 38.5%. This is intriguing as the collaborative research between advanced and developing country scientists in the field of science in general and diabetes in particular has shown an increase over the past few decades [12]. One reason could be the decreasing knowledge gradient that generally drives the interest of poorer partner [12]. Additionally, the researchers from developed countries may not consider authors from developing countries as partners in the real research tasks such as theoretical synthesis and new theoretical configurations [12].

An important finding of our analysis is the wide gap in pediatric T1D research between India and some of the developed countries both quantitatively and qualitatively. The primary reason is lack of focus on pediatric T1D research of the stakeholders despite an alarming increase in disease burden over the past several years [1, 2]. Several stakeholders such as the Indian government, research organizations, professional bodies, and researchers' groups need to collaborate to develop research capacity necessary to deal with T1D in Indian children. As quality research requires funding, the government needs to increase investment through research organizations such as the ICMR, DBT, and DST, similar to what other countries with high pediatric T1D burden have done over the last few decades [33]. Taking a clue from the developed countries, the government can facilitate the formation of a task force which can identify priority research areas and guide further research in T1D [33]. The professional bodies such as Research Society of Study of Diabetes in India (RSSDI), Endocrine Society of India (ESI), and Indian Society for Pediatric and Adolescent Endocrinology (ISPAE) can develop research focus on childhood-onset T1D, similar to professional bodies in the developed countries with declared T1D research missions [33]. Indian researchers in pediatric T1D should develop better collaboration on research, similar to the collaborative approaches in developed countries such as The Pediatric Diabetes Consortium in the USA [34]. The increase in research activity will result in improvement in care of children with T1D as well as increase the quantity and quality of Indian publication output in this field in future. A journal for pediatric diabetes or a dedicated section to pediatric diabetes in journals such as IJDDC or IJEM may also help improve the publication output in pediatric T1D in India.

There are some limitations of the current bibliometric analysis. Although we standardized the names of the authors to avoid spelling errors in names and initials, and resolved the issue of synonyms or homonyms in authors' names by using other specific fields such as affiliations, all the data was probably not captured. For example, the PubMed search showed 39 T1D articles authored by Dayal D while Scopus retrieved only 27 at the time of current analysis. Secondly, we could have missed some publication data by restricting of our search to only the Scopus database. We chose Scopus as it provides a much larger content coverage, search analysis tools, and funding information as compared to other medical databases such as PubMed and Web of Science [35, 36]. A simultaneous exhaustive search in the three major databases, as suggested by some authors, may have allowed us to capture additional data [12]. But even combined search involving all these databases may not capture all publications from developing countries [12, 36]. It is well known that scientific articles in developing countries are under-represented in international databases and even the developing country targeted indexing services such as the WHO's ExtraMed have not succeeded to overcome this problem [12]. Despite these limitations, the current study provides an insight into the gaps in T1D research in India and may serve as an important framework for national research initiatives in this field.

In conclusion, Indian research in T1D is lagging behind several other countries having similar disease burden, both in quantity and quality. There is a need for Indian researchers to foster national and international collaboration in pediatric T1D research.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-021-00919-7.

Compliance with ethical standards

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

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):412–418
https://doi.org/10.1007/s13410-020-00902-8

ORIGINAL ARTICLE

Prevalence and risk factors associated with diabetes in Meru County, Kenya: a cross-sectional study

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Received: 28 July 2020 / Accepted: 8 December 2020 / Published online: 14 January 2021

Abstract

Background Diabetes has emerged as a leading global health problem associated with severe morbidity, mortality, and health-system costs. This is attributed to population growth, aging, urbanization, physical inactivity, and obesity. The increased prevalence of diabetes particularly in rural settings creates a public health challenge for prevention and treatment. However, there is currently a dearth of data supporting planning and implementation of programs for prevention and management of diabetes in rural communities.

Purpose of the study The objective of this study was to estimate the prevalence of diabetes and its associated risk factors among a rural population in Meru County, Kenya.

Methods A descriptive cross-sectional study was conducted in Imenti South, rural areas in Meru County between September and November 2019. Data from 435 respondents comprising 263 (60.5%) females and 172 (39.5%) males were analyzed. Prevalence ratios were calculated using Poisson regression models with robust variance to explore factors associated with the prevalence of diabetes.

Results The prevalence of diabetes was higher among women (16.35%, 95% CI: 12.3–21.4) compared to that among men (13.95%, 95% CI: 9.5–20) and significantly increases with advancing age, BMI, previous diagnosis of hypertension, and high cholesterol. Our findings showed an overall diabetes prevalence of 15.4% (95% CI: 12.3–19.1) in the study area. Age, hypertension, BMI, physical inactivity, alcohol consumption, and tobacco use were significantly associated with a higher risk of diabetes.

Conclusion Preventive intervention strategies should aim to address the modifiable correlates so as to reduce the burden of diabetes in rural communities in Kenya.

Keywords Diabetes · Prevalence · Risk factors · Rural · Kenya

Introduction

In recent years, diabetes has emerged as a leading global health problem associated with severe morbidity, mortality, and health-system costs. Population growth, aging, urbanization, physical inactivity, and obesity are driving the diabetes epidemic globally. The International Diabetes Federation estimates that diabetes currently affects 463 million adults worldwide, and by 2030, approximately 578 million people will live with diabetes [1]. The prevalence of diabetes has increased particularly in low- and middle-income countries [2]; estimates suggest that in 2019, 19 million Africans had diabetes; this number is anticipated to rise to 29 million by 2030 [1].

Kenya like many other African countries is experiencing a transition from infectious diseases to non-communicable diseases (NCDs) as the leading cause of morbidity and mortality. Previous studies [3, 4] have found that sedentary behavior, excessive caloric intake, processed diets, and low physical activity are associated with an increased risk of diabetes. The prevalence of diabetes was 3.6% in 2013 and is projected to increase to 4.4% by 2035 [5]. Earlier studies in Kenya have reported a prevalence range of 5.3–6.6% [6, 7], with a

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varying prevalence across different rural and urban communities. An additional contributor to the burden of the disease is the prevalence of undiagnosed diabetes which further complicates the situation. About two-thirds of people with diabetes are unaware of their condition; hence, these numbers may underestimate the overall prevalence [8]. Previous studies have also demonstrated the substantial health burden for patients, families, and the health system in rural communities [9]. The County morbidity records indicate that in 2018, there were 60,275 cases of its adult population enrolled in diabetic and hypertension treatment clinics with Imenti South recording 4296 diabetes-related morbidity. Despite the high incidences of diabetes-related mortality, there is limited data to support planning and implementation of programs for prevention and management of diabetes in rural populations. Therefore, this study aims to determine the prevalence of diabetes and its associated risk factors among a rural population in Meru County, Kenya.

Methodology

Materials and methods

This was a descriptive cross-sectional study conducted in Imenti South, Meru County, between September and November 2019. A sample size of 435 participants was estimated using the Yamane formula:

$$n = \frac{N}{1 + N(e)^2}$$

where N is the estimated number of households, which was 111,591. This was estimated from the population size of 458,362, given a household size of 4, while e^2 is the error limit, adding a 10% non-response rate.

A multistage sampling method was applied in the selection of participants for the research. The stages involved the following: stage 1, simple random sampling to select 30% of the six wards out of the eight wards in Imenti Sub-county; stage 2, 10% of the villages in each of the three wards were then selected from the communities in the wards by simple random sampling. Proportionate allocation of the sample size ensured that a site with a higher population was allocated with a larger portion of respondents.

In each village, research assistants standing at the center of the village spun a bottle to determine the direction of the first house. Next, the researchers took the right direction from the first participant household to the next household. The procedure was followed until the required sample size was met. On visiting the house, the research assistants obtained informed consent from the household head or spouse (pregnant women were not included) to participate in the survey. The data was collected using a pretested questionnaire that captured information on sociodemographic characteristics, habits, lifestyles, and self-assessment of one's state of health. The outcome variable of interest was self-reported diabetes, according to positive answers to the questions "Has a doctor ever told you that you have diabetes?" and "Are you taking medication for diabetes?" Similarly, individuals were considered to be hypertensive or have high cholesterol if they had been previously diagnosed by a physician.

The researchers were assisted by trained nurses and community health volunteers (CHVs) to measure the participants' blood pressure and anthropometric measurements (weight and height) using standard methods and calibrated devices. Body mass index (BMI) was then calculated as weight in kilograms divided by the squares of height in meters. Overweight was defined as a BMI $\geq$25–29.9 kg/m^2 and obesity was defined as BMI $\geq$30 kg/m^2 using WHO defined cut-off points.

Statistical analysis

The prevalence of self-reported diabetes was calculated as the proportion of those participants classified as diabetic against the total numbers of the participants. Gender-standardized prevalence proportion and its 95% confidence intervals (CIs) were estimated on all the known diabetes risk factors. Descriptive statistics in the form of means and proportions were calculated for continuous and discrete variables, respectively. Univariate analysis was done using the chi-square test, to find the association between various factors and diabetes status.

Poisson regression models with robust variance were fitted to explore factors associated with the prevalence of diabetes and presented as both crude and risk ratios (RRs) with 95% confidence intervals (CIs) adjusted to age, BMI, hypertension, physical activity, use of alcohol, and smoking status. Analyses were performed using STATA version 13 (StataCorp, College Station, USA).

Results

A total of 435 respondents participated in the study, with a mean age of 49.09 years (range 19–99 years). There was a predominance of women in the study sample (60.5%, 263). Only 24% had received post-primary education and

15% had never been to school. Table 1 describes the demographic characteristics and prevalence and risk factors of diabetes stratified by sex. Overall, 15.4% (95% CI: 12.30–19.12) of the respondents had diabetes; prevalence of diabetes was higher among women (16.35%) compared to that among men (13.95%).

Diabetes prevalence was found to significantly increase with advancing age, BMI, previous diagnosis of hypertension, and high cholesterol. The prevalence of diabetes increased among both men and women by age group; those aged 60 years and older had the highest prevalence of diabetes of 19.05% (95% CI: 10.97–30.99) for men while females aged

Table 1 Prevalence of diabetes by sex, according to sociodemographic and other risk factors

Characteristics	Total (%)	95% CI	Male (%)	95% CI	Female (%)	95% CI	*p* value*
Total	*15.40*	12.30–19.12	*13.95*	9.51–20.01	*16.35*	12.34–21.35	0.458
Age group							
18–29 years	1.52	0.21–10.17	0.000		2.27	0.29–15.48	0.001
30–44 years	12.20	7.46–19.31	12.2	4.97–26.94	12.2	6.60–21.44	
45–59 years	21.74	15.09–19.31	15.22	7.22–29.28	26.09	16.90–37.98	
60 years and above	19.85	13.85–27.61	19.05	10.97–30.99	20.59	12.42–32.15	
Mean age	49.09	47.43–50.74	51.86	49.29–54.43	47.27	45.13–49.42	0.008
Marital status							
Married	15.90	12.07–20.66	16.26	10.67–23.99	15.63	10.74–22.18	0.384
Separated/divorced	15.38	5.76–35.09	0.000		30.77	10.21–63.46	
Single	8.47	3.54–18.96	9.09	2.02–32.65	8.11	2.49–23.34	
Widowed	19.40	11.55–30.74	14.29	2.92–48.01	20.75	11.65–34.22	
Level of education							
None	14.58	7.04–27.78	8.33	0.82–50.08	16.67	7.38–33.43	0.828
Primary education	16.54	12.48–21.59	16.16	10.06–24.94	16.77	11.71–23.43	
Secondary education	14.02	8.60–22.03	12.5	5.55–25.76	15.25	7.97–27.21	
Tertiary	10.00	2.41–33.31	7.69	0.78–46.89	14.29	0.95–74.33	
High cholesterol (yes)	53.85	27.21–78.45	75	17.75–97.66	44.44	16.46–76.46	0.000
Hypertension (yes)	36.02	28.95–43.77	40	25.94–54.06	34.23	25.27–43.20	0.000
BMI							
Normal weight (18.5–24.9)	12.24	8.33–17.66	11.34	6.32–19.50	13.13	7.70–21.49	0.040
Underweight (< 18.5)	2.78	0.38–17.76	5.56	0.62–35.45	0.00		
Overweight (25.0–29.9)	20.88	13.67–30.53	19.23	7.73–40.37	21.54	13.01–33.51	
Obesity (≥ 30.0)	17.54	9.64–29.80	7.14	0.75–44.07	20.93	10.96–36.27	
Tobacco[1]							
Never used	17.28	13.40–21.99	16.22	9.32–26.71	17.62	13.17–23.18	0.223
Yes—but stopped using	8.11	2.59–22.66	9.52	2.10–34.02	6.25	0.68–39.31	
Yes—currently using	12.37	7.13–20.61	12.99	7.03–22.75	10.00	2.19–35.51	
Physical activity							
Low (< 150 min/week)	14.81	11.58–18.73	13.46	8.90–19.85	15.72	11.53–21.07	0.338
High (≥ 150 min/week)	20.00	11.02–33.53	18.75	5.33–48.59	20.59	9.75–38.37	
Currently take alcohol (yes)	10.64	5.79–18.74	12.86	6.72–23.22	4.17	0.50–27.35	0.148
Bad fat intake[2]	10.26	5.18–19.30	7.50	2.32–21.71	13.16	5.35–28.87	0.165
High salt intake[3]	8.76	5.64–13.35	6.33	2.61–14.57	10.14	6.06–16.50	0.000
Total (*N*)	435	435	172	172	263	263	

[1] Tobacco use was defined as self-reported within the past 30 days of smoked products (cigarettes, hand-rolled, cigars, water pipes/shisha, or pipes/kiko) and smokeless tobacco products (snuff, chewing tobacco, kuber, and pan)

[2] Bad fat intake was defined as self-reported use of saturated fats, e.g., solid fat, margarine, butter, and vegetable fat for cooking

[3] High salt intake was defined as answering often or always to questions regarding salt intake (adding salt to the plate before tasting, adding salty seasoning or a salty sauce, and eating processed food high in salt)

* *p* values derived from the chi-square test

45 to 59 years had the highest prevalence (26.09%, 95% CI: 16.90–37.98) for women.

As compared to those with normal weight, those overweight reported higher prevalence of diabetes (20.88% vs. 12.24%). The frequency of diabetes was higher among those who reported a previous diagnosis of hypertension (36.02%, 95% CI: 28.95–43.77) and high cholesterol (53.85%, 95% CI: 27.21–78.45). By education attainment, prevalence was highest among respondents who had primary-level education at 16.54% and was lowest among respondents who had tertiary-level education at 10%, although not statistically significant.

Men, compared with women, were more likely to demonstrate risky behaviors such as current tobacco use (13% vs. 10%) and current alcohol use (12.86% vs. 4.17%), whereas women had a substantially higher proportion with a high salt intake (10.14% vs. 6.33%) and bad fat intake (13.16% vs. 7.50%).

Table 2 shows the results of the robust Poisson regression model of the analysis between the prevalence of diabetes and the various potential risk factors. The coefficients represent crude and adjusted prevalence ratios (PR); their interpretation is the same as for the risk ratios. The analysis revealed that age (> 60 years or older), hypertension, BMI (overweight, BMI = 25.0–29.9), and alcohol consumption had significantly higher prevalence risk ratios (PRR) for diabetes. Compared to the 18–29-year age group, prevalence of diabetes was significantly higher among those in the 45–59 years with a PRR of 6.23 (95% CI = 0.88–44.21) and highest among those aged 60 years or older with a PRR of 6.82 (95% CI = 0.97–48.10).

Hypertension had the strongest association with diabetes of any measured variable. Respondents with hypertension had a sevenfold increased crude prevalence risk ratio for diabetes (PRR = 7.02; 95% CI: 3.37–14.64). Compared to participants with BMI of 18.5–24.9 kg/m^2, the prevalence of diabetes among overweight participants with BMI between 25 and 29.9 kg/m^2 was higher with an adjusted PRR = 1.94 (95% CI = 1.18–3.20). The risk among obese respondents (BMI ≥ 30 kg/m^2) was 1.27 (95% CI = 0.67–2.41) although not statistically significant. Higher physical activity (duration and intensity) showed an inverse association with risk of diabetes, implying that physical activity offered protection against diabetes.

Furthermore, vegetable consumption (3–4 vegetable servings) significantly lowered the risk of developing diabetes (RR 0.41, 95% CI = 0.17–0.96). Our findings are congruent with those of previous studies which suggest that high consumption of fruit and vegetable can lower blood pressure and hence reduce the incidence of diabetes.

Alcohol consumption and smoking have a strong association with diabetes, either as independent risk factors or confounding lifestyle risk factors [10]. The results indicate that higher frequency of alcohol consumption (several days each month) was significantly associated with approximately 3.83 (95% CI = 1.98–7.43) increased risk of diabetes, whereas tobacco use cessation lowered the risk by 0.16 (95% CI = 0.02–1.13).

The model was further adjusted for demographic and modifiable characteristics, as presented in Table 2. The prevalence of diabetes was highest among those aged 60 years or older with an adjusted PRR of 8.83 (95% CI = 1.16–67.43) followed by those aged 45–59 years with an adjusted PRR of 7.79 (95% CI = 1.05–57.67). Older age was the only un-modifiable factor found to be associated with diabetes in the study. Further, hypertension, being overweight, and frequency of alcohol consumption were significantly more likely to predict a high risk of diabetes. The inverse association between physical activity and diabetes remained the same after the adjustments.

Discussion

Our findings showed an overall diabetes prevalence of 15.4% in the study area. The high prevalence corresponds with that observed in other rural communities in Kenya. El-busaidy et al. [11] found a 16% diabetes prevalence rate in Isiolo County, associated with obesity, poor diet, physical inactivity, and other lifestyle behaviors. Christensen et al. [4] reported a prevalence of 4.2% among various rural and urban ethnic communities in Kenya. Similar to our findings, other studies reported that advanced age, high BMI, hypertension, low-level physical activity, and recurrent alcohol intake were probable risk factors [4, 11–13].

Our results align with previous findings that women have a higher prevalence of diabetes than men in Kenya [14, 15]. Our reported prevalence was 16.35% among women compared to men (13.95%). The higher rates of diabetes among women have been attributed to malnutrition, obesity, sedentary lifestyles, and other risk factors which contribute to the higher prevalence of diabetes [16, 17]. Our analysis has showed that mean BMI across all age groups and insufficient physical activity were higher among women in our study.

Several studies suggest that prevalence of diabetes increased with age [19, 20]. This is consistent with our findings with the disease being more prevalent among women aged 45–59 years old and men aged 60 years and older. The results of this study provide a reliable and meaningful snapshot of the current situation against which the impacts of interventions for the prevention and control of diabetes in Meru County may be measured.

Table 2 Crude and adjusted prevalence risk ratio estimates for diabetes

Variables	PR	95% CI	*p* value	Adjusted PR	95% CI	*p* value
Sex						
Female	Ref.			Ref.		
Male	1.246 (0.325)	0.748–2.077	0.398			
Age group						
18–29 years	Ref.			Ref.		
30–44 years	5.592 (5.484)	0.818–38.222	0.079	6.072 (6.185)	0.825–44.698	0.077
45–59 years	6.235 (6.231)	0.8793–44.209	0.067	7.785 (7.954)	1.051–57.669	0.045
60 years and above	6.819 (6.797)	0.9667–48.102	0.054	8.831 (9.159)	1.156–67.431	0.036
Marital status						
Married	Ref.					
Separated/divorced	0.709 (0.325)	0.289–1.739	0.452			
Single	0.632 (0.332)	0.234–1.803	0.369			
Widowed	1.061 (0.332)	0.575–1.958	0.850			
Hypertension (yes)	7.020 (2.633)	3.366–14.643	0.000	7.761 (2.799)	3.827–15.738	0.000
BMI						
Normal weight (18.5–24.9 kg/m^2)	Ref.			Ref.		
Underweight (< 18.5 kg/m^2)	0.366 (0.313)	0.068–1.960	0.240	0.387 (0.314)	0.079–1.894	0.241
Overweight (25.0–29.9 kg/m^2)	1.938 (0.494)	1.175–3.195	0.010	1.997 (0.521)	1.198–3.329	0.008
Obesity (≥ 30.0 kg/m^2)	1.273 (0.414)	0.673–2.407	0.458	1.241 (0.388)	0.672–2.292	0.490
Tobacco						
Never used	Ref.			Ref.		
Yes—but stopped using	0.157 (0.158)	0.022–1.132	0.066	0.152 (0.149)	0.022–1.036	0.054
Yes—currently using	0.814 (0.285)	0.410–1.617	0.557	0.810 (0.280)	0.411–1.594	0.542
Alcohol						
Never consumed alcohol	Ref.			Ref.		
Consuming alcohol several days each month	3.831 (1.294)	1.976–7.428	0.000	4.065 (1.400)	2.070–7.984	0.000
Fruit servings days per week						
None	Ref.					
1–3 days	1.116 (0.369)	0.588–2.134	0.740			
4–5 days	1.612 (0.685)	0.701–3.706	0.261			
6–7 days	1.794 (0.830)	0.725–4.440	0.206			
Vegetable servings days per week						
None	Ref.			Ref.		
1–2 servings	0.613 (0.204)	0.319–1.178	0.142	0.697 (0.225)	0.370–1.313	0.264
3–4 servings	0.409 (0.178)	0.174–0.960	0.040	0.521 (0.197)	0.249–1.093	0.084
≥ 5 servings	1.137 (0.479)	0.498–2.598	0.760	1.440 (0.526)	0.704–2.947	0.318
Physical activity						
Time spent vigorous activities (min)	0.997 (0.0015)	0.994–1.000	0.023	0.997 (0.001)	0.994–0.999	0.013
Time spent moderate activities (min)	0.997 (0.0023)	0.992–1.001	0.173	0.997 (0.002)	0.993–1.002	0.242
Salt						
Never	Ref.					
Often after tasting	0.577 (0.201)	0.292–1.142	0.114			
Often before tasting	1.117 (0.422)	0.533–2.342	0.770			
Constant	0.0135 (0.0129)	0.002–0.087	0.000	0.010 (0.009)	0.001–0.064	0.000

CI confidence interval; standard errors in parentheses. Adjusted RR (adjusted for age, BMI, hypertension, physical activity, alcohol, and tobacco consumption)

According to the robust Poisson model, age, hypertension, overweight, and alcohol consumption were significant predictors of disease prevalence. Pattern of higher incidences of diabetes with advancing age has been observed in previous studies [4, 18, 19]. This association could be due to the cumulative effect of the aging process, co-occurrence of multiple medical conditions, and prior exposure to various determinants of diabetes [20]. This underscores the needed public health interventions that emphasize the need for routine regular screening of blood sugar among older adults and early lifestyle modification among young individuals.

An extensive body of literature suggests that diabetes and hypertension are closely linked in etiology and disease mechanisms [21–23]. The diseases are closely interlinked because of similar risk factors resulting in a predisposition to risk of developing diabetes. Our findings indicate that hypertension was the main predictor of risk for diabetes; hence, it should be of primary consideration when assessing the priorities of diabetics. These findings emphasize the importance of adequate control of blood pressure through lifestyle modification and pharmacological therapy so as to reduce the risk of diabetes [24].

Association between obesity and risk of diabetes was observed in the study. Similar associations have reported a high BMI indicative of overweight/obesity and increased disease prevalence in Kenya [15, 19]. Increased physical activity is recommended as an effective lifestyle intervention for the management and prevention of diabetes [25].

Conclusion and recommendations

In conclusion, the high prevalence of diabetes in Imenti South, Meru County, underscores the need for interventions to prevent, control, and reduce the burden of diabetes in the future. Age, hypertension, BMI, physical inactivity, alcohol consumption, and tobacco use were associated with a higher risk of diabetes.

Based on our study findings, we make the following recommendations: (1) Foster positive behavior change through targeted social and behavior change communication (SBCC) to address the modifiable correlates as points for intervention to improve diabetes outcomes; (2) develop diabetes prevention program targeting women to reduce the burden of diabetes; and (3) strengthen the health system to enhance regular screening, early detection, and treatment of diabetes.

Acknowledgments The study team would like to thank the Project implementation team from the KRCS, Health and Social Services department, for their support and coordination. We would also like to extend our thanks to the participants for their participation in the study.

Authors' contributions SK and ASH conceptualized, wrote, and revised the manuscript. Data analysis, interpretation, and writing were conducted by SK, ASH, and RM. RM and LA were involved in data acquisition and analysis, manuscript preparation, editing, and review.

Funding This research study was funded by the Danish Red Cross (DRC) and is based on baseline findings from the DRC and Kenya Red Cross Society (KRCS) Prevention and Control of Non-Communicable Diseases (Diabetes and Hypertension) project.

Data availability The data analyzed and presented to support the study findings are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Code availability Not applicable.

Ethical consideration Informed verbal consent was sought from all participants following a detailed explanation of the study in local dialect to ensure that they understood the information to make the consent. Participation was voluntary without any coercion or penalty for refusal to participate in the study. Confidentiality was assured by undertaking the interviews in a private setting; anonymity and privacy of all information were guaranteed at all the levels of this study. During the survey, any person with high blood pressure was counseled and referred to the nearest health facility to get appropriate care and attention if they were not currently receiving any treatment.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):419–426
https://doi.org/10.1007/s13410-020-00910-8

ORIGINAL ARTICLE

Prevalence and correlates of pre-diabetes and diabetes among a national population-based sample of adults in Zambia: results of the first national STEPS survey in 2017

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Received: 19 June 2020 / Accepted: 22 December 2020 / Published online: 5 January 2021

Abstract

Background/purpose Diabetes has been on the rise in Africa. This study aimed to estimate for the first time the national prevalence and its correlates of pre-diabetes and diabetes among individuals aged 18–69 years in Zambia.

Methods Nationally representative cross-sectional data were analyzed from 3608 persons aged 18–69 years (median age: 31 years) that participated in the "2017 Zambia STEPS survey," with complete blood glucose measurements.

Results Results indicate that 8.8% of 18–69 year-olds had pre-diabetes and 7.2% diabetes.

In adjusted multinomial logistic regression analysis, rural residence (adjusted relative risk ratio = ARRR: 2.01, 95% confidence interval = CI: 1.40–2.89), and raised total cholesterol (ARRR: 1.78, 95% CI: 1.08–2.94) were positively, and high physical activity (ARRR: 0.57, 95% CI: 0.39–0.83) was negatively associated with pre-diabetes. Being 50–69 years old (ARRR: 3.03, 95% CI: 2.03–4.52), having central obesity (ARRR: 1.90, 95% CI: 1.20–3.03), and hypertension (ARRR: 2.24, 95% CI: 1.61–3.13) were positively associated with diabetes.

In addition, in the unadjusted analysis, female sex, lower education, alcohol family problems, and alcohol dependence were associated with pre-diabetes and/or diabetes. Only 8.4% of the study sample reported that they ever had their blood glucose examined by a health care professional. Having had blood glucose measured was higher among women (9.6%) than men (7.2%) were but not significant ($p = 0.08$). Residents in urban areas (11.8%) had significantly more often their blood glucose ever measured than residents in rural areas (5.4%) ($p < 0.001$). Among study participants with diabetes, 22.3% were aware, 9.4% were currently taking treatment, and 17.1% had controlled their diabetes (< 7.0 mmol/L).

Conclusion Almost one in ten participants had pre-diabetes and diabetes and several associated variables were detected which can aid in designing intervention strategies.

Keywords Diabetes · Pre-diabetes · Prevalence · Risk factors · Adults · Zambia

Background and purpose

Almost one-third (29%) of all deaths in 2016 in Zambia was attributed to non-communicable diseases (NCD); the mortality contribution from diabetes was 1% [1]. According to the World Health Organization (WHO), diabetes was estimated to be the seventh largest cause of mortality in 2016 worldwide [2]. Globally, the prevalence of diabetes increased significantly from 1980 to 2014 (in women from 5.0 to 7.9%, and in men from 4.3 to 9.0%) [3]. Compared to high-income countries, the prevalence of diabetes has been rising more rapidly in low- and middle-income in recent years [2]. To prevent and control diabetes, it is important that national population-based surveys are conducted periodically [3]. There is a lack of national data on the prevalence of pre-diabetes and diabetes and associated factors in Zambia, a lower-middle-income country in Southern Africa.

In a large study among adults in 16 communities from five of 10 provinces in 2010 in Zambia, the prevalence of diabetes was 3.5% [4]. In the 2008 STEPS survey in Lusaka district, Zambia, among participants 25 years or older, the combined prevalence for pre-diabetes or diabetes was 4.0% [5], and in an

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investigation of bank employees ($N = 121$) in Ndola, Zambia, the prevalence of diabetes mellitus was 15% [6]. In other African countries, the national prevalence of diabetes was 5.8% in Burkina Faso [7], 3.3% in Ethiopia [8], 5.7% in Guinea [9], 5.6% (pre-diabetes 4.2%) in Malawi [10], and 1.4% (pre-diabetes 2.0%) in Uganda [11].

In several African countries, a high proportion of undiagnosed diabetes has been shown, e.g., 56% in Guinea [9], 70.5% in Uganda [12], 68.0% in Benin [12], 34.5% in Zambia [4], and 91.7% in Burkina Faso [12]. The proportion of diabetics treated and controlled has been low, e.g., 32.0% treated and 21.7% controlled in Benin, 7.3% treated and 6.9% controlled in Burkina Faso, 27.7% treated and 18.4% controlled in Kenya, and 40.1% treated and 21.4% controlled in South Africa [12].

Some factors associated with the risk of type 2 diabetes may include, as reviewed by Vonglokham et al. [13], sociodemographic factors (older age, male sex, lower education, and rural or urban residence), health status (central obesity, overweight, hypertension, and hypercholesterolemia), and health risk behaviors (poor dietary pattern, sedentary behavior, physical inactivity and substance use). In addition, psychosocial distress, such as depression, [14] suicidal behaviors [15, 16], stress [17, 18], and passive smoking [19] may be associated with pre-diabetes and/or diabetes.

The investigation aimed to estimate the prevalence and its correlates of pre-diabetes and diabetes among 18–69 year-old persons in Zambia.

Materials and methods

Study design and procedures

Cross-sectional nationally representative data from the "2017 Zambia STEPS Survey" were analyzed [20]. Using a multistage cluster sampling technique, a nationally representative sample of adults (18–69 years) in Zambia was produced [21]. "In the first stage of sampling, Standard Enumeration Areas (SEAs) were selected from each province using a probability proportional to size (PPS). In the second stage, 15 households in rural SEAs and 20 households in urban SEAs were selected systematically using an appropriate sampling interval based on the number of households in that SEA" [21], and in the third stage, one member from the eligible household members (18–69 years, residing in household) was selected by simple random sampling [21]. Data collection followed the WHO three STEPS methodology: step 1 included administration of a structured questionnaire, step 2 consisted of blood pressure and anthropometric measurements, and step 3 included biochemical tests (blood glucose and blood lipids) [21]. A pilot study was conducted to check the content validity of the questions after translation [21]. The main fieldwork started on July 22, 2017, and ended on October 15, 2017 [21]. Each field investigator team included one supervisor for planning and checking the completeness of questionnaires [21]. The "overall response rate was 74.3%" [21].

Measures

Outcome variable: pre-diabetes and diabetes

Fasting (≥ 10 h) blood sugar measurements were conducted and the history of diabetes assessed (see Supplementary file 1) [21]. "Testing was performed using a portable rapid diagnostic device (Cardiochek™) machine which used test strips for both blood glucose and lipid profiles (total cholesterol and HDL cholesterol)." [21]. Blood samples were collected using a finger prick [21]. Pre-diabetes was defined as "fasting plasma glucose levels 6.1 to< 7 mmol/L and diabetes as fasting plasma glucose levels ≥ 7.0 mmol/L, and/or currently taking insulin or oral hypoglycemic drugs and/or having been diagnosed with diabetes by a health care professional." [3]. Diagnosed diabetes was defined as self-reported health care provider diagnosis and/or currently taking insulin or oral hypoglycemic drugs, and undiagnosed diabetes was defined as fasting plasma glucose levels ≥ 7.0 mmol/L and no self-reported health care provider diagnosis and/or currently taking insulin or oral hypoglycemic drugs.

Sociodemographic information included sex, age, work status, education, ethnic affiliation, residence status, and marital status.

Psychosocial distress variables included having alcohol family problems in the past 12 months, family members ever died from suicide, suicidal ideation in the past 12 months and passive smoking (at home and/or at work) in the past 30 days (details in Supplementary file 1) [21].

Health status variables included measured central obesity (waist circumference > 88 cm in females and > 102 cm in males); body mass index (measured < 18.5 kg/m^2 underweight, 18.5–24.4 kg/m^2 normal weight, 25–29.9 kg/m^2 overweight and ≥ 30 kg/m^2 obesity); hypertension based on blood pressure (BP) measurements (average of the last two of three readings) defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg or currently on antihypertensive medication; raised total cholesterol (TC) ("fasting TC ≥ 5.0 mmol/L or currently on medication for raised cholesterol") [21].

Health risk behavior variables included daily tobacco use (smoking and/or smokeless tobacco, alcohol dependence, inadequate fruit and vegetable intake) (< 5 servings/day), and based on the "Global Physical Activity Questionnaire" low, moderate, or high physical activity and sedentary behavior (≥

8 h/day) [21]. Alcohol dependence was assessed with three questions of the "Alcohol Use Disorder Identification Test (AUDIT)" (items 4–6), e.g., "How often during the last year have you found that you were not able to stop drinking once you had started?" Response options ranged from "0 = never to 4 = daily or almost daily"; total scores of 4 or more indicate alcohol dependence [22]. Physical activity was categorized by the median metabolic equivalent (METs) of performed activities as low ("total physical activity METs minutes per week is < 600"), moderate ("3 or more days of vigorous-intensity activity of at least 20 min per day OR; 5 or more days of moderate-intensity activity or walking of at least 30 min per day OR; 5 or more days of any combination of walking, moderate or vigorous intensity activities achieving a minimum of at least 600 MET-min per week") and high ("vigorous-intensity activity on at least 3 days achieving a minimum of at least 1500 MET-min per week OR; 7 or more days of any combination of walking, moderate or vigorous intensity activities achieving a minimum of at least 3000 MET-min per week.") physical activity [23, 24].

Data analysis

Statistical analyses were done with "STATA software version 15.0 (Stata Corporation, College Station, Texas, USA)," taking into account the complex study design. The data were weighted "to make the sample representative of the target population (adults in Zambia aged 18 to 69 years)." [21]. Pearson Chi-square tests are used to calculate differences in proportions. Unadjusted and adjusted multinomial logistic regression was used to assess predictors of pre-diabetes and diabetes (with no pre-diabetes/diabetes as the reference category). Variables significant in the unadjusted analysis were included in the multivariable logistic regression model. The missing values were not included in the analysis. $p < 0.05$ was accepted as significant.

Results

Sample and diabetes status characteristics

The sample comprised of 3657 18–69 year-old persons (31 years median age, 18 years interquartile range) with complete blood glucose measurement. More than half of the participants (61.8%) were female, 48.0% had more than primary education, 41.0% were never married, separated, divorced, or widowed, 50.4% were employed, 33.8% were Tonga by ethnicity, and 64.0% lived in rural areas. More than one in seven participants (14.7%) reported alcohol family problems, 6.2% had a close family member who died from suicide, 7.8% had past 12-month suicidal ideation, and 26.8% were exposed to passive smoking.

Almost one in four participants (22.8%) were overweight or obese, 12.0% had central obesity, 18.8% had hypertension, and 7.4% raised total cholesterol. Regarding health risk behaviors, 11.0% used tobacco daily, 7.4% depended on alcohol, 91.2% ate insufficient fruit and vegetables, 18.5% were physically inactive, and 8.1% engaged in sedentary behavior. Almost one in ten 18–69 year-olds had pre-diabetes (8.8%) and 7.2% diabetes, 5.6% had undiagnosed diabetes, and 1.6% had diagnosed diabetes (see Table 1).

Associations with pre-diabetes and diabetes

In adjusted multinomial logistic regression analysis, rural residence (adjusted relative risk ratio = ARRR: 2.01, 95% confidence interval = CI: 1.40–2.89), and raised total cholesterol (ARRR: 1.78, 95% CI: 1.08–2.94) were positively, and high physical activity (ARRR: 0.57, 95% CI: 0.39–0.83) was negatively associated with pre-diabetes. Being 50–69 years old.

(ARRR: 3.03, 95% CI: 2.03–4.52), central obesity (ARRR: 1.90, 95% CI: 1.20–3.03), and hypertension (ARRR: 2.24, 95% CI: 1.61–3.13) were positively associated with diabetes.

In addition, in the unadjusted analysis, female sex, lower education, alcohol family problems, and alcohol dependence were associated with pre-diabetes and/or diabetes (see Table 2).

Diabetes awareness, treatment, and control

Only 8.4% of the study sample reported that they ever had their blood glucose measured by a health care professional. Having had blood glucose measured was higher among women (9.6%) than men (7.2%) were but not significantly ($p = 0.08$). Residents in urban areas (11.8%) had significantly more often their blood glucose measured than residents in rural areas (5.4%) ($p < 0.001$). Among the study participants with diabetes, 22.3% were aware, 9.4% were currently taking treatment, and 17.1% had controlled their diabetes (< 7.0 mmol/L). Awareness, treatment, and control status of diabetes did not significantly differ by sex. Urban dwellers with diabetes were significantly more often aware, treated, and controlled their diabetes than rural dwellers. Awareness, treatment, and control of diabetes increased with age, but this was only significant for the treatment of diabetes (see Table 3).

Discussion

The investigation aimed to estimate the prevalence and correlates of pre-diabetes and diabetes in a national population-based survey among 18–69 year-old persons in Zambia. The prevalence of diabetes (overall 7.2%, 7.5% in women and 6.9% in men) and pre-diabetes (8.8%) was similar among women globally (7.9%) and lower among men

Table 1 Sample and diabetes status characteristics among 18–69 year-old persons in Zambia, 2017

Variable	Sample *N* (%)	No diabetes *N* (%)	Pre-diabetes *N* (%)	Undiagnosed diabetes *N* (%)	Diagnosed diabetes *N* (%)
Socio-demographics					
All	3608	(84.0)	363 (8.8)	296 (5.6)	55 (1.6)
Age (years)					
18–34	1810 (50.2)	1559 (87.8)	165 (7.8)	75 (3.6)	11 (0.8)
35–49	1085 (30.1)	880 (81.3)	115 (9.9)	79 (7.2)	11 (1.6)
50–69	713 (19.8)	510 (71.7)	83 (11.5)	87 (11.5)	33 (5.3)
Gender					
Male	1379 (38.2)	1146 (85.6)	119 (7.5)	91 (5.3)	23 (1.6)
Female	2229 (61.8)	1803 (82.4)	244 (10.2)	150 (5.9)	32 (1.6)
Education					
< Primary	1324 (29.3)	1045 (80.4)	155 (11.5)	114 (7.6)	10 (0.5)
Primary	859 (22.8)	702 (81.7)	90 (10.4)	58 (6.5)	9 (1.4)
> Primary	1424 (48.0)	1201 (87.2)	118 (6.5)	69 (3.9)	36 (2.4)
Marital status					
Married/cohabiting	2203 (59.0)	1798 (82.5)	226 (9.6)	146 (6.2)	33 (1.7)
Nev. married/separated/divorced/widowed	1398 (41.0)	1146 (86.1)	137 (7.8)	95 (4.7)	20 (1.4)
Employment status					
Employed	1792 (50.4)	1489 (83.8)	169 (8.4)	108 (6.1)	26 (1.6)
Nonpaid	677 (20.1)	562 (87.6)	64 (7.2)	39 (3.8)	12 (1.4)
Unemployed	1134 (29.4)	893 (81.7)	130 (10.6)	94 (5.9)	17 (1.7)
Residence					
Urban	1300 (36.0)	1091 (87.0)	103 (5.9)	73 (4.6)	33 (2.5)
Rural	2308 (64.0)	1858 (81.4)	260 (11.4)	168 (6.5)	22 (0.8)
Ethnic group					
Bemba	1048 (32.3)	886 (86.9)	93 (7.1)	60 (4.7)	9 (1.3)
Tonga	1033 (33.8)	828 (83.0)	108 (9.3)	81 (6.3)	16 (1.3)
Other	1238 (33.8)	1010 (83.0)	128 (9.3)	75 (5.4)	25 (2.3)
Psychosocial distress					
Alcohol family problem	442 (14.7)	349 (80.7)	51 (8.8)	32 (7.9)	10 (2.6)
Family member died from suicide	217 (6.2)	174 (82.8)	24 (11.1)	12 (3.9)	7 (2.2)
Suicidal ideation	286 (7.8)	232 (84.9)	33 (10.5)	16 (3.4)	5 (1.2)
Passive smoking	880 (26.8)	729 (85.5)	86 (8.2)	52 (4.5)	13 (1.8)
Health status					
Central obesity	501 (12.0)	361 (73.7)	66 (11.3)	56 (10.8)	18 (4.2)
Body mass index					
Normal	2402 (70.2)	2015 (85.8)	224 (8.3)	143 (4.9)	20 (0.8)
Underweight	231 (6.9)	186 (84.7)	25 (9.5)	17 (4.9)	3 (1.0)
Overweight	567 (15.6)	445 (79.3)	65 (9.6)	39 (7.2)	18 (3.9)
Obesity	280 (7.2)	190 (73.1)	40 (12.2)	36 (10.8)	14 (3.9)
Hypertension	739 (18.8)	539 (75.1)	86 (11.1)	85 (9.9)	29 (3.8)
Raised total cholesterol	333 (7.4)	228 (71.0)	49 (14.6)	39 (9.4)	17 (5.0)
Health risk behavior					
Daily tobacco use	389 (11.0)	310 (82.4)	34 (8.2)	42 (9.0)	3 (0.4)
Alcohol dependence	197 (7.4)	157 (81.0)	15 (6.2)	21 (10.5)	4 (2.4)
Fruit and vegetable intake (< 5 servings/day)	3045 (91.2)	2502 (84.3)	302 (8.7)	196 (5.3)	45 (1.6)
Physical activity					
Low	706 (18.5)	539 (78.6)	89 (12.4)	59 (6.9)	19 (2.2)
Moderate	370 (13.0)	302 (84.6)	35 (8.1)	23 (4.6)	10 (2.7)
High	2178 (68.5)	1812 (85.3)	209 (8.1)	136 (5.4)	21 (1.3)
Sedentary behavior	312 (8.1)	245 (79.2)	34 (11.5)	26 (6.5)	7 (2.7)

CI confidence interval

globally (9.0%) [3], and was higher than in local studies in Zambia (3.5% diabetes [4] and pre-diabetes or diabetes 4.0% [5]) and in Malawi (5.6% diabetes and pre-diabetes 4.2%) [10], in Uganda (1.4% diabetes and 2.0% pre-diabetes 2.0%) [11], Burkina Faso (5.8%) [7], Ethiopia (3.3% diabetes) [8], and in Guinea (5.7%) [9]. The increased rate of diabetes found in Zambia (a lower-middle income country) may be explained by a greater change of lifestyle, older age structure, and greater urbanization than in low-income other African countries (Burkina Faso, Ethiopia, Guinea, and Malawi) and older studies in Zambia [13, 25].

Table 2 Associations with pre-diabetes and diabetes

Variable	Pre-diabetes Unadjusted RRR (95% CI)	Diabetes Unadjusted RRR (95% CI)	Pre-diabetes Adjusted RRR (95% CI)	Diabetes Adjusted RRR (95% CI)
Socio-demographics				
Age (years)				
18–34	1 (reference)	1 (reference)	1 (reference)	1 (reference)
35–49	1.37 (1.03, 1.83)*	2.17 (1.39, 3.40)***	1.21 (0.87, 1.67)	1.64 (1.06, 2.54)*
50–69	1.81 (1.29, 2.55)***	4.66 (3.19, 6.81)***	1.23 (0.84, 1.80)	3.03 (2.03, 4.52)***
Gender				
Male	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Female	1.40 (1.07, 1.85)*	1.12 (0.79, 1.58)	1.33 (0.97, 1.83)	0.86 (0.54. 1.37)
Education				
< Primary	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Primary	0.88 (0.62, 1.26)	0.97 (0.64, 1.48)	0.97 (0.65, 1.45)	0.93 (0.59, 1.45)
> Primary	0.52 (0.38, 0.71)***	0.73 (0.50, 1.05)	0.74 (0.51, 1.10)	0.93 (0.63., 1.37)
Marital status				
Married/cohabiting	1 (reference)	1 (reference)	–	–
Never married/separated/ divorced/widowed	0.78 (0.59, 1.02)	0.74 (0.53, 1.04)		
Employment status				
Employed	1 (reference)	1 (reference)	–	–
Nonpaid	0.82 (0.57, 1.19)	0.64 (0.41, 1.01)		
Unemployed	1.29 (0.96, 1.74)	1.01 (0.72, 1.43)		
Residence				
Urban	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Rural	2.05 (1.50, 2.82)***	1.09 (0.77, 1.56)	2.01 (1.40, 2.89)***	1.19 (0.80, 1.78)
Ethnic group				
Bemba	1 (reference)	1 (reference)	–	–
Tonga	1.37 (0.94, 2.01)	1.34 (0.79, 2.28)		
Other	1.37 (0.96, 1.96)	1.34 (0.86, 2.10)		
Psychosocial distress				
Alcohol family problem	1.04 (0.66, 1.62)	1.66 (1.04, 2.64)*	1.21 (0.75, 1.95)	1.42 (0.84, 2.39)
Family member died from suicide	1.30 (0.72, 2.35)	0.87 (0.49, 1.55)	–	–
Suicidal ideation	1.19 (0.75, 1.88)	0.62 (0.36, 1.06)	–	–
Passive smoking	0.88 (0.63, 1.22)	0.82 (0.56, 1.20)	–	–
Health status				
Central obesity	1.52 (1.01, 2.27)*	2.78 (1.75, 4.43)***	1.26 (0.79, 2.02)	1.90 (1.20, 3.03)**
Body mass index				
Normal	1 (reference)	1 (reference)	Not included because of overlap with central obesity	
Underweight	1.16 (0.67, 2.00)	0.99 (0.57, 1.70)		
Overweight	1.25 (0.87, 1.78)	2.03 (1.29, 3.21)**		
Obesity	1.72 (1.04, 2.82)*	2.92 (1.72, 4.98)***		
Hypertension	1.53 (1.14, 2.06)**	2.72 (1.99, 3.73)***	1.38 (0.97, 1.95)	2.24 (1.61, 3.13)***
Raised total cholesterol	2.10 (1.39, 3.16)***	2.61 (1.80, 3.77)***	1.78 (1.08, 2.94)*	1.40 (0.89, 2.20)
Health risk behavior				
Daily tobacco use	0.93 (0.58, 1.52)	1.38 (0.89, 2.14)	–	–
Alcohol dependence	0.71 (0.37, 1.36)	1.98 (1.07, 3.68)*	0.77 (0.39, 1.51)	1.68 (0.89, 3.17)
Inadequate fruit and vegetable intake	0.97 (0.61, 1.55)	0.72 (0.45, 1.15)	–	–
Physical activity				
Low	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Moderate	0.61 (0.36, 1.02)	0.75 (0.42, 1.31)	0.75 (0.44, 1.28)	0.98 (0.53, 1.81)
High	0.60 (0.43, 0.85)**	0.68 (0.46, 0.99)*	0.57 (0.39, 0.83)**	0.86 (0.56, 1.32)
Sedentary behavior	1.40 (0.88, 2.24)	1.46 (0.88, 2.44)	–	–

RRR relative risk ratio, *CI* confidence interval; ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

The investigation showed a high prevalence of undiagnosed diabetes (77.3%), which seems to be higher than in Guinea (56%) [9], in Uganda (70.5%) [12], in Benin (68.0%) [12], and in Zambia (34.5%) [4] and lower than in Burkina Faso (91.7%) [12]. The prevalence of treated diabetics in this study (9.4%) was lower than in most other African countries, e.g., Benin (32.0%), Kenya (27.7%), and South Africa (40.1%), except for Burkina Faso (7.3%) [12].

Table 3 Diabetes awareness, treatment, and control ($N = 296$)

Variable	Of diabetics aware	Of diabetics treated	Of diabetics controlled (< 7.0 mmol/l)
	N (%)	*N* (%)	*N* (%)
Total	55 (22.3)	28 (9.4)	42 (17.1)
Sex			
Male	23 (23.4)	11 (9.3)	16 (17.2)
Female	32 (21.3)	17 (9.5)	26 (17.0)
p value[1]	0.816	0.955	0.991
Residence			
Rural	22 (11.0)	10 (4.7)	17 (9.3)
Urban	33 (35.7)	18 (14.9)	25 (26.3)
p value[1]	< 0.001	< 0.004	< 0.002
Age group			
18–34	11 (18.8)	3 (2.5)	9 (14.4)
35–49	11 (18.3)	7 (9.1)	12 (18.5)
50–69	33 (31.5)	18 (18.4)	21 (18.9)
p value[1]	0.125	0.005	0.613

[1] *Mmol* millimol, based on Chi-square test statistics

The prevalence of controlled diabetes among diabetics (17.1%) in this study was lower than in Benin (21.7%) and South Africa (21.4%), similar to Kenya (18.4%) and higher than in Burkina Faso (6.9%) [12]. The study found that urban dwellers had greater awareness, treatment, and control of their diabetes than rural dwellers, while there were no sex differences. The lack of awareness, treatment, and control among rural dwellers may be attributed to poorer health services access. "Most primary care facilities in Zambia do not routinely screen for cholesterol or diabetes." [21]. By enhancing primary facilities to conduct blood glucose tests, especially in rural Zambia [21], diabetes awareness, treatment, and control may improve.

Consistent with former research [4, 8–11], in this investigation, pre-diabetes and diabetes increased with age. In unadjusted analysis, the study showed that female sex was associated with pre-diabetes, but no significant sex differences were found in the adjusted analysis for pre-diabetes and diabetes. In a systematic review on sex differences of the prevalence of diabetes in Africa, in most countries, no sex differences were identified [26]. Residing in rural areas and in unadjusted analysis, lower education increased the odds for pre-diabetes. Some previous studies confirmed the association between rural residence [27], and lower education in high-income and not low- or middle-income countries [25, 27] with diabetes, while some other studies [8, 28, 29] found a higher prevalence in urban areas. It appears, however, that diabetes has penetrated into both urban and rural areas in Zambia. Another possible reason for the higher rate of pre-diabetes in rural compared to urban areas in Zambia is that rural dwellers are significantly older than urban residence ($p < 0.001$), as pre-diabetes increases with age. A diabetes-screening program may be introduced, particularly targeting the older age high-risk groups [29].

Some studies found an association between psychosocial distress, such as suicidal behavior [15, 16], stress [17, 18], and passive smoking [19], increased the likelihood of diabetes, while in this study, only in unadjusted analysis alcohol family problem was associated with diabetes, while the stress of family members that died from suicide, suicidal ideation, and passive smoking were not significantly different with pre-diabetes and/or diabetes.

This survey found an association between hypertension, central obesity, and raised total cholesterol with pre-diabetes or diabetes. These findings are consistent with previous investigations [4, 6, 8, 9, 11], showing major "modifiable cardiometabolic risk factors" [9]. This "combination of cardiometablic risk factors calls for a multiple rather than a single risk intervention approach in this population." [13].

Several health risk behaviors, such as unhealthy diet, sedentary behavior, physical inactivity, tobacco use, and alcohol misuse, have been found to increase the risk for diabetes [6, 11, 30–33], while in this study, only physical inactivity and in the unadjusted analysis alcohol dependence were associated with pre-diabetes, and in unadjusted analysis, physical inactivity was associated with diabetes, and no significant association between sedentary behavior, daily tobacco use, and inadequate fruit and vegetable intake and pre-diabetes and/or diabetes was found.

Study limitations

Diagnosed diabetes was based on participant's recall and not medical records, which may have led to underreporting. Participants were also not asked to specify whether they had type 1, type 2, or gestational diabetes. Only fasting capillary blood glucose was used for diagnosis of pre-diabetes and diabetes which in the absence of HbA_{1C} and a post glucose load value would result in an underestimate. The variable of household income had many missing values and could therefore not be included in the analysis.

Conclusion

The study found among a nationally representative population of 18 to 69 years in Zambia that almost one in ten participants had pre-diabetes and diabetes. Less than one in five Zambians were aware, treated, and controlled their diabetes. Several risk factors for pre-diabetes and/or diabetes were identified, including older age, rural residence, central obesity (or

overweight or obesity), hypertension, raised total cholesterol, and physical inactivity, and in unadjusted analysis, female sex, lower education, alcohol family problems, and alcohol dependence, which can assist in guiding interventions to prevent pre-diabetes and diabetes in the Zambian population.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13410-020-00910-8.

Acknowledgments The data source, the World Health Organization NCD Microdata Repository (URL: https://extranet.who.int/ncdsmicrodata/index.php/catalog), is hereby acknowledged.

Authors' contributions All authors fulfil the criteria for authorship. SP and KP conceived and designed the research, performed statistical analysis, drafted the manuscript, and made a critical revision of the manuscript for key intellectual content. All authors read and approved the final version of the manuscript and have agreed to the authorship and order of authorship for this manuscript.

Data availability The data for the current study are publicly available at the World Health Organization NCD Microdata Repository (URL: https://extranet.who.int/ncdsmicrodata/index.php/catalog).

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethical considerations The study was approved by the University of Zambia (UNZA) Research Ethics Committee (REC). After written informed consent was obtained from participants, field investigators gathered infomation using an e-STEPS Android-based data collection tool.

Abbreviations STEPS, STEPwise approach to surveillance; STATA, statistics and data

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):427–434
https://doi.org/10.1007/s13410-020-00912-6

ORIGINAL ARTICLE

Prevalence and associated factors of metabolic syndrome among a national population-based sample of 18–108-year-olds in Iraq: results of the 2015 STEPS survey

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Received: 16 June 2020 / Accepted: 23 December 2020 / Published online: 8 January 2021

Abstract

Background This study aimed to assess the prevalence and associated factors of the metabolic syndrome (MetS) among 18–108-year-old persons in Iraq.

Method Nationally representative cross-sectional data were analysed from 3703 18–108-year-old persons (32 years median age) that participated in the "2015 Iraq STEPS survey," with complete MetS measurements.

Results Results indicate that 39.4% of 18–108 year-olds had MetS (harmonized definition), 39.8% among women and 39.0% among men, and the mean number of MetS components was 2.4 (SD=1.4), 2.4 (SD=1.4) among women and 1.5 (SD=1.4) among men. In adjusted logistic regression analysis, aged 60–108 years (adjusted odds ratio (AOR) 6.69, 95% confidence interval (CI) 4.82–9.29), current smoking (AOR 1.38, 95% CI 1.01–1.90), past smoking (AOR 1.54, 95% CI 1.00–2.36), general overweight (AOR 4.87, 95% CI 3.07–5.63) and obesity (AOR: 8.33, 95% CI: 6.27–11.07) were associated with MetS. In adjusted linear regression analysis, aged 60–108 years (beta 1.21, 95% CI 1.06 to 1.37), male sex (beta 0.23, 95% CI 0.12 to 0.34), overweight (beta 0.77, 95% CI 0.64 to 0.90) and obesity (beta 1.27, 95% CI 1.13 to 1.40) were positively and having more than primary education (beta −0.22, 95% CI −0.34 to −0.09) was negatively associated with greater number of MetS components.

Conclusion Two in five participants had MetS and several associated indicators were found which could be supportive in designing intervention activities.

Keywords Metabolic syndrome · Prevalence · Risk factors · Adults · Iraq

Background

Noncommunicable diseases (NCDs) are "estimated to account for 55% of all deaths in Iraq in 2016," which include 27% cardiovascular diseases and 4% diabetes [1]. Compared with people without metabolic syndrome (MetS), individuals with MetS have a twofold higher risk for cardiovascular disease and a fivefold higher risk for type 2 diabetes [2–4]. "A cluster of risk factors for cardiovascular disease and type 2 diabetes mellitus, which occur together more often than by chance alone, have become known as the metabolic syndrome." [5] "The risk factors include raised blood pressure, dyslipidemia (raised triglycerides and lowered high-density lipoprotein cholesterol), raised fasting glucose, and central obesity." [5] Globally, it is estimated that "25% of the adult population can be characterized as having MetS." [2, 6] The prevalence of MetS is increasing in low- and middle-income countries with "improvement in economic situation, increasing urbanization, nutrition transition, and reduced physical activity." [7] To prevent and control MetS, it is important that national population-based surveys are conducted periodically [8]. There is a lack of national population-based data on the prevalence and associated factors of MetS in Iraq, an upper middle-income country in the Middle East.

In a cross-sectional study among adults recruited from different institutions (19–80 years) (N=566) in Erbil City, Northern Iraq, the prevalence of MetS (ATP IV criteria) was 30.6% [9], in a hospital outpatient sample (N=300) (30–75 years) in Baghdad in Iraq the prevalence of MetS (IDF criteria) was 42% [10] and among 320 hospital outpatients

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(25–85 years) in Baghdad in Iraq the prevalence of MetS (ATP III criteria) was 37.8% [11]. In national surveys in countries of the Eastern Mediterranean region, the national prevalence of MetS (NCEP-ATPIII definition) in persons 35–74 years in 2004–2005 in Tunisia was 30.0% [12], in 2012 in Qatar (18–64 years) (IDF definition) 37% [13], in Iran (24–64 years) (IDF definition) in 2007 37.4% [14] and in 2005 in Saudi Arabia (15–64 years) (IDF definition) 28.3% [15]. In comparison, the prevalence of MetS in 2009 in China (18 years and older) (Revised NCEP ATPIII definition) was 21.3% [16] and in 2015 in a low-income country Ethiopia (15–69 years) (IDF definition) 4.8% [17].

Factors that are associated with the prevalence of MetS include sociodemographic, health status and health risk behavior/s related variables. Sociodemographic factors associated with MetS may include, female sex [18, 19–21], older age [12, 13, 18, 19, 21], higher education [19], lower education [13, 15], higher income [15] and urban residence [12, 18, 19]. Health status variables associated with MetS may include higher body mass index, general overweight or obesity [19, 20] and abnormal waist-to-hip ratio [19, 20]. Health risk behavior/s variables associated with MetS may include physical inactivity [20, 21], low leisure-time physical activity [22], sedentary behaviour [23], combined physical inactivity and inadequate fruit and vegetable intake [24], low intake of fruits and dairy foods [25] and inadequate fruit and/or vegetable consumption [26–28]. In addition, frequent smoking [16], current smoking [29, 30] and former smoking [31] are associated with a higher risk of MetS. Regarding alcohol use, some studies found that mild to moderate alcohol use decreased and heavy alcohol increased the risk of MetS [32, 33], while other studies showed a positive association between current alcohol use and MetS [16]. The study using the Iraq STEPwise approach to noncommunicable disease risk factor surveillance (STEPS) 2015 data aimed to assess the prevalence and associated factors of MetS among 18–108 year-old persons in Iraq.

Methods

Participants and procedures

This is a secondary analysis conducted using nationally representative population-based and cross-sectional data from the "2015 Iraq STEPS survey" [34]. "STEPS focus on obtaining population-based data on the established risk factors that determine the major disease burden" [34]. "STEPS surveys collect data at three levels: Step 1- Questionnaire-based assessment includes socio-economic data, data on tobacco and alcohol use, nutritional status, and physical inactivity; Step 2- includes simple physical measurements, such as height, weight, waist circumference, and blood pressure; and Step 3- includes biochemical measurements" [34]." The 2015 Iraq STEPS survey data and more detailed survey methods can be accessed; the overall response rate for STEP III was 93.5%, STEP II 98.6% and STEP I 98.8% [34, 35]. Briefly, a "multistage cluster sampling was used with stratification to urban and rural areas. Primary sampling units (PSUs) (N=412) were the blocks, which consisted of 70 households or more before selection. One person from each household was randomly selected." In total, 4071 persons 18 years or older were potentially eligible in this study. However, 368 individuals were excluded from this analysis since they did not have complete MetS measurements so that 3703 participants were included in the final data analysis. Comparing participants with complete and without complete MetS measurements, there were no significant differences in terms of educational background, residence status, physical activity level, sedentary behavior/s, fruit and vegetable consumption, passive smoking, alcohol use and body weight status. However, compared to participants with complete MetS measurements, participants without complete MetS measurements were younger (p=0.018), women (p<0.001) and smokers (p=0.016).

Sample size calculation In three local studies in different institutions and hospital outpatients in Iraq [9–11], the average prevalence of MetS was 36%. Based on this information, the sample size was calculated with an expected MetS prevalence of 36%, acceptable margin of 5% and clusters 412; the minimum sample for each cluster is 2, the minimum sample is 824. In this study, we used all 3703 participants for the analysis.

Measures

Outcome variable: metabolic syndrome

The harmonized definition of MetS was used, including three or more of any of the following five risk factors [5]: (1) "Elevated waist circumference (waist ≥97 cm in men, ≥99 cm in women) [36] [=High WC]; (2) Elevated blood pressure (systolic BP ≥130 or diastolic BP ≥85 mmHg and/or on anti-hypertensive medication) [=High BP]; (3) Elevated fasting blood glucose (≥100 mg/dL and/or currently taking insulin or oral hypoglycaemic drugs) [=High FBG]; (4) Elevated triglycerides (≥150 mg/dL and/or currently on medication for raised cholesterol) [=High TG] and (5) Reduced high-density lipoprotein (HDL) cholesterol (<40 mg/dL in men; <50 mg/dL in women and/or currently on medication for raised cholesterol) [Low HDL]."

Body mass index (measured <18.5 kg/m^2 underweight, 18.5–24.4 kg/m^2 normal weight, 25–29.9 kg/m^2 overweight and ≥30 kg/m^2 obesity) was measured; blood pressure (BP) measurements (average of the last two of three readings) were conducted with an electronic blood pressure monitor Spengler® ES 60. Blood samples were drawn in the morning

(after 10–14 h fasting, respondents on medication for diabetes were asked to postpone taking the medication until after drawing the blood sample) and centrifuged. Levels of "fasting plasma glucose and fasting total cholesterol and lipid profile were determined using the enzymatic method (glucose oxidase for fasting blood glucose and cholesterol oxidase for total cholesterol)." [35] "Absorption were read utilizing (Visible Light Spectrophotometer) instrument [35]."

Health risk behaviour variables included current and past smoking, past months passive smoking in home and/or at closed spaces at work, ever alcohol use, inadequate fruit and vegetable intake (<5 servings/day) and based on the "Global Physical Activity Questionnaire" low, moderate or high physical activity and sedentary behavior/s (≥8 h/day) [35].

Data analysis

Statistical analyses were done with the STATA software version 15.0 (Stata Corporation, College Station, TX, USA), taking into account the complex study design. The data were weighted to make the sample representative of the target population in Iraq (by sex and by age group, 18–39, 40–59, 60 and over). [35] Prior to data analyses, the normal distribution of the study variables was examined. The P-P-plot analyses and K-S tests of normal distribution showed that the study variables fulfilled the postulation of normal distribution. Chi-square tests were used to test for differences in proportions. Unadjusted and adjusted logistic regression was used to assess predictors of MetS and linear regression for the number of MetS components. Covariates were included based on a previous literature review [12,13,16,18–35]. Explanatory variables are statistically significant at $p<0.05$ and are free from multicollinearity as measured by the variance inflation factor (VIF < 1.8). Missing values (<1.2% for any study variable) were excluded from the statistical analysis. $p<0.05$ was accepted as significant.

Results

Sample and MetS status characteristics

The sample comprised of 3703 18–108-year-old persons (32 years median age, 22 years interquartile range) with complete MetS measurements. More than one in five of the participants (59.5%) were female, 37.6% had more than primary education and 75.9% lived in urban areas. More than one in ten participants (21.3%) reported current smoking, 7.3% past smoking, 60.3% past month passive smoking, 2.5% ever alcohol use, 79.5% inadequate fruit and vegetable intake, 52.3% low physical activity, 26.3% sedentary behaviour and 34.0% obesity. The prevalence of MetS was 39.4%, 39.8% among women and 39.0% among men, and the mean number of MetS components was 2.4 (SD=1.4), 2.4 (SD=1.4) among women and 2.5 (SD=1.4) among men (see Table 1).

Associations with MetS

In adjusted logistic regression analysis, aged 60–108 years (adjusted odds ratio (AOR) 6.69, 95% confidence interval (CI) 4.82–9.29), current smoking (AOR 1.38, 95% CI 1.01–1.90), past smoking (AOR 1.54, 95% CI 1.00–2.36), general overweight (AOR 4.87, 95% CI 3.07–5.63) and obesity (AOR 8.33, 95% CI 6.27–11.07) were associated with MetS. In addition, in unadjusted analysis, having lower education, ever alcohol use and low physical activity were associated with MetS. In adjusted linear regression analysis, aged 60–108 years (beta 1.21, 95% CI 1.06 to 1.37), male sex (beta 0.23, 95% CI 0.12 to 0.34), overweight (beta 0.77, 95% CI 0.64 to 0.90) and obesity (beta 1.27, 95% CI 1.13 to 1.40) were positively and having more than primary education (beta −0.22, 95% CI −0.34 to −0.09) was negatively associated with greater number of MetS components (see Table 2).

MetS components

Overall, high WC was 43.8%, high BP 51.0%, high FBC 31.8%, high TG 35.4% and low HDL 54.5%. Low HDL was significantly higher in women than in men, and high TG was significantly higher among men than women, while high WC, high BP and high FBG did not differ significantly between the sexes. All five MetS components did not significantly differ by residence status. Between both sexes, all five MetS components significantly increased with age. Among men, high WC, high BP and high FBG increased with age, high TG increased from the 18–39 year-old age group to the 40–59 year-old age group and decreased among the 60 years and older age group. Low HDL did not significantly differ among age groups in men. Among men, high BP, high FBG, high TG and low HDL increased with age, while high WC increased from the 18–39-year-old age group to the 40 to 59-year-old age groups and slightly decreased among the 60 years and older age group (see Table 3).

Discussion

The investigation aimed to estimate the prevalence and correlates of MetS in a national population-based survey among 18–108-year-old persons in Iraq. The prevalence of MetS (harmonized definition) (39.4%) in 2015 seems higher than global estimates (25%) [2, 6], and similar to different local studies in Iraq, in different institutions in Erbil City (30.6%, ATP IV criteria) [9], in a hospital outpatient sample (30–75 years) in Baghdad (42%, IDF criteria) [10] and among outpatients (25–85 years) in Baghdad (37.8%, ATP III criteria) [11], and probably similar to

Table 1 Prevalence of metabolic syndrome (MetS) and mean number of MetS components according to sociodemographic and health characteristics among adults in Iraq, 2015

Variable (# missing values)	Sample *N* (%)	MetS (≥3 components) % (95% CI)	Number of MetS components *M* (SD)
Sociodemographics			
All	3703	1726 (39.4)	2.42 (1.4)
Age (years) (# 10)			
18–39	1777 (48.1)	479 (24.1)	1.82 (1.2)
40–59	1311 (35.5)	796 (62.9)	2.85 (1.3)
60–108	605 (16.4)	447 (72.3)	3.27 (1.2)
Gender (# 0)			
Female	2204 (59.5)	1004 (39.8)	2.41 (1.4)
Male	1499 (40.5)	722 (39.0)	2.45 (1.4)
Education (# 20)			
<Primary	1622 (37.8)	842 (46.2)	2.61 (1.4)
Primary	933 (24.6)	414 (39.9)	2.37 (1.3)
>Primary	1128 (37.6)	460 (32.1)	2.20 (1.4)
Residence (# 0)			
Rural	801 (24.1)	366 (40.0)	2.39 (1.4)
Urban	2902 (75.9)	1360 (39.2)	2.43 (1.4)
Health variables			
Smoking status (# 0)			
Never	2796 (71.4)	1249 (37.3)	2.36 (1.4)
Past	298 (7.3)	195 (60.3)	2.99 (1.3)
Current	609 (21.3)	282 (39.2)	2.42 (1.4)
Passive smoking (# 7)			
No	1658 (39.7)	793 (41.6)	2.45 (1.4)
Yes	2038 (60.3)	932 (38.0)	2.41 (1.4)
Ever alcohol use (# 2)			
No	3611 (97.5)	1668 (39.0)	2.41 (1.4)
Yes	90 (2.5)	58 (57.1)	2.84 (1.1)
Inadequate fruit and vegetable intake (# 16)			
No	808 (20.5)	382 (37.2)	2.43 (1.4)
Yes	2879 (79.5)	1338 (40.00	2.42 (1.4)
Physical activity (# 3)			
Low	2065 (52.3)	1014 (42.5)	2.50 (1.4)
Moderate	856 (22.8)	412 (43.3)	2.47 (1.3)
High	779 (24.9)	300 (29.5)	2.17 (1.3)
Sedentary behaviour (# 41)			
No	2617 (73.7)	1166 (38.1)	2.35 (1.3)
Yes	1045 (26.3)	545 (42.9)	2.61 (1.4)
Body mass index (# 13)			
Underweight/normal	949 (34.4)	177 (13.4)	1.54 (1.2)
Overweight	1200 (31.6)	533 (43.0)	2.37 (1.3)
Obesity	1541 (34.0)	1012 (62.4)	3.01 (1.2)

national estimates in 2004–2005 in Tunisia (30.0%, NCEP-ATPIII definition) [12], in 2012 in Qatar (37%, IDF definition) [13], in 2007 in Iran (24–64 years) (37.4%, IDF definition) [14], and higher than in 2005 in Saudi Arabia (28.3%, IDF definition) [15], in 2009 in China (21.3%, Revised NCEP ATPIII definition) [16] and in 2015 in Ethiopia (4.8%, IDF definition) [17]. Some of the country differences in the prevalence of MetS may be related to the different definitions used for MetS, different age groups are analysed and some studies are older with probably having lower rates of MetS. For example, in a recent study (2017) among adults (18–100 years) in Morocco, the prevalence of MetS (using the harmonized definition) was 40.0% [37],

Table 2 Associations with metabolic syndrome (MetS) and number of MetS components among adults in Iraq, 2015

Variable	MetS		Number of MetS components
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted beta (95% CI)
Sociodemographics			
Age (years)			
18–39	1 (Reference)	1 (Reference)	Reference
40–59	5.34 (4.31, 6.62)***	3.40 (2.68, 4.31)***	0.82 (0.69 to 0.95)***
60–108	8.22 (6.20, 10.98)***	6.69 (4.82, 9.29)***	1.21 (1.06 to 1.37)***
Gender			
Female	1 (Reference)	–	Reference
Male	0.97 (0.82, 1.14)		0.23 (0.12 to 0.34)***
Education			
<Primary	1 (Reference)	1 (Reference)	Reference
Primary	0.77 (0.63, 0.95)*	1.03 (0.80, 1.34)	−0.06 (−0.18 to 0.06)
>Primary	0.55 (0.45, 0.68)***	0.83 (0.64, 1.07)	−0.22 (−0.34 to −0.09)***
Residence			
Rural	1 (Reference)	–	Reference
Urban	0.97 (0.74, 1.26)		−0.05 (−0.18 to 0.09)
Health variables			
Smoking status			
Never	1 (Reference)	1 (Reference)	Reference
Past	2.55 (1.84, 3.54)***	1.54 (1.00, 2.36)*	0.08 (−0.13 to 0.29)
Current	1.08 (0.84, 1.39)	1.38 (1.01, 1.90)*	0.03 (−0.13 to 0.19)
Passive smoking			
No	1 (Reference)	–	Reference
Yes	0.86 (0.71, 1.04)		0.009 (−0.10 to 0.11)
Ever alcohol use			
No	1 (Reference)	1 (Reference)	Reference
Yes	2.09 (1.23, 3.53)**	1.56 (0.77, 3.19)	0.13 (−0.16 to 0.43)
Inadequate fruit and vegetable intake			
No	1 (Reference)	–	Reference
Yes	1.13 (0.89, 1.43)		0.01 (−0.11 to 0.13)
Physical activity			
Low	1 (Reference)	1 (Reference)	Reference
Moderate	1.03 (0.83, 1.29)	1.07 (0.80, 1.44)	0.04 (−0.09 to 0.17)
High	0.57 (0.44, 0.73)***	0.90 (0.66, 1.22)	−0.12 (−0.26 to 0.03)
Sedentary behaviour			
No	1 (Reference)	–	Reference
Yes	1.22 (0.98, 1.52)		−0.04 (−0.17 to 0.10)
Body mass index			
Underweight/Normal	1 (Reference)	1 (Reference)	Reference
Overweight	4.87 (3.67, 6.46)***	5.16 (3.07, 5.63)***	0.77 (0.64 to 0.90)***
Obesity	10.73 (8.21, 14.03)***	8.33 (6.27, 11.07)***	1.27 (1.13 to 1.40)***

OR odds ratio, *CI* confidence interval

***$p<0.001$, **$p<0.01$, *$p<0.05$

similar to our study in Iraq (39.4%). The high prevalence of MetS may be attributed to "improvement in economic situation, increased urbanization, nutrition transition, and reduced physical activity." [7].

The study found that the most prevalent MetS components were low HDL, high BP and high WC. Similar results were found in national surveys in Iran [14] and in Nepal [19]. In this study, we saw a decline of two MetS components (high WC

Table 3 Characteristics of components of metabolic syndrome among adults in Iraq, 2015

Variable	High WC %	High BP %	High FBG %	High TG %	Low HDL %
Total	43.8	51.0	31.8	35.4	54.5
Sex					
Female	43.1	48.8	32.1	32.0	64.9
Male	44.4	47.1	31.6	38.3	45.4
p value	<0.375	0.060	0.994	0.002	<0.001
Residence					
Rural	44.4	53.1	28.6	33.1	57.5
Urban	43.7	50.1	32.6	36.1	53.5
p value	0.990	0.645	0.217	0.334	0.450
Age group all					
18–39	30.2	35.5	23.6	27.4	51.3
40–59	67.1	73.1	43.6	48.1	58.8
60–108	67.3	88.5	51.9	51.5	62.5
p value	<0.001	<0.001	<0.001	<0.001	<0.001
Age group male					
18–39	32.4	40.9	24.7	31.5	43.3
40–59	62.8	72.5	43.3	53.9	49.8
60–108	70.1	87.7	50.8	48.8	49.6
p value	<0.001	<0.001	<0.001	<0.001	0.081
Age group female					
18–29	27.3	28.4	22.2	22.2	61.9
30–59	66.1	73.6	43.9	43.1	66.6
60–108	64.2	89.4	46.8	54.4	76.5
p value	<0.001	<0.001	<0.001	<0.001	<0.001

High WC waist circumference (waist ≥89 cm in men, ≥91 cm in women), *High BP* blood pressure (systolic BP ≥130 or diastolic BP ≥85 mmHg and or on anti-hypertensive medication), *High FBG* fasting blood glucose (≥100 mg/dL or on antidiabetic medication), *High TG* triglycerides (≥150 mg/dL and/or on anti-cholesterol medication), *Low HDL* high-density lipoprotein cholesterol (<40 mg/dL in men; <50 mg/dL in women and/or on anti-cholesterol medication)

among women and high TG among men) in persons 60 years and older. One possible explanation for this could be mortality prior to 60 years [18].

Consistent with former research [18, 19, 12, 13, 21], this investigation showed an association between older age and MetS. While several studies found a higher prevalence of MetS among women than men [18, 19, 20, 21], this study did not show any significant sex differences. In fact, men seemed to have a greater number of MetS components than women in this study. Several studies showed an increased risk of MetS in people with lower education [13, 15], which was confirmed in our study in unadjusted analysis and in the adjusted analysis in terms of a greater number of MetS components. Persons with lower education may have lesser knowledge on health risk behavior/s that are implicated in the development of MetS [10]. While several previous research studies showed an association between urban residence and MetS [12, 18, 19], this survey did not find significant rural-urban differences. This could mean that MetS risk behavior/s (sedentary lifestyle, stress and diet changes) have penetrated rural areas as well as urban areas.

In agreement with previous research findings [19, 20], this survey showed that having general overweight or obesity increased the odds for MetS. Consistent with previous studies [20–23], this investigation showed in unadjusted analysis an inverse association between high physical activity and MetS. Several studies and reviews [25–28] found a significant association between inadequate fruit and vegetable consumption and MetS, while this survey did not find any significant association between the two.

This study found in unadjusted analysis that ever alcohol use and in the adjusted analysis that current and past smoking were associated with MetS. Regarding alcohol use, our findings confirm former research conducted in China [16]. Since the proportion of current alcohol users was too small in this study population, we are not able to distinguish heavy from moderate alcohol users. In terms of smoking, our findings are in line with former research showing a positive association between active and past smoking and MetS [16, 29–31]. In a recent review, the following lifestyle changes are recommended to prevent and manage MetS: stop smoking, engage in physical activity (30–60 min daily), moderate intake of red wine and beer, a healthy diet for weight loss and fruit and vegetable consumption as part of a healthy diet [38].

Study limitations

The strength of the study was to cover a nationally representative adult sample in Iraq, but was limited because of its cross-sectional design as well as the self-report of the interview data. The variable of household income was not available on the publically available dataset and could therefore not be included in the analysis.

Conclusion

The 2015 Iraq STEPS survey found among a nationally representative population of adults that two in five participants had MetS. Several risk factors for MetS were identified, including older age, current and past smoking and general overweight and obesity, which can facilitate in aiding interventions to prevent and control MetS in the general population in Iraq.

Acknowledgments The data source, the World Health Organization NCD Microdata Repository (URL: https://extranet.who.int/ncdsmicrodata/index.php/catalog), is hereby acknowledged.

Authors' contributions All authors fulfil the criteria for authorship. SP and KP conceived and designed the research, performed statistical analysis, drafted the manuscript and made critical revision of the manuscript for key intellectual content. All authors read and approved the final version of the manuscript and have agreed to authorship and order of authorship for this manuscript.

Data availability The data for the current study are publicly available at the World Health Organization NCD Microdata Repository (URL: https://extranet.who.int/ncdsmicrodata/index.php/catalog).

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethics approval and consent to participate Ethical approval for the study was obtained from the "Republic of Iraq Ministry of Health/ Environment Public Health Directorate" and written informed consent was obtained from participants prior to the study [35].

Consent for publication Not applicable.

Abbreviations BP, Blood pressure; FBG, Fasting blood glucose; HDL, High-density lipoprotein cholesterol; MetS, Metabolic Syndrome; STEPS, STEPwise approach to surveillance; STATA, Statistics and data; TG, Triglycerides; WC, Waist circumference

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):435–440
https://doi.org/10.1007/s13410-020-00906-4

ORIGINAL ARTICLE

Molecular basis of non-alcoholic fatty liver disease and metabolic syndrome in a subset of South Asians

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Received: 15 July 2020 / Accepted: 8 December 2020 / Published online: 14 January 2021

Abstract

Background Metabolic syndrome (MetS) and non-alcoholic fatty liver disease (NAFLD) are emerging threats in Pakistan. The prevalence of MetS is reported to be 18–46% in general population. Adipokines and their genetic polymorphisms are now considered as risk factors for these conditions. Chemerin modulates glucose and lipid homeostasis, nesfatin-1 acts as an anorexigenic peptide, desnutrin regulates adipose tissue fatty acid oxidation, adipocyte fat content, and size. Therefore, this study was designed to identify the molecular and genetic differences in the above-mentioned biomarkers in metabolic syndrome positive and negative individuals.

Methods This cross-sectional study was carried out at the Aga Khan University during October 2018 till August 2019. MetS positive ($n = 92$) versus negative ($n = 208$) adults aged between 18 and 50 years of age were recruited. MetS was diagnosed on the basis of National Cholesterol Education Program Adult Control Panel III criteria. Serum adipokine levels, lipid profile, blood glucose, and insulin were measured. Fatty liver was detected by ultra-sonographic scans. Chemerin rs17173608 polymorphism was determined by tetra-arm polymerase chain reaction.

Results Higher chemerin (37.87 ± 13.60 vs. 24.03 ± 12.32 pg/ml), low desnutrin (103.08 ± 5.84;270.19 ± 25.67 pg/ml), and nesfatin levels (276.49 ± 31.09; 754.34 ± 57.77 pg/ml) were seen in Mets/NAFLD positive versus negative individuals, respectively. Serum chemerin showed a moderate positive correlation with MetS and fatty liver while desnutrin showed weak correlation with MetS, whereas nesfatin showed a moderate correlation with MetS and fatty liver ($p < 0.05$). Each unit increases in chemerin, and decrease in desnutrin was associated with higher odds of developing NAFLD ($p < 0.05$). The variant rs17173608 showed association with MetS phenotype (1.841 (1.101–3.078); $p = 0.020$). Presence of the minor "G" allele was seen to increase the risk of developing MetS by 1.567 ($p < 0.012$).

Conclusion High chemerin and low desnutrin are linked to fatty liver changes and MetS in a subset of local population. The presence of chemerin "G" allele increases the risk of developing MetS even further in the same individuals. Further studies are required for an in-depth analysis of the mechanism through which these biomarkers cause the effect.

Keywords Metabolic syndrome · Non-alcoholic fatty liver disease · Adipokine · Nesfatin · Desnutrin · Chemerin

Introduction

"Global time bomb" is the term used by the International Diabetes Federation (IDF) for metabolic syndrome (MetS), a leading contributor of global mortality [1]. Over 25% of the total adult population is affected by metabolic syndrome (MetS); however, the likelihood of an increase is apparent with each passing day [1]. Roughly 20% adult population of the Asia-Pacific region [2] and specifically 18 to 46% of Pakistan population is suffering from MetS [3]. MetS is associated with the progression of complications such as atherosclerosis, dyslipidemia, hypertension, and pro-inflammatory conditions leading to the development of type II diabetes

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and cardiovascular disease. Moreover, conditions like fatty liver, gallstones, and polycystic ovarian syndrome have also shown a correlation with MetS [4]. On the other hand, many obese individuals do not develop any comorbid and are considered healthy (metabolically healthy obese-MOH) while others, who appear to be of normal weight, develop certain comorbid and are labeled as metabolically unhealthy normal weight (MUHNW) [5–7]. Therefore, identification of the difference in circulatory biomarkers such as nesfatin, desnutrin, and chemerin may help in differentiating this phenotype.

Nesfatin is known to regulate hunger by increased feeling of satiety [8] and possesses anti-inflammatory properties with enhanced lipid breakdown characteristics [9]. Due to these actions, nesfatin-1 can play a crucial role in preventing the development of T2DM in obesity [10]. Similarly, Desnutrin, an adipose triglyceride lipase, has been identified as the major triacylglycerol (TAG) hydrolase expressed mainly in adipose tissues [11]. Lower desnutrin levels can contribute to ectopic TAG accumulation in various tissues, especially in the liver and muscle. Desnutrin decline can cause TAG buildup in islets, leading to significant hyperglycemia, and has been linked to metabolic syndrome and insulin resistance [12]. Chemerin is characterized as an adipokine due to its involvement in glucose reuptake and adipocyte differentiation. Since it has chemoattractant properties, it is assumed to be involved in chronic inflammation. Many genetic variants are studied in this regard including chemerin, which has a single nucleotide polymorphism and rs17173608; however, very few of these studies indicate chemerin's role in MetS [13].

Therefore, this study was designed to identify the molecular and genetic differences of these factors in metabolically healthy and unhealthy subjects. The findings can be used to assess their risk of developing disease severity in future.

Materials and methods

This cross-sectional study recruited 300 individuals, patients, and volunteers from the outpatient department of Aga Khan University between the ages of 18 and 50 years from October 2018 to August 2019. They were classified as MetS-positive ($n = 92$) and MetS-negative ($n = 208$) adults diagnosed on the basis of National Cholesterol Education Program Adult Control Panel III criteria [14]. Patients with chronic diseases (renal, cardiovascular, hepatic, cancer, etc.) taking anti-inflammatory medicine and pregnant women were excluded from the study. Sample size was calculated using OpenEpi version 3 software. At 95% confidence interval and 5% absolute precision, the sample size was calculated to be 92 considering the prevalence of T2DM in Pakistan and the anticipated frequency as 50% (unknown anticipated frequency).

A 10-ml venous blood sample was obtained after an overnight fasting of 12–14 h. Fasting blood glucose (FBG), insulin, and lipid profile were measured by automation. Serum adipokine levels of chemerin, desnutrin, and nesfatin were measured by sandwich enzyme linked immunosorbent assay method (Kit cat no. GR106262, Genorise Scientific Inc.; USA; GR111420, Genorise Scientific Inc; USA and C12393, Glory Science Co, Belgium, respectively). The inter-assay coefficient of variance (CV) for chemerin and desnutrin was 9%, intra-assay was 6%, inter-assay CV for nesfatin was 10%, and intra-assay was 8%. Body fat percentage was measured by bioelectrical impedance analysis (BG55 Buerer, Germany). Fatty liver was detected by ultrasonographic scans by a trained radiologist. Chemerin rs17173608 polymorphism was determined by tetra arm polymerase chain reaction (Fig. 1). The primer sequence used was as follows:

a. Forward inner (G allele): ATTGCTATAGTCCA GTGCCCTTCG (base pair 262 G allele)
b. Reverse inner (T allele): CCAGTTCCCTCTGT CGGCTTAA (base pair 332 T allele)
c. Forward outer: GTCAGACCCATGCAGTTTTCAAAC
d. Reverse outer: GAGTTCCTCTCTCAAGCATCAGGG (base pair 549 control)

Data obtained was coded in IBM Statistical Package for the Social Sciences (IBM SPSS; IBM Corp Inc., Armonk, NY) version 21. The distribution of continuous variables was determined, and Mand standard deviations were reported, respectively. For categorical variables, descriptive analysis was presented in terms of frequencies and percentages. Mann-Whitey U test and t test for quantitative variables and Pearson chi-square test for categorical variables were applied. Pearson correlation and logistic regression were applied as appropriate. A p value less than 0.05 was considered significant in all cases.

Operational definition

The NCEP ATP III criteria [14] define MetS as the presence of any three of the following five traits:

a. Abdominal obesity defined as a waist circumference ≥ 102 cm (40 in) in men and ≥ 88 cm (35 in) in women
b. Serum triglycerides ≥ 150 mg/dL (1.7 mmol/L) or drug treatment for elevated triglycerides
c. Serum HDL cholesterol < 40 mg/dL (1 mmol/L) in men and < 50 mg/dL (1.3 mmol/L) in women or drug treatment for low HDL cholesterol
d. BP ≥ 130/85 mmHg or drug treatment for elevated BP
e. FPG ≥ 100 mg/dL (5.6 mmol/L) or drug treatment for elevated blood glucose

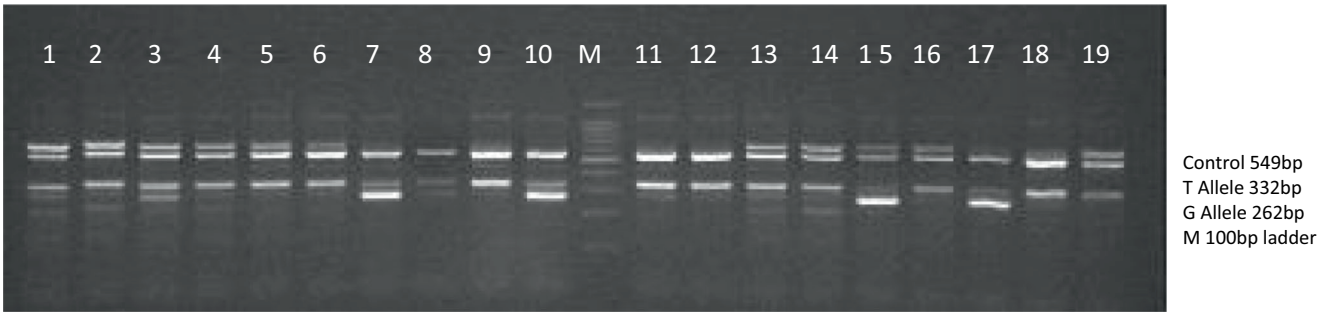

Fig. 1 Gel electrophoresis of the chemerin gene polymorphism

Results

Higher triglycerides, fasting glucose, and insulin levels were seen in MetS-positive individuals as compared with the MetS-negative group ($p < 0.05$). Similarly, higher chemerin was observed in MetS positive versus negative (37.87 ± 13.60 vs. 24.03 ± 12.32 pg/ml) individuals while low desnutrin and nesfatin levels were seen in Mets positive versus negative individuals (103.08 ± 5.84;270.19 ± 25.67 pg/ml) (276.49 ± 31.09; 754.34 ± 57.77 pg/ml), respectively (Table 1). Chemerin showed a moderate correlation with MetS (r = 0.507; $p < 0.00$), fatty liver (0.305; $p < 0.05$), and weak correlation with FBG (r = 0.152; p = 0.17); desnutrin showed weak correlation with MetS (r = 0.490; $p < 0.00$) and a negative correlation with FBG ($r = -0.159$; p = 0.103), whereas nesfatin showed a moderate correlation with MetS (r = 0.652; p = 0.00), fatty liver (r = 0.334; p = 0.003), and a weak negative correlation with FBG ($r = -0.273$; p = 0.032). Further, every unit rise in chemerin and a unit decrease in desnutrin were associated with higher odds of developing NAFLD (p<0.05). Sixty percent of obese individuals versus 27% of normal weight subjects had fatty liver changes, whereas 17.9% normal weight subjects suffered from metabolic syndrome compared with 32.1% of obese subjects (p<0.05) (Table 2). Genotype analysis of the Chemerin variant rs17173608 showed association with MetS phenotype (1.841 (1.101–3.078); p=0.020). The TG genotype was predominantly seen in MetS-positive (46.7% versus 38.9%) versus MetS-negative subjects (Table 3). Presence of this minor "G" allele was seen to increase the risk of developing MetS by 1.567 (p<0.012) (Table 4).

Table 1 Descriptive data of study subjects

	MetS negative (n = 208)	MetS positive (n = 92)	p value
Age (year)	29.80 ± 11.19	32.20 ± 14.70	0.024
Weight (kg)	76.28 ± 16.26	73.43 ± 27.38	0.712
BMI (kg/m^2)	24.59 ± 6.34	27.38 ± 8.84	< 0.001
Body fat (%)	26.47 ± 8.00	31.30 ± 6.80	< 0.001
Waist circumference (cm)	88.51 ± 10.78	95.67 ± 11.61	< 0.001
Hip circumference (cm)	98.96 ± 10.32	100.95 ± 10.07	0.123
Waist-to-hip ratio	0.90 ± 0.10	0.92 ± 0.11	0.025
Systolic blood pressure (mmHg)	125.36 ± 8.36	131.16 ± 13.75	< 0.001
Diastolic blood pressure (mmHg)	78.57 ± 9.07	85.11 ± 9.99	< 0.001
Cholesterol (mg/dL)	163.06 ± 47.93	172.20 ± 52.12	0.206
Triglyceride (mg/dL)	142.51 ± 61.65	171.39 ± 77.56	0.002
High-density lipoprotein (mg/dL)	38.59 ± 9.03	36.66 ± 9.40	0.007
Low-density lipoprotein (mg/dL)	89.45 ± 51.94	93.24 ± 52.91	0.597
Fasting glucose (mg/dL)	80.42 ± 22.32	129.03 ± 68.91	< 0.001
Fasting insulin (mIU/L)	23.25 ± 15.05	15.51 ± 17.47	< 0.001
Chemerin (pg/mL)	24.03 ± 12.32	37.87 ± 13.60	< 0.005
Desnutrin (pg/mL)	103.08 ± 5.84	270.19 ± 25.67	0.024
Nesfatin (pg/mL)	276.49 ± 31.09	754.34 ± 57.77	0.031

Mann-Whitney U test was applied to assess the difference between groups. A p value of < 0.05 was considered significant

Table 2 Distribution of fatty liver stratified as per body mass index and MetS

	Normal weight (n = 140)	Overweight (n = 79)	Obese (n = 81)	p value
Fatty liver on sonogram				
Yes	38 (27.1)	28 (35.4)	49 (60.5)	0.017
No	102 (72.9)	51 (64.6)	32 (39.5)	
Metabolic syndrome				
Yes	25 (17.9)	41 (51.9)	26 (32.1)	< 0.001
No	115 (82.1)	38 (48.1)	55 (67.9)	

Independent sample t test was applied to assess the difference between groups. A p value of < 0.05 was considered significant

Discussion

MetS is associated with the progression of obesity-related complications such as atherosclerosis, dyslipidemia, hypertension, and pro-inflammatory conditions leading to the development of type II diabetes and cardiovascular disease. Research indicates the role of cytokines and adipokines in mediating and often promulgating, the pathways that facilitate progression of this condition. In line with this trajectory, we explored the use of biomarkers such as chemerin, nesfatin, and desnutrin in the diagnosis of MetS. We found that subjects having NAFLD and MetS expressed significantly higher levels of chemerin and lower levels of nesfatin and desnutrin. Moreover, our data suggests that MetS patients had significantly greater levels of TGs, insulin, and fasting blood glucose when compared with the control group. Additionally, MetS patients with higher body fat positively correlated with increased chemerin levels when compared with their counterparts. The correlation of obesity and NAFLD found in our study is in agreement with previous studies, where a higher prevalence was observed among those with elevated BMI ($p < 0.0005$) as compared with non-obese individuals [15]. Similarly, Romero-Velarde et al. demonstrated that 37.5–54.5% of obese individuals had MetS [16].

We concurrently performed genetic analysis of a specific chemerin polymorphism involved in MetS. The chemerin G allele polymorphism has been investigated previously in an Iranian population via an observational case-control study, with minor G allele reported to be significantly associated with MetS ($p = 0.012$) [13]. Another study conducted in Egypt reported G allele frequency in rs17173608 of chemerin gene to be significantly associated with patients suffering from MetS [17]. Remarkably, these observations are constant within Pakistani samples as reported in this study. Moreover, our observations also suggest that there is a predisposition for NAFLD in patients with a rise in chemerin levels or polymorphism.

Furthermore, studies correlating nesfatin-1 and T2DM demonstrate significantly less serum nesfatin-1 in those receiving T2DM treatment as compared with controls and recently diagnosed diabetics [18]. Ding et al. also supported the finding that individuals with T2DM and peripheral arterial disease (PAD) had lower nesfatin-1 levels than those with T2DM only [19] . Moreover, Mirzaei et al. showed that obese individuals with high peroxisome proliferator-activated receptor gamma (PPARγ), a key player in adipocyte synthesis and maturation, was associated with significant risk of developing MetS and abdominal fat [20]. In contrast, some contradicting studies that indicate high nesfatin levels are associated with IR [18]. This may be due to the body's compensatory mechanism to decrease adipose tissue mass in order to decrease insulin resistance secondary to nesfatin's anorexic effects. Our results are in line with the former—that nesfatin-1 is reduced in MetS patients. Similarly, desnutrin has been found to be suppressed in individuals who had elevated glucose levels leading to insulin resistance [21]. Moreover, it also plays a crucial role in the activation of PPARδ to stimulate production of insulin in

Table 3 Genotype frequencies and association with MetS

Genotype	MetS negative (n = 208)	MetS positive (n = 92)	p value	Odds ratio (95.0% CI)	p value
rs17173608				Additive model	
TT	98 (47.1)	30 (32.6)	0.05	1.734 (0.999–3.010)	0.049
TG	81 (38.9)	43 (46.7)		Dominant model	
GG	29 (13.9)	19 (20.7)		1.841 (1.101–3.078)	0.020
HWE	0.071	0.620		Recessive model	
				0.543 (0.325–0.908)	0.143

Genotype frequencies are given as absolute values with percentage given in parenthesis. The Hardy Weinberg equilibrium (HWE) stats for the study cohort was > 0.05. In the logistic regression model, genotype of normal allele homozygote (T/T), heterozygote (T/G), and minor allele homozygote (G/G) were coded as 0, 1, and 2, respectively. OR with $p < 0.05$ considered as significant

Table 4 Association of chemerin rs17173608 SNP alleles with MetS

SNP allele	Group	Allele data		
		Frequency	OR (95%CI)	*P* value
Mets				0.012
T	Mets negative	0.6659	0.638 (0.447–0.910)	
	Mets positive	0.5598		
G	Mets negative	0.3341	1.567 (1.099–2.236)	
	Mets positive	0.4402		

p value < 0.05 calculated by Pearson's x^2 square test. Allele frequencies are given as absolute values

islet β cells [22]. Our study has indicated that lower desnutrin levels are positively associated with MetS/NAFLD which explains increased insulin resistance and spiked blood glucose levels in MetS patients.

Certain limitations arose during the course of our study. The NAFLD/fatty liver was diagnosed by ultra-sonography which has a poor sensitivity in diagnosing this condition when fat accumulation is under 30%. Moreover, this is subjected to observer bias as ultra-sonography is dependent on the operator of the procedure. Thus, the possibility of false negatives cannot be ignored. Further, due to funding restrictions in this current study, we were unable to perform liver function tests to correlate with the fatty liver changes observed. The association between the presence of T2DM and low serum nesfatin levels could not be generalized due to the small sample size and selection bias that is often inherent with a tertiary care setting. In addition, the ratio of MetS-positive to MetS-negative patients was low, which increases the likelihood of type 1 errors in our analysis. Nevertheless, our study was able to identify subtle differences in MetS-positive and -negative individuals. Further studies in non-tertiary care settings, heterogeneous populations, and/or larger sample groups can help solidify these findings.

Conclusion

High chemerin and low desnutrin are linked to fatty liver changes and MetS in a subset of local population. The presence of chemerin "G" allele increases the risk of developing MetS even further in the same individuals. Further studies are required for an in-depth analysis of the mechanism through which these biomarkers cause the effect. More so, liver biopsies and protein expression studies may also be beneficial to compare differences in MetS-positive versus -negative individuals.

Author contributions SSF conceptualized the project, received the funds, and analyzed the data. SL, UE, ZN, and KA were involved in sample processing and manuscript writing. All authors wrote the manuscript and approved the final version before publication.

Funding This work is funded by the UGME research course fund and the Biological and Biomedical Sciences (BBS) Department, Aga Khan University, for providing the research module funds to conduct this student lead research work.

Compliance with ethical standards

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

Ethics approval number ERC-4951-BBS-17.

Informed consent An informed written consent was obtained from participants. Institutional ethics committee approval was sought before initiating the study (ERC-4951-BBS-17)

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):441–446
https://doi.org/10.1007/s13410-021-00921-z

ORIGINAL ARTICLE

Transcription factor 7-like 2 (*TCF7L2*) rs12255372 variant and the risk of type 2 diabetes mellitus among the Kurdish population in Erbil, Iraq

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Received: 14 June 2020 / Accepted: 8 January 2021 / Published online: 26 January 2021

Abstract

Background The genetic predispositions related to type 2 diabetes (T_2D) in the Middle East are poorly understood. One of the most common single-nucleotide polymorphisms (SNP) located in the transcription factor 7-like-2 (*TCF7L2*) gene is rs12255372 variant which elucidated a significant role in developing T_2D, especially in the European population. The current study is the first to have examined the association between *TCF7L2* rs12255372 variant and T_2D among the Iraqi Kurdish population from Iraq.

Methods Three hundred participants were enrolled in this study: 150 were type 2 diabetic patients and 150 were normoglycemic controls. For genotyping rs12255372 (G > T) variant, Tetra ARMS-PCR was used which is high-throughput, cost and time effective technique.

Results The genotypic frequencies in the additive genetic model (co-dominant) for GG, GT, and TT were 62.7%, 24%, and 13.3% in the normoglycemic controls, respectively, and for the diabetic patients were 50.7%, 20%, and 29.3%, respectively. The TT genotype was found considerably higher in cases when matched to the normoglycemic controls in both co-dominant and recessive models with OR (95% CI) = 2.64 (1.29–5.41) (*p value* = 0.006) and OR (95% CI) = 1.531 (1.232–1.902) (*p value* = 0.001), respectively. These frequencies indicated that the carriers of the TT genotype were more susceptible to T_2D compared to other genotypes. The T allele showed a high significant frequency in T_2D patients compared to the normoglycemic controls with OR (95% CI) = 1.38 (1.16–1.59) (*p value* = 0.000).

Conclusion Our results suggest rs12255372 T allele as a putative risk factor that increases the susceptibility of T_2D among the Iraqi Kurdish population.

Keywords rs12255372 G > T · Single-nucleotide polymorphism · Tetra ARMS-PCR · *TCF7L2* · Type 2 diabetes (T_2D)

Introduction

The dramatic increase of type 2 diabetes (T_2D) incidence across the globe has led to intense surveillance of the genetic predispositions of this disorder. According to the World Health Organization report about the prevalence of diabetes in the Eastern Mediterranean Region, the prevalence of diabetes in Iraq in 2000 was 668,000, and it could elevate to as high as 2,009,000 by 2030 [1]. The International Diabetes Federation (IDF) in 2008 estimated 5–8% of the population in Erbil city to have diabetes [2]. This rate has noticeably accelerated so far as due to the reduction of physical activity and the sedentary lifestyle. While the specific mechanisms underlying the induction and progression of T_2D have not been revealed, it is generally accepted that T_2D is a complex disease, and a combination of numerous genetic and environmental factors can lead to disease initiation and its evolution [3]. Even though genome-wide association studies (GWAS) had identified 143 loci associated with T_2D [4], to date, transcription factor 7-like 2 (*TCF7L2*) was recognized as the most potent known candidate gene to confer susceptibility to T_2D [5, 6]. One of the most common T_2D-specific single-nucleotide polymorphisms (SNPs) present in the *TCF7L2* gene is rs12255372. There are many specific single-nucleotide polymorphisms (SNPs) of the *TCF7L2* found associated with T_2D e.g., rs7903146, rs12255372, rs7901695, rs11196205, and rs7895340. In the present study, the association between rs12255372 variant and type 2 diabetes was

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investigated. Genetic variants within the *TCF7L2* gene were in the recent time specified to be related to type 2 diabetes,;*TCF7L2* gene is known to encode a transcription factor involved in the Wnt signaling cascade, which plays an imperative role in the development and adipogenesis of pancreatic islet [7, 8]. *TCF7L2* polymorphisms have been associated with dysregulated glucose production, insulin secretion, and glucose tolerance through direct influences on pancreatic islet beta cells [9, 10]. The correlation between the *TCF7L2* rs12255372 SNP and T_2D has never been studied among Iraqi Kurdish from Iraq, where T_2D is very prevalent; therefore, we decided to set bases with this research by examining the association between the *TCF7L2* rs12255372 (G > T) polymorphism and T_2D among Kurdish population in Erbil, Kurdistan Region of Iraq.

Material and methods

Study population

A case-control study contained three hundred subjects: 150 healthy controls (79 males and 71 females) and 150 diabetics (76 males and 74 females) with the age range between 49 and 57. Enrolled subjects were of the same ethnic group, Iraqi Kurdish. Diabetic subjects were selected from the Layla Qasim Diabetic Center in Erbil, Kurdistan Region of Iraq. Patients were selected after fulfilling the diagnostic criteria of type 2 diabetes agreed by the American Diabetes Association (ADA): greater than 6.5% (48 mmol/mol) of glycated hemoglobin (Hb A1C) which is an average of blood glucose over the last 2 to 3 months, after an 8-h fasting overnight and on two different occasions, fasting plasma glucose (FPG) level of 126 mg/dL or greater, and the plasma glucose (PG) level in the 2-h sample is greater than 200 mg/dL (11.1 mmol/L) in the 2-h oral glucose tolerance test (OGTT) [11]. Control subjects were recruited randomly after being checked negative for any kind of diabetes or other medical diseases. Subjects who had heart failure, type 1 diabetes mellitus, cancer, liver disease, renal failure, pregnancy, and breastfeeding were excluded.

Biochemical measurements

Using standard enzymatic analysis techniques, fasting blood glucose (FBG), triglycerides (TG), and total cholesterol (TC) for serum samples were analyzed [12]. After precipitation of apolipoprotein B-containing lipoproteins, high-density lipoprotein (HDL) was measured [13], and depending on the method of Hatch and Lees, low-density lipoprotein (LDL-C) was calculated [14]. Turbidometric inhibition immunoassay was used to determine glycated hemoglobin (Hb A1c) levels. Insulin sensitivity was measured using an indirect HOMA-IR index [15]. The chemiluminescent technique (DPC kit on Immulite 1000) was used to assess Fasting serum insulin [16].

DNA extraction

Wizard Genomic DNA purification kit (Promega, USA) was used to extract genomic DNA from whole blood (200 μl), according to the manufacturer's instructions. The purity of DNA and its concentration were evaluated by Nanodrop 1000 spectrophotometer (Thermo Scientific, UK), and the presence of the DNA bands was confirmed by 2% agarose gel electrophoresis (Cleaver, UK).

Genotyping of *TCF7L2* rs12255372 SNP

TCF7L2 rs12255372 variant was identified using the tetra amplification refractory mutation system polymerase chain reaction (Tetra primer ARMS- PCR). PCR reactions were executed in 25 μl volumes comprising of 12.5 μl master mix 2X (GoTaq®Green Master Mix, Promega, USA), 1 μl of each primer, a template DNA (35–50 ng), nuclease-free water up to 25 μl. The PCR conditions were as follows: at 95 °C; the initial denaturation step was carried out for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 53 °C for 1 min, and extension at 72 °C for 1 min. The final extension was conducted for 10 min at 72 °C. PCR was performed in an Alpha Thermal Cycler (TECHNE TC-512, UK) by using four primers (GeNet Bio): forward outer primer [5`-GGGCAATAGATACATTTTAAGA-3`], reverse outer primer [5`-GAGATAGATGATAG GCTGTT-3`], forward inner primer (for G allele) [5`-GGAATATCCAGGCAAGAATG-3`], and reverse inner primer (for T allele) [5`-CCTGAGTAATTATC AGAATATGGTA-3`] [17]. The amplicons were put through 2% agarose gel electrophoresis (Cleaver, UK), stained with safe dye (prime safe Dye, GeNet Bio) and visualized by Gel documentation system (Proxima 2500, iSO GEN life science, Netherland).

Statistical analysis

For data processing, the Statistical Package of Social Science (SPSS, version 15) was used. Quantitative variables were correlated using mean ± SD, while genotype and allele frequencies were compared using the Fisher's exact test and odds ratio with 95% confidence intervals (CI). Data distributions were checked by the normality test. A statistical power analysis program G*Power (version 3.1.9.2) was used to calculate the power of the study. A *p value* of < 0.05 was measured to be significant.

Results

The anthropometric and clinical characteristics of type 2 diabetic patients and normoglycemic subjects are presented in Table 1. The patients and the control groups showed no significant variance regarding age, gender, and body mass index. Type 2 diabetes cases showed higher level of fasting blood glucose (*p value* = 0.03), serum insulin level (*p value* = 0.04), and hemoglobin A1c% (*p value* = 0.01) when compared with the controls. Triglyceride (TG), low-density lipoprotein (LDL), and total cholesterol (TC) levels were significantly higher in the T_2D cases compared to the control subjects (*p value* = 0.007, 0.009, and 0.02, respectively). While high-density lipoprotein (HDL) cholesterol was significantly higher in the control subjects when compared to the T_2D cases with ranges of 54.92 ± 1.12 and 43.83 ± 1.07, respectively, (*p value* = 0.03). Tetra primer ARMS-PCR, which is a high-throughput and economical technique, was successfully used for amplification of rs12255372 SNP and resulted in the production of three genotypes; GG, GT, and TT (Fig. 1). The expected product sizes of the rs12255372 (G > T) variant were 760 bp for the non-specific band, 494 bp for the G allele, and 310 bp for the T allele. The frequencies of genotypes and alleles of rs12255372 polymorphism are assessed based on three models, co-dominant, dominant, and recessive model, and are documented in Table 2. The genotypic frequencies of GG, GT, and TT in T_2D cases were 50.7%, 20%, and 29.3%, respectively, and in the controls were 62.7, 24%, and 13.3%, respectively. The TT genotype was significantly higher in T_2D cases when compared to the controls in the co-dominant model (OR 2.64, 95% CI = 1.29–5.41, *p value* = 0.006). Moreover, in the recessive model, the TT genotype was markedly higher among T_2D cases vs GG + GT genotypes (OR 1.531, 95% CI = 1.232–1.902, *p value* = 0.001). However, the GG genotype, which is dominant over GT + TT, showed significantly higher frequency in the controls when compared to the cases (OR 2.84, 95% CI = 1.010–1.631, *p value* = 0.024). The T allele displayed a significant association with the increased risk of type 2 diabetes. The frequency of T allele in T_2D was 39.3% and in the controls was 25.3% (OR 1.38, 95% CI = 1.16–1.59, *p value* = 0.000). The genotypic frequencies of rs12255372 GG, GT, and TT are assessed among T_2D patients in relation to some anthropometric and clinical variables (Table 3). There were no significant variances between frequencies of GG, GT, and TT genotypes regarding age, gender, BMI, FBG, TC, HDL, and LDL variables. The type 2 diabetes patients who carry the TT genotype had a significantly higher serum insulin and hemoglobin A1c% levels when compared to other genotypes (GG and GT) (*p value* 0.01 and 0.03, respectively). Moreover, the triglyceride (TG) level in T_2D carriers of TT genotype was markedly higher than T_2D patients who carry GG or GT genotypes (*p value* 0.03).

Discussion

Type 2 diabetes (T_2D) has been a problematic condition over the globe. Despite the high prevalence of this disorder, there is a lack of information about genetic predispositions that increase T_2D incidence. There are numerous genetic susceptible risk factors responsible for T_2D pathogenicity, the most common one being the *TCF7L* gene. A study conducted by Yazdi et al. showed the significant association between the *TCF7L2* gene and the risk of T_2D among the Iranian population from Tehran province [18]. Understanding the mechanism of *TCF7L2* genetic polymorphisms associated with the pathogenicity of type 2 diabetes could improve our understanding of genetic predispositions of the disease and help physicians to consider these variants for early diagnosis of T_2D especially in highly vulnerable individuals. Huang et al. addressed several mechanisms by which *TCF7L2* variants increased type 2 diabetes induction [19]. An appropriate understanding of these mechanisms could assist in the development of more effective therapies against

Table 1 Anthropometric and clinical parameters of the subjects

Variable	Control *n* = 150	Case *n* = 150	*p value*
Age/year	53.32±1.16	57.11±1.24	NS
Sex M/F	79 (52.66%)/71(47.33%)	76 (50.67%)/74 (49.33%)	NS
BMI Kg m^2	29.73±1.08	31.98±1.41	NS
FBG mg/dL	91.84±1.09	234.53±1.17	0.03
Serum insulin/μlU/mL	8.32±1.43	15.59±2.01	0.04
HbA1c%	5.57±1.21	8.82±1.08	0.01
TC mg/dL	126.28±1.08	218.34±1.13	0.007
TG mg/dL	102.13±1.14	176.13±1.14	0.009
HDL mg/dL	54.92±1. 12	43.83±1.07	0.03
LDL mg/dL	101.73±1.08	152.12±1.04	0.02

NS not significant, *BMI* body mass index, *HbA1c* hemoglobin A1c, *FBG* fasting blood glucose, *TG* triacylglyceride, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

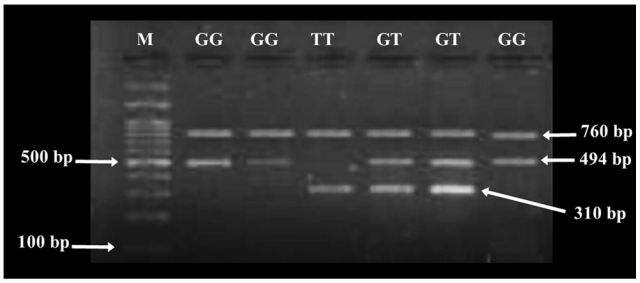

Fig. 1 Agarose gel electrophoresis (2%) showing patterns for rs12255372 (G > T) genotypes. M: molecular DNA marker, GG: homozygous genotype (wild type), GT: heterozygous genotype, TT: homozygous genotype (mutant type)

this complex disorder. The current study is the first to consider the relationship between *TCF7L2* rs12255372 variant and T_2D among the Iraqi Kurdish population. Our results showed that participants who harbor TT genotype were more vulnerable to affect by T_2D compared to other genotypes. Anthropometric and biochemical parameters of T_2D cases and normoglycemic controls are evaluated and documented in Table 1. Type 2 diabetic patients demonstrated higher levels of FBG, serum insulin, and HbA1c% when compared with non-diabetic controls. This finding was in accord with that of Nanfa et al., who revealed a significant difference between diabetic patients and the controls regarding these parameters [20]. Lipid profiles can be considered as major risk factors for type 2 diabetes. In T_2D cases the triglyceride (TG), low-density lipoprotein (LDL) and total cholesterol (TC) levels were significantly higher when compared with the controls. Cui et al, conducted a study and demonstrated a significant relationship between these parameters and the increased risk of T_2D [21]. Our results revealed a strong association between rs12255372 variant and T_2D among the Iraqi Kurdish population. Peng et al. addressed 155 studies in a comprehensive meta-analysis and found a strong association between T_2D and six single-nucleotide polymorphisms including rs12255372 SNP with an odds ratio (OR) = 1.33 and 95% confidence interval (CI) of 1.27–1.40 [22]. Similar results were observed in several studies that admitted a strong effect of *TCF7L2* rs12255372 variant on the increased risk of type 2 diabetes [23, 24]. In contrast to our study, Mandour et al. reported a lack of association of rs12255372 genotypes and alleles to the risk of type 2 diabetes in the Egyptian population, with no statistically significant difference of the frequency of T allele between diabetic patients and the controls with an odd ratio (OR) = 0.602, 95% CI = 0.361–1.005 and *p value = 0.052* [25]. The lack of association between T_2D pathogenicity and rs12255372 SNP was found in some other studies such as among Pima Indian population [26], the Saudi population [27], and the Chinese population [28]. The rs12255723 SNP was analyzed by using a high-throughput Tetra primer ARMS-PCR assay. Genotypic and allelic frequencies of rs12255372 (G > T) in T_2D patients and normoglycemic controls are assessed based on three genetic models: dominant, recessive, and co-dominant (Table 2). According to our results, the co-dominant and recessive models best explained the effect of the rs12255372 variant on the risk of type 2 diabetes among the studied population. Our result was in line with that of Nanfa et al. [20], who chose the co-dominant model to best fit the influence of *TCF7L2* variant on T_2D susceptibility. The frequency of the TT genotype was considerably higher in diabetic cases when compared to the controls in both co-dominant and recessive models. Carriers of the TT genotype could be more susceptible to T_2D and could predict future incidence. The T allele was considerably more frequent in the diabetics than in the controls, which indicate that the T allele could be implicated with an increased risk of type 2

Table 2 Association between TCF7L2 rs12255372 polymorphisms of control and case subjects based on co-dominant, dominant, and recessive models

rs12255372 SNP	Controls *n* = 150	%	Cases *n* = 150	%	OR (95% CI)	*p value*
Genotypes frequencies						
Co-dominant						
GG	94	62.7	76	50.7	–	NS
GT	36	24	30	20		
TT	20	13.3	44	29.3	2.64 (1.29–5.41)	0.006
Dominant						
GG	94	62.7	76	50.7	1.284 (1.010–1.631)	0.024
GT + TT	56	37.3	74	49.3		
Recessive						
GG + GT	130	86.7	106	70.7		
TT	20	13.3	44	29.3	1.531(1.232–1.902)	0.001
Alleles						
G	224	74.7	182	60.7		
T	76	25.3	118	39.3	1.38 (1.16–1.59)	0.000

OR odd ratio, *95% CI* 95% confidence interval, *NS* not significant

Table 3 Genotypic assessment of rs12255372 (G > T) among type 2 diabetes cases in relation to some anthropometric and clinical parameters

Variables	Genotypes			*p value*
	GG	GT	TT	
Age/year	58.32±1.47	56.11±1.28	56.91±1.31	NS
Sex M/F	49/45	19/17	8/12	NS
BMI Kg m^2	31.52±1.24	32.91±2.08	31.51±1.63	NS
FBG mg/dL	218.93±2.07	250.26±2.01	234.42±1.83	NS
Serum insulin/μlU/mL	14.86±1.21	15.27±2.07	17.73±1.47	0.01
HbA1c%	8.51±2.16	8.03±1.61	9.91±1.87	0.03
TC mg/dL	218.42±2.03	218.91±2.071.18	217.71±1.14	NS
TG mg/dL	168.42±1.06	173.65±2.03	186.33±1.12	0.03
HDL mg/dL	45.93±1.32	44.89±1.07	40.67±1.83	0.04
LDL mg/dL	152.28±2.36	151.83±1.84	152.24±1.12	NS

NS not significant, *BMI* body mass index, *HbA1c* hemoglobin A1c, *FBG* fasting blood glucose, *TG* triglyceride, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

diabetes disorder in the Iraqi Kurdish ethnicity. Shokouhi et al. conducted a study on the most relevant *TCF7L2* polymorphisms related to the risk of T_2D and concluded the statistically significant difference of the T allele frequency between diabetic patients (18%) and the normoglycemic controls (10%) (*p value* 0.001). Moreover, the GT and TT genotypes were considerably higher in T_2D patients compared with the controls (*p value* 0.005 and 0.046, respectively) [29]. Similar results were demonstrated in a number of studies that determined the rs12255372 T allele as a possible risk factor associated with the increased incidence of T_2D [23, 30–32]. Wang et al. conducted a meta-analysis and suggested different effect size of rs12255372 polymorphism in different ethnicities, as a result of variable frequencies of the risk allele showed among diverse populations [33]. The relationship between anthropometric and clinical covariates with rs12255372 genotypes was examined in the T_2D patients group to determine the influence of these variables on rs12255372 (G > T) genotypes. Genotypic frequencies of GG, GT, and TT showed no differences in age, gender, BMI, FBG, TC, HDL, and LDL variables. However, carriers of TT genotype had elevated levels of serum insulin, HbA1c%, and TG compared to other genotypes (Table 3) which indicated a strong association of TT genotype with the increased risk of the development of T_2D in the studied population.

Conclusion

The polymorphism rs12255372 in the *TCF7L2* gene is highly associated with an increased incidence of type 2 diabetes among the Iraqi Kurdish population, and the T allele could be a risk factor for T_2D. The significant association of this variant with T_2D could predict the future occurrence of this disorder in the studied population and could be applied to other populations. More studies are encouraged to reinforce our findings and analyze the effect of rs12255372 polymorphism in the susceptibility of type 2 diabetes in different populations.

Acknowledgments The authors would like to thank the General Directorate for Scientific Research, Salahaddin University- Erbil for its financial support and to the technicians in the Layla Qasim Diabetic Center for their assistance.

Financial support General Directorate for Scientific Research, Salahaddin University- Erbil, Iraq.

Authors' contribution Conceptualization: Suhad Asaad Mustafa
Performed research: All authors contributed to this part
Formal analysis and investigation: All authors contributed to this part
Writing the paper: All authors contributed to this part
Supervision: Suhad Asaad Mustafa
Approval of the final manuscript: All authors read and approved the final manuscript

Compliance with ethical standards

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

Ethical approval and informed consent to participate and publication All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation set by the committee of Ethical Standards of Salahaddin University, Erbil- Iraq. Informed consent for it was obtained from all patients for being included in the study and for the data to be published.

The present study was authorized and approved by the Human Ethics Committee of the College of Science, Salahaddin University, Erbil. All patients and healthy participants provided written, informed consent to participate and for the publication of data in this study.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):447–455
https://doi.org/10.1007/s13410-020-00898-1

ORIGINAL ARTICLE

SIRT1 functional polymorphisms (rs12778366, rs3758391) as genetic biomarkers of susceptibility to type 2 diabetes mellitus in Iranians: a case-control study and computational analysis

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Received: 7 September 2020 / Accepted: 25 November 2020 / Published online: 7 January 2021

Abstract

Introduction Genetic background is an important risk factor for type 2 diabetes mellitus (T2DM). We designed this study to examine the role of rs12778366 and rs3758391, two functional *SIRT1* gene polymorphisms, on the risk of T2DM in an Iranian population.

Material and methods In this case-control study, a total of 813 subjects were enrolled. SNPs were genotyped via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Multiple computational analyses were also performed to examine the potential effects of the studied variants.

Results and conclusion We found a significant association between rs12778366 polymorphism and an enhanced risk of T2DM under allelic C vs. T (OR = 1.50), codominant TC vs.TT (OR = 1.86), dominant CC + TC vs. TT (OR = 1.65), and over-dominant TC vs. CC + TT (OR = 1.80) genetic models. In contrast, codominant CT vs. CC (OR = 0.54) and dominant CT + TT vs. CC (OR = 0.68) models of rs3758391 polymorphism were correlated with decreased risk of T2DM. Compared to the TC haplotype, we have found that the CC combination significantly enhanced the risk of T2DM by 1.86-fold. Computational analyses indicated that the C allele of rs12778366 might disrupt the binding site of the CEBP transcription factor. *SIRT1* rs12778366 and rs3758391 polymorphisms might be associated with T2DM susceptibility in our population. Replications in different races with larger sample sizes are necessary to yield more accurate results.

Keywords Diabetes mellitus · Type 2 · Polymorphism · Single nucleotide · Sirtuin 1 · Restriction fragment length polymorphism

Introduction

Type 2 diabetes mellitus (T2DM) is a progressive disease with increasing prevalence [1]. As a medical catastrophe of worldwide dimensions, T2DM is characterized by co-existing insulin deficiency and insulin insensitivity [2–4]. Obesity, along with defects in insulin-sensitive glucose transporter 4 (GluT-4) and insulin receptor, may lead to insulin resistance [5]. Insulin resistance is considered the primary cause of T2DM [6]. Genetic background, environmental factors, age, unhealthy diet, high blood pressure, obesity, sedentary lifestyle, increased basic metabolic index (BMI), and oxidative stress are more important risk factors for T2DM onset and progression [3, 7–10]. To better understand the genetic architecture of T2DM and other complex diseases, the candidate gene approach has come under focus together with the powerful genome-wide association studies [11]. To this day, numerous genetic loci have been identified as risk factors for T2DM [12–14].

Sirtuin 1 (SIRT1), as an insulin sensor, reduces body weight and increases insulin in skeletal muscle and adipose tissue, increasing insulin secretion and sensitivity [7, 15, 16]. This NAD^+-dependent histone deacetylase is mainly activated by calorie restriction and is tightly linked to lifespan extension under this situation. SIRT1 modulates glucose/lipid metabolism via its histone

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deacetylase function on various substrates. To name a few, SIRT1 enhances insulin secretion in pancreatic β cells and, therefore, plays a crucial role in the regulation of insulin signaling [17]. Moreover, this protein can protect cells against reactive oxygen species [18], which induce insulin resistance [19].

Single-nucleotide polymorphisms (SNPs) within non-coding DNA regions can impact essential biological processes [20]. The *SIRT1* encoding gene is located on chromosome 10q21.3, encoded in 11 exons interrupted by 10 introns [21]. Two functional SNPs, rs12778366 T>C and rs3758391 T>C, are located in the *SIRT1* gene promoter, with the minor allele frequencies between 0.097 and 0.5 (for rs12778366 T>C) and 0.146 and 0.494 (for rs3758391 T>C) [22]. Lately, few studies in the different geographic areas investigated the effects of SNPs in the *SIRT1* gene on T2DM susceptibility. Among these, Faradonbeh et al. [23], Rai et al. [24], and Cruz et al. [25] have shown the association of these polymorphisms with T2DM. However, studies performed by Peng et al. [26] and Han et al. [11] found no relationship between rs12778366 and rs3758391 polymorphisms and risk of T2DM. In the current study, we aimed to replicate these inconsistent findings and examined such relationships in an Iranian population.

Methods

Subjects

In this case-control study, a total of 813 age- and sex-matched participants were enrolled between 2017 and 2019. The participants consisted of 403 patients with T2DM (referred to Ali Asghar Hospital, Zahedan, Iran) and 410 healthy individuals (admitted to the reference laboratory of Zahedan, Iran). T2DM diagnosis was made by at least two diabetes specialist physicians based on fasting blood sugar (FBS) > 126 mg/dl, 2 h post-prandial (2hpp) > 140 mg/dl, and glycated hemoglobin A1C (HbA1c) > 6.5 levels. The controls were in a healthy condition, with FBS < 99 mg/dL and HbA1c < 5.7%, without any family history of diabetes. Patients with T1DM, thalassemia, gestational diabetes mellitus (GDM), iron deficiency anemia, acute/chronic blood loss, and pregnant women were excluded. Participants were interviewed face to face, and demographic data were collected.

Analysis of biochemical markers

Venous blood was drawn in 3-ml samples following at least 13 h of fasting (between 7:00 pm and 9:00 am). Measurement of plasma level of biochemical parameters, including triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL cholesterol), blood urea nitrogen (BUN), and glycemic indices (FBS, HbA1C) was done by an automated clinical chemistry analyzer (BT1500, Italy) using standard biochemical kits (Pars Azmoon, Tehran, IR Iran) [27].

Genomic DNA isolation

For each participant, 2-ml venous whole blood was collected in an ethylenediaminetetraacetic acid (EDTA)-contained tubes. Genomic DNA was isolated from nucleated blood cells using the simple salting-out method [28]. The quantity and quality of isolated DNA were assessed via spectrophotometric A260/280 ratio and electrophoresis, respectively. Extracted DNAs were kept at − 20 °C until further use.

Genotyping

Genotyping of *STRT1* gene polymorphisms was performed via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The *STRT1* genomic sequences (Gene ID: 23411) were retrieved from the NCBI database (available at http://www.ncbi.nlm.nih.gov). Primers were designed using Gene Runner 3.05 (http://www.generunner.com) and synthesized by Pishgam Company (Tehran, Iran). The primers used are as follows: forward primer 5′-TAAGGCTTCTAGGA CTGGAGATGA-3′ and reverse primer 5′-GTCC CTTAAGCCTAGTATGGGTTC-3′ (for rs12778366) and forward primer 5′-GTCACGCAGGTAATTGATGCAG-3′ and reverse primer 5′-GGCTTAGTGGAAAGCCCTTC-3′ (rs3758391). Each PCR reaction was prepared in 20 μL volume which contained ~ 90 ng (0.6 μL) of genomic DNA, 5 pmol of each primer (1 μL), 10.4 μL of PCR master mix (Ampliqon, Denmark), and 7 μL of sterile water. The optimal PCR conditions were as follows: initial denaturation at 95 °C for 5 min, 30 cycles each consisted of denaturation at 95 °C for 35 s, annealing (62.3 °C for rs12778366 and 67.6 °C for rs3758391) for 35 s, and an extension at 72 °C for 30 s. A final extension followed these steps at 72 °C for 10 min.

The PCR products (10 μL) were digested using *TaaI* (for rs12778366) and *Hin1II* (for rs3758391) restriction enzymes. Then, digested PCR products were run on 2% agarose gel (Kawsar Biotech Company) containing 0.5 μg/ml ethidium bromide. The bands were visualized via an ultraviolet transilluminator. For rs12778366, the allele C was digested and produced 170 and 42 bp fragments, while the T allele was undigested and created a 212 bp amplicon. Regarding rs3758391, the allele C was undigested and created a 241 bp band, while the T allele was digested and produced 146 and 94 bp fragments. To confirm the genotyping results, 30% of the samples were randomly re-genotyped.

Computational analysis

We used AliBaba2.1 online tool (available at http://generegulation.com/pub/programs/alibaba2) and PROMO (as described previously [29]) to evaluate the impact of the studied variants on the binding sites of transcription factors through sequencing the local DNA [30, 31]. The ElemeNT web tool was utilized to predict the transcription start sites (TSS) and core promoter elements of the region contained studied variations [32]. Finally, both variants were processed using WebLogo tool versions 2.8.2 (available at https://weblogo.berkeley.edu) to assess the conservation of their DNA sequences [33].

Statistical analysis

Continuous and categorical data were reported as mean ± standard deviation (SD) and frequencies (percentages), respectively. The Chi-square test, Mann-Whitney *U* test, and binary logistic regression analysis were used to determine the differences among the data sets. Odds ratios (ORs) and 95% confidence intervals (CIs) were also estimated. Haplotype analysis was performed using the SHEsis software platform [34]. The statistical analyses were carried out using IBM SPSS v22.0 at the 5% level of significance.

Results

Patient's characteristics

Table 1 summarizes demographic characteristics and laboratory measurements of patients with T2DM and the control group. There was no significant difference regarding age ($p = 0.64$) and sex ($p = 0.31$) between the studied groups. Significant increases in serum levels of FBS ($p < 0.001$), HbA1C ($p < 0.001$), TG ($p < 0.001$), TC ($p = 0.001$), BUN ($p < 0.001$), creatinine ($p < 0.001$), BMI ($p < 0.001$), and decreased levels of HDL cholesterol ($p = 0.02$) were found in cases as compared with controls.

Genetic association analysis

Table 2 shows the genotypes and allele frequencies of the studied polymorphisms. We found a significant association between rs12778366 polymorphism and the risk of T2DM under codominant (TC vs. TT; OR 1.86; CI 1.2–2.88; *p* value = 0.005), dominant (CC + TC vs. TT; OR 1.65; CI 1.17–2.32; *p* value = 0.004), and over-dominant (TC vs. CC + TT; OR 1.80; CI 1.17–2.77; *p* value = 0.007) contrasted genetic models. For this SNP, the TC genotype was more common in T2DM patients compared with the control group (15.1% vs. 9%). The C allele of rs12778366 conferred an increased risk

Table 1 Demographic characteristics of patients with T2DM and healthy controls

Parameters evaluated	T2DM (mean ± SD)	Controls (mean ± SD)	*p* Value
Age (year)	52.25 ± 12.41	52.47 ± 9.68	0.64
Gender (female/male)	295/108	287/123	0.31
Disease period (years)			
1–4.9	95 (23.6%)	-	
5–9.9	130 (32.2%)	-	
10–14.9	104 (25.8%)	-	
> 15	74 (18.4%)	-	
History (yes/no)			
Thyroid diseases	4/346	-	
Kidney diseases	13/337	-	
Cardiovascular diseases	44/306	-	
FBS (mg/dL)	217.79 ± 79.54	87.85 ± 8.25	*< 0.001*
HbA1C (%)	9.04 ± 1.67	5.22 ± 0.54	*< 0.001*
BUN (mg/dL)	33.51 ± 14.75	14.22 ± 5.19	*< 0.001*
Creatinine (mg/dL)	1.10 ± 0.34	0.84 ± 0.23	*< 0.001*
TC (mg/dL)	173.67 ± 45.27	162.46 ± 40.51	*0.001*
TG (mg/dL)	173.03 ± 102.90	116.72 ± 54.79	*< 0.001*
HDL-C (mg/dL)	48.09 ± 10.12	50.48 ± 12.78	*0.02*
BMI (kg/m2)	27.81 ± 4.32	26.13 ± 2.4	*< 0.001*

FBS fast blood sugar, *TC* total cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *BMI* body mass index, *T2DM* type 2 diabetes mellitus. The Mann-Whitney test and Chi-square test were used for quantitative and qualitative variations, respectively. $p < 0.05$ was considered statistically significant (italicized *p* values)

Table 2 Genotypic and allelic frequencies of *SIRT1* polymorphisms in T2DM patients and healthy controls

Polymorphism	Model	T2DM, *n* (%)	Control, *n* (%)	OR (95%CI)	*p* Value
rs12778366					
	Codominant				
	TT	301 (74.7%)	340 (82.9%)	1 [reference]	
	TC	61 (15.1%)	37 (9.0%)	1.86 (1.20–2.88)	*0.005*
	CC	41 (10.2%)	33 (8.1%)	1.40 (0.87–2.28)	0.17
	Allele				
	T	663 (82.3%)	717 (87.4%)	1 [reference]	
	C	143 (17.7%)	103 (12.6%)	1.50 (1.14–1.98)	*0.004*
	Dominant				
	TT	301 (74.7%)	340 (82.9%)	1 [reference]	
	CC + TC	102 (25.3%)	70 (17.1%)	1.65 (1.17–2.32)	*0.004*
	Recessive				
	TT + TC	362 (89.8%)	377 (92.0%)	1 [reference]	
	CC	41 (10.2%)	33 (8.0%)	1.29 (0.80–2.09)	0.29
	Over-dominant				
	CC + TT	342 (84.9%)	373 (91.0%)	1 [reference]	
	TC	61 (15.1%)	37 (9.0%)	1.80 (1.17–2.77)	*0.007*
rs3758391					
	Codominant				
	CC	145 (35.7%)	113 (28.0%)	1 [reference]	
	CT	205 (52.8%)	251 (63.1%)	0.64 (0.47–0.87)	*0.004*
	TT	53 (11.4%)	46 (8.9%)	0.90 (0.56–1.43)	0.96
	Allele				
	C	495 (61.4%)	477 (58.2%)	1 [reference]	
	T	311 (38.6%)	343 (41.8%)	0.87 (0.72–1.07)	0.18
	Dominant				
	CC	145 (36.0%)	113 (27.6%)	1 [reference]	
	CT + TT	258 (64.0%)	297 (72.4%)	0.68 (0.50–0.91)	*0.01*
	Recessive				
	CC + CT	350 (86.8%)	364 (88.8%)	1 [reference]	
	TT	53 (13.2%)	46 (11.2%)	1.20 (0.79–1.83)	0.40
	Over-dominant				
	CC + TT	198 (49.1%)	159 (38.8%)	1 [reference]	
	CT	205 (50.9%)	251 (61.2%)	0.66 (0.50–0.87)	*0.003*

CI confidence interval, *OR* odds ratio, *T2DM* type 2 diabetes mellitus. The Chi-square test and logistic regression were used to analyze the association between genotypes and risk of T2DM. $p < 0.05$ was considered statistically significant (italicized *p* values)

of T2DM by 1.50-fold (C vs. T; OR 1.50; CI 1.14–1.98; *p* value = 0.004).

As regards rs3758391, codominant (CT vs. CC; OR 0.64; CI 0.47–0.87; *p* value = 0.004), dominant (CT + TT vs. CC; OR 0.68; CI 0.50–0.91; *p* value = 0.01), and over-dominant (CT vs. CC + TT; OR 0.66; CI 0.50–0.87; *p* value = 0.003) markedly decreased the risk of T2DM. Compared with healthy controls, the frequency of CC genotype of this SNP in the case group was higher than that of CC genotype (35.7% versus 28%). Meanwhile, the presence of the T allele was not associated with T2DM susceptibility (T vs. C; OR 0.87; CI 0.72–1.07; *p* value = 0.18). The results of the haplotype analysis indicated that the haplotype frequencies were different between studied groups. Haplotypes CC and CT were more frequent in cases compared with the healthy subjects. We also found that the CC haplotype increased T2DM incidence by 1.86-fold (*p* value = 0.001)(Table 3). A significant association was also observed between total cholesterol levels and CC vs. TT + TC genetic model in controls, but not in T2DM patients. No other significant associations were noticed between *SIRT1*

Table 3 Haplotype analysis of *SIRT1* gene polymorphisms in patients with T2DM and healthy controls

rs12778366	rs3758391	T2DM (%)	Control (%)	OR (95%CI)	*p* Value
T	C	413 (51.2%)	431 (52.6%)	1 [reference]	
C	C	82 (10.2%)	46 (5.6%)	1.86 (1.27–2.73)	*0.001*
C	T	61 (7.6%)	57 (7.0%)	1.12 (0.76–1.64)	0.57
T	T	250 (31.0%)	286 (34.9%)	0.91 (0.73–1.13)	0.41

OR odds ratio, *CI* confidence interval, *T2DM* type 2 diabetes mellitus. The Chi-square test was recruited. $p < 0.05$ was considered statistically significant (italicized *p* value)

polymorphisms and clinical-demographic characteristics of T2DM patients and healthy controls (Table 4).

Computational analyses

The sequence of *SIRT1* gene promoter, in which rs12778366 and rs3758391 are located was retrieved from the NCBI server (accession number NC_000010.11). Our findings using the AliBaba2.1 transcription factor binding site prediction software showed that T to C substitution in rs12778366 interrupts the binding site of CCAAT/enhancer-binding protein alpha (CEBPA) transcription factor on the *SIRT1* promoter region. Nevertheless, the T to C substitution in rs3758391 did not affect transcription factor binding sites in this region (data not shown). Additionally, the results of the PROMO server demonstrated that rs12778366 is located at the core of recognition and binding site of the GR-beta transcription factor. The T to C substitution at this position can disrupt the GR-beta's binding site (Fig. 1a). As regards rs3758391, the T allele creates two binding sites for the YY1 transcription factor.

In contrast, the presence of the other allele did not influence the binding of any transcription factor to this region (Fig. 1b). The ElemeNT server predicted that the allele C of rs3758391 might slightly affect the *SIRT1* transcription by adding a new start site (Fig. 2 b and b′). Finally, the server showed that none of the studied variants is located in a well-conserved region across several mammalian species (Fig. 3).

Discussion

T2DM is a complex multifactorial disease associated with hyperglycemia, hypertension, and dyslipidemia [35]. Lately, many studies have recommended multifactorial treatment for T2DM. Still, T2DM patients have an increased incidence of early mortality [36]. In this regard, increasing knowledge and identifying risk factors of T2DM can reduce its complications.

In our study, *SIRT1* rs12778366 and rs3758391 gene polymorphisms were significantly associated with T2DM susceptibility. Our results indicated that the rs12778366 polymorphism enhanced the risk of T2DM. Instead, the other *SIRT1* variant, rs3758391, conferred a protective role against T2DM under different genetic models. Also, the CC haplotype enhanced T2DM susceptibility in our population. By performing computational analyses, we found that rs12778366 polymorphism may affect the DNA binding potential of CEBPA, a transcription factor that modulates adipogenesis and adipose morphology, and might have a role in glucose metabolism [37]. Furthermore, our computational analyses predicted that rs3758391 might slightly affect the *SIRT1* transcription start site in mammals.

Kovanen et al. found no association between rs12778366 and T2DM risk [38]. Rai et al. showed that carriers of TT genotype might have a high risk for developing T2DM, which

Table 4 Association between *SIRT1* polymorphisms and clinical-demographic characteristics of T2DM patients and healthy controls

SNP	Genotype	FBS (mg/dL)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	BUN (mg/dL)	Creatinine (mg/dL)	BMI (kg/m^2)
rs12778366								
T2DM	TT (reference)	218.14 ± 81.14	173.48 ± 45.66	175.35 ± 104.315	47.88 ± 10.14	33.70 ± 15.00	1.12 ± 0.35	27.70 ± 4.37
	CC + TC	214.66 ± 64.30	175.31 ± 42.32	152.97 ± 88.56	49.97 ± 9.86	31.85 ± 12.57	1.02 ± 0.19	28.71 ± 3.78
	p Value	0.74	0.98	0.13	0.16	0.42	0.28	0.25
Control	TT (reference)	87.80 ± 8.30	162.08 ± 40.87	116.90 ± 55.03	50.33 ± 12.78	14.24 ± 5.24	0.84 ± 0.24	26.30 ± 1.48
	CC + TC	89.70 ± 6.57	175.00 ± 24.31	110.80 ± 48.44	55.30 ± 12.23	13.60 ± 3.60	0.80 ± 0.20	26.20 ± 13.13
	p Value	0.48	0.23	0.78	0.18	0.86	0.77	0.86
rs3758391								
T2DM	CC (reference)	203.67 ± 67.10	172.23 ± 43.38	161.70 ± 123.79	47.10 ± 8.89	30.82 ± 9.66	1.05 ± 0.20	27.40 ± 3.73
	TT + TC	219.62 ± 80.93	173.86 ± 45.60	174.56 ± 99.90	48.22 ± 10.30	33.87 ± 15.30	1.12 ± 0.36	27.86 ± 4.40
	p Value	0.24	0.74	0.09	0.65	0.32	0.68	0.52
Control	CC (reference)	85.87 ± 9.26	176.32 ± 32.21	133.00 ± 67.50	48.90 ± 8.86	14.06 ± 4.07	0.87 ± 0.27	26.26 ± 1.29
	TT + TC	88.05 ± 8.14	161.07 ± 41.05	115.08 ± 53.20	50.64 ± 13.11	14.24 ± 5.30	0.84 ± 0.24	26.30 ± 1.49
	p Value	0.24	*0.03*	0.14	0.58	0.97	0.69	0.94

FBS fast blood sugar, *TC* total cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *BUN* blood urea nitrogen, *BMI* body mass index, *kg* kilogram, m^2 square meter, *T2DM* type 2 diabetes mellitus. The Mann-Whitney test was used for analyzing the association between studied genotype and demographic data. $p < 0.05$ was considered statistically significant (italicized *p* value)

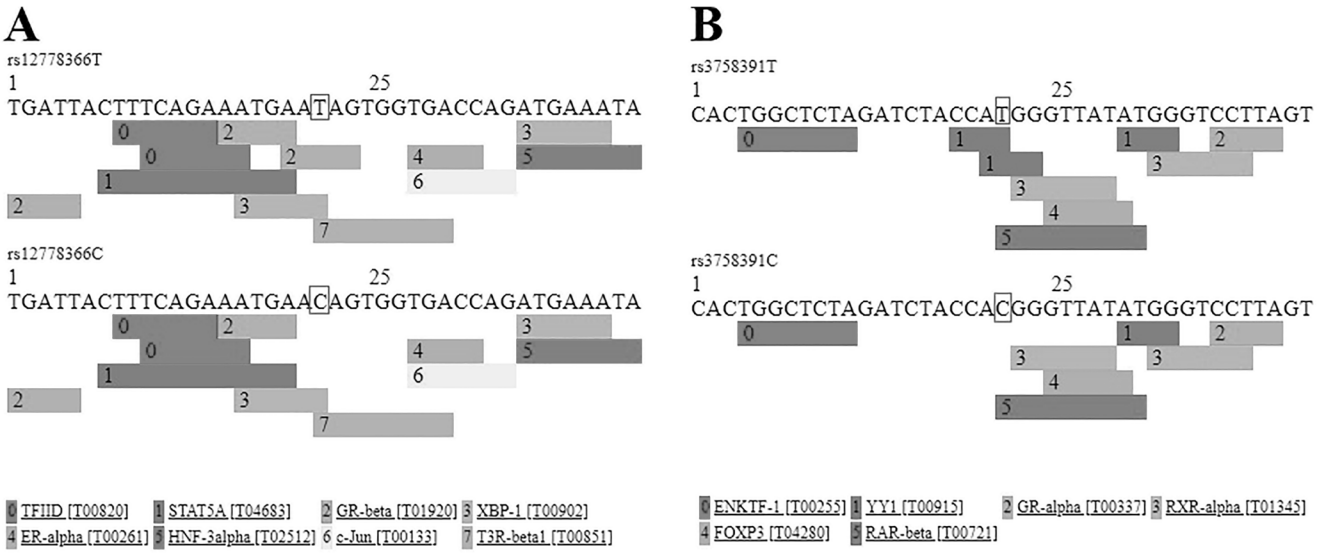

Fig. 1 Impacts of **a** rs12778366 and **b** rs3758391 polymorphisms on the binding potential of various transcription factors. Regarding both studied variants, the T to C substitution caused the loss of some transcription factor's binding sites

was not entirely consistent with our results [24]. In agreement with our findings, Han et al. showed that the allele C of this variant increases the incidence of T2DM [11]. Cruz et al. [25] and Naqvi et al. [39] showed that both the alleles of rs3758391 were associated with T2DM risk, respectively. In another study by Tavakoli et al., the rs3758391 TC and TT genotypes were linked to diabetic nephropathy [23], which was not consistent with our findings. On the contrary, Peng et al. failed to find any significant association between rs3758391 polymorphisms and T2DM [26]. Dong and colleagues found a significant association between this SNP and obesity [16]. However, studies performed by Naqvi et al. [39] and Faradonbeh et al. [23] have not reported such association. Rose and his research team revealed that CT genotype carriers of rs3758391 had a lower risk of cardiovascular mortality [40]. One year later, Zillikens and colleagues showed that the heterozygous carriers of the rs3758391 minor allele had a lower but yet insignificant risk for cardiovascular mortality. According to their results, rs3758391 is in high linkage disequilibrium with rs1467568 ($R^2 = 0.96$), an intronic *SIRT1* polymorphism [41].

Sirtuins have substantial roles in adapting the physiology of white adipose tissue [42]. *SIRT1* activates peroxisome proliferator-activated receptors gamma

A nt 15-... ATGAA**T**AGTGG ...+15 nt

Element	Start position	Sequence	PWM score	Consensus Match
Mammalian Initiator	4	TTACTTT	0.1371	7 out of 7
Mammalian Initiator	30	CCAGATG	0.0852	6 out of 7
Mammalian Initiator	10	TCAGAAA	0.0170	5 out of 7
Mammalian Initiator	1	TGATTAC	0.0107	5 out of 7
Drosophila Initiator	10	TCAGAA	0.0270	4 out of 6
Drosophila Initiator	1	TGATTA	0.0105	4 out of 6

B nt 15- ... TACCA**T**GGGTT ... +15 nt

Element	Start position	Sequence	PWM score	Consensus Match
Mammalian Initiator	9	CTAGATC	0.1218	7 out of 7
Mammalian Initiator	25	TTATATG	0.0156	6 out of 7
TATA box	26	TATATGGG	0.0204	6 out of 8

A' nt 15-... ATGAA**C**AGTGG ...+15 nt

Element	Start position	Sequence	PWM score	Consensus Match
Mammalian Initiator	4	TTACTTT	0.1371	7 out of 7
Mammalian Initiator	30	CCAGATG	0.0852	6 out of 7
Mammalian Initiator	10	TCAGAAA	0.0170	5 out of 7
Mammalian Initiator	1	TGATTAC	0.0107	5 out of 7
Drosophila Initiator	20	ACAGTG	0.0446	4 out of 6
Drosophila Initiator	10	TCAGAA	0.0270	4 out of 6
Drosophila Initiator	1	TGATTA	0.0105	4 out of 6

B' nt 15-... TACCA**C**GGGTT ...+15 nt

Element	Start position	Sequence	PWM score	Consensus Match
Mammalian Initiator	9	CTAGATC	0.1218	7 out of 7
Mammalian Initiator	25	TTATATG	0.0156	6 out of 7
Mammalian Initiator	15	CTACCAC	0.0130	5 out of 7
TATA box	26	TATATGGG	0.0204	6 out of 8

Fig. 2 The prediction of core TSS and core promoter elements for (**a** and **a′**) rs12778366 and (**b** and **b′**) rs3758391 polymorphisms. The results demonstrated that the substitution of the wild allele with its counterpart does not have a significant influence on the pattern of TSS and the core promoter element

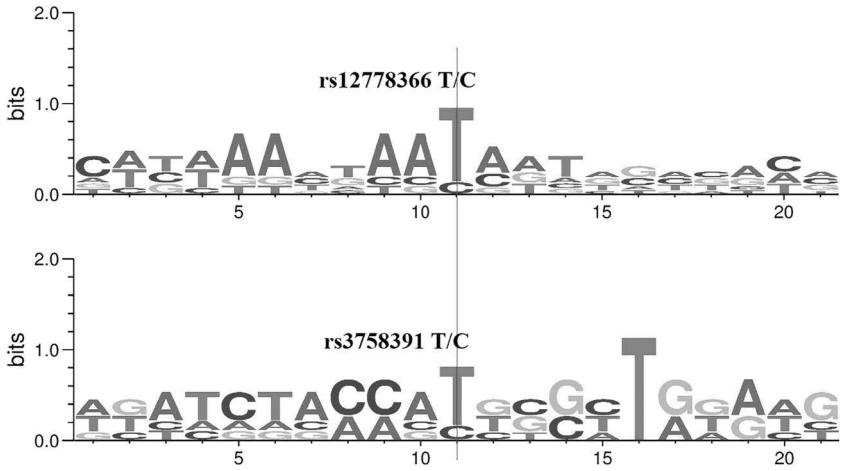

Fig. 3 The analysis of the local DNA region containing rs127778366 and rs3758391 polymorphisms. The blue vertical line shows the precise location of these variations

(PPARγ) coactivator 1α (PGC-1α) and forkhead box O1 (FOXO1) that contribute to glucose and fatty acid metabolism [43]. *SIRT1* gene plays an essential role in lipid and glucose metabolism, response to oxidative stress factors, and many other intracellular processes [44]. *SIRT1* gene variations affect the development of some diseases such as pituitary adenoma, autoimmune thyroid disease, and cancer and also T2DM [23, 45–47]. Besides, silencing mediators of thyroid hormone receptors are cofactors of SIRT1 [48]. Hence, functional polymorphisms in *SIRT1* can potentially alter the function of its cofactors, and many contribute to thyroid disorders. Differential expression of *SIRT1*, geographical differences, lifestyle, and environmental factors might be responsible for the inconclusive effects of these variations among different populations.

On the other hand, we found that rs12778366 and rs3758391 polymorphisms influence the binding of glucocorticoid receptor beta (GR-beta) and Yin Yang 1 (YY1) transcription factors, respectively. Liu and colleagues reported that increased glucocorticoid receptor β might contribute to the phenotype of T2DM [49]. In this scenario, the pancreatic beta cells overexpressing these receptors are primary targets for the diabetogenic action of glucocorticoids [50]. Furthermore, Verdeguer and coworkers showed that diminished genetic dosage of YY1 in mice liver leads to insulin resistance, dyslipidemia, and the onset of diabetic-like symptoms [51]. Yet, the precise role of these transcription factors in the development of T2DM is not fully understood.

Despite all the efforts made, our work had some limitations. Our sample size was relatively small. We believe that such population-based genetic studies associate with population size; thus, larger samples would lead to more accurate results. Moreover, we did not investigate the possible effects of *SIRT1* polymorphisms on *SIRT1* gene expression. More studies in this area may enlighten the role of these functional *SIRT1* polymorphisms in T2DM development.

Conclusion

In summary, the results of this study showed that *SIRT1* gene rs12778366 and rs3758391 might be associated with T2DM susceptibility in a population of Iranian ancestry. Replication in different races with larger sample sizes is needed to yield more accurate results.

Acknowledgments The authors are grateful to Dr. Maryam Piri and Dr. Hamed Taheri, the two diabetes specialist physicians, for their assistance.

Funding This work was financially supported by a dissertation grant (M.Sc. thesis of Mohammad Bagher Sadeghi project no. 7949) from Zahedan University of Medical Sciences.

Compliance with ethical standards

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

Ethical considerations Written informed consent was taken from all participants for obtaining a sample. The local ethics committee of Zahedan University of Medical Sciences (Zahedan, Iran) approved the present study (Ethical code: IR.ZAUMS.REC.1395.131). This experiment was carried out in compliance with the Declaration of Helsinki.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):456–468
https://doi.org/10.1007/s13410-020-00900-w

ORIGINAL ARTICLE

Ethanol extract of *Tephrosia bracteolata* leaves and its fractions ameliorates alloxan-induced diabetes and its associated complications in Wistar rat model

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Received: 23 June 2020 / Accepted: 26 November 2020 / Published online: 7 January 2021

Abstract

Background *Tephrosia bracteolata* Guill. & Perr. (Leguminosae-Papilionoideae) is a traditional Nigerian medicinal plant used for the treatment of whitlow, toothache, wounds, and diabetes.

Aim This study investigated the biochemical and histopathological effects of the ethanol extract of *T. bracteolata* leaves (EETB) and its fractions on alloxan-induced diabetic rats.

Methods EETB was fractionated successively with n-hexane, chloroform, ethyl acetate, methanol, and water to yield the respective fractions (nHF, CF, EAF, MF, and AF). The antidiabetic activities of EETB and its fractions at 250 and 500 mg/kg body weight (groups 4–15) were investigated in comparison to the normal control (group 1), the diabetic control (group 2), and the standard (150 mg/kg b.w. metformin, group 3) on alloxan-induced diabetic Wistar rats (200–220 g) for 28 days. Alterations in some biochemical parameters and histopathology of major organs were assessed.

Results Induction of diabetes triggered significant ($p < 0.05$) alterations in biochemical indices of the diabetic control relative to the normal control. Following treatments, EAF recorded the most potent effect by restoring altered biochemical parameters examined as indices of diabetic complications. Histopathological examination indicated a rapid regeneration of beta cells, hepatocytes, and nephrotic cells necrotized by alloxan, with EAF producing the best histo-architecture relative to EETB and other fractions.

Conclusions EAF indicated the most potent antidiabetic effect at the doses investigated, as it reversed complications associated with diabetes in Wistar rats, thus suggesting its potential for future development of potent antidiabetic drugs. Further studies on the characterization of the bioactive principles in EAF are underway.

Keywords Diabetes mellitus · Medicinal plants · Histology · *Tephrosia bracteolata* · Biochemical alterations · Alloxan · Wistar rats

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Abbreviations

AF	Aqueous fraction
ALB	Albumin
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
BC	Beta cells
CAT	Catalase
CF	Chloroform fraction
CV	Central vacuole
Dbil	Direct bilirubin
DM	Diabetes mellitus
EAF	Ethyl acetate fraction
EETB	Ethanol extract of *Tephrosia bracteolata* leaves

FBS	Fasting blood sugar
G	Glomerulus
H	Hepatocytes
IDF	International Diabetes Foundation
IST	Islet
MDA	Malondialdehyde
MF	Methanol fraction
nHF	n-Hexane fraction
RT	Renal tubules
SOD	Superoxide dismutase
Tbil	Total bilirubin
TP	Total protein
WBC	White blood cell

Introduction

Medicinal plants and herbal medicine continue to be an important area of investigation to researchers globally. Scientifically, these plants are usually first screened for the presence of bioactive compounds which are known to possess therapeutic potentials. Different parts of these plants such as the leaves, bark, and roots are highly rich in phytochemicals such as phenols, alkaloids, flavonoids, terpenes, and glycogens with the inherent capacity to protect against diseases and attenuate toxicities [1]. The plant *Tephrosia bracteolata* Guill. & Perr. (Leguminosae-Papilionoideae) is a glabrous shrub, usually 2 to 8 ft in height, with long straight thinly silky branches and bright pink or purple flowers. The fruits are narrowly linear, about 5 to 6 long and 4 mm broad. They appear erect and are usually curved upwards. The seeds are contained in pods, and the leaflets are 4 to 8 cm long, 3 to 5 cm broad, linear, silky, and pubescent beneath, with bracteoles broadly ovate, 5 mm enclosing the buds [2]. The plant has been widely used for various purposes in African folklore medicine. The root is used as medicine for venereal diseases, for example, treatment of pregnant women with syphilis. The aerial parts of the plant serve as a source of food for grazing animals [3] and have been reported to possess analgesic anti-inflammatory and antipyretic properties [4]. Information obtained through personal communications in herbal markets around Lokoja and Bassa Local Government Areas of Kogi State, North Central Nigeria, confirmed that *T. bracteolata* leaf preparation is used for the treatment of whitlow, toothache, earache, open wounds, and diabetes. A study by Egharevba et al. [5] reveals the presence of phyto-compounds such as alkaloids, steroids, tannins, flavonoids, and terpenoids in the n-hexane and ethyl acetate extracts of the leaves of *T. bracteolata*. Scientific validation of several plant species has proven that some of these phytochemicals are responsible for antidiabetic activities of many medicinal plants.

Diabetes mellitus (DM) is one of the most significant and life-threatening complex disorders. According to recent statistics by the International Diabetes Foundation (IDF), the prevalence of diabetes mellitus stands at about 425 million persons worldwide, with an estimated 50% of these cases undiagnosed, of which developing countries in Africa and Asia constitute the most significant numbers [6]. In Nigeria alone, the 2017 prevalence of diabetes mellitus among adults between the age of 20 and 69 years was reported to be 1.7% [6]. The United Nation estimated the population of Nigeria as of September 2017 to be 193.3 million [7]. A recent meta-analysis conducted by Uloko et al. [8] identified a significant increase in the prevalence of DM in Nigeria, with a pooled DM prevalence of 5.77%, suggesting that 11.2 million Nigerians (about 1 in 17 adults) are living with DM, and this affects all regions of the country, with the southern region having the highest prevalence of DM. Some predisposing risk factors include urban dwelling, aging, lack of physical activity, and unhealthy diet [8]. Hence, there is a need for more studies on the pathophysiology, management, and treatment options for DM and its associated complications especially from plant sources. One scientific way to accomplish this is by induction of a similar disease-like condition in the experimental animal models which mimic the human system, and to investigate the effects of potential alternative therapies on the biological and pathological aspects of the disease with a view to discovery of safer, more potent, easily affordable, and effective treatment options.

Alloxan monohydrate is one of the most common diabetogenic agents used for induction of diabetes in rodents. It is a toxic analogue of glucose, which when administered to vertebrates, results in the destruction of the β-cells. Consequently, an insulin-dependent type of diabetes mellitus develops in the animals. Alloxan is selectively toxic to insulin-producing cells of the pancreas (β-cells) because it preferentially accumulates in the β-cells through uptake via the glucose transporter-2 (GLUT2) [9]. In the presence of intracellular thiols, alloxan generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The β-cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. The action of the ROS coupled with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells, decreasing insulin secretion, which in turn increases blood glucose concentration [10]. This results in the elevation of serum glucose concentration (hyperglycemia), which could persist overtime, and is characteristic of diabetes mellitus. Other than the persistent elevation of blood glucose level associated with diabetes, there are also observed abnormalities in the metabolic pattern of lipids, carbohydrates, proteins, and electrolytes, as well as the elevation in hepatic enzyme activities, and the subsequent failure of multiple organs due to complications associated with the diabetic condition. DM is also associated with increased

oxidative stress, which could be supported by enhanced accumulation of lipid peroxides in the plasma of diabetic patients [11]. The present study was therefore set up to investigate the biochemical and histopathological effects of the ethanol extract of *Tephrosia bracteolata* leaves (EETB) and its fractions on alloxan-induced diabetes in Wistar rats.

Materials and methods

Materials

Collection, identification, and authentication of plant material

Fresh leaves of *T. bracteolata* Guill. & Perr. were collected from the natural habitat along the River Niger area of Lokoja, Kogi State, Nigeria, in April 2017. The plant sample was identified and authenticated at the Herbarium of the Department of Biological Sciences, Federal University, Lokoja, Kogi State, Nigeria, by Mr. Gbenga Akanni, an ethnobotanist. The leaf sample was deposited at the herbarium for future reference and was assigned a voucher number (FULH/0765).

Chemicals, drugs, and reagents

All chemical and reagents used for the conduct of this study were of analytical grade. Dragendorff's reagent, Wagner's reagent, Mayer's reagents, 5% iron (III) chloride, 1% aluminum (III) chloride, bromine water, aqueous ammonia, and concentrated tetraoxosulphate (VI) acid were procured from British Drug House, England. Concentrated hydrochloric acid, α-naphthol and lead acetate solution, ethanol (≥ 99.5% purity), n-hexane (≥ 98.5% purity), chloroform (≥ 98.5% purity), ethyl acetate (≥ 99.8% purity), and methanol (≥ 99.8% purity) were purchased from Sigma-Aldrich, St. Louis, Mo, USA. Aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) assay kits were procured from Randox® diagnostics Laboratories, UK. Total cholesterol, triacylglycerol (TAG), and low-density lipoprotein (LDL) test kits were purchased from Agappe® Diagnostics Ltd., India. Total bilirubin (Tbil), total protein, albumin, catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) test kits were purchased from Randox® diagnostics Laboratories, UK. Precoated silica gel $60F_{254}$ on aluminum sheets, silica gel (60–120 mesh) for column chromatography, and alloxan monohydrate were products of Sigma-Aldrich, St. Louis, MO, USA. The drug metformin (Glucophage®) was purchased from Bristol Myers Squibb Company, New York City. Distilled water was obtained from the National Centre for Energy Research and Development (NCERD), University of Nigeria, Nsukka.

Animals

Twelve-week-old adult male Wistar rats weighing between 200 and 220 g, purchased from the Animal House Facility of Salem University, Lokoja, Kogi State, Nigeria, were used for the study. They were kept in well-ventilated stainless steel rat cages under standard laboratory conditions guaranteed of 12-h dark/light cycle and were fed standard rodent feed and potable drinking water ad libitum. The animals were acclimatized to the laboratory environment for a period of 7 days prior to use and accorded humane care in line with the recommendations of the Institutional Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka, with Approval No. UNN/FBS/EC/1037, together with the International Guidelines for Handling of Laboratory Animals [12].

Methods

Preparation and processing of plant material

The leaves of *T. bracteolata* were washed with clean water and freed from sand and debris. The leaves were drained completely and shade-dried for 15 days to a constant weight. The dried plant material was pulverized into powdered form using an electric blender (Rashnik®, RN-1001, China) and stored in airtight containers for crude plant extraction.

Crude plant extraction

A known weight, 2000 g, of the pulverized plant material was macerated in ethanol (99.5%) using cold maceration method and allowed to stand for 72 h after which it was filtered with a muslin sieve, with further filtration done using the Whatman No. 1 filter paper (Whatman, Maidstone, England). The filtrate was concentrated with a rotary evaporator at 40 °C to obtain crude extract known as ethanol extract of *T. bracteolata* (EETB).

Fractionation of EETB

EETB was fractionated according to the method of Uzor et al. [13]. Briefly, a weighed quantity (200 g) of EETB was triturated with 400 g of silica gel in a glass column to increase the surface area and then partitioned successively with different solvents according to increasing order of polarity starting with n-hexane, chloroform, ethyl acetate, methanol, and water respectively. The collected samples were concentrated to afford the corresponding fractions, which were labeled n-hexane (nHF), chloroform (CF), ethyl acetate (EAF), methanol (MF), and aqueous fraction (AF) respectively.

Phytochemical analyses of EETB and its fractions

Phytochemical analyses of EETB and its fractions were carried out to determine the presence of different classes of phyto-compounds present in the extract and fractions. This was done according to the method of Trease and Evans [14].

Induction of experimental diabetes in rats

At the end of the 7 days' acclimatization, the animals were subjected to an overnight fast, after which experimental diabetes was induced using alloxan monohydrate (150 mg/kg b.w.) via intra-peritoneal route [15]. Rats having fasting blood sugar (FBS) level greater than 200 mg/dl 72 h post induction were considered diabetic and included in the study.

Experimental design: a curative study

Ninety healthy adult male rats of Wistar strain having weights between 200 and 220 g were randomized into 15 groups of 6 rats each and treated orally for 28 days as follows: Group 1 (normal control) received distilled water, while rats in group 2 served as diabetic control, with the diabetes induced using 150 mg/kg b.w. alloxan and untreated. Animals in groups 3 to 15 were induced with diabetes and treated with EETB and the fractions. Group 3 served as the standard control and was treated with 150 mg/kg b.w. metformin hydrochloride.

Groups 4 and 5 rats were treated with 250 and 500 mg/kg b.w. EETB respectively, while groups 6 and 7 received treatment with 250 and 500 mg/kg b.w. nHF respectively. Group 8 and 9 rats were treated with 250 and 500 mg/kg b.w. CF respectively. Groups 10 and 11 received EAF at doses of 250 and 500 mg/kg b.w. EAF, while groups 12 and 13 received MF at doses of 250 and 500 mg/kg b.w. MF respectively. Groups 14 and 15 rats were treated with 250 and 500 mg/kg b.w. AF respectively following confirmation of diabetes.

At the end of the 28 days' treatment regimen with standard drug, EETB, and the fractions, the rats were subjected to an overnight after which they were anesthetized and decapitated following exposure to chloroform vapor and scarified one after the other. Blood samples were collected into plain sample tubes and centrifuged at 3000 rpm for 15 min using a Beckman Centrifuge (CS-15, Germany). The serum was carefully transferred into another set of sterile plain tubes labeled accordingly and subsequently used for biochemical analysis.

Determination of biochemical indices

Weekly FBS was determined using a FineTest® glucometer with the corresponding test strips (FineTest®, Milpitas, USA). The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel [16]. Alkaline phosphatase (ALP) activity was assayed according to the method of Kind and King [17]. The concentrations of serum total bilirubin (Tbil), direct bilirubin (Dbil), total protein (TP), albumin (ALB), and malondialdehyde (MDA) were determined according to the procedures described in their respective Randox Diagnostic Laboratories test kit. Serum catalase (CAT) and superoxide dismutase (SOD) activities were also assayed by following the procedures outlined in their respective Randox Diagnostic assay kits. Serum urea and creatinine concentrations were determined using the method of Bartels and Bohmer [18], while electrolyte concentrations were determined using an electrolyte analyzer (OPTI® LION electrolyte analyzer, OPTI Medical Systems Inc., GA, USA).

Histopathological examination

The histopathological conditions of the pancreas, liver, and kidney of rats drawn from each study group were examined according to the method of Drury et al. [19].

Statistical analysis

The data obtained from this study were analyzed using IBM Statistical Product and Service Solution (SPSS), version 21 (Chicago, IL). Significant differences in the means were established by the one-way analysis of variance (ANOVA), followed by Duncan's post hoc multiple comparison test. The results were expressed as mean ± standard deviation of replicate measurements. Mean values with $p < 0.05$ when compared were considered statistically significant.

Results

Phytochemical constituents of EETB and its fractions

Table 1 presents the phytochemical constituents of EETB and its fractions. The results indicate the presence of phytochemical classes such as phenols, terpenoids, saponins, steroids, glycosides, flavonoids, tannins, and alkaloids in EETB, EAF, and MF. Traces of saponins, glycosides, flavonoids, and tannins were present in AF, while terpenoids, steroids, flavonoids, and alkaloids were present in CF and nHF.

Effect of EETB and its fractions on the fasting blood sugar level of alloxan-induced diabetic rats

Table 2 shows the effect of EETB and its fractions on FBS levels of alloxan-induced diabetic rats. The results indicated a significant ($p < 0.05$) rise in FBS level of the diabetic control from day 7 to day 28 as compared to the normal control rats which recorded no significant ($p > 0.05$) changes in FBS level

Table 1 Phytochemical constituents of EETB and its fractions

Phytochemicals	Bioavailability of crude extract and fractions					
	EETB	nHF	CF	EAF	MF	AF
Phenols	+++	−	−	++	++	−
Terpenes	+	+	+	++	+	−
Saponins	++	−	−	+	+	+
Steroids	+	+	+	+	+	−
Glycosides	+++	−	−	+	+	+
Flavonoids	+++	+	+	++	+	+
Tannins	+	−	−	+	+	+
Alkaloids	++	+	+	+	+	−

Key: slightly present (+), moderately present (++), highly present (+++), not detected (−)

EETB ethanol extract of *T. bracteolate* leaves, *nHF* n-hexane fraction, *CF* chloroform fraction, *EAF* ethyl acetate fraction, *MF* methanol fraction, *AF* aqueous fraction

for the duration of the experiment. Treatment with metformin, EETB, and its fractions (groups 3–15) resulted in a significant ($p < 0.05$) decrease in FBS levels of the diabetic rats across the different time intervals, with day 28 recording the highest decline in FBS concentration. It is noteworthy to highlight that at doses of 250 and 500 mg/kg b.w. respectively, EETB and EAF recorded the most drastic and dose-dependent decline in FBS level relative to the diabetic control and other treated groups.

Effect of EETB and its fractions on lipid peroxidation and antioxidant status of alloxan-induced diabetic rats

Table 3 shows the effect of EETB and its fractions on lipid peroxidation and antioxidant status of alloxan-induced diabetic rats. The data obtained shows the MDA concentration of the diabetic control rats to be significantly ($p < 0.05$) higher when compared to the normal control rats. After 28 days of treatment with the standard drug, EETB, and its fractions, only EETB and EAF resulted in a significantly ($p < 0.05$) lower MDA concentration relative to the diabetic control. The decline in MDA concentration was observed to be dose-dependent and comparable to that of the normal control group as shown in Table 3. The results also indicated a significant ($p < 0.05$) decline in SOD and CAT activities of the diabetic control as compared to the normal control. Most striking from the treatment groups are the significant ($p < 0.05$) increases in the activities of SOD and CAT of the rats treated with EETB (groups 4 and 5) and EAF (groups 10 and 11) when compared to the diabetic control and other treatment groups.

Table 2 Effect of EETB and its fractions on the fasting blood sugar levels of alloxan-induced diabetic rats

Post-treatment time in days					
Groups	0	Day 7	Day 14	Day 21	Day 28
1	80.8 ± 12.19	81.2 ± 9.47	79.2 ± 11.34	81.0 ± 8.12	80.6 ± 10.67
2	371.8 ± 114.75	380.0 ± 166.03	337.6 ± 123.64	385.2 ± 110.92	431.5 ± 116.30
3	368.2 ± 107.57*	347.2 ± 104.19*	289.2 ± 113.60*,#	221.4 ± 80.00*,#	171.0 ± 22.89*,#
4	367.4 ± 106.45*	347.6 ± 97.38*	280.2 ± 88.87*,#	174.4 ± 68.58*,#	129.6 ± 26.23*,#
5	363.8 ± 103.47*	339.8 ± 92.57*	227.8 ± 46.53*,#	175.2 ± 70.51*,#	104.0 ± 8.09*,#
6	362.2 ± 99.31*	351.6 ± 100.57*	340.0 ± 93.83*	316.6 ± 92.28*,#	304.8 ± 93.22*,#
7	362.0 ± 97.70*	338.4 ± 81.62*	326.2 ± 94.13*	303.2 ± 101.60*,#	292.2 ± 106.30*,#
8	362.0 ± 99.20*	347.6 ± 94.17*	336.6 ± 95.41*	312.4 ± 93.00*,#	295.8 ± 87.94*,#
9	359.8 ± 101.67*	339.4 ± 92.44*	322.6 ± 91.58*	308.4 ± 92.11*,#	293.6 ± 88.10*,#
10	353.8 ± 106.39*	296.6 ± 82.85*	272.0 ± 88.66*,#	169.4 ± 72.29*,#	140.0 ± 57.72*,#
11	350.0 ± 104.29*	298.4 ± 91.81*	236.4 ± 73.07*,#	169.4 ± 67.50*,#	108.4 ± 38.99*,#
12	359.6 ± 103.44*	345.2 ± 100.06*	322.0 ± 95.55*	298.6 ± 85.13*,#	287.2 ± 82.51*,#
13	355.4 ± 108.45*	331.6 ± 105.19*	302.4 ± 101.54*	294.4 ± 98.91*,#	282.0 ± 103.59*,#
14	355.8 ± 111.13*	339.8 ± 103.11*	324.8 ± 102.49*	299.6 ± 87.67*,#	273.0 ± 83.76*,#
15	354.8 ± 111.58*	344.0 ± 106.95*	335.4 ± 115.9*	312.1 ± 110.39*,#	273.0 ± 127.61*,#

Results are presented as mean ± standard deviation, $n = 6$. Mean values with "*" are significantly different from the normal control, while those with "#" are significantly different from the diabetic control at $p < 0.05$. (1) Normal control. (2) Diabetic control. (3) D + 150 mg/kg b.w. metformin. (4) D + 250 mg/kg b.w. EETB. (5) D + 500 mg/kg b.w. EETB. (6) D + 250 mg/kg b.w. nHF. (7) D + 500 mg/kg b.w. nHF. (8) D + 250 mg/kg b.w. CF. (9) D + 500 mg/kg b.w. CF. (10) D + 250 mg/kg b.w. EAF. (11) D + 500 mg/kg b.w. EAF. (12) D + 250 mg/kg b.w. MF. (13) D + 500 mg/kg b.w. MF. (14) D + 250 mg/kg b.w. AF. (15) D + 500 mg/kg b.w. AF. *D* diabetic)

Table 3 Effect of EETB and its fractions on lipid peroxidation and antioxidant status of alloxan-induced diabetic rats

Groups	Treatments	MDA (nmol/ml)	SOD (U/l)	CAT (U/l)
1	Normal control	15.07 ± 3.51	24.15 ± 3.53	33.85 ± 3.26
2	Diabetic control	30.23 ± 4.18	9.93 ± 1.97	10.58 ± 1.26
3	Diabetic + 150 mg/kg b.w. Metformin	28.42 ± 5.02*	16.32 ± 2.33*,#	26.35 ± 3.76*,#
4	Diabetic + 250 mg/kg b.w. EETB	18.91 ± 1.13#	20.53 ± 1.69#	28.39 ± 3.79#
5	Diabetic + 500 mg/kg b.w. EETB	17.63 ± 1.37#	23.34 ± 2.67#	30.79 ± 6.70#
6	Diabetic + 250 mg/kg b.w. nHF	29.65 ± 2.73*	12.44 ± 3.64*	16.99 ± 4.06*,#
7	Diabetic + 500 mg/kg b.w. nHF	26.37 ± 3.99*	14.80 ± 2.75*,#	11.62 ± 1.89*
8	Diabetic + 250 mg/kg b.w. CF	25.10 ± 4.25*	13.20 ± 2.7*	14.88 ± 2.62*
9	Diabetic + 500 mg/kg b.w. CF	25.31 ± 5.00*	15.30 ± 3.87*,#	14.91 ± 4.20*
10	Diabetic + 250 mg/kg b.w. EAF	17.13 ± 3.65#	22.64 ± 1.73#	29.73 ± 2.59#
11	Diabetic + 500 mg/kg b.w. EAF	15.65 ± 2.72#	23.40 ± 1.72#	30.18 ± 3.70#
12	Diabetic + 250 mg/kg b.w. MF	25.50 ± 7.01*	14.25 ± 4.33*,#	14.80 ± 2.84*
13	Diabetic + 500 mg/kg b.w. MF	27.25 ± 4.28*	12.75 ± 2.18*	14.02 ± 2.46*
14	Diabetic + 250 mg/kg b.w. AF	28.50 ± 5.01*	15.77 ± 4.42*,#	14.17 ± 3.43*
15	Diabetic + 500 mg/kg b.w. AF	23.58 ± 6.26*	13.33 ± 1.98*	15.43 ± 2.16*

Results are presented as mean ± standard deviation, $n = 6$. Mean values with "*" are significantly different from the normal control, while those with "#" are significantly different from the diabetic control at $p < 0.05$

Effect of EETB and its fractions on liver function indices of alloxan-induced diabetic rats

Table 4 shows the effect of EETB and its fractions on biomarkers of liver functions of alloxan-induced diabetic rats. From the data obtained, we observed a significant ($p < 0.05$) increase in the activities of serum AST, ALT, and ALP, with a corresponding increase in direct bilirubin and total bilirubin concentrations of the diabetic control when compared to the normal control and the treated groups. However, EETB- and EAF-treated groups registered significant ($p < 0.05$) declines in the liver enzyme activities relative to the diabetic control and other treated groups. Also, induction of alloxan diabetes in rats led to a significant ($p < 0.05$) depletion in the total protein and albumin concentrations recorded by the diabetic control rats. Treatment with the standard drug, EETB, and EAF significantly ($p < 0.05$) elevated the altered protein concentrations of the treated rats when compared to the diabetic control (untreated group) and other treatment groups.

Effect of EETB and its fractions on renal function parameters of alloxan-induced diabetic rats

The effect of EETB and its fractions on renal function parameters of alloxan-induced diabetic rats is shown in Table 5. The results showed significant ($p < 0.05$) increases in serum electrolytes (Na^+, K^+, Cl^-, and HCO^-_3) concentrations, as well as urea and creatinine concentrations of the diabetic control when compared to those recorded by the normal control rats and rats in the treated groups. Administration of EETB and its fractions led to significant ($p < 0.05$) declines in the concentrations of these renal function parameters in the treated groups. Most importantly, the EAF-treated groups (10 and 11) recorded the most potent effect as it displayed serum Na^+, K^+, Cl^-, HCO^-_3, urea, and creatinine concentrations, which is comparable to the normal control rats.

Histomorphology of organs of alloxan-induced diabetic rats treated with EETB and its fractions

Histopathological examination of the pancreas, liver, and kidney of the normal control rats showed the presence of normal islets and beta cell population, normal hepatocytes and central vein, and renal tubules respectively (A). Induction of diabetes by alloxan caused necrosis of the beta cells with reduced dimension of islets, necrosis of pancreatic acini and beta cell degeneration, hepatic necrosis, and congestion of renal tubules indicating damage to the liver and kidney architecture of the diabetic control rats (B). Treatment with nHF (F, G), CF (H, I), MF (L, M), and AF (N, O) indicated scanty regeneration and gradual restoration of beta cells and islet size in different sections of the pancreatic tissue (Fig. 1). However, the standard control (C), EETB (D, E)- and EAF (J, K)-treated groups presented remarkable improvements in the pancreatic histology of the treated groups relative to the untreated groups (B). Furthermore, the histopathological condition of the liver following treatments with the standard drug, crude extract, and fractions indicated the presence of the central

Table 4 Effect of EETB and its fractions on liver function indices of alloxan-induced diabetic rats

Groups	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	Tbil (μmol/l)	Dbil (μmol/l)	TP (g/dl)	ALB (g/dl)
1	41.47 ± 5.03	37.67 ± 6.47	35.60 ± 9.55	6.26 ± 0.97	2.39 ± 0.50	73.73 ± 7.52	42.54 ± 6.49
2	91.67 ± 13.80	100.87 ± 6.88	94.87 ± 3.72	11.81 ± 0.82	8.05 ± 0.53	38.00 ± 5.12	20.47 ± 2.54
3	51.47 ± 3.78*,#	48.93 ± 13.52*,#	57.73 ± 5.66*,#	8.37 ± 0.86*,#	3.11 ± 1.10#	60.07 ± 3.54*,#	35.33 ± 3.66#
4	46.73 ± 5.12#	47.93 ± 7.68*,#	42.40 ± 6.81#	8.04 ± 0.60*,#	3.41 ± 1.35#	66.20 ± 7.75#	38.53 ± 3.48#
5	44.20 ± 4.48#	43.73 ± 5.40#	42.47 ± 5.61#	7.18 ± 0.49#	2.80 ± 0.46#	67.60 ± 5.27#	40.27 ± 4.33#
6	80.07 ± 9.41*,#	72.60 ± 13.52*,#	74.80 ± 2.50*,#	8.47 ± 0.88*,#	3.31 ± 0.62#	43.53 ± 5.87*	28.00 ± 4.61*
7	79.80 ± 10.27*, #	70.60 ± 7.66*,#	72.90 ± 2.20*, #	8.31 ± 0.78*,#	3.44 ± 0.62#	46.20 ± 4.68*	26.27 ± 7.16*
8	76.53 ± 9.05*,#	73.00 ± 8.12*,#	72.13 ± 7.12*,#	8.20 ± 1.44*,#	3.91 ± 0.92#	40.33 ± 7.13*	24.00 ± 4.51*
9	78.87 ± 11.12*, #	72.26 ± 6.72*,#	71.93 ± 2.61*,#	7.66 ± 0.63#	2.82 ± 0.31#	40.40 ± 6.39*	25.93 ± 1.79*
10	42.87 ± 7.12#	42.07 ± 4.59#	41.73 ± 2.46#	8.02 ± 0.91*,#	3.26 ± 0.92#	66.40 ± 6.72#	37.59 ± 6.20#
11	42.80 ± 5.94#	42.13 ± 5.16#	39.00 ± 6.31#	7.38 ± 1.01#	2.54 ± 0.37#	67.33 ± 8.69#	37.53 ± 5.08#
12	72.33 ± 6.55*,#	42.67 ± 13.79#	57.00 ± 5.60*,#	7.82 ± 1.51#	3.34 ± 0.58#	43.13 ± 8.05*	35.20 ± 9.31#
13	72.46 ± 9.62*,#	42.00 ± 11.85#	51.60 ± 6.36*,#	8.06 ± 1.44*,#	3.85 ± 1.11#	41.87 ± 6.90*	36.27 ± 5.63#
14	72.40 ± 8.92*,#	62.33 ± 3.85*,#	67.53 ± 4.30*,#	7.84 ± 0.77#	3.89 ± 0.99#	39.20 ± 7.85*	26.33 ± 3.73*
15	61.73 ± 4.94*,#	61.20 ± 6.44*,#	55.66 ± 3.55*,#	7.71 ± 1.79#	2.85 ± 0.48#	41.13 ± 5.39*	25.33 ± 4.99*

Results are presented as mean ± standard deviation, $n = 6$. Mean values with "*" are significantly different from the normal control, while those with "#" are significantly different from the diabetic control at $p < 0.05$. (1) Normal control. (2) Diabetic control. (3) D + 150 mg/kg b.w. metformin. (4) D + 250 mg/kg b.w. EETB. (5) D + 500 mg/kg b.w. EETB. (6) D + 250 mg/kg b.w. nHF. (7) D + 500 mg/kg b.w. nHF. (8) D + 250 mg/kg b.w. CF. (9) D + 500 mg/kg b.w. CF. (10) D + 250 mg/kg b.w. EAF. (11) D + 500 mg/kg b.w. EAF. (12) D + 250 mg/kg b.w. MF. (13) D + 500 mg/kg b.w. MF. (14) D + 250 mg/kg b.w. AF. (15) D + 500 mg/kg b.w. AF. *D* diabetic)

vein (CV) and regeneration of hepatocytes (H) in different tissue sections (Fig. 2). Also, the kidney histopathology of the treated rats all showed improvements in the renal histopathology with the presence of normal renal tubules (RT) and glomerulus (G) visible except the diabetic control group, which showed congested renal tubules (Fig. 3).

Table 5 Effect of EETB and the fractions on renal function parameters of alloxan-induced diabetic rats

Groups	K^+ (mmol/l)	Na^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mmol/l)	Urea (mg/dl)	Creatinine (mg/dl)
1	3.84 ± 0.30	136.8 ± 3.49	100.2 ± 1.92	38.44 ± 5.12	2.09 ± 0.24	59.47 ± 2.61
2	7.34 ± 0.83	169.0 ± 8.00	121.6 ± 3.21	58.74 ± 11.81	5.05 ± 0.29	100.00 ± 6.74
3	4.32 ± 1.01#	142.2 ± 4.15#	104.4 ± 5.50#	42.12 ± 9.16#	2.96 ± 0.46#	66.20 ± 10.79#
4	3.98 ± 0.53#	143.6 ± 9.81#	103.2 ± 4.27#	44.38 ± 8.77#	2.52 ± 0.63#	67.59 ± 6.30#
5	3.84 ± 0.30#	141.4 ± 7.82#	103.6 ± 4.72a#	41.54 ± 11.70#	2.53 ± 0.61#	61.67 ± 5.58#
6	6.00 ± 1.35*	155.0 ± 6.36*, #	114.8 ± 2.77*,#	46.20 ± 7.96#	3.79 ± 1.12*	75.13 ± 12.06*,#
7	5.80 ± 0.48*,#	147.0 ± 8.06*, #	110.0 ± 7.82*,#	42.80 ± 9.97#	4.03 ± 1.32*	76.13 ± 9.40*,#
8	5.78 ± 0.61*,#	148.0 ± 3.16*, #	115.0 ± 1.87*,#	41.66 ± 5.51#	3.91 ± 0.75*	77.00 ± 4.68*,#
9	5.94 ± 0.27*,#	148.8 ± 2.17*, #	112.2 ± 4.15*,#	42.66 ± 5.07#	4.07 ± 0.75*	76.67 ± 15.42*,#
10	3.96 ± 0.54#	140.8 ± 6.36#	104.0 ± 4.57#	40.70 ± 8.44#	2.50 ± 0.75#	66.80 ± 9.40#
11	3.80 ± 0.19#	141.6 ± 4.72#	103.4 ± 3.05#	42.00 ± 2.79#	2.48 ± 0.47#	61.80 ± 2.19#
12	6.02 ± 0.36*	155.8 ± 5.76*,#	110.4 ± 5.13*,#	49.22 ± 9.06*,#	3.97 ± 1.11*	74.80 ± 10.04*,#
13	6.04 ± 0.74*	147.2 ± 8.47*,#	110.4 ± 4.62*,#	42.84 ± 11.43#	3.82 ± 1.12*	69.27 ± 6.87*,#
14	5.68 ± 0.61*,#	153.8 ± 7.46*,#	104.2 ± 9.31#	55.28 ± 13.56*	3.82 ± 0.47*	82.07 ± 9.05*,#
15	5.80 ± 0.21*,#	143.4 ± 10.59#	111.8 ± 4.15*,#	37.40 ± 13.05#	3.84 ± 1.40*	79.27 ± 11.19*,#

Results are presented as mean ± standard deviation, $n = 6$. Mean values with "*" are significantly different from the normal control while those with "#" are significantly different from the diabetic control at $p < 0.05$. (1) Normal control. (2) Diabetic control. (3) D + 150 mg/kg b.w. metformin. (4) D + 250 mg/kg b.w. EETB. (5) D + 500 mg/kg b.w. EETB. (6) D + 250 mg/kg b.w. nHF. (7) D + 500 mg/kg b.w. nHF. (8) D + 250 mg/kg b.w. CF. (9) D + 500 mg/kg b.w. CF. (10) D + 250 mg/kg b.w. EAF. (11) D + 500 mg/kg b.w. EAF. (12) D + 250 mg/kg b.w. MF. (13) D + 500 mg/kg b.w. MF. (14) D + 250 mg/kg b.w. AF. (15) D + 500 mg/kg b.w. AF. *D* diabetic

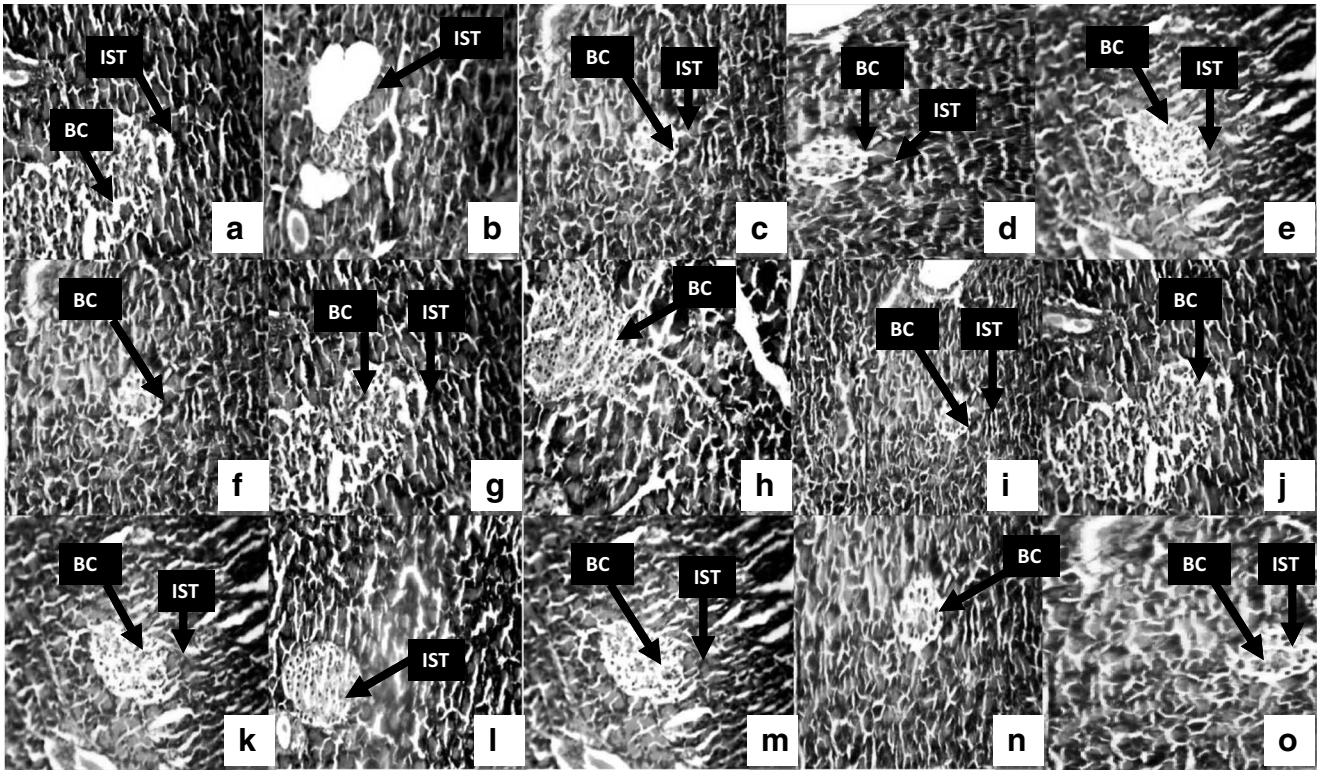

Fig. 1 Histopathological section of the pancreas: **a** normal control; **b** diabetic control; **c** 150 mg/kg b.w. metformin; **d** 250 mg/kg b.w. EETB; **e** 500 mg/kg b.w. EETB; **f** nHF 250 mg/kg b.w nHF; **g** 500 mg/kg b.w. nHF; **h** 250 mg/kg b.w. CF; **i** 500 mg/kg b.w. CF; **j** 250 mg/kg b.w. EAF; **k** 500 mg/kg b.w. EAF; **l** 250 mg/kg b.w. MF; **m** 500 mg/kg b.w. MF; **n** 250 mg/kg b.w. AF; **o** 500 mg/kg b.w AF (H&E staining, × 250 magnification). IST islet, BC beta cell

Discussion

Medicinal plants which possess antihyperglycemic and antidiabetic properties usually contain certain phytochemicals such as sterols, terpenoids, alkaloids, tannins, phenols, and flavonoids [20]. From the data presented in Table 1, EETB and EAF indicated higher bioavailability of these phytochemical classes, and their high presence could be responsible for the sharp decline in hyperglycemia of rats treated with EETB and EAF from days 7 to 28. This increased bioavailability of therapeutic phytochemicals in EETB and EAF could be accrued to the type and percentage of solvent used for extraction, the solvent-to-solid ratio, size of the plant material, nature of the leaves, time of harvesting, percentage of leaf acidification, temperature at which the plant material was dried, storage conditions, extraction temperature, and duration of extraction.

Since DM is characterized by sustained hyperglycemia for prolonged periods of time, the measurement of fasting blood glucose concentration becomes paramount, as it is usually the first biomarker investigated for the diagnosis of diabetic condition. In the present study, induction of diabetes in Wistar rats using alloxan monohydrate triggered massive disruption of the beta cells, leading to poor glucose uptake into the cells, thereby creating a state of hyperglycemia as seen in the diabetic control (Table 2). Following 28 days of treatment, the EETB and EAF produced the most potent and statistically significant ($p < 0.05$) reduction in FBS level when compared to diabetic control and the groups treated with nHF, CF, MF, and AF (Table 2). Many polyphenolic compounds such as flavonoids and phenolics found in plant extracts and fractions possess significant antioxidant properties. The high bioavailability of these antioxidant phytochemicals in the EETB and EAF could have played a role in inhibiting and scavenging the free radicals generated by alloxan administration, consequently leading to the regeneration of the beta cells and the subsequent release of insulin (pancreatotrophic action) and decrease in the blood glucose concentration. It could also be that the EETB and EAF facilitated the uptake of glucose by the peripheral cells, resulting in a decrease in the blood glucose concentration [21], or promoting insulin secretion by closure of K^+ ATP channels, membrane depolarization, and stimulation of calcium influx into the cells, which is an important step in secretion of insulin [22].

Studies have implicated oxidative stress in the pathogenesis of diabetes due to alterations in enzymatic systems, lipid peroxidation, impaired glutathione metabolism, and decrease in vitamin C levels [23]. In diabetic condition, free radicals are generated from different sources ranging from host immune response to alloxan action, and breakdown of red blood cells. These free radicals cause oxidative degradation of lipid

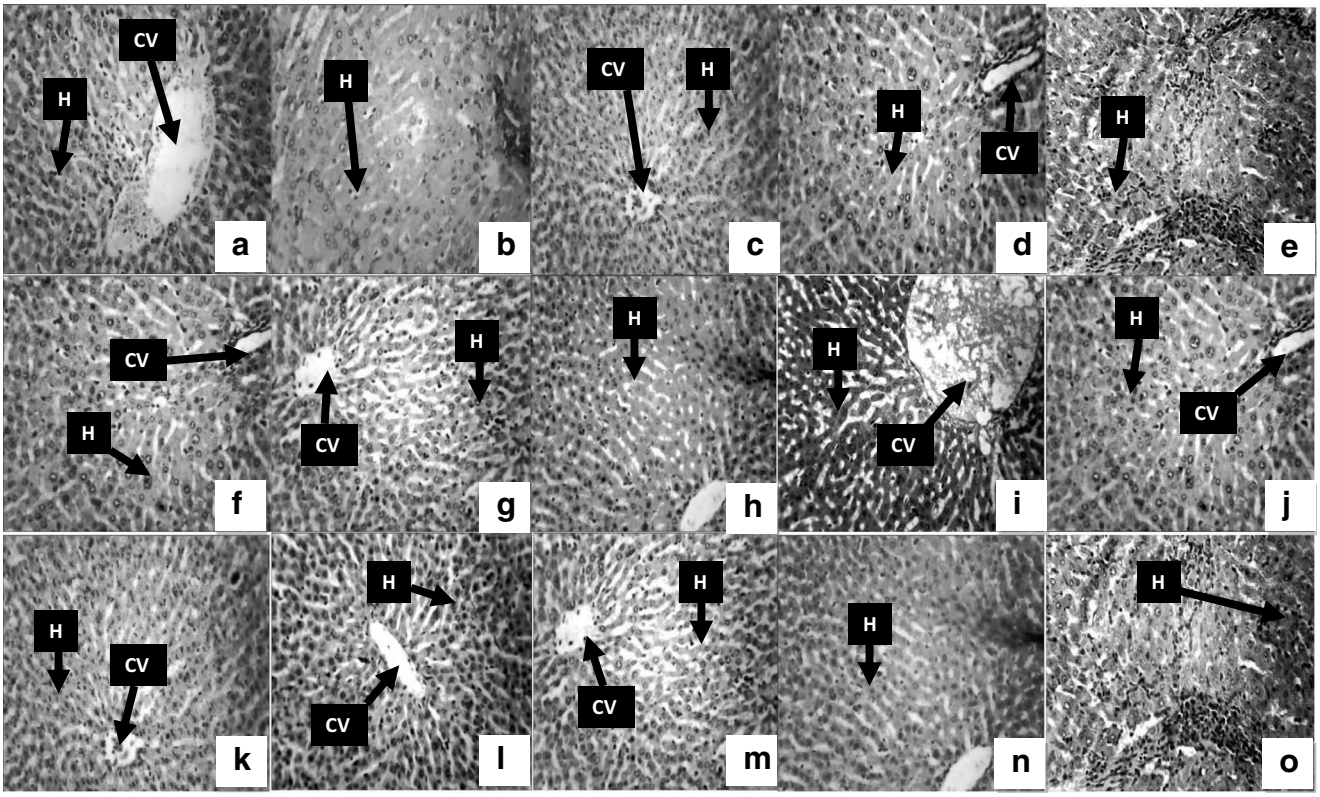

Fig. 2 Histopathological section of the liver: **a** normal control; **b** diabetic control; **c** 150 mg/kg b.w. metformin; **d** 250 mg/kg b.w. EETB; **e** 500 mg/kg b.w. EETB; **f** nHF 250 mg/kg b.w nHF; **g** 500 mg/kg b.w. nHF; **h** 250 mg/kg b.w. CF; **i** 500 mg/kg b.w. CF; **j** 250 mg/kg b.w. EAF; **k** 500 mg/kg b.w. EAF; **l** 250 mg/kg b.w. MF; **m** 500 mg/kg b.w. MF; **n** 250 mg/kg b.w. AF; **o** 500 mg/kg b.w AF (H&E staining, × 250 magnification). CV central vacuole, H hepatocytes

peroxides and deplete the endogenous glutathione concentration and the antioxidant enzyme (CAT and SOD) levels as well. When the production of free radicals generated supersedes the body's antioxidant defense system, oxidative stress; a condition known as oxidative stress which results in damage to cells and tissues results. The results of this study (Table 3) showed a significant ($p < 0.05$) reduction in serum MDA concentration of diabetic rats treated with graded doses of EETB and EAF. This indicates that administration of the extracts led to a reduction in the level of lipid peroxidation. This result agrees with the findings of Obasi et al. [24] who showed that the aqueous leaf extract of *Vitex doniana* reduced MDA concentration of alloxan-induced diabetic rats. Also, treatment of diabetic rats, particularly with EETB and EAF, led to significant ($p < 0.05$) elevations in the activities of SOD and CAT when compared to the diabetic control (Table 3). This finding also correlates well with Obasi et al. [24] who reported that aqueous leaf extract of *Vitex doniana* increased SOD and CAT activities of diabetic rats. The observed increase in the antioxidant status of the treated rats could be attributed to the presence of polyphenolic phyto-compounds such as flavonoids, tannins, and phenolic classes highly present in EETB and EAF. The antidiabetic and antioxidant activities of many plants have been linked to the presence of alkaloids, flavonoids, tannins, saponins, steroids, and other phenolic compounds [23, 25]. A possible mechanism by which the extract and the fractions increased the activities of these enzymes could be at molecular level by increasing the expression of messenger RNA of these enzymes contrary to what was obtainable in the diabetic condition as reported by Sindhu et al. [26].

The liver is an important site for clearance of insulin and production of inflammatory cytokines which help to maintain normal fasting and post-prandial glucose concentrations [22]. Alteration in liver functions leading to liver diseases is a complication of diabetes mellitus. In our study, a significant ($p < 0.05$) increase in the activities of serum AST, ALT, and ALP, as well as an increase in the total and direct bilirubin concentrations, was observed in diabetic control (Table 4). This may be an indication of damage to the hepatic tissue resulting in the leakage of these cellular enzymes from the cytosol into the bloodstream [15]. Also, the increase in the total and direct bilirubin concentration recorded by the diabetic treated rats may be as a result of a decrease in liver uptake, conjugation, or increased bilirubin production from hemolysis. The increase in bilirubin concentrations indicates alteration in liver function as confirmed by the changes in the activities of plasma enzymes. Treatment with the EETB and

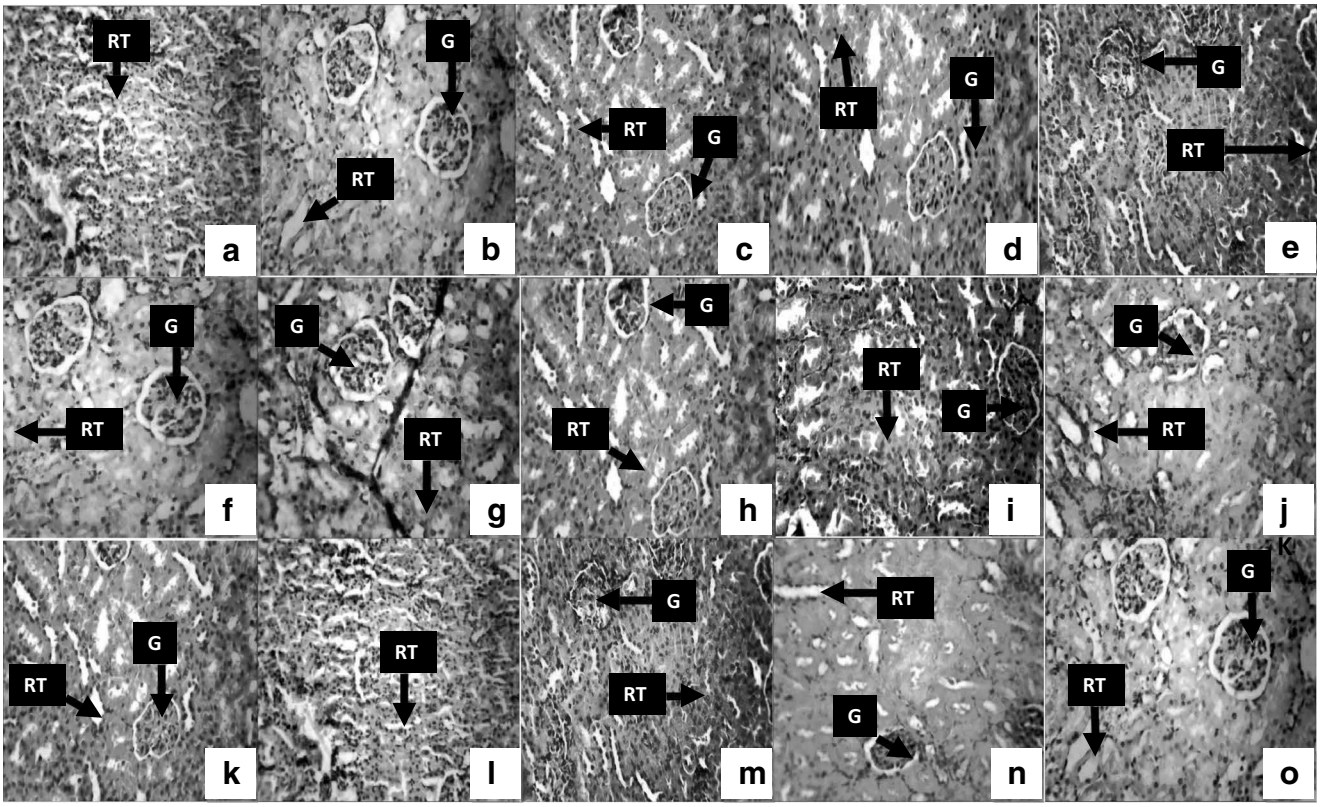

Fig. 3 Histopathological section of the kidney: **a** normal control; **b** diabetic control; **c** 150 mg/kg b.w. metformin; **d** 250 mg/kg b.w. EETB; **e** 500 mg/kg b.w. EETB; **f** nHF 250 mg/kg b.w nHF; **g** 500 mg/kg b.w. nHF; **h** 250 mg/kg b.w. CF; **i** 500 mg/kg b.w. CF; **j** 250 mg/kg b.w. EAF; **k** 500 mg/kg b.w. EAF; **l** 250 mg/kg b.w. MF; **m** 500 mg/kg b.w. MF; **n** 250 mg/kg b.w. AF; **o** 500 mg/kg b.w AF. (H&E staining, × 250 magnification). G glomerulus, RT renal tubules

the fractions resulted in remarkable declines in ALT, AST, and ALP activities as well as total and direct bilirubin concentrations, with EETB- and EAF-treated groups showing the best effect observed when compared to the diabetic control. This decline in the activities of the liver enzymes and reduction in the total and direct bilirubin concentrations indicates a return to normal kidney function, and this effect could be attributed to the hepatoprotective and antioxidant activity of *T. bracteolata* since antioxidants are known to reduce the development of chemically induced liver damage. Polyphenolic phytochemicals have demonstrated powerful antioxidant property and have been suggested to be potent in the management of DM and inflammations. Also, they may act as inhibitors on biological targets such as enzymes like α-glucosidase and α-amylase dipeptidyl peptidase IV, which are all involved in DM [27]. The results presented in Table 4 also indicated a significant ($p < 0.05$) decrease in the serum albumin and total protein concentrations of the diabetic control relative to the normal control. This reduction in the protein concentration could be accrued to the increased rate of amino acid conversion to glucose [28] and increased conversion rate of glucogenic amino acids to carbon (IV) oxide and water. Treatment of diabetic rats with the EETB and its fractions triggered marked ($p < 0.05$) increases in the serum total protein and albumin concentrations. The EETB and EAF showed the most promising effects on total protein and albumin levels relative to the other fractions. This rise in TP and ALB levels could be attributed to the increase in the hepatic uptake of amino acids, stimulation of amino acid incorporation into protein, and decreased proteolysis by activating the enzyme that catalyzes the transamination of amino acids.

Electrolytes are important for the normal functioning of many bodily processes ranging from control of fluid levels, acid-base balance (pH), nerve conduction, and blood clotting to muscle contraction. An imbalance in electrolyte concentrations usually results from kidney failure, dehydration, fever, and vomiting, all of which have also been implicated as some of the culprits responsible for complications that are usually associated with DM and other endocrine disorders. The link between glycemia and serum electrolytes is intricate and also a function of other factors such as age and associated conditions [29]. From the results obtained in our study, we observed significant ($p < 0.05$) increases in serum K^+, Na^+, Cl^-, and HCO_3^- concentrations of the diabetic control when compared to normal control following alloxan administration (Table 5). This could be due to the characteristic hyperglycemia in diabetic conditions, which is majorly responsible for serum electrolyte imbalance, as the body through homeostatic measures

tries to rid itself of the excess blood glucose by the way of increasing the urinary output. Consequently, water and electrolytes are lost through this process in the urine, thus giving rise to derangements in homeostatic balance with respect to electrolytes [30]. However, rats in the EETB- and EAF-treated groups registered significantly ($p < 0.05$) lower serum K^+, Na^+, Cl^-, and HCO_3^- concentrations relative to the diabetic control and groups treated with nHF, CF, MF, and AF. This effect could be attributed reno-modulatory effect of EETB and EAF which resulted in an improvement in glucose homeostasis and reduction in fluid and electrolyte loss. Urea is a nitrogenous end product of protein catabolism and is a waste product of the body passed into the bloodstream for onward removal by the kidneys through urine, while creatinine is a waste product formed from spontaneous dehydration of the kidneys and is usually produced in proportion to body mass [31]. The determination of serum urea and creatinine concentrations are useful markers for assessing the function of the kidney in a diseased condition such as DM. Table 5 also indicated significant ($p < 0.05$) increases in serum urea and creatinine concentrations following alloxan administration, thus indicating an impairment in kidney function. Following treatment with the extract and fractions, EETB- and EAF-treated groups recorded significant ($p < 0.05$) dose-dependent declines in urea and creatinine concentrations relative to the diabetic control. This finding could be accrued to the presence of pharmacologically active phyto-constituents such as phenols, tannins, and flavonoids, which were highly detected in EETB and EAF and are known to protect the kidney tissues from the damaging effects of radical species generated by alloxan action.

Histological studies of organs revealed that alloxan caused necrosis of the β-cells of the pancreas and damage to the hepatocytes and kidney architecture. This observation is in agreement with Thakran et al. [32] who reported considerable reduction in the pancreatic islets and degenerative changes in the histology of the liver and kidney of diabetic rats following alloxan induction. In the present study, the pancreas of the diabetic rats showed considerable reduction in islet size and depleted islets. The cytotoxic action of alloxan is mediated through the generation of radical species which undergo dismutation to hydrogen peroxide. This action of the radical species coupled with a simultaneous increase in calcium concentration leads to destruction in beta cells [22]. The diabetic rats showed pancreatic islet regeneration following treatment with EETB and its fractions, particularly the EAF at the doses investigated (Fig. 1). Antioxidants play an important role in inhibiting and scavenging free radicals. From our preliminary phytochemical studies, EETB and EAF indicated the presence of antioxidant phytochemicals such as phenols, tannins, and flavonoids which possess relatively high antioxidant activity and could have scavenged the free radicals generated by alloxan, leading to the regeneration of the β-pancreatic cells.

The liver section of the diabetic rats (Fig. 2) showed marked structural alterations in the liver architecture as a result of the diabetic condition. The major visible alteration was periportal fatty infiltration and necrosis of the hepatocytes. This damage was partially reversed by the administration of the EETB and particularly the EAF. This could be attributed to the ability of EETB and EAF to normalize altered hepatic enzymes and antioxidant status of the treated groups, thereby leading to an improved liver histology. The kidney is a major organ susceptible to oxidative damage following alloxan induction. In the present study, the histopathology of the kidney of alloxan-induced diabetic rats showed marked tubular damage and hemorrhage in Bowman's space due to glomerular damage (Fig. 3). The results indicated both primary and secondary effects of the diabetes on the histology of the rat kidneys which could be associated with persistent hyperglycemia resulting in dilatation of proximal and distal tubules in the cortex. Diuresis, a common feature associated with DM, could also be a reason for the structural changes observed with the glomerulus. The restoration of normal renal histology following treatments with EETB and EAF could be attributed to the regenerative ability of the renal tubules in the presence of regenerative factors such as bioactive alkaloids, terpenoids, and polyphenolic phytochemicals in the plant extract and fraction. Observations from histological studies corroborated with the data from serum biochemistry, implying that alloxan did not only affect the functional integrity of these cellular tissues but also caused structural changes to the pancreatic tissues as seen in the elevation of fasting blood glucose and necrosis of the β-pancreatic cells; elevation in serum aminotransferases, bilirubin levels, and necrosis of the hepatocytes; and elevation in serum electrolytes, urea and creatinine concentrations, and the damage to the kidney architecture. Following treatment, EETB and EAF best normalized alterations in biochemical and histological architecture affected by the action of alloxan in Wistar rats.

Conclusions

This study demonstrated that EETB and EAF exhibited a significant antidiabetic activity in the model studied. This justifies the traditional use of *T. bracteolata* leaves in the management of diabetes. We attribute this effect to the presence of pharmacologically active constituents in EETB and EAF which may have acted independently or in synergy with one another to exert the antidiabetic activity observed. In addition, EETB and EAF also improved the endogenous antioxidant enzymatic defense system overwhelmed by the pancreatotrophic action of alloxan, restored altered histology of major organs examined, and also offered protection against oxidative stress and lipid peroxidation associated with diabetic conditions. We accrue these effects to the presence of phytochemical classes such as

saponins, tannins, flavonoid glycosides, and other polyphenolic bioactive compounds present in the EETB and EAF. The isolation and the characterization of the antidiabetic principles in the most potent fraction are being carried out with the aim of identifying the active ingredients, which could be subjected to clinical trials so as to facilitate the development of an active biopharmaceutical agent for management and treatment of diabetes and its associated complications.

Acknowledgments The authors are thankful to Mr. Onugwu Ernest Okonkwo of the Department of Biochemistry, Salem University, Lokoja, Kogi State, Nigeria, for assisting with animal management and facility procurement. We are also grateful to Mr. Akanni T. Gbenga of the Department of Botany, Federal University, Lokoja, Kogi State, Nigeria, and Mr. Okoyomoh Christian of the Department of Histopathology, Federal Medical Centre, Lokoja, Kogi State, Nigeria, for conducting the microscopic histological assessment.

Authors' contributions P.A. Idakwoji, P.E. Joshua, O.U. Njoku, and O.F.C. Nwodo were responsible for the conceptualization and design of the study. P.A. Idakwoji and D.E. Ekpo conducted the experiments. P.A. Idakwoji, D.E. Ekpo, and P.E. Joshua collected the data set, performed statistical analyses, and interpreted the data. D.E. Ekpo wrote the first manuscript draft and revised it critically for intellectual content. P.E. Joshua, O.U. Njoku, and O.F.C. Nwodo supervised the work. All authors read and approved the final manuscript.

Data availability Dr. Parker Elijah Joshua and Dr. Precious Adejoh Idakwoji are the curators of the data set, which is available on request.

Compliance with ethical standards

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

Ethical approval All procedures performed involving the use of experimental animals in this study were in accordance with the International Guidelines for Handling of Laboratory Animals [12]. The Institutional Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria, with Ethics Committee Approval No.: UNN/FBS/EC/1037.

Consent to participate Not applicable

Consent for publication Not applicable

Code availability Not applicable

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):469–475
https://doi.org/10.1007/s13410-020-00915-3

ORIGINAL ARTICLE

Efficacy and safety of a new insulin infusion protocol adapted for the target glycemic range of 140–180 mg/dl in adult critical care units: a tertiary care centre experience

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Received: 15 April 2020 / Accepted: 23 December 2020 / Published online: 8 January 2021

Abstract

Introduction In critical care setting, insulin infusion rather than subcutaneous insulin is recommended to maintain the blood glucose (BG) level in the target range. We have devised an insulin infusion protocol which requires monitoring of blood glucose and insulin dose adjustment one hourly. In this study, we have studied the efficacy of our insulin infusion protocol (IIP) in terms of bringing and maintaining blood glucose to target range, and its safety in terms of preventing hypoglycemia.

Research design and methods It is a retrospective review of all patients who received insulin infusion from the 1st of July 2017 to the 30th of June 2018. A total of 231 were included and data pertaining to demographic details and related to insulin infusion was documented in a predefined questionnaire.

Results Insulin infusion was continued for a mean duration of 26.3 ± 13.7 h taking 5.7 ± 3.1 h to reach the target BG level < 180 mg/dl. Once the BG was in the target range, 44.3% of blood glucose readings were between 140 and 180 mg/dl, 62.52% of readings were within the safe range, i.e. 110–180 mg/dl while hypoglycemia (< 70 mg/dl) was observed in only 0.44% times with only one episode of severe hypoglycemia (< 40 mg/dl). The 31.65% of BG readings were found to be above the target BG level (> 180 mg/dl) but the mean BG remains 168.95 ± 45.52 within the desired target range.

Conclusion Our insulin infusion protocol not only achieved and maintained the blood glucose in the target range, it is safe with a very low risk of hypoglycemia.

Keywords Hyperglycemia · Insulin infusion · Blood glucose · Hypoglycemia

Introduction

Diabetes mellitus including gestational diabetes continues to be a major cause of morbidity worldwide, along with traditional risk factors like hyperlipidemia and physical inactivity [1–3]. The importance of management of hyperglycemia in critical care setting has been emphasized in the later part of the previous century while the targets of blood glucose have been evolved over the first decade of this century. The landmark study of the Van Den Burghe from Belgium in 2001 suggested a very tight glycemic target of 80–110 mg/dl, showing a significant reduction in intensive care unit (ICU) mortality and morbidity [4]. This has led to the recommendation by the American Association of Clinical Endocrinologist (AACE) in 2002 that critically ill patients should be kept as close to a blood glucose of 110 mg/dl as possible. However, in 2006 Van Den Burgher's same protocol was when applied to medical ICU, it failed to reproduce similar results [5, 6]. These controversies and barriers in achieving a very tight glycemic

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control led to the start of Normoglycemia in Intensive Care Evaluation–Survival Using Glucose Algorithm Regulation (NICE-SUGAR) study, published in 2009. This was conducted in 42 different centres and it showed that a slight relaxed target of 140–180 mg/dl is acceptable in intensive care units and highlighted the fact that targeting blood glucose to < 110 mg/dl resulted in increased risk of hypoglycemia, prolonged hospital stay and increased mortality [7]. After this trial, AACE and American Diabetes Association (ADA) also recommended the same targets of 140–180 mg/dl for critically ill patients. However, these societies still recommend to consider lower target blood glucose in selected patients [8].

Continuous variable rate intravenous insulin infusion has become the standard of care for the management of hyperglycemia in critical care units. An ideal insulin infusion is the one which can be easily administered and managed and maintains the blood glucose in target range without the risk of significant hypoglycemia [8]. As a quality improvement initiative, we developed an insulin infusion protocol in 2015 (Fig. 1), to be used across all critical care units of our institution including medical and surgical intensive care units (ICU), coronary care units (CCU) and special care units (SCU). Before developing this protocol, we were using different types of insulin infusions in medical and surgical ICUs, which were complicated, with a different target set for blood glucose and multiple hypoglycemic episodes were reported. This new protocol is nurse's driven with a target blood glucose of 140–180 mg/dl. It requires checking blood glucose through point of care test (POCT) and dose adjustment of insulin according to the blood glucose levels one hourly. The initial insulin bolus is calculated by dividing the current blood glucose in mg/dl by 100. The resultant value is used as bolus and starting infusion rate. Subsequently, the infusion rate is titrated as per protocol shown in Fig. 1. This protocol was modelled after the original Yale University protocol, [9]. We have also developed an insulin infusion dose calculator in our electronic health system and an application for the android and iOS system (AKUH Insulin Calculator) to facilitate the insulin dose calculation and dose adjustment accordingly.

In this study, we have studied the efficacy of our modified insulin infusion protocol in terms of bringing blood glucose to target range and maintaining it, and its safety in terms of preventing hypoglycemia.

Methodology

This study was conducted at Aga Khan University Hospital. It is a tertiary care centre in Karachi, Pakistan, with a 545-bed capacity. Because of the retrospective nature of the study, we got an exemption from our intuitional ethical review committee (ERC) before starting it. We removed the names and hospital record number of patients and coded them with a study ID, to maintain confidentiality. A list of 1206 patients was generated from the pharmacy system, who were started on intravenous insulin infusion during the period of July 1, 2017, to June 30, 2018. Out of these, we selected 231 patients who received insulin infusion for at least 12 h and were either taking nothing per oral (NPO) or continuous enteral or total parenteral nutrition. We excluded patients taking regular meals or intermittent feeding through nasogastric tube or received insulin infusion for less than 12 h. We reviewed the medical records of these patients and collected the data regarding age, gender, weight and location of patient while on insulin infusion. The prior diagnosis of type 1 or type 2 diabetes was documented along with the use of insulin before hospital admission. We also noted the on-admission diagnosis and feeding status whether receiving continuous enteral, total parenteral or nothing per oral. The treatment entities that may affect the glycemic status like the use of steroids, vasopressors, dextrose infusion and mechanical ventilation were also noted. We reviewed the blood glucose level through point of care testing (POCT) at the start of insulin infusion and then one hourly thereafter along with insulin doses adjusted according to the protocol.

The efficacy of insulin infusion protocol was assessed by the time required to achieve the blood glucose of < 180 mg/dl. After this initial target, the percentage of readings was recorded as within a target range of 140–180 mg/dl, in a safe range of 110–180 mg/dl and above the target range of > 180 mg/dl. The safety was assessed by documenting the percentage of readings between 70 and 109 mg/dl, hypoglycemia range < 70 mg/dl or in severe hypoglycemia range < 40 mg/dl.

Frequencies with percentages were reported for all independent categorical variables including the demographic and clinical variables as shown in Tables 1 and 2.

Mean with standard deviation (SD) was reported for continuous categorical variables since the normality assumption was met as shown in Tables 1 and 2.

All analysis was performed on SPSS 16.

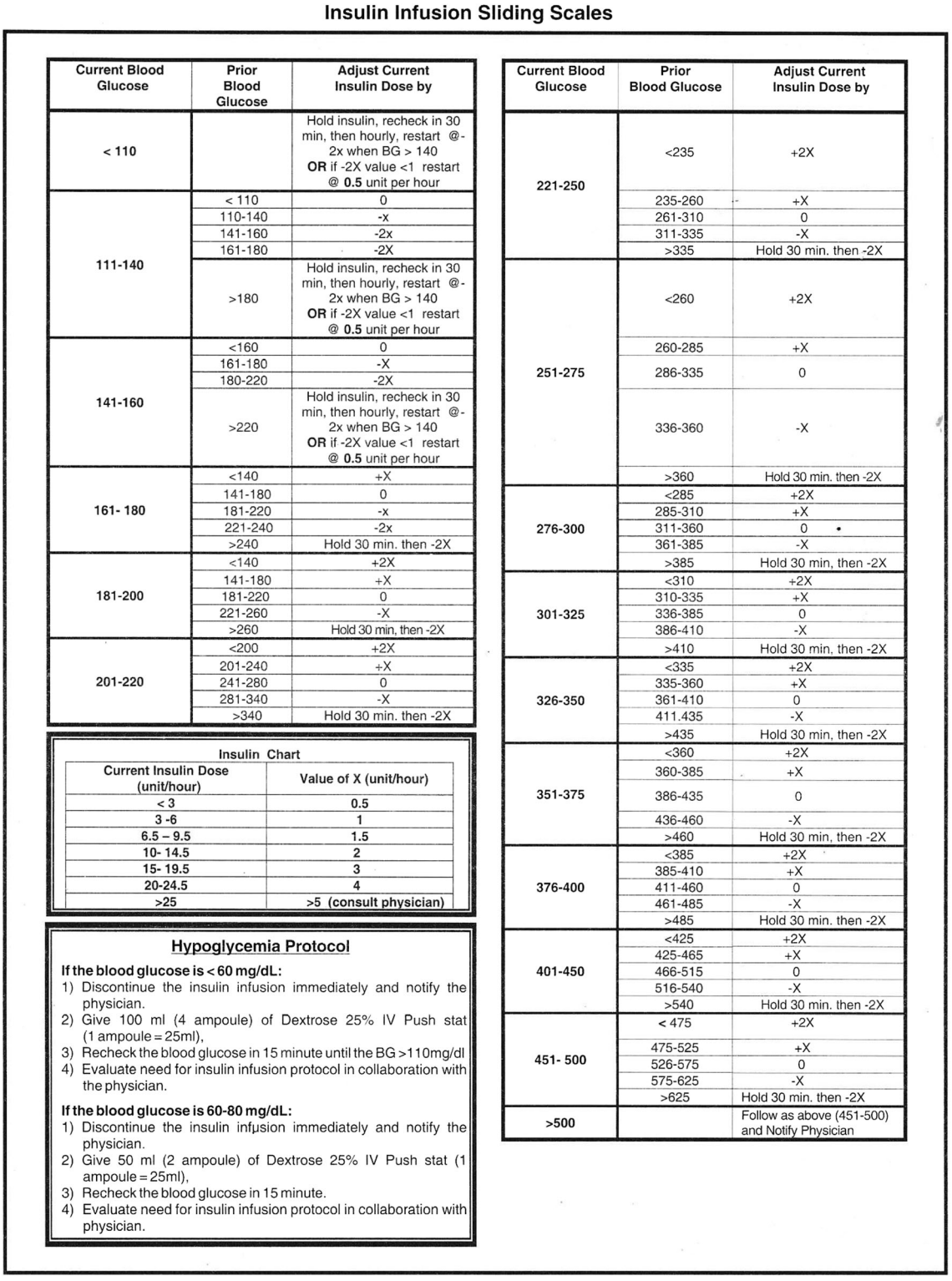
Results

During the study period, 1206 participants were started on intravenous insulin infusion and 231 patients met the inclusion criteria and were included in the final analysis.

The baseline characteristics of the patients are shown in Table 1.

The majority of patients were known to have diabetes i.e. 78.4%, and 30% of these were taking insulin prior to hospitalization. Only five patients had type 1 diabetes. At the time of initiation of insulin infusion, 67% of patients were on mechanical ventilation and 50.6% and 33.3% were receiving corticosteroids and vasopressors respectively.

Feeding status was documented in all patients with approximately 40% had NPO status and remaining were receiving



Insulin Infusion Sliding Scales

Current Blood Glucose	Prior Blood Glucose	Adjust Current Insulin Dose by
< 110		Hold insulin, recheck in 30 min, then hourly, restart @-2x when BG > 140 **OR** if -2X value <1 restart @ **0.5** unit per hour
111-140	< 110	0
	110-140	-x
	141-160	-2x
	161-180	-2X
	>180	Hold insulin, recheck in 30 min, then hourly, restart @-2x when BG > 140 **OR** if -2X value <1 restart @ **0.5** unit per hour
141-160	<160	0
	161-180	-X
	180-220	-2X
	>220	Hold insulin, recheck in 30 min, then hourly, restart @-2x when BG > 140 **OR** if -2X value <1 restart @ **0.5** unit per hour
161- 180	<140	+X
	141-180	0
	181-220	-x
	221-240	-2x
	>240	Hold 30 min. then -2X
181-200	<140	+2X
	141-180	+X
	181-220	0
	221-260	-X
	>260	Hold 30 min, then -2X
201-220	<200	+2X
	201-240	+X
	241-280	0
	281-340	-X
	>340	Hold 30 min. then -2X

Current Blood Glucose	Prior Blood Glucose	Adjust Current Insulin Dose by
221-250	<235	+2X
	235-260	+X
	261-310	0
	311-335	-X
	>335	Hold 30 min. then -2X
251-275	<260	+2X
	260-285	+X
	286-335	0
	336-360	-X
	>360	Hold 30 min. then -2X
276-300	<285	+2X
	285-310	+X
	311-360	0
	361-385	-X
	>385	Hold 30 min, then -2X
301-325	<310	+2X
	310-335	+X
	336-385	0
	386-410	-X
	>410	Hold 30 min. then -2X
326-350	<335	+2X
	335-360	+X
	361-410	0
	411.435	-X
	>435	Hold 30 min, then -2X
351-375	<360	+2X
	360-385	+X
	386-435	0
	436-460	-X
	>460	Hold 30 min. then -2X
376-400	<385	+2X
	385-410	+X
	411-460	0
	461-485	-X
	>485	Hold 30 min. then -2X
401-450	<425	+2X
	425-465	+X
	466-515	0
	516-540	-X
	>540	Hold 30 min. then -2X
451- 500	< 475	+2X
	475-525	+X
	526-575	0
	575-625	-X
	>625	Hold 30 min. then -2X
>500		Follow as above (451-500) and Notify Physician

Insulin Chart

Current Insulin Dose (unit/hour)	Value of X (unit/hour)
< 3	0.5
3 -6	1
6.5 – 9.5	1.5
10- 14.5	2
15- 19.5	3
20-24.5	4
>25	>5 (consult physician)

Hypoglycemia Protocol

If the blood glucose is < 60 mg/dL:

1) Discontinue the insulin infusion immediately and notify the physician.
2) Give 100 ml (4 ampoule) of Dextrose 25% IV Push stat (1 ampoule = 25ml),
3) Recheck the blood glucose in 15 minute until the BG >110mg/dl
4) Evaluate need for insulin infusion protocol in collaboration with the physician.

If the blood glucose is 60-80 mg/dL:

1) Discontinue the insulin infusion immediately and notify the physician.
2) Give 50 ml (2 ampoule) of Dextrose 25% IV Push stat (1 ampoule = 25ml),
3) Recheck the blood glucose in 15 minute.
4) Evaluate need for insulin infusion protocol in collaboration with physician.

Fig. 1 Insulin infusion protocol

continuous enteral nutrition. Only two participants were given total parenteral nutrition.

Matrix of glycemia is shown in Table 2. The mean blood glucose at the start of infusion was 317 (SD 94.2) mg/dl; it took 5.7 (SD 3.1) h to achieve the target blood glucose level < 180 mg/dl and the infusion was continued for a mean duration of 26.3 (SD 13.7) h.

Once the blood glucose was in the target range, 44.3% (2133/4805) of blood glucose readings were within the target i.e. between 140 and 180 mg/dl and 62.52% (3004/4805) of

Table 1 Baseline characteristics of participants

Variables	*n* (%)
*Age mean (SD)	59.1 (12.9) years
Male	148 (64.1)
Female	83 (35.9)
Weight mean (SD)	72.8 (12.89) kg
Location of participants	
i. MICU/SICU	153 (66.2)
ii. CCU	35 (15.2)
iii. HDU	43(18.6)
History of diabetes	
i. Yes	181(78.4)
ii. No	50 (21.6)
Use of insulin before admission	
i. Yes	70 (30.3)
ii. No	161 (69.7)
Concomitant interventions affecting blood glucose	
i. Corticosteroids	117(50.6)
ii. Vasopressors	77 (33.3)
iii. Mechanical ventilation	155 (67.1)
iv. Dextrose infusion	48 (20.8)
Nutrition	
i. NPO	93 (40.3)
ii. Continuous enteral nutrition	136 (58.9)
iii. Parenteral nutrition	02 (0.9)

MICU, medical intensive care unit; *CCU*, coronary care unit; *HDU*, high dependency unit

*Continuous variables with normality assumption met, mean with standard deviation is reported

readings were within the safe range i.e. 110–180 mg/dl. Only 5.37% (258/4805) of readings were between 70 and 109 mg/dl while hypoglycemia (< 70 mg/dl) was observed in only 0.44% (21/4805) times with only one episode of severe hypoglycemia (< 40 mg/dl).

31.65% (1521/4805) of BG readings were found to be above the target BG level (> 180 mg/dl) with the mean blood glucose of 168.95 (SD 45.52).

Discussion

Out of 1206 patients, only 231 were included for further evaluation. The reason for excluding many participants was either a short duration of infusion i.e. less than 12 h or intermittent feeding either orally or through nasogastric (NG) route. For a very small duration of infusion, it is difficult to assess its efficacy in achieving or maintaining blood glucose levels within targets. Moreover, the intermittent feed through NG or regular meals will make the interpretation of insulin infusion adjustment difficult because of post prandial rise in blood glucose. In critically ill patients, pump-assisted continuous feeding is generally accepted practice and may help with diarrhoea and dumping [10, 11]. Patients who are stable enough to eat regular meals or getting bolus enteral feeding every 4–6 h are generally shifted to subcutaneous basal bolus insulin regimen. In some protocols, the intravenous insulin infusion is supplemented with subcutaneous rapid or short-acting insulin before meal to control post prandial glucose excursion [12]. In our protocol, there is no provision of extra bolus insulin in the infusion or subcutaneously to cover the carbohydrate content of the meal. Hence, these patients were excluded as the true

Table 2 Matrix of glycemia

Variables	Results
*BG level at the start of insulin infusion, mean (SD)	317 (94.2) mg/dl
*Infusion rate at the start of infusion, mean (SD)	3.1 (1.3) units/h
*Time to reach target blood glucose < 180 mg/dl, mean (SD)	5.7 (3.1) h
Median	
Interquartile range	5
	5
*Duration of infusion, mean (SD)	26.39 (13.76) h
BG readings in target range (140–180 mg/dl)	44.39% (2133/4805)
BG readings in safe range (110–180 mg/dl)	62.52% (3004/4805)
BG readings between 70 and 109 mg/dl	5.37% (258/4805)
Hypoglycemia (< 70 mg/dl)	0.4% (21/4805)
Severe hypoglycemia (< 40 mg/dl)	0.02% (1/4805)
BG readings above target range (> 180 mg/dl)	31.65% (1521/4805)
*Mean BG once the target of < 180 mg/dl is achieved, mean (SD)	168.95 (45.52)

BG, blood glucose

*Continuous variables with normality assumption met, mean with standard deviation is reported

performance of intravenous insulin protocol cannot be tested in this setting.

The mean age and gender distribution of our study population were similar to the participants in other studies (nice-sugar, modified Yale and john Hopkins) [7, 13, 14]. Our study patients had a lower mean body weight; this may be because of ethnic differences (nice-sugar, modified Yale). However, we did not calculate the BMI of our patients. Most of our patients were in medical, surgical or coronary care ICUs, except 18% of patients were in special care units (SCU). In our SCU, patients can be on vasopressors or non-invasive positive pressure ventilation. The known history of diabetes was similar in our patients and in the Shetty et al. study, 77.8% and 76% respectively. The use of insulin prior to admission was lower in our patients by 30.3% as compared to 45.2% in the modified Yale protocol study [13].

The mean BG at the start of infusion was 317(SD 94.2) mg/dl which is comparable to the basal blood glucose (BG) found in modified Yale infusion protocol study 306 (SD 89.8) mg/dl) [13]. Although most of the ICU protocols recommend starting the insulin infusion at the blood glucose of 180–200 mg/dl, our mean blood glucose at the start of insulin infusion was quite high. This has also been observed in other infusion protocols as well. This points to the fact that there is still resistance from the physician's side in starting the insulin infusion early. The time required to achieve the target BG was 5.7 (SD 3.1) h which is earlier than required by modified Yale protocol (7 h) or by old Yale protocol with 10.1 (SD 4.6) h. However, the infusion protocol used in coronary care units of Italy with a target BG of 110–140 mg/dl achieved the target in just 03 h [13, 15, 16]. The different target blood glucose used in these protocols might be a reason for a difference in time duration.

The mean duration of insulin infusion in our study was 26.39 (SD 13.76) h while in the modified Yale insulin infusion protocol, the median duration was 59 (25–127) h [13]. This points to the fact that in our setup, there is still reluctance to use insulin infusion protocol (IIP) for a long duration and physicians try to convert it to a subcutaneous regimen as early as possible and many participants were excluded from this study for the same reason, as they received IIP for only few hours. The main reason for this practice is our limitations in terms of resources and shortage of nursing staff. Because of these, one hourly blood glucose monitoring is difficult to conduct and is costly, as the patient requires to be in SCU. This results in early switching from intravenous insulin infusion to subcutaneous insulin which require less monitoring of blood glucose.

The target BG defined in our insulin infusion protocol was 140–180 mg/dl and after achieving this target; 44.39% of BG values were in this range (which was 42% in modified Yale IIP) while 62.52% of them were within a safe range of 110–180 mg/dl (found to be 76% in modified Yale IIP) [13]. In another study of cardiothoracic ICU using IIP with a target of 100–139 mg/dl, 94% of BG was within the acceptable limit of 80–199 mg/dl [17]. Only 5.37% of BG was found between 70 and 110 mg/dl in our participants and hypoglycemia was observed in 0.4% of readings which was dealt with immediately. This is comparable to the modified Yale IIP and notably only one episode of severe hypoglycemia (< 40 mg/dl) was observed in our study which occurred in 1/5000 BG readings in modified Yale protocol and 0.2% in cardiothoracic ICU [13, 17]. Although the tight glycemia control in Leuven study strongly advocated benefits both in terms of mortality and morbidity, subsequent ones were unable to demonstrate similar benefits. The most widely accepted explanation is that the higher incidence of hypoglycemia and its complications balance the benefits achieved with strict BG control. The BG < 40 mg/dl can cause neuron damage irreversibly and can lead to autonomic instability and cardiac arrhythmias [18]. In the NICE-SUGAR study, 3.7% of participants had severe hypoglycemia (< 40 mg/dl) while moderate hypoglycemia (41–70 mg/dl) occurred in another 45% of them, mostly observed in an intensive insulin therapy group. The severe hypoglycemia was associated with twofold increase in mortality [7]. In our study, there was only one episode of severe hypoglycemia observed and moderate hypoglycemia rate was also very low which did not lead to any significant adverse clinical outcome. Our protocol advises to hold insulin infusion at blood glucose of $\leq$ 110 mg/dl and thus prevents the blood glucose to decline any further. This established the safety of our insulin infusion protocol in terms of preventing hypoglycemia.

Another important finding observed was that 31.96% of times BG value was > 180 mg/dl. This is relatively high as compared to another infusion protocol with the same target blood glucose of 140–180 mg/dl, which showed 80.3% of readings in the range of 80–199 mg/dl [14], while in the modified Yale infusion protocol, 76% of BG readings were < 180 mg/dl. An important factor here is the slight difference in the target ranges of blood glucose in most of the protocols which make it difficult to compare them. In critical care units, the use of steroids, vasopressors and mechanical ventilation is very common. We found that steroids were administered to a significant number of participants i.e. 43% while vasopressors and mechanical ventilation were employed in 33.3% and 67.1% cases respectively. The corticosteroids were used in patients with exacerbation of asthma and chronic obstructive pulmonary disease and in patients with severe sepsis. Moreover, 38% were receiving IV dextrose infusion for variable reasons; most of the time it was used as diluent for intravenous antibiotic and captured from the medication list. The use of these considerably affects the BG levels. The use of glucocorticoids leads to significant hyperglycemia especially

post prandial surge in blood glucose level. Moreover, 58.9% of participants were receiving continuous enteral nutrition at variable rates. All these factors might be responsible for high blood glucose levels above the target range. However, the endocrine society and ADA still recommend continuous IV insulin infusion rather than subcutaneous insulin in patients receiving high dose steroids [19]. The same is true with vasopressor use and mechanical ventilation.

Notably, the mean blood glucose in our study was within target range i.e. 168.95 (SD 45.52) mg/dl and is comparable to other studies with a mean BG of 167 mg/dl and 155.9 (SD 22.9) mg/dl (modified Yale Infusion protocol) [13]. This signifies that insulin infusion protocol being used in our institution effectively controls hyperglycemia overall, without significant risk of hypoglycemia.

The main limitation of our study is the retrospective design because of time and resource constraints; this resulted in our inability to control factors like the use of intravenous dextrose and corticosteroids. Another important limitation is that the less sick patients who were eating regular meals or receiving intermittent bolus feeds were excluded as there was no provision in the protocol to provide additional bolus insulin.

Conclusion

Despite the difference in the targets for blood glucose levels in acute illness in different centres, intravenous insulin infusion remains the preferable mode for hyperglycemia management. Different insulin infusion protocols are there, but most of them advocate for moderate glycemia control with the lowest possible risk of hypoglycemia. Our IIP was able to maintain blood glucose in target range for most of the time with an acceptable mean BG without the evidence of major hypoglycemia.

Acknowledgments We would like to acknowledge the administrative support from Dr. Saadia Farooqui, and also to Mr. Asif Ansari, Mr. Uzair Danish and Mr. Kashif Siddiqui for the software development of insulin infusion calculator and their support.

Authors contribution All authors have read and approved the final manuscript.

Data availability All data is available and can be provided on request.

Compliance with ethical standards

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

Ethics approval This study was approved by the ethical review committee of our institution and throughout the study, core ethical principles of research were followed.

Code availability Not applicable.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):476–483
https://doi.org/10.1007/s13410-020-00918-0

ORIGINAL ARTICLE

What makes poor diabetic control worse? A cross-sectional survey of biopsychosocial factors among patients with poorly controlled diabetes mellitus in Malaysia

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Received: 19 May 2020 / Accepted: 23 December 2020 / Published online: 23 January 2021

Abstract

Background Diabetes mellitus (DM) is an increasingly prevalent condition that is associated with significant complications, especially when glycemic control is poor. This study explored what biopsychosocial factors are associated with poor vs. very poor glycemic control among Malaysian patients with sub-optimal glycated hemoglobin (HbA1c) levels.

Methods This cross-sectional study recruited diabetic patients with poor glycemic control (HbA1c ≥ 7.0%) from the Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Malaysia. The Generalized Anxiety Disorder (GAD)-7, Beck Depression Inventory (BDI) and Big Five Inventory (BFI) questionnaires assessed for anxiety, depression and personality traits, respectively. Multivariate logistic regression analysis was performed to evaluate associations between biopsychosocial factors and the occurrence of poor vs. very poor glycemic control, using the median HbA1c level of the study sample as the cutoff point.

Results The median age of participants ($n = 176$) was 61.5 years. The median duration of diabetes was 15.0 years, and the median HbA1c level was 8.5%. Very poor control of diabetes (HbA1c > 8.5%) was associated with younger age, single status, retirement, hypertension and dyslipidemia in bivariate analysis. However, in the final multivariate regression model, only agreeableness was associated with very poor glycemic control ($p < 0.01$). There were no differences in depression and anxiety scores between the poor and very poor glycemic control groups.

Discussion This study suggests that the agreeableness personality trait may be associated with very poor glycemic control. The effects of personality traits on glycemic control might be influenced by sociocultural factors. Further investigations are needed to provide a better understanding of this area.

Keywords Agreeableness · Big Five model · Diabetic control · HbA1c

Introduction

Diabetes mellitus (DM) is one of the most common chronic conditions worldwide, affecting 451 million people in 2017 [1]. Although DM is an international health crisis, the prevalence of DM is increasing at a more rapid rate in lower- and middle-income countries, such as Malaysia [2]. The major costs associated with DM are from the treatment of its sequelae, including nephropathy and retinopathy; however, indirect costs, such as government benefits or days absent from work, are important costs that are often unaccounted for.

Patients with DM often experience a broad range of disease-related distresses, including concerns about serious complications, unease about treatment regimens and guilt when disease management is poor [3]. Indeed, the prevalence of depression and anxiety among patients with DM is two [3] and five [4] times higher than the general population, respectively. As a result, the American Diabetes Association recommends regular mental health screening for patients with DM [5]. Previous research has demonstrated that both depression

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and anxiety are associated with very poor glycemic control [6–8]. It is important to consider the relationship between glycemic control and mental illness so that optimal management of both conditions can be achieved.

Studies exploring associations between personality traits and glycemic control remain inconclusive. Lane et al. observed that neuroticism was associated with better glycemic control, while altruism was associated with poor glycemic control [9]. However, other studies using the five-factor model of personality suggest that there are no significant relationships between personality traits and HbA1c levels [10, 11].

Poor glycemic control is associated with macrovascular and microvascular complications; therefore, maintaining HbA1c levels within recommended ranges is a primary focus of DM management. HbA1c levels greater than 7.0% are associated with a significantly increased risk of cardiovascular and microvascular complications, including myocardial infarction, stroke, nephropathy, retinopathy and neuropathy [5]. Though the relationship is not linear, the risk of future complications varies by HbA1c level. For example, the United Kingdom Prospective Diabetes Study showed that reducing HbA1C by 1% was associated with a 37% reduction in microvascular complications in patients over a 10-year follow-up period [12]. A meta-analysis of 14 studies on DM showed that reductions in HbA1c levels decreased the incidence of macrovascular events in both type 1 and type 2 DM [13].

Previous research has identified risk factors for poor glycemic control. However, it remains unclear what biopsychosocial factors contribute to worsening glycemic control among patients who already struggle to maintain optimal HbA1c levels, particularly in low- and middle-income countries. We studied a population of patients with poor glycemic control (HbA1c > 7.0%) to determine if mental health comorbidities, personality traits or sociodemographic factors are associated with very poor glycemic control. Knowledge of these mediating factors may allow clinicians to prioritize psychosocial interventions that can help patients with poor glycemic control more effectively manage their disease.

Methods

Study design and participant selection

This was a cross-sectional study conducted at the Universiti Kebangsaan Malaysia Medical Centre (UKMMC), a tertiary referral centre in Kuala Lumpur, Malaysia. The study site was chosen for its large patient load with socioeconomic and demographic characteristics that are representative of the Metropolitan Kuala Lumpur region, as well as its high-quality multi-disciplinary DM management program. Patients receive treatment at heavily subsidized rates as UKMMC is a government-owned university hospital controlled by the Ministry of Education. The target population of the study was patients who attended the outpatient Endocrine Clinic of UKMMC, which has an estimated patient roster of 4500 based on the 2018 clinic census. Approval for the study was obtained from the Research Ethics Committee of the Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM FPR.SPI 800-2/28/166/FF-2019-342).

Patients were recruited using convenience sampling. The sample size was not calculated as this study was conducted as part of a larger study that has been reported elsewhere. Participants were included if they were older than 18 years, had a confirmed diagnosis of type 1 or type 2 DM, had a measured HbA1c reading of ≥ 7.0%, did not have impaired capacity and provided verbal and written consent to participate. Patients with impaired mental capacity, such as those with dementia or cognitive impairment and those with psychotic symptoms, were excluded from the study.

Data collection

Participants were asked to complete a total of six questionnaires under the supervision of a researcher. Demographic data were collected, including age, gender, marital status, employment and monthly income. Clinical data were also obtained, including BMI, type of DM and whether the patient was on insulin therapy. These clinical data were supplemented with information obtained from each participant's electronic medical record, including blood test results processed in the ISO-certified laboratory of UKMMC. The laboratory readings recorded included the most recent HbA1c and lipid profile. The HbA1c of ≥ 7.0% was used as an indicator of poor diabetic control [5]. The study participants were subsequently divided into two groups: those with poor diabetic control (HbA1c 7–8.5%) and those with very poor diabetic control (HbA1c ≥ 8.5%). The cutoff point of this division was determined by the median HbA1c level of the study sample. Three validated instruments were also used to evaluate personality traits, anxiety and depression.

Big Five Inventory

The BFI is a 44-item, self-rated tool that measures each of the domains described in the five-factor model of personality: openness to experience, conscientiousness, extraversion, agreeableness and neuroticism [14]. The BFI has been shown to have good internal consistency (Cronbach's α average > 0.80) [15]. The Malay version of the BFI has also been shown to be reliable, with a Hancock and Mueller coefficient H ranging from 0.70 to 0.77 for all domains, except for openness (coefficient H of 0.60) [16].

Generalized Anxiety Disorder-7

The GAD-7 is a 7-item, self-rated screening tool and severity indicator for generalized anxiety disorder (GAD) in the primary healthcare setting [17]. Possible total scores range from 0 to 21, with higher scores indicating greater severity of GAD symptoms. The tool has a diagnostic cutoff point of ≥ 8, with a sensitivity of 92% and a specificity of 76% [17]. The GAD-7 has been shown to have a good internal consistency as measured by Cronbach's α value of 0.85 [18]. The Malay version of the GAD-7 has demonstrated acceptable internal consistency (Cronbach's α of 0.74) [19].

Beck Depression Inventory-II

The BDI-II is a 21-item, self-rated questionnaire that assesses cognitive and somatic symptoms of depression, with higher scores indicating more severe depressive symptoms. A cutoff point of ≥ 20 indicates moderate to severe depressive symptoms and was diagnostic of clinical depression. The BDI-II is reported to have excellent internal consistency with Cronbach's α of 0.90 [20]. The Malay version of the BDI-II also has good internal consistency (Cronbach's α of 0.80) [21].

Data analysis

Statistical analysis was conducted using the Statistical Package for Social Science (SPSS) version 20 (IBM Corp., Armonk, NY, USA). For descriptive statistics, categorical variables were reported in frequency and percentages, while continuous variables were reported in median and interquartile range (IQR). Continuous variables were not normally distributed as demonstrated by the Kolmogorov-Smirnov test ($p < 0.05$). Comparisons were made between respondents in the poor control group (defined as HbA1c 7.0–8.5%) and the very poor control group (defined as HbA1c > 8.5%). Pearson's chi-square test and Fisher's exact test were used to compare demographic, social and clinical characteristics with anxiety and depression. The associations between GAD-7 and BDI-II scores were evaluated with the Mann-Whitney U test. Furthermore, multivariate logistic regression analysis was performed to determine which factors were significantly associated with the occurrence of poor and very poor glycemic control among participants. In the logistic regression analysis, missing values were adjusted for using the multiple imputation method [22, 23]. Analysis of missing value patterns revealed no consistent pattern, suggesting the missing values occurred at random. Statistical significance for all analyses was set to $p < 0.05$.

Results

Sociodemographic and clinical characteristics

Sociodemographic and clinical characteristics of participants ($n = 176$) are reported in Table 1. The mean age of participants was 61.5 years (IQR: 52.0–69.0 years), and slightly more than half were male (53.4%). The majority of participants were married (78.4%) and in a low-income bracket (54.0%). The median duration of DM was 15.0 years (IQR: 10.0–20.3 years) and the median HbA1c level was 8.5% (IQR: 7.6%–10.0%). A minority of participants fulfilled the criteria for generalized anxiety disorder according to the GAD-7 (10.2%), while 7.4% met the criteria for moderate to severe depression according to the BDI-II (Table 2).

Bivariate analysis

Comparisons were made between respondents in the poor control group (HbA1c 7.0–8.5%) and the very poor control group (HbA1c ≥ 8.5%; Tables 3 and 4). In the bivariate analysis, the very poor control group was younger ($p = 0.006$, Table 4). Single respondents had a higher risk of very poorly controlled diabetes (OR = 3.24, 95% CI: 1.10–9.48, Table 3). Very poor control of diabetes was also associated with hypertension (OR = 2.60, 95% CI: 1.26–5.36) and dyslipidemia (OR = 1.91, 95% CI: 1.05–3.47).

Logistic regression analyses

Further logistic regression analyses were conducted (Table 5). In the full model incorporating sociodemographic, clinical and psychological variables, agreeableness was associated with very poor diabetic control ($p < 0.01$). Depression and anxiety were not associated with very poor glycemic control in this analysis.

Discussion

We studied a group of patients with DM in Malaysia with poor glycemic control (HbA1c ≥ 7.0%). The aim of this study is to assess whether certain biopsychosocial factors are associated with significantly very poor glycemic control among patients whose HbA1c levels are already outside of recommended clinical ranges. Very poor control of diabetes (HbA1c > 8.5%) was associated with younger age, single status, retirement, hypertension and dyslipidemia in bivariate analysis. However, only agreeableness was associated with very poor glycemic control in the final multivariate regression model ($p < 0.01$). There were no differences in depression and anxiety scores between the poor and very poor glycemic control groups.

Table 1 Sociodemographic and clinical characteristics of subjects (N = 176)

Variables	N	%
Age	61.5[a]	52.0–69.0[b]
Gender		
Male	94	53.4
Female	82	46.6
Marital status		
Married	138	78.4
Single	19	10.8
Divorced/separated	3	1.7
Widowed	14	8.0
Missing	2	1.1
Employment		
Employed	46	26.1
Unemployed	60	34.1
Retired	68	38.6
Missing	2	1.1
Household income		
< RM 3000	95	54.0
RM 3000–6000	37	21.0
> RM 6000	34	19.3
Missing	10	5.7
DM type		
Type 1	19	10.8
Type 2	157	89.2
Duration of diabetes (years)	15.0[a]	10.0–20.3[b]
Insulin therapy		
Yes	108	61.4
No	56	31.8
Missing	12	6.8
HbA1c level (%)	8.5[a]	7.6–10.0[b]
Diabetic control		
Poor control (7.0–8.5%)	88	50.0
Very poor control (> 8.5%)	88	50.0
Obesity		
BMI < 25	37	21.0
BMI 25–30	51	29.0
BMI > 30	51	29.0
Missing	37	21.0
Hypertension		
Yes	133	75.6
No	43	24.4
Dyslipidemia		
Yes	94	53.4
No	82	46.6

[a] Median

[b] Interquartile range

Table 2 Psychological measures of subjects (N = 176)

Variables	Median	IQR
GAD-7 score	1.0	0.0–4.0
BDI-II score	4.0	2.0–9.0
BFI subscales		
Extraversion	3.38	3.00–3.75
Agreeableness	3.89	3.56–4.11
Conscientiousness	3.67	3.44–4.00
Neuroticism	2.50	2.13–2.88
Openness	3.30	3.00–3.60
Variables	N	%
Anxiety		
Yes (GAD-7 score ≥ 8)	18	10.2
No (GAD-7 score < 8)	158	89.8
Depression		
Yes (BDI-II score ≥ 20)	13	7.4
No (BDI-II score < 20)	163	92.6

Younger age was found to be associated with very poor glycemic control, which reflects trends that have previously been observed in various regions of the world. In a study of American patients with DM (n = 1200), adults under 65 years were more likely to have greater HbA1c levels than their elderly counterparts [24]. Similarly, in a study of over 2500 patients with DM in Atlanta, younger age was independently associated with higher HbA1c levels at the time of referral [25]. In a study in Singapore, both mean HbA1c and LDL-cholesterol were higher among adults when compared to elderly patients, which may be in part due to lower doses of prescribed medications or decreased frequency in the use of combination drug regimens [26]. These findings may suggest a need to provide early interventions that more effectively manage glycemic control among younger populations with DM.

Very poor control of DM was also found to be associated with hypertension and dyslipidemia [27–30]. DM, hypertension and dyslipidemia are linked by common mechanisms at play in metabolic syndrome [24, 28]. The majority of patients with metabolic syndrome is older, obese and sedentary, and have at least some degree of insulin resistance [27, 28]. The pathophysiology of metabolic syndrome is complex and has been only partially elucidated [27, 28]. However, endothelial dysfunction, vascular microangiopathy, oxidative stress and diet have all been proposed as either biological mechanisms or lifestyle factors that contribute to metabolic syndrome and each of its component disorders [12, 13].

Upon performing logistic regression analyses, the personality trait of agreeableness, as measured by the BFI, was found to be associated with very poor diabetic control. According to the Big Five personality theory, an agreeable individual typically prioritizes others' interests over their own, which may in part explain the relationship between agreeableness and poor

Table 3 Comparisons of categorical variables between participants with poor diabetic control (HbA1c 7.0–8.5%) and very poor diabetic control (HbA1c > 8.5%) ($N = 176$)

Variables	Poor control ($N = 88$)		Very poor control ($N = 88$)		Crude ORs	95% CIs
	N	%	*N*	%		
Gender ($N = 176$)						
Male†	51	58.0	43	48.9		
Female	37	42.0	45	51.1	1.44	0.80–2.62
Marital status ($N = 174$)						
Married†	74	84.1	64	74.4		
Single	5	5.7	14	16.3	3.24*	1.10–9.48
Divorced/separated	1	1.1	2	2.3	2.31	0.21–26.10
Widowed	8	9.1	6	7.0	0.87	0.29–2.63
Employment ($N = 174$)						
Employed†	18	20.7	28	32.2		
Unemployed	24	27.6	36	41.4	0.96	0.43–2.12
Retired	45	51.7	23	26.4	0.33**	0.15–0.71
Household income ($N = 166$)						
< RM 3000†	48	59.3	47	55.3		
RM 3000–6000	17	21.0	20	23.5	1.20	0.56–2.57
> RM 6000	16	19.8	18	21.2	1.14	0.52–2.52
Obesity ($N = 139$)						
BMI < 25†	12	17.9	25	34.7		
BMI 25–30	30	44.8	21	29.2	0.34	0.14–0.82
BMI > 30	25	37.3	26	36.1	0.50	0.21–1.20
Hypertension ($N = 176$)						
Yes	14	15.9	29	33.0	2.60**	1.26–5.36
No†	74	84.1	59	67.0		
Dyslipidemia ($N = 176$)						
Yes	34	38.6	48	54.5	1.91*	1.05–3.47
No†	54	61.4	40	45.5		

† Reference group

*$p < 0.05$

**$p < 0.01$

Table 4 Comparisons of continuous variables between participants with poor and very poor diabetic control ($N = 176$)

Variables	Poor control ($N = 88$)		Very poor control ($N = 88$)		*p* values
	Median	IQR	Median	IQR	
Age	64.0	54.0–72.0	59.0	59.0–67.5	0.006[a]*
GAD-7 score	0.5	0.0–3.0	1.0	0.0–5.0	0.089[a]
BDI-II score	4.0	2.0–9.0	5.0	2.0–8.5	0.566[a]
BFI subscales					
Extraversion	3.4	3.1–3.8	3.4	3.0–3.6	0.336[a]
Agreeableness	3.8	3.6–4.0	3.9	3.6–4.2	0.189[a]
Conscientiousness	3.7	3.4–4.0	3.7	3.4–4.0	0.902[a]
Neuroticism	2.5	2.1–2.8	2.5	2.1–2.9	0.846[a]
Openness	3.2	3.0–3.6	3.3	3.0–3.7	0.594[a]

[a] Mann-Whitney *U* test

*Statistically significant

Table 5 Multivariate logistic regression analysis of sociodemographic, clinical and psychological factors for poor diabetic control

Variables	Adjusted OR	95% CI for OR	
		Lower	Upper
Age	0.98	0.93	1.02
Gender			
Male†	1.00		
Female	0.69	0.27	1.78
Marital status			
Married†	1.00		
Single	2.86	0.49	16.64
Divorced/separated	1.54	0.06	42.97
Widowed	1.25	0.17	9.17
Employment			
Employed†			
Unemployed	0.65	0.15	2.8
Retired	0.31	0.09	1.07
Household income			
< RM 3000†	1.00		
RM 3000–6000	0.92	0.31	2.78
> RM 6000	0.73	0.21	2.56
Obesity			
BMI < 25†	1.00		
BMI 25–30	0.24*	0.08	0.74
BMI > 30	0.43	0.14	1.31
Hypertension			
No†	1.00		
Yes	2.38	0.77	7.33
Dyslipidemia			
No†	1.00		
Yes	1.12	0.46	2.7
GAD-7 score	1.12	0.95	1.32
BDI-II score	0.96	0.89	1.04
BFI scores			
BFI extraversion	0.43	0.16	1.17
BFI agreeableness	6.39*	1.78	22.96
BFI conscientiousness	0.58	0.16	2.15
BFI neuroticism	1.09	0.43	2.77
BFI openness	1.1	0.41	2.95

Note. The logistic regression model was statistically significant, χ^2 = 37.030, df = 20, p = 0.012; Nagelkerke R^2 = 0.324

† Reference groups for categorical independent variables

*Statistically significant ($p < 0.05$)

glycemic control. These patients may place greater value on the well-being of others, at the cost of maintaining their own optimal health. While previous studies have reported conflicting results regarding the relationship between agreeableness and glycemic control [30, 31], it is important to consider the sociocultural context in which these studies take place.

Unlike previous studies in this field, our current study was conducted within the sociocultural landscape of Malaysia where conformity to community and family norms is often prioritized [10, 32]. As the country has rapidly developed over the past few decades, culinary trends have shifted from starch-based, high-fibre ingredients to processed foods with high sugar and fat content [33]. Agreeable patients who value conformity may be more likely to comply with these general dietary trends that have the potential to worsen glycemic control.

However, agreeableness could also benefit patients struggling to adhere to their treatment plan. By working closely with their healthcare provider, agreeable patients may be more likely to overcome psychological barriers and accept the changes required to improve poor diabetic control. For example, one study reported that when depression was recognized as a barrier and antidepressant treatment was recommended, agreeableness further promoted enhancements in blood pressure, Hb1Ac and lipid control [34]. Thus, by continually monitoring adherence and identifying potential barriers to adherence, agreeableness may facilitate the implementation of barrier-specific interventions that can be used to ultimately improve disease management.

There were no significant differences in depression or anxiety scores between participants with poor diabetic control and participants with very poor diabetic control. One possibility is that the relationship between poor glycemic control and mental illness is not linear and is instead mediated by a multitude of different factors. However, previous studies have shown that sociodemographic factors, empathy and psychological stress influence glycemic control among patients with DM [35, 36]. Therefore, by screening for mental illness and employing the appropriate psychosocial interventions, both DM and its mental health comorbidities can be optimally managed.

The study findings should be interpreted in light of several limitations. The study employed a cross-sectional design, preventing us from observing any causal relationships between factors and glycemic control. Second, the sample size was relatively small. Furthermore, we did not collect data on diabetes-related wounds, amputations or physical activity levels, which could be pertinent to glycemic control. Lastly, the study was conducted at a single tertiary centre in Kuala Lumpur and may not be generalizable to the entire diabetic population in Malaysia. Future research should aim for multicentre subject recruitment to confirm findings.

Conclusion

This study investigated biopsychosocial factors in relation to very poor glycemic control among patients who struggle to maintain optimal HbA1c levels. It highlights the possible role

of personality in glycemic control and provides insight into how agreeableness may be applied in a clinical setting to facilitate adherence to treatment plans. It is important to consider that this relationship between personality and glycemic control may be mediated by sociocultural factors. Ultimately, this study emphasizes the importance of evaluating glycemic control in the greater context of each patient's life, as numerous biopsychosocial factors can influence the effectiveness of diabetes management.

Acknowledgments The authors would like to thank Dr. Nurul Hazwani Hatta, Dr. Puteri Arnawati and Dr. Amelia Yasmin Zulkifli for their involvement in data collection for this study.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Paula Junggar Gosse, Emily Samantha Kaunismaa, Roslyn Laurie Mainland and Luke Sy-Cherng Woon. The first draft of the manuscript was written by Luke Sy-Cherng Woon and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was funded by the Young Lecturers Incentive Grant (GGPM-2019-024) from the Research University Fund of the National University of Malaysia, as well as the University of Toronto Comprehensible Research Experience for Medical Students (CREMS) and Medial Alumni Association (MAA).

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

Compliance with ethical standards

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

Ethics approval The questionnaire and methodology for this study were approved by the Research Ethics Committee of the Faculty of Medicine, Universiti Kebangsaan Malaysia (Ethics approval number: UKM FPR.SPI 800-2/28/166/FF-2019-342).

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

Consent to publish Not applicable.

Code availability Not applicable.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):484–490
https://doi.org/10.1007/s13410-020-00916-2

ORIGINAL ARTICLE

Detection of early diabetic retinopathy using visual electrophysiological tests

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Received: 19 April 2020 / Accepted: 23 December 2020 / Published online: 7 January 2021

Abstract

Background Detection of functional impairment of vision in pre-clinical stages helps early identification of diabetic retinopathy. We aimed to determine the functional integrity of retina and post retinal pathways using electro-oculography (EOG), pattern electroretinography (PERG) and pattern-reversal visual evoked potentials (PR-VEP) in newly diagnosed diabetic patients who have not developed fundoscopic features of diabetic retinopathy.

Methods Twenty-five adults with newly diagnosed diabetes mellitus without fundoscopic evidence of retinopathy and a control group of healthy adults were subjected to visual electrophysiological assessment. Retinal pigment epithelium (RPE)-photoreceptor interaction, photoreceptors and ganglion cells of the macula and post retinal pathways were assessed by EOG, PERG and PR-VEP, respectively.

Results Fourteen of the 25 diabetic patients, i.e. 56% (95% confidence intervals 34.9%, 75.6%), had LP:DT (light peak to dark trough) ratio less than 1.7, to the cut-off defined by the International Society for Clinical Electrophysiology of Vision (ISCEV). All control group subjects had LP:DT ratios above 1.7. The median LP:DT ratio in diabetic group (1.62 ± IQR 0.27) was significantly lower than that of the controls (1.8 ± IQR 0.21). Four patients had prolonged PR-VEP P100 latencies, and seven had prolonged PERG P50 latencies as per the ISCEV cut-offs, whereas none of the control group had abnormal PERG or PR-VEP measures.

Conclusion With a limited sample, we found that 56% of newly diagnosed diabetic patients with normal fundoscopy had defective RPE-photoreceptor interaction. Further studies are needed to obtain more precise point estimates of these EOG abnormalities, and to determine the conversion rates into more advanced stages of diabetic retinopathy.

Keywords Early diabetes · Electro-oculography · Pattern electroretinography · Pattern-reversal visual evoked potentials

Introduction

Diabetic retinopathy leads to significant morbidity in diabetes mellitus. Early detection and timely treatment is important to avoid serious visual impairment [1]. Though mechanisms of diabetic retinopathy are being extensively researched, only few studies have systematically assessed the functional integrity of the retina and post retinal pathways in early diabetic patients [2–6]. Visual electrophysiological tests are used to assess functional integrity of the visual system, where electro-oculography (EOG) is used to assess retinal pigment epithelium (RPE)-photoreceptor interaction, pattern electroretinography (PERG) to assess the functional integrity of macular photoreceptors and ganglion cells and pattern-reversal visual evoked potentials (PR-VEP) to assess the visual conduction along the post retinal pathways [7, 8].

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Fundus autofluorescence imaging and optical coherence tomography show structural changes in RPE in early diabetic patients even before they developed fundoscopic changes [9, 10]. Two previous studies show RPE abnormalities in newly diagnosed diabetes without fundoscopic changes, one using fast oscillation EOG and other one using non photic EOG [2, 3] . Breakdown of RPE barrier causes excessive water influx to the retina and leads to the development of some forms of diabetic macular edema. This macular edema is considered to be a major cause of visual impairment in diabetic retinopathy [2, 4]. Few studies with small samples have assessed the post retinal pathways using PR-VEP and macular photoreceptors and ganglion cells using PERG separately [4–6]. However, none had systematically assessed the retina and post retinal pathways in a combined approach.

In this background, the present study aimed to systematically assess the functional integrity of RPE, macular photoreceptors and ganglion cells and post retinal pathways using battery of visual electrophysiological tests, viz. EOG, PERG and PR-VEP in newly diagnosed diabetes mellitus with normal fundoscopy, and to compare the findings with those of a healthy control group.

Materials and methods

Study setting and participants

This was a cross-sectional study in which we compared visual electrophysiological parameters in a test group of adults who had newly diagnosed diabetes mellitus but had not developed fundoscopic features of diabetic retinopathy with those of a healthy adult control group. The study was carried out from September 2017 to October 2019, at the Ophthalmology Department of the National Hospital Kandy and the General Medicine and Neurophysiology Departments of the Teaching Hospital Peradeniya—two tertiary care referral centres in Sri Lanka.

Diagnosis of diabetes mellitus was done by one of the co-authors of this study (MP) who is a specialist physician, adhering to the International Diabetes Federation guidelines [11]. Patients presented to a physician for the first time with symptoms, and had random plasma glucose level above 200 mg/dL (11.1 mmol/L), and subsequent fasting plasma glucose level above 126 mg/dL (7.0 mmol/L) was classified as having newly diagnosed diabetes mellitus. Range of HbA1c level was also measured after the diagnosis of diabetes mellitus; but the level of HbA1c was not used to confirm the diagnosis as different patients might have had different durations of onset of the condition. After the diagnosis, patients underwent a neuro-ophthalmological assessment which included monocular measurements of visual acuity, visual field assessment, monocular measurement of colour vision by Ishihara chart, pupillary reflexes, fundoscopy and ocular motor examination by a co-author who is a specialist ophthalmologist (SS). Patients with diabetes with diabetic retinopathy on fundoscopic examination, those who have undergone ocular surgeries, coexistent optic nerve disorders, coexistent retinal disorders, and patients who did not give informed consent were excluded from the study. Finally, 25 patients ≥ 18 years of age with normal fundoscopy, who were diagnosed having diabetes mellitus within the last 2 months, were consecutively recruited to the present study. The total planned sample size was 50 (n = 25 in diabetes group and n = 25 in the control group). The control group comprised 25 healthy subjects of age ≥ 18 years identified by the co-author SS as having normal or corrected-to-normal vision in neuro-ophthalmologic examination, and without neurological or ophthalmological diseases. The principal author (PD) and the co-author SS examined the patients, and recorded the clinical details and the risk factors in structured data sheets. All the investigation findings and treatment details were also recorded.

Visual electrophysiological assessment

The visual electrophysiological tests were performed at the Clinical Neurophysiology Laboratory of the Teaching Hospital Peradeniya in the following order:

Pattern-reversal visual evoked potentials

A Natus EMG/NCV/EP machine (Natus Neurology Inc. USA) was used to produce visual stimuli and to record and average the PR-VEP waveforms. The recording technique conformed to the International Society for Clinical Electrophysiology of Vision (ISCEV) guidelines [12]. PR-VEPs were recorded in response to 2 Hz pattern-reversal checkerboard stimuli presented on a cathode ray tube monitor with a black and white alternating checkerboard pattern. The field size was 15° of visual angle at the smallest point. The contrast between black and white squares was ≥ 80% as defined by Michelson contrast. Silver/silver chloride electrodes were used. An active electrode was fixed at the Oz position, reference electrode at Fz position, and ground electrode at Cz position. The electrode impedances were maintained below 5 kΩ. Mean photopic luminance was 50 cdm^{-2}. The mean luminance of the stimulus screen was constant during checkerboard reversals and varied less than 30% between the centre and periphery of the visual field. Each eye was tested separately. A band-pass filter with low and high cut-off frequencies of 1 Hz and 100 Hz was used. One hundred sweeps were averaged, and two averaged waveforms were recorded for reproducibility. The peak amplitudes and latencies of the N75, P100 and N145 components were measured.

Pattern electroretinography

Active electrodes were placed on the lower eyelids. These were preferred to corneal electrodes because of the complications associated with the latter [13]. Silver/silver chloride electrodes were used. The reference electrode was placed near the ipsilateral, outer canthus of each eye. The ground electrode was located on Cz position. A Nicolet Viking Quest EMG/NCV/EP machine (Natus Neurology, USA) was used to record the PERG waveforms. The stimuli were a black and white reversing checkerboard pattern with check size for the PERG set to 0.8° (± 0.2°). The stimulus field size was 15°. Transient PERG stimuli had a reversal rate of 4 per second (2 Hz). The band-pass filters were set to 1 Hz and 100 Hz. A photopic luminance of the white areas was greater than 80 cdm^{-2}. The mean luminance of the stimulus screen was constant during checkerboard reversals. The sweep time was 250 ms. Pupils were not dilated. A minimum of 100 artifact-free sweeps were averaged. At least two trials for each stimulus condition confirmed reproducibility. The peak amplitudes and latencies of the N35, P50 and N95 peaks were measured.

Electro-oculography

The EOG waveforms were recorded using the Natus EMG/NCV/EP machine. Pupils were not dilated as it was shown that application of pupillary dilatation does not influence the quality or the results of electro-oculograms [14]. The bitemporal method was used to record EOG wave forms. Silver/silver chloride electrodes were used. The active and reference electrodes were attached to the outer canthi of each eye, thus collecting a compound potential difference resulting from both eyes. The ground electrode was placed at Cz. Band-pass filters were set to 0.1 Hz and 30 Hz. A Ganzfeld dome provided the stimulation. Two fixating lights were located in the Ganzfeld dome, 15° apart left and right of the centre. The patient was kept in stable indoor lighting for at least 30 min before the test. Fixation lights in the Ganzfeld dome were set to alternate at a frequency of once per second, for 10 s out of every minute. The EOG potentials were recorded for once per second for 10 s every minute as the eyes moved to left and right according to the alternating lights in the Ganzfeld dome. Auditory cues were used during the recordings. The procedure of making saccades was practiced with the recording system before dark adaptation, to familiarize the patient with the task and to check on the stability and quality of the recorded saccades. The same procedure was used to test the control subjects. EOG recording began with the beginning of the dark adaptation; the EOG potentials being recorded once a minute for 10 s. The dark phase of the EOG potentials lasted for 15 min. Then, the room lights and adapting light of Ganzfeld dome were switched on. The adapting light of Ganzfeld dome was a white light with a luminance of 100 cdm^{-2}. The light phase recording lasted for another 15 min [7]. The patient was positioned in the headrest of the Ganzfeld stimulator throughout the procedure, with eyes open to maintain retinal illumination. Gentle coaching and reminders were done throughout the procedure to minimize the effects of poor compliance. Raw EOG traces were visually inspected by the principal author (PD) to place the cursors accurately to measure the saccadic amplitude in each epoch. The EOG amplitudes were measured in microvolts (μV) manually after visual inspection. When measuring, the effects of overshoot or irregular saccades were discounted.

The average of the EOG amplitudes within each 10-s recording epoch was taken and plotted against time. Then, the underlying physiologic curve was drawn using computer-based curve fitting algorithms to derive reliable light peak (LP) and dark trough (DT) amplitudes. Then, the LP:DT ratio was calculated by dividing the light peak amplitude of the curve by dark trough amplitude.

Data analysis

PR-VEP latencies and amplitudes, PERG latencies and amplitudes and EOG amplitudes of light peak and dark trough and LP:DT ratio showed skewed distributions: they are reported as medians and interquartiles ranges (IQR), and were compared between groups using Mann-Whitney *U* test. LP:DT ratio in EOG < 1.7, P100 latency in PR-VEP > 110 ms, P50 latency in PERG > 60 ms, N95 latency in PERG > 100 ms were considered abnormal as per the ISCEV cut-off values. As there were no abnormalities found in controls, odds ratios could not be calculated and the abnormalities in visual electrophysiological parameters in diabetes study group are reported as percentages. The comparisons were interpreted as significant at a cut-off p value < 0.05. IBM SPSS Statistics for Windows, version 22.0. was used to analyze the data.

Results

Demographic and clinical characteristics

The test group had 25 patients (14 males) with diabetes mellitus diagnosed within 2 months preceding visual electrophysiology testing (median age: 52 ± IQR 17; range: 30–79 years). None of them complained of visual disturbances in either eye. The median duration of diagnosis of diabetes mellitus to the date of visual electrophysiological examination was 30 ± IQR 18 days (range: 5–60 days). The median level of glycosylated hemoglobin (HbA1c) level in patients was 7.0% ± IQR 1.2 (range 5–8.2). None of them had ocular pain or positive relative afferent pupillary defect in either eye

at the time of visual examination. Corrected visual acuity ranged from 6/6 to 6/9 by 6-m notation in both eyes. Colour vision was normal in all of them. No one had fundoscopic changes of diabetic retinopathy or any other ocular abnormalities. The control group consisted of 25 healthy adults (6 males) (median age: 45 ± IQR 11; range: 32–62 years).

Visual electrophysiological assessment

As per the ISCEV cut-off limits, 14 out of 25, i.e. 56% (95% CI of 34.9%, 75.6%) in the diabetes mellitus group, but none in the control group had significant reduction in LP:DT ratio. Intergroup comparisons of visual electrophysiological outcome measures are summarized in Table 1. Median LP:DT ratio was significantly lower and median dark trough was significantly higher in newly diagnosed diabetes mellitus group compared to the controls (Table 1, Fig. 1). However, four patients had prolonged PR-VEP P100 latency in both eyes and seven patients had prolonged P50 latency in PERG at least in one eye as per the ISCEV cut-offs, indicating abnormality in post retinal pathways and photoreceptors in macular region respectively (Table 1). PR-VEP and PERG measures were normal in all control subjects.

Table 1 Intergroup comparison of outcome measures of visual electrophysiological assessment

Outcome measure (tested function) (Latencies were measured in ms, and amplitudes in μV)	Number of subjects with abnormal values according to the ISCEV norms* [7, 12, 15]		Median ± IQR		*p* value
	Diabetes mellitus group (*n* = 25)	Controls (*n* = 25)	Diabetes mellitus group (*n* = 25)	Controls (*n* = 25)	
Integrity of RPE					
LP:DT ratio (RPE-photoreceptor interaction)	14	0	1.62 ± 0.27	1.8 ± 0.21	**< 0.001**
Light peak	-	-	1785.7 ± 1178.6	1500 ± 857.1	0.24
Dark trough	-	-	1071.4 ± 625	850 ± 395	**0.01**
Integrity of macula region					
Right P50 latency (photoreceptors)	3	0	54.0 ± 11.6	52 ± 11.8	0.14
Left P50 latency (photoreceptors)	5	0	54.7 ± 16.4	53 ± 10.3	0.33
Right P50 amplitude (photoreceptors)	-	-	0.98 ± 1.61	1.24 ± 1.8	0.29
Left P50 amplitude (photoreceptors)	-	-	0.70 ± 1.5	1.4 ± 1.3	0.07
Right N95 latency (ganglion cells)	0	0	90.0 ± 8.0	90.5 ± 5.0	0.31
Left N95 latency (ganglion cells)	0	0	88.0 ± 4.8	90.0 ± 4.3	0.12
Right N95 amplitude (ganglion cells)	-	-	1.22 ± 2.5	1.70 ± 2.2	0.73
Left N95 amplitude (ganglion cells)	-	-	1.65 ± 1.1	1.52 ± 0.8	0.52
Right N35 latency	-	-	39.25 ± 17.1	35.0 ± 6.8	0.14
Left N35 latency	-	-	38.0 ± 7.8	37.0 ± 5.8	0.19
Right N35 amplitude	-	-	0.2 ± 0.4	0.2 ± 0.4	0.79
Left N35 amplitude	-	-	0.45 ± 0.8	0.35 ± 0.8	0.64
Integrity of post retinal pathways					
Right N75 latency	-	-	72.0 ± 17.4	75.0 ± 7.5	0.38
Left N75 latency	-	-	68.25 ± 18.3	76.0 ± 6.8	0.08
Right N75 amplitude	-	-	0.96 ± 0.5	1.00 ± 0.9	0.21
Left N75 amplitude	-	-	1.0 ± 0.4	1.0 ± 0.8	0.32
Right P100 latency (post retinal pathways)	4	0	101.0 ± 14.6	104.0 ± 10.8	0.54
Left P100 latency (post retinal pathways)	4	0	98.0 ± 13.0	100.0 ± 12.3	0.77
Right P100 amplitude (post retinal pathways)	-	-	4.25 ± 3.0	5.1 ± 6.7	0.09
Left P100 amplitude (post retinal pathways)	-	-	2.9 ± 3.3	4.0 ± 4.2	0.23
Right N145 latency	-	-	141.7 ± 10.1	140.0 ± 7.0	0.65
Left N145 latency	-	-	139.0 ± 12.0	140.0 ± 10.0	1.00
Right N145 amplitude	-	-	2.95 ± 2.0	4.0 ± 5.0	0.23
Left N145 amplitude	-	-	3.62 ± 3.7	4.0 ± 7.9	0.20

Values in bold are statistically significant

*Only for the measures where ISCEV norms are available

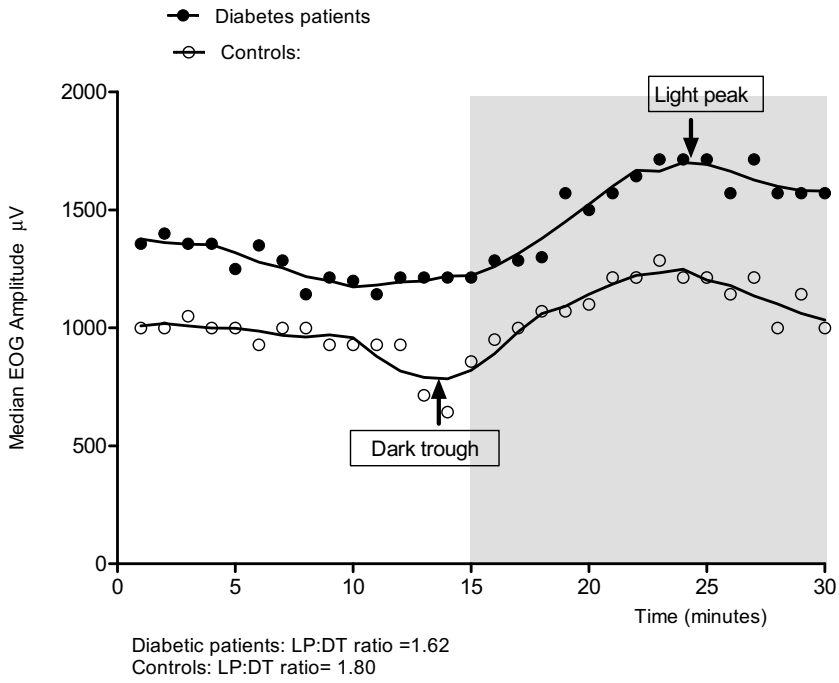

Fig. 1 Median EOG waveforms in dark and light phases over 30 min in diabetes mellitus group and the controls

Discussion

In this study, our aim was to systematically examine the functional integrity of RPE, macular region and post retinal pathways in newly diagnosed diabetes mellitus patients who had not developed clinically evident retinopathy as indexed by fundoscopic changes. In our sample, 56% of the patients had EOG abnormalities that signified deranged RPE-photoreceptor interactions while none in the control group had EOG abnormalities. With the limited sample of patients, the 95% CI ranged from 34.9 to 75.6%, indicating even going by the lowest estimate one in three patients who do not show fundoscopic abnormalities have functional abnormalities of the retina.

The median LP:DT ratio was lower in diabetic patients compared to that of the control group. This reduction of LP:DT ratio was accounted by elevation of dark trough. The EOG findings specifically indicate defective RPE-photoreceptor interaction. Limited number of studies show different mechanisms underlying such disintegration. These include compromisation of tight junctions in both endothelial and RPE barriers and degeneration of photoreceptors and RPE—as observed in experimental rats [16, 17]. Such loss of RPE integrity may result in alterations in the transepithelial potential which is the main contributor to the standing potential of the eye that in turn electrophysiologically recorded as EOG. The mechanism for the light rise in EOG has been studied since the introduction of the concept of EOG [18]. The current understanding is that a substance is released from the rod outer segment to set off a rise in inositol triphosphate through an apical membrane receptor [19]. This in turn causes a subsequent rise in intracellular free calcium. Then, calcium-activated chloride channels will be operated leading to depolarization of the basal membrane of the RPE resulting in initiation of light rise [19–21]. Though many mechanisms have been postulated to explain light rise, the mechanism of dark trough has not been well explained. Given the role of changes in basolateral chloride conductance regulating the light peak, dark trough also seems to be generated by a decrease in basolateral chloride conductance [22]. This is relevant to the present findings because LP:DT ratio reduction that we observed was accounted by a rise in dark trough amplitude. Schneck et al. (2008) have shown that light peak and dark trough amplitudes of fast oscillation EOG were significantly reduced in early diabetic patients. They have postulated that these changes could be due to reduction of activity of the chloride ion channels of the RPE leading to hyperpolarization of the basolateral RPE membrane [2].

There were no significant intergroup differences in median comparisons of PERG measures or PR-VEP measures. However, as per the ISCEV cut-offs, 4 out of 25 diabetic patients had delayed P100 latencies in PR-VEP indicating abnormal post retinal pathways; and 7 out of 25 diabetic patients had prolonged P50 latencies in PERG indicating abnormal photoreceptor cell function in the macula region. Therefore, we propose to perform all three tests—EOG, PERG and PR-VEP—in electrophysiological testing for early diabetic retinopathy.

The main limitation of our study was the small sample size. However, as seen in Table 2, previous studies [2–6, 23] are also based on small samples, except one PERG study where the prevalence rate is not reported. Despite these limited samples, our findings concur with the previous EOG studies that report 40–68% prevalence of EOG abnormalities [2, 3]. Prevalence of PR-VEP abnormalities in our study was less than the previous two studies [4, 5]. We observed 28% had

Table 2 Prevalence of visual electrophysiological test abnormalities in studies in diabetic patients with normal fundoscopy

Study	Prevalence of EOG abnormalities	Prevalence of PERG abnormalities	Prevalence of PR-VEP abnormalities
Present study	56% (n = 25)	28% (n = 25)	16% (n = 25)
Schneck et al. (2008) [2]	68% (n = 11)		
Heravian et al. (2012) [4]			40% (n = 20)
Shirao and Kawasai (1998) [3]	40% (n = 42 eyes)		
Algan et al. (1989) [5]			28% (n = 19)
Caputo et al. (1990) [23]		NM[a] (n = 42)	
Coupland (1987) [6]		0% (n = 14)	

[a] Not mentioned

PERG abnormalities and one previous study has not reported the prevalence rate of PERG abnormalities [23] and whereas Coupland et al. (1987) report no PERG abnormalities in 14 early diabetic patients [6].

Conclusions

According to our findings, we estimate one-third to three-fourths of patients with newly diagnosed diabetes mellitus show electrophysiological evidence of RPE dysfunction even before developing clinically evident retinopathy or visual impairment. Future studies—with larger samples—should explore this subgroup of patients with early functional changes of retina to see whether they are at a higher risk of conversion to overt retinopathy and clinically significant visual dysfunction.

Acknowledgments The authors wish to thank all the subjects in the study and Consultant Physicians who referred patients for the study.

Compliance with ethical standards

Ethical clearance for the study was obtained from the Ethical Review Committee, Faculty of Medicine, University of Peradeniya, Sri Lanka. The study design and protocols complied with the code of ethics of the World Medical Association Declaration of Helsinki [24]. The procedures were explained, and informed written consent obtained from all participants.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):491–497
https://doi.org/10.1007/s13410-020-00911-7

ORIGINAL ARTICLE

Expanding the collation of urinary biomarkers in improving the diagnosis of diabetic nephropathy

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Received: 11 August 2020 / Accepted: 22 December 2020 / Published online: 6 January 2021

Abstract

Background Diabetic nephropathy (DN) is the foremost cause of chronic kidney disease (CKD), which may lead to end-stage renal disease. Due to the inconsistent modifications in urine albumin, the conventional immunoassays underestimate urine albumin, thereby delaying the diagnosis. This study was designed to find an alternative urinary marker for the early detection of DN.

Methods Urine neutrophil gelatinase-associated lipocalin (NGAL) was estimated along with routine tests. This cross-sectional study recruited 180 healthy controls (group I), 103 diabetics without microalbuminuria (group II), and 102 diabetics with microalbuminuria (group III).

Results There was an increasing trend for urinary NGAL from group I to III in both the females and males ($p = 0.003$, 0.004, respectively).With the urine NGAL cut-off of 70.87 ng/ml, there was 66.7% sensitivity and 66.8% specificity, and the odds of diabetic patients having nephropathy was 4.02. Combining the urine albumin creatinine ratio (UACR) and U NGAL, the AUC improved to 1.000 ($p = 0.000$) for UACR and U NGAL had AUC of 0.992 ($p = 0.004$).

Conclusion As tubular damage occurs prior to glomerular damage in DN, we conclude that measurement of urinary NGAL predicts DN better than microalbumin alone.

Keywords Microalbuminuria · Urine albumin creatinine ratio · Diabetes mellitus · Neutrophil gelatinase-associated lipocalin · NGAL · Tubular involvement · T2DM

Introduction

Diabetic nephropathy (DN) is the foremost cause of chronic kidney disease (CKD), which may lead to end-stage renal disease (ESRD) with consequential reduction of patients' quality of life and survival [1, 2]. Progressive renal injury in diabetes is predominantly considered to be due to glomerular changes. However, lesser than one-third of these patients with microalbuminuria have histological alterations. And importantly, long-term outcomes of most renal disease depend on the extent of tubular involvement [3, 4]. Early detection and easy monitoring of any renal damage before and during treatment using urinary marker allow deferment of nephropathy. Microalbuminuria is presently the most dependable predictor of diabetic nephropathy [5]. But most of the times its estimation is complicated by immunoassays used, because urinary albumin exists in many forms [6, 7]. The immunochemically non-reactive nature of this albumin is attributed to the alteration of epitope by conformational change either due to incomplete processing by the lysosomal pathway which is compromised in diabetes but not in healthy individuals [8–10], or due to the attachment of ligands such as glucose or fatty acids, which are increased in diabetes. Because of these inconsistent modifications in urine albumin and the fact that antibodies in immunoassays for urine albumin are from serum albumin, there is a definite underestimation of urine albumin measurement [11]. Moreover, by the time microalbumin is detected, nephropathy could already be existing in type 2 diabetes [5, 12]. Though, at this late phase, injury to the glomeruli may be irreversible and consequently, therapy ineffective and non-curable [13]. Therefore, it is imperative to find alternative urinary markers for early detection of diabetic nephropathy.

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The proteomic/peptidomic analyses done in uncomplicated diabetes direct that early stimulation of fibrotic pathways in the kidney ensues before the commencement of microalbuminuria. In incipient nephropathy, by the time albumin excretion rates are increased, the glomerular permselectivity and tubular reabsorption change. In overt nephropathy additional proteins involved in wound healing, ongoing fibrosis, and inflammation are excreted [10, 11]. Neutrophil gelatinase-associated lipocalin [NGAL), the most investigated biomarker kidney involvement, is a glycoprotein from the lipocalin superfamily. Tubular injury leads to an increase in NGAL secretion by tubular cells [14].

Though it is over a decade now about our knowledge and understanding of the urinary proteomics and the pathogenesis of diabetic nephropathy, detection of microalbuminuria continues to be the earliest marker for the same. Till date, there is a paucity of reports on the clinical performance of novel biomarkers for the diagnosis of DN and diabetic albuminuria. Furthermore, it is still not clear regarding the utility of their detection before the onset of microalbuminuria. Hence, this research study was designed to estimate and compare urine NGAL microalbumin in diabetes mellitus (T2DM) patients without and with microalbuminuria and in healthy controls.

Material and methods

Study design

It was a cross-sectional comparative study on ambulatory T2DM subjects conducted in a tertiary care center in Eastern India after the approval of the Institutional Ethics Committee. The cases were from our non-communicable diseases (NCD) clinic and controls were apparently healthy individuals among our staff and their family members or from the group of subjects who came to our out-patient departments (OPDs) for routine health check-up.

Sample size

A total of 205 patients of diabetes mellitus and 180 controls.

Inclusion criteria

- Controls were taken as group I: 180 nondiabetic healthy subjects were recruited after taking written consent.
- Out of the 221 T2DM patients recruited, 205 were included in the study and 16 were rejected for having microalbuminuria > 300 mg/l. They were classified into two groups: Group II had urine albumin creatinine ratio (UACR) < 30 mg/g and group III had 30–300 mg/g.

Exclusion criteria

Subjects excluded were those not willing, or were hypertensive, or had proteinuria, or had any other comorbidities like thyroid disorders, disorders requiring the need for long-term use of anti-inflammatory drugs or steroids, pregnancy, and type 1 diabetes mellitus.

Sample collection and biochemical assay

1. Urine samples from both cases and controls were collected as a midstream portion of a mid-morning void, and were stored in 3 separate 1-ml aliquots at − 20 °C till assay of the following: The ELISA method used for NGAL was performed as per instructions provided by the manufacturer. The quantitative human urine NGAL ELISA kit Cat# KT883 used was from Epitope Diagnostic, Inc., CA, USA. Urine albumin by immunoturbidimetric method and urine creatinine by Jaffe's kinetic method in an autoanalyzer from Beckman Coulter Chemistry Analyzer AU5800 (Beckman Coulter, Brea, USA). The urine samples of controls (group I) were checked for qualitative proteinuria using dipstick, before storage.
2. Blood was collected for estimation of the following: serum creatinine, glycated hemoglobin (HbA1c), fasting (FBS), and post-prandial blood sugar (PPBS). All the colorimetric estimations were done the same day using the Beckman Coulter Chemistry Analyzer AU5800 (Beckman Coulter, Brea, USA).

Calculated parameters

1. The degree of early DN was determined using the urinary albumin-to-creatinine ratio (UACR) and expressed as mg/g; microalbuminuria range for UACR 30–300 mg/g is considered as a marker for DN; < 30 is normal and > 300 as overt proteinuria.
2. The estimated glomerular filtration rate (eGFR) is by the following formula:

$$\text{eGFR (ml/min)} = [(140-\text{age}) \times \text{Wt}/(0.814 \times \text{S.Cr in } \mu\text{mol/l})] \times (0.85 \text{ if female}).$$

Table 1 General characteristics of the study group compared according to gender

	Female				Male			
GROUPS	I	II	III	p [a]	I	II	III	p [a]
N	85	34	38		95	69	64	
Age (years)[b]	48 (15)	50.5 (13)	48.5 (12)	0.083	46 (22)	53 (17)	54.5 (18)	0.011
BMI [b]	25.6 (6)	25.3 (4.3)	24.6 (5.5)	0.755	25 (6.6)	25.2 (4.5)	25.9 (5.7)	0.865
WHR [b]	0.9 (0.08)*	0.92 (0.06)*	0.88 (0.06)*	0.215	0.95 (0.05)	0.95 (0.05)	0.96 (0.06)	0.268
eGFR (ml/min) [b]	82.5 (38)	72.3 (23)	79 (26.8)	0.059	90.6 (33.5)	77.5 (27)	83.5 (29.8)	*0.002*
ACR [b] (mg/g of creatinine)	4.1 (9.2)	21.5 (25)	74 (115.6)	*0*	5.45 (9.8)	15.5 (35.6)	52.5 (102.8)	*0*
U NGAL [b] (ng/ml)	33.9 (49.5)	73.9 (102)	144.7 (163)*	*0.003*	39.9 (54)	66.4 (92.7)	106.5 (186.2)	*0.004*

[a] Kruskal-Wallis test

[b] All parameters are given as median (IQR)

[*] Comparison by Mann-Whitney *U* test between females and males within a group had $p < 0.05$

Statistical analysis was used: SPSS version 19.0 was used for the statistical calculations.

Results

Clinical characteristics

The study recruited 180 healthy controls (group I), 103 diabetics without microalbuminuria (group II), and 102 diabetics with microalbuminuria (group III) whose data were used for statistical analysis.

Table 1 shows the general characteristics of the three study groups compared according to gender. The data for some parameters were skewed according to Kolmogorov-Smirnov test; hence, all data were represented as median and interquartile range (IQR). The comparison of groups among females and males done by Kruskal-Wallis H test showed no difference in age, BMI and WHR. The eGFR showed statistically significant difference within groups in males ($p = 0.002$) but not so among the females. The ACR increased in cases as compared controls, highest being in group III in both females and males ($p = 0$). Similarly, there was an increasing trend for urinary NGAL from group I to III in both the females and males ($p = 0.003$, 0.004, respectively). Mann-Whitney *U* test was used to compare the females with males in cases and controls. There was statistical difference in WHR in all three groups ($p < 0.05$) between the two sexes. The U NGAL was higher in females in group III ($p < 0.05$).

The area under the curve (AUC) as shown in Table 2 from ROC curve (Fig. 1) shows the urine biomarkers for diabetic nephropathy, the most suitable being ACR (0.891, $p = 0.000$) which was good, followed by NGAL (0.699, $p = 0.000$) being a fair marker.

Upon combining the two urine parameters (Table 3), ACR and U NGAL, the AUC improved for both and status as a diagnostic tool improved from good and fair, respectively, to excellent markers. ACR had AUC of 1.000 ($p = 0.000$) and U NGAL had AUC of 0.992 ($p = 0.004$).

The U NGAL correlated positively with ACR ($r = 0.301$) in our study groups ($p = 0.000$) and negatively

Table 2 Area under curve (AUC) in ROC for the urine parameters

Urine parameters	AUC	Std. error[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
ACR	0.891	0.017	0.000	0.858	0.925
NGAL	0.699	0.032	0.000	0.636	0.761

[a] Under the nonparametric assumption

[b] Null hypothesis: true area = 0.5

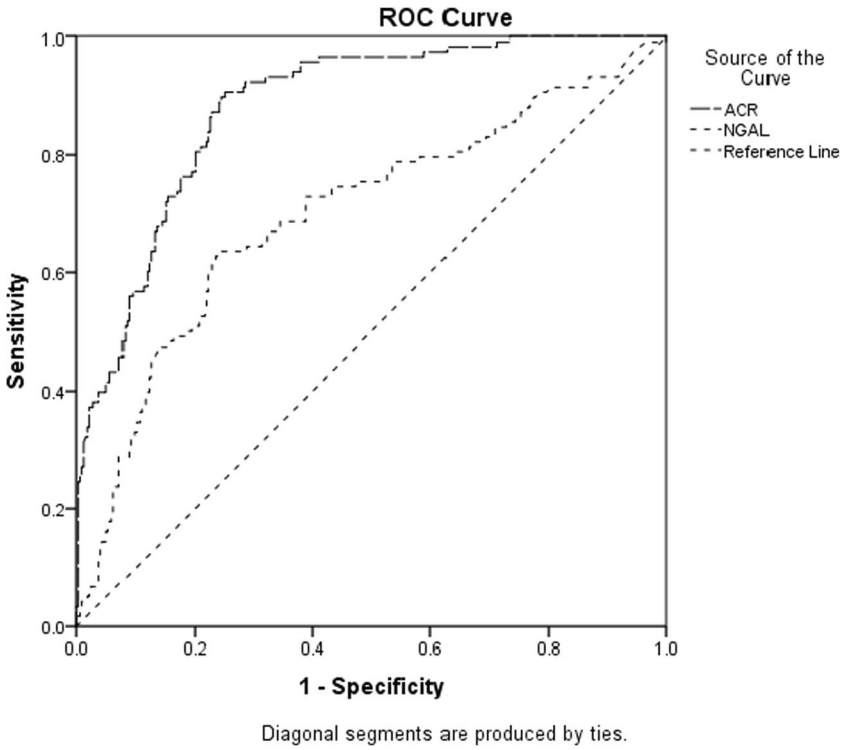

Fig. 1 ROC curve to show the AUC for the urine parameters

with the eGFR (Table 4). The risk assessment was done using 70.87 ng/ml as cut-off for U NGAL as obtained from our ROC curve. As shown in Table 5, the odds of diabetic patients having nephropathy was 4.02 (95% CI of 2.5–6.5) for those having U NGAL more than 70.87 ng/ml ($p = 0.000$).

Discussion

According to recent proteomic studies, it is seen that though the diabetic kidney involvement is detected and measured by the albumin excretion, a glomerular function marker, there is tubular injury during the incipient stages of DN. Not all diabetics, but 20–30% of them develop DN, initiated and activated by long standing hyperglycemia [5]. The rate of kidney injury and tissue recovery depends on the immune response, regeneration, and scarring [15]. Any kind of epithelial damage induces a high expression of NGAL, leading to an increase in the baseline serum level. In normal conditions, the NGAL filtered by the glomerulus is reabsorbed by proximal tubules and only a minimal amount is excreted in the urine. Tubular injury resulting from ischemia, inflammation, and hyperglycemia leads to a decrease in NGAL reabsorption and an increase in NGAL secretion by tubular cells [14].

In our study, there was an increase in urinary biomarkers, UACR, and U NGAL in the diabetics as compared to controls and further rise was seen in patients with

Table 3 AUC for combined ROC for ACR and U NGAL in predicting DN

Area under the curve					
Test result variable(s)	Area	Std. error[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
NGAL	0.992	0.004	0.089	0.983	1.000
ACR	1.000	0.000	0.084	1.000	1.000

[a] Under the nonparametric assumption

[b] Null hypothesis: true area = 0.5

Table 4 The correlation of the parameters used for DN detection

		eGFR	ACR
eGFR	*r*		− 0.101*
	p		0.049
ACR	*r*	− 0.101*	
	p	0.049	
U NGAL	*r*	− 0.062	0.301**
	p	0.223	0

DN as compared to those diabetics without DN. Similar results were shown by other cross-sectional comparative studies and the brief findings are represented in the table below:

Reference	Type of study	Cases of T2DM	Controls	Result of U NGAL in DN	U NGAL cut-off level. Our study, 70.87 ng/ml with a 66.7% sensitivity (sn) and 66.8% specificity (sp)	Correlation with ACR (r). Our study: $r = 0.301$ ($p = 0.000$)	Inference with respect to our study
2009, Bolignano [16]	Observational study	56	18	Significantly elevated in patients with micro- and macroalbuminuria	22 ng/ml; 75% sn and 100% sp	Not done	Similar
2013, Assal et al. [17]	Cohort	70	20	Significantly elevated in patients with micro- and macroalbuminuria	13.5 ng/ml; 70.6% sn and 83.3% sp	0.707 ($p < 0.001$)	Similar
2014, Al-Refai [18]	Observational study	46	15	log UNGAL/Creatinine ratio was different in cases and controls but not in groups with different urine albumin levels	Not applicable	No significant correlation	Different calculated parameter used
2015, Garg et al. [12]	Observational study	91	–	Significantly elevated in prediabetes and T2DM patients with normo- and microalbuminuria	Not mentioned	$r = 0.820$ ($p = 0.000$)	Partly similar
2017, Zeng [14]	Observational cohort study	146	30	Significantly elevated in patients with microalbuminuria	85.0 ng/ml; 66.7% sn, and 87.5% sp.	0.563 ($p = 0.000$)	Similar
2018, Vijay et al. [19]	Cross-sectional comparative study	126; 63 each in groups without and with microalbumin	30	Significantly elevated in patients with microalbuminuria	146.28 ng/ml; 82.5% sn and 72% sp	0.85 ($p = 0$)	Similar
2019, Sueud [20]	Observational study	90	90	Significantly elevated in patients with normo-, micro-, and macroalbuminuria	21.4 ng/ml; 94.67% sn, 26.67% sp.	0.009 ($p = 0.983$)	Partly similar

The NGAL which is a proximal and distal tubular protein is increased along with the albumin which denotes a glomerular dysfunction. Abbasi et al. suggested that NGAL and retinol binding protein-4 (RBP-4) both tubular proteins can be measured complementary to albumin as they appear before microalbuminuria, the glomerular marker [21]. There were reports which suggest that the presence of microalbuminuria alone does not indicate DN [22] and additional markers like non-albumin protein (NAP) [23], cystatin C [17], clusterin [14], sialic acid [24], and the most validated marker that is NGAL [12, 14, 19–21, 25] have to be included. The limitation of the present study was that we could not complete the recall of duration of disease for all our cases and most of our subjects were not aware of the family history of DM, so we could not analyze the same variables to assess the association of urinary biomarkers with DN. We suggest cohort studies to confirm the diagnostic utility of NGAL in DN.

Table 5 The risk assessment of U NGAL cut-off obtained from ROC curve

		Diabetic nephropathy		
		Absent	Present	Total
U NGAL (ng/ml)	< 70.87	84.80%	15.20%	100%
	> 70.87	57.80%	42.20%	100%
Total (n= 385)		73.50%	26.50%	100%
Risk estimate	Value	95% Confidence interval		
		Lower	Upper	p*
Odds ratio for U NGAL > 70.87 ng/ml	4.086	2.527	6.607	0

*For Fisher's exact test computed from the 2 × 2 table

Conclusion

As tubular damage occurs prior to glomerular damage in DN, we conclude that measurement of urinary NGAL predicts DN better than microalbumin alone.

Acknowledgments We acknowledge the support of the patients and healthy controls who consented to participate and cooperate during the data collection. We are grateful to our institute for funding the research. We thank Mr. Mahesh Sahu, Mr. John Joseph, and Miss Abigail Joseph for their help in data entry.

Authors' contributions Dr. Suchanda Sahu, SS; Dr. Manish Taywade, MT; Dr. Balamurugan Ramadass, BR; Dr. Gautom Kumar Saharia, GKS.

SS conceived and designed the project, and MT, BR, and GKS edited the same. SS and MT executed the study and acquired the data. SS was involved in the analysis and interpretation of the data. SS, MT, BR, and GKS helped in the preparation of the manuscript and approved the final draft.

Funding This study was funded by All India Institute of Medical Sciences (AIIMS), Bhubaneswar, Odisha, India, as part of the intramural research grant for faculty; the grant number was T/IM-F/18-19/32.

Availability of data and material Dataset: NGAL and UACR in DN.

File: data repository; https://doi.org/10.17632/28r5bwb6pn.1#file-5c2be2ac-dbe7-4107-bd42-d0cfedceca8e

Sahu, Suchanda (2020), "NGAL and UACR in DN," Mendeley Data, v1 https://doi.org/10.17632/28r5bwb6pn.1

Compliance with ethical standards

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

Ethics approval This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Full board review was sought for by the Institutional Ethics Committee (IEC) of AIIMS, Bhubaneswar, India, and the IEC approval granted was T/IM-F/18-19/32 dated 22nd December 2018.

The study was approved by the Institutional Ethics Committee.

Consent to participate Written consent was obtained from each participant after explaining the nature of study to each in the language that each follows.

Consent for publication Our participants have consented to publish the compiled data, keeping their identity confidential.

Code availability Not applicable.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):498–503
https://doi.org/10.1007/s13410-020-00901-9

ORIGINAL ARTICLE

Use of the online Framingham platform for the evaluation of the cardiovascular risk in diabetes mellitus and systemic arterial hypertension patients in primary health care

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Received: 1 July 2020 / Accepted: 26 November 2020 / Published online: 7 January 2021

Abstract

Background Cardiovascular disease (CVD) is influenced by several factors. In this context, identifying cardiovascular risk (CVR) may contribute to taking action on modifiable risk factors especially in the population with diabetes mellitus (DM) and systemic arterial hypertension (SAH) in primary health care, where laboratory tests are often difficult to access.

Objective The objective of this study was to evaluate the risk of developing cardiovascular disease in the next 10 years in diabetic and hypertensive primary healthcare patients using the online Framingham platform.

Material and methods This is a cross-sectional study. Were evaluated 246 individuals by medical records, from the Center for Specialized Medicine in Diabetes and Hypertension. The Framingham Heart Study online table was used to assess CVR. The variables collected were blood pressure and body circumferences.

Results Sixty-five (26.42%) were diabetic, 67 (27.23%) were hypertensive, and 114 (46.34%) had DM and SAH. Significant values of CVR were observed in the SAH (19.76%) and DM + SAH (33.79%) groups when compared with the DM group (10.68%).

Conclusion In conclusion, the online Framingham platform tool was able to identify the CVR. Additionally, SAH seems to be a more powerful factor to increase CVR, and the coexistence of DM and SAH increases this risk even more.

Keywords Diabetes mellitus · Systemic arterial hypertension · Cardiovascular risk · Risk score calculator

Introduction

Diabetes mellitus (DM) is now considered a worldwide endemic, especially in poorer countries [1]. The literature is robust in showing the link between DM and cardiovascular disease (CVD). Thus, DM is expected to be a major driver of CVD worldwide [2]. Systemic arterial hypertension (SAH) is a clinical condition characterized by increased pressure levels and affects the world population significantly [3]. Its prevalence has steadily increased even with the expanded use of antihypertensive drugs. It is already well established in the literature that hypertension is associated with increased all-cause mortality, independent of other risk factors [4].

SAH, DM, and dyslipidemia are important collective health problems in Brazil, due to their high prevalence, the acute and chronic complications that they give rise to, and because they represent risk factors associated with CVD, conditioning high morbidity and mortality rates [5], social and economic costs arising from the use of health services. Together, DM and SAH are the major contributors to the global burden of disease [6].

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In an attempt to assess and predict long-term cardiac risk, vascular age has been shown to be a valuable indicator/predictor [7]. In this context, as the vascular age increases, so does the progression of arterial stiffness [7]. Some well-known factors such as age [8], DM [9], and SAH [10] are associated with vascular aging, sometimes prematurely.

Thus, the aim of this study was to establish the risk of developing cardiovascular disease in the next 10 years in diabetic and hypertensive patients, especially in primary health care, as access to laboratory tests is often extremely difficult. Additionally, we further assessed the impact of coexistence of DM and SAH over 10 years, as we hypothesized that this coexistence would increase this cardiovascular risk when compared with the population with the disease alone. Finally, as secondary objectives, we evaluated the vascular age in these conditions in isolation (DM or SAH) and in their coexistence (DM + SAH), as well as making correlations between the waist, wrist, neck and calf circumferences, and CVR and vascular age.

Methods

Study design and ethical aspects

This is a retrospective, descriptive, quantitative study aiming to analyze the profile of patients enrolled at the Center for Specialized Medicine in Diabetes and Hypertension in São Luís (Maranhão, Brazil). The inclusion criteria adopted for the medical records were individuals of both genders, aged between 30 and 74 years, with a medical diagnosis of DM and SAH, undergoing treatment for both, diagnosed within a maximum of 5 years. Medical records with missing, unreadable, or erased data were excluded.

Analysis of medical records

The data contained in the medical records were recorded in an identification form containing the following items: name, gender, age, medical diagnosis, and time since diagnosis, medications in use, systolic blood pressure (SBP), diastolic blood pressure (DBP), height, weight, body mass index (BMI), perimeter (abdominal, right wrist, right ankle, right calf), and capillary blood glucose.

Cardiovascular risk assessment

To assess cardiovascular risk, we used the online platform Gencardio based on the study conducted by D'Agostino et al. [11], available on The Framingham Heart Study website: https://www.framinghamheartstudy.org/fhs-risk-functions/cardiovascular-disease-10-year-risk. In this platform is calculated, through multivariate regression using some predictors, the risk of the individual developing cardiovascular disease within the next ten years. It is noteworthy that the use of this tool is encouraged in the strategy for modifying risk factors, as well as for calculating the estimate of vascular or cardiometabolic age by the Brazilian Society of Cardiology [12].

The Gencardio platform was fed with the following data: gender (male or female); age (between 30 and 74 years), SBP, if being treated for hypertension (yes or no), if you were a smoker (yes or no), if you had diabetes (yes or no), and BMI (15 to 50 kg/m^2). After the data entry, through the calculations performed by the software, it was possible to obtain the percentage of CVR and the vascular age in years.

Statistical analysis

Histograms were created to test data normality, and all outcomes had normal distributions. The data were expressed as mean and standard deviation (SD) values. Multivariate analysis of variance (MANOVA) analysis was used, and groups that differed significantly were compared at a pair level using the Tukey test. Pearson's correlation coefficient was used to correlate waist, wrist, ankle, and calf circumferences with CVR and vascular age. The SPSS program, version 17.0 (Chicago, IL, USA), was used for all analyses, with a 5% significance level established for comparisons.

Results

A total of 2113 medical records were reviewed. After applying the eligibility criteria, the medical records of 246 patients were included, 145 (58.94%) women and 101 (41.05%) men, with a medical diagnosis of DM and hypertension. Data were obtained by reviewing the medical records of patients treated at the service between 2017 and 2018.

Differences in the sample diagnoses showed that 65 (26.42%) of the patients were diabetic, 67 (27.23%) were hypertensive, and 114 (46.34%) were associated with diabetes and hypertension.

Table 1 presents the sample characterization data, according to the division by group. It was observed that age, weight, BMI, SBP, DBP, waist, and wrist circumferences were significantly higher ($p < 0.05$) in the SAH and DM + SA groups when compared with the DM group. In addition, as expected, blood glucose was significantly ($p < 0.05$) elevated in the DM and DM + SAH groups when compared with the SAH group.

Table 1 Sample characterization according to the groups

Variables	All ($n = 246$)	DM (n = 65)	SAH (n = 67)	DM + SAH ($n = 114$)
Age (years)	54.10 (11.84)	48.69 (11.80)	54.61 (11.53) [a]	56.88 (11.08) [a]
Gender (female)	145 (58.94%)	41 (63.07%)	34 (50.74%)	70 (61.40%)
Height (m)	1.56 (0.09)	1.55 (0.08)	1.57 (0.09)	1.55 (0.08)
Weight (kg)	69.57 (15.88)	62.41 (11.64)	73.27 (17.39) [a]	71.47 (15.89) [a]
BMI (kg/m^2)	28.42 (5.84)	25.80 (3.59)	29.47 (7.36) [a]	29.29 (5.44) [a]
Glycemia (mg/dL)	187.06 (92.14)	223.66 (107.85)	111.40 (23.46) [a, b]	210.66 (82.28)
SBP (mmHg)	133.25 (26.40)	112.15 (7.80)	140.74 (27.54) [a]	140.87 (26.19) [a]
DBP (mmHg)	81.74 (13.29)	74.61 (7.51)	87.16 (15.35) [a]	86.22 (12.36) [a]
Tobacco (no)	238 (96.74%)	61 (94.28%)	62 (93.58%)	109 (95.48%)
Body composition				
Underweight	8 (3.3%)	2 (3.08%)	2 (2.99%)	4 (3.51%)
Eutrophic	58 (23.6%)	26 (40%)	13 (19.40%)	19 (16.66%)
Overweight	100 (40.7%)	29 (44.61%)	28 (41.79%)	43 (37.72%)
Obese I	53 (21.5%)	8 (12.31)	12 (17.91%)	33 (28.95%)
Obese II	19 (7.7%)	0	8 (11.94%)	11 (9.65%)
Obese III	8 (3.3%)	0	4 (5.97%)	4 (3.51%)
Circumferences (cm)				
Abdominal	95.36 (12.29)	88.93 (10.06)	96.15 (13.47) [a]	98.57 (11.38) [a]
Wrist	16.91 (1.58)	16.30 (1.15)	17.11 (1.41) [a]	17.14 (1.79) [a]
Ankle	21.59 (3.16)	20.91 (1.76)	22.53 (3.79) [a]	21.43 (3.27)
Calf	34.61 (4.58)	33.43 (3.52)	35.49 (5.64) [a]	34.76 (4.31)

DM: diabetes mellitus; SAH: systemic arterial hypertension; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure

BMI according to by Word Health Organization: <18.5 underweight; 18.5–24.9 Normal or eutrophic; 25; 29.9 Overweight or pre-obesity; 30–34.9 Obesity class I, 30–39.9 Obesity class II and, ≥40.0 Obesity class III

[a] Differs from DM group ($p < 0.05$, MANOVA post hoc Tukey);

[b] Differs from DM + HAS group ($p < 0.05$, MANOVA post hoc Tukey)

Data regarding CVR and vascular age are presented in Table 2. For CVR, significantly ($p < 0.05$) higher values were observed in the SAH and DM + SA groups when compared with the DM group. However, the DM + SAH group also presented significantly higher ($p < 0.05$) CVR values than the SAH group. Regarding vascular age, the DM + SAH group presented values significantly ($p < 0.05$) higher than the SAH and DM group.

The correlations of waist, wrist, ankle, and calf circumferences with CVR and vascular age are shown in Table 3. Significant ($p < 0.05$), positive, and weak magnitude ($0.140 \leq r \leq 0.367$) correlations were observed between the abdominal and wrist circumferences and the CVR and vascular age.

The drugs used by the patients are described in Table 4, showing the greater use of biguanides (70.76%) and sulfonylureas (44.66%) in the DM group; angiotensin antagonists (76.62%), and diuretics (31.34%) in the SAH group; and biguanides (81.57%), sulfonylureas (58.77%), angiotensin antagonists (55.26%), and diuretics (28.94%) in the DM + SAH group.

Table 2 Prediction of cardiovascular risk and vascular age according to group division

Cardiovascular risk prediction	All (n = 246)	DM ($n = 65$)	SAH ($n = 67$)	DM + SAH ($n = 114$)
Cardiovascular risk (%)	23.86 (19.11)	10.68 (8.88)	19.76 (15.93) [a, b]	33.79 (19.75) [a]
Vascular age (years)	72.13 (13.80)	66.69 (14.75)	67.79 (13.41) [b]	80.00 (8.33) [a]

DM: Diabetes mellitus; SAH: systemic arterial hypertension

[a] Differs from DM group ($p < 0.05$, MANOVA post hoc Tukey);

[b] Differs from DM + HAS group ($p < 0.05$, MANOVA post hoc Tukey)

Table 3 Correlation of abdominal, wrist, ankle and calf circumferences with cardiovascular risk and vascular age

Circumferences (cm)	Cardiovascular risk	Vascular age
Abdominal	r = 0.367. $p < 0.001$ [a]	r = 0.314. p < 0.001 [a]
Wrist	r = 0.323. p < 0.001 [a]	r = 0.140. $p = 0.015$ [a]
Ankle	r = 0.102. $p = 0.112$	r = 0.012. $p = 0.844$
Calf	r = 0.037. $p = 0.563$	r = 0.047. $p = 0.438$

[a] Significant correlation ($p < 0.05$, Pearson correlation coefficient)

Discussion

The main findings of this study showed (i) SAH, even if controlled, seems to be a major risk factor for the onset of cardiovascular disease when compared to DM alone; ii) the coexistence of DM and SAH significantly increases the CVR; iii) vascular age is higher when DM and SAH coexist; iv) the higher the abdominal and wrist circumference, the greater the CVR and vascular age.

SAH and CVR

Recently, a systematic review was conducted by Petrie et al. [13] in order to clarify the pathophysiological mechanisms of vascular complications and concluded that DM is associated with a higher CVR, which is increased when there is a coexistence of SAH. Our findings showed that being hypertensive results in a higher CVR (7.05% increase) than DM itself. However, our study agrees to show that the coexistence of both diseases significantly increases CVR when compared with the isolated presence of DM or SAH.

In addition, results from the Trial Evaluating Cardiovascular Outcomes with Sitagliptin —TECOS trial— show that, although SAH is one of the leading modifiable causes of cardiovascular events in adults with diabetes, it remains with suboptimal values worldwide. In addition, around 40% of individuals with high CVR have SBP values of ≥ 140 mmHg, which corroborates our study [14].

Coexistence of DM, SAH, and CVR

The literature is robust as to the higher CVR in the presence of DM or SAH, it also shows that these two diseases are the strongest predictors for CVD [15, 16]. The control of both is linked to decreased future cardiovascular events [17, 18]. However, it is noteworthy, according to the results of the present study that even blood pressure levels for the SAH group being within the levels considered acceptable for hypertensive individuals, the CVR of these patients was higher than in the DM group.

However, the literature is unclear about the CVR in the coexistence of DM and SAH. Thus, we need to start from a point even earlier than this, because, according to data from a large study called the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus (SAVOR)—Thrombolysis in Myocardial Infarction (TIMI) 53 trial—it is uncertain at this time how to define the optimal blood pressure value for the prevention of cardiovascular

Table 4 Medicines used according to groups

Medicines	DM ($n = 65$)	SAH ($n = 67$)	DM + SAH ($n = 114$)
Oral antidiabetics			
Sulfaniureas	29 (44.66%)	0	67 (58.77%)
Biguanides	46 (70.76%)	0	93 (81.57%)
Antidislipidemic	11 (16.92%)	18 (29.84%)	35 (30.7%)
Insuline	16 (24.61%)	0	12 (10.52%)
Thiazolidinediones	0	0	2 (1.75%)
Combination	9 (13.84%)	0	5 4.38%)
Antihypertensives			
Diuretics	0	21 (31.34%)	33 (28.94%)
Beta blockers	0	14 (20.89%)	14 (12.28%)
Angiotensin antagonist	0	50 (74.62%)	63 (55.26%)
Calcium blocker	0	10 (14.92%)	16 (14.03%)
ACE inhibitor	0	4 (5.97%)	15 (13.15%)
Antiplatelet/Anticoagulant	0	17 (25.37%)	13 (11.4%)
Cardioglycosides	0	1 (1.49%)	1 (0.87%)
Combination	0	1 (1.49%)	4 (3.5%)

DM diabetes mellitus, *SAH* systemic arterial hypertension, *ACE* angiotensin-converting-enzyme

Data presented in absolute values (%). Combination: Patients taking more than one drug for the same purpose

events in patients with DM [19]. However, the findings of Bergmark et al. partially corroborate the findings of this study, as the authors found a persistent association between subclinical myocardial injury and risk of myocardial infarction in diabetic patients with elevated CVR [19].

A study conducted by Böhm et al. aimed to evaluate the relationship of blood pressure in the CVR of individuals with and without DM and found that in patients without DM, high blood pressure, i.e., systolic > 160 or diastolic > 90 mmHg, was associated with higher CVR and death, and levels considered low/normal (< 120 or < 70 mmHg) had the same cardiovascular outcome, except for stroke and death, whereas patients with DM have higher risks across the full range of blood pressure consistently [20]. This fact was not identified in our study since patients with only DM had blood pressure within the normal limits.

Vascular age and CVR

Arterial aging is characterized by increased arterial stiffness, which can be assessed by pulse wave velocity [21], which is considered the gold standard for such diagnostic purposes [22]. On the other hand, it is understood that performing this exam in an accessible manner is still a distant reality due to its high cost [23]. From this perspective, other tools for calculating vascular age have been investigated and indicated in an attempt to fill this gap, such as the tool used in this study [12].

Body circumferences and CVR

The literature is robust with regard to abdominal circumference showing a positive correlation with negative cardiovascular outcomes [24, 25]. According to a study conducted by Rezende et al., which corroborates our findings, overweight, especially above-expected abdominal circumference, has a major impact on increased CVR, as shown by the positive correlation between the abdominal circumference and CVR.

Currently, another anthropometric measure, wrist circumference, has been suggested to be associated with insulin resistance in both obese children and adolescents, as reported by a study conducted by Capizzi et al. [26].

In this context, an interesting cohort study conducted by Noudeh et al. aiming to analyze whether wrist circumference was associated with the incidence of DM, independently of other adiposity measures such as BMI or waist circumference of an Iranian adult population, found that in a 20-year-old population, wrist circumference was significantly associated with DM and its risk factors in both sexes [27]. The results observed in the present study reinforce the findings of Noudeh et al. since a positive correlation was found between wrist circumference and CVR; however, it should be remembered that the individuals in this study already had a clinical diagnosis of DM. Thus, wrist measurement could also be considered an additional predictor for the development of CVD in individuals with a definite diagnosis of DM and SAH, especially in the primary care as is the case in our study.

This study has some limitations that should be cited, such as the lack of complementary laboratory tests (e.g., glycated hemoglobin). The lack of correct completion of medical records was another limiting factor, which we tried to remedy by excluding incomplete medical records. The DM group had a BMI statistically lower than the others, which is an important limitation. However, all groups had mean BMI within the same category, i.e. overweight (25 to 29.9 kg/cm^2).

Conclusion

In view of the above, we can conclude that SAH seems to be a more powerful factor for increased CVR, and the coexistence of DM + SAH further increases this risk. Online CVR prediction tools should be encouraged especially in the primary health care. Thus, secondary prevention measures could be adopted to prevent the coexistence of a more severe cardiovascular disease in these patients. However, the groups have heterogeneity and differences in some clinical aspects.

Acknowledgments This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

Compliance with ethical standards

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

Ethical consideration This study was submitted to the research ethics committee of the Ceuma University (São Luís, Brazil) in accordance with the Declaration of Helsinki and approved under protocol number 2.524.515.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):504–510
https://doi.org/10.1007/s13410-020-00891-8

ORIGINAL ARTICLE

Development and testing of Diabetes Complications Risk Educational Tool (DiREcT) for improving risk perception among patients with diabetes mellitus: a mixed method study

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Received: 17 April 2020 / Accepted: 28 October 2020 / Published online: 6 January 2021

Abstract

Background The problem of type 2 diabetes mellitus and its complications are threateningly increasing in the existing health scenario. The awareness and perception about risk of developing complications and role of self-care practices for glycemic control are very important. This study aimed to develop and validate an educational tool for improving perception about risk of getting complications among patients with diabetes mellitus.

Methodology This mixed method study was conducted in a tertiary hospital. Known risk factors of diabetic complications were identified from literature and drafted based on their strength of association. A panel of eight experts from different departments was constituted to evaluate the risk factors, and the tool was finalised after three rounds using Delphi technique. Content and construct validity of the developed tool were also assessed by the panel. A pilot study among 50 patients with diabetes mellitus was done to assess the effectiveness of the tool.

Results The DiREcT tool developed had 11 risk factors of diabetic complications, each categorised into low, moderate and high risk. On baseline risk assessment, 60% of the participants were in high-risk category for glycemic control. Patient's perception about risk of developing complications increased from 26 to 60% by using the tool. More than 80% of the participants reported that the tool is simple and easy to understand.

Conclusion The DiREcT tool was effective in changing perception of developing complications among patients with diabetes mellitus. It can be used by the healthcare provider at the primary healthcare level for both educating and monitoring of patients with high risk.

Keywords Diabetes-related complications · Risk factors · Delphi technique · Educational tool

Introduction

Diabetes mellitus and its related complications show a large increase in prevalence all around the world [1]. The international diabetic federation in 2015 released data on global prevalence of diabetes mellitus. This report shows that around 5 million deaths occurred due to diabetes-related complications and around 673 billion US dollars spent on the treatment of diabetes and its complications [2]. Indians are more susceptible to develop diabetes and its related complications because of the variations in cultural practices, food habits and behavioral factors [3]. These complications may be either micro-vascular or macro-vascular. The main risk factors for all micro- and macro-vascular complications were age, duration of disease, hypertension, hyperlipidemia, family history, smoking, physical activity, BMI and diet [4]. Financial burden associated with the treatment of complications is also high. Based on the recent studies in India, around 25% of the total family income is spent on treatment of patients with diabetes mellitus in a low-income family [5].

Education is one of the key components in the treatment of diabetes and prevention of complications. Evidences are showing that an increase in knowledge about diabetes will

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help to improve the outcome and thus ultimately will help to reduce the complications and problems related to it [6]. Creating more awareness and providing knowledge about diabetes and its complications are very necessary to face the threat of diabetes and related complications. Health belief model for behavior change shows that people will be ready to adopt some practices if they perceive the susceptibility and severity of the disease.

The Indian diabetes risk score (IDRS) is a popular screening tool for risk of developing diabetes mellitus [7]. The American Diabetes Association risk scores for the early prediction of pre-diabetes and diabetes have also been studied [8]. However, due to multiplicity of risk factors and diabetes-related complications, screening tools to assess the risk of diabetes complications in Indian context are very rare.

There are few studies in India, which developed an educational tool related to obesity, smoking cessation and improvement of physical activities in school settings using Delphi's technique [9–11]. In disease like diabetes mellitus, where a major part of management includes self-care practices, individuals with diabetes mellitus must perceive their susceptibility to develop complications to adopt behavioral and lifestyle modifications. In this context, improving the awareness and perception about complications are very necessary, which is poor in the Indian setting [6, 12]. There are some screening tools available like AHA/ACC (American Heart Association/ American College of Cardiology) calculator to predict the cardiovascular risk status among patients with diabetes [13]. But stratification of risk factors for all types of complications of diabetes mellitus is yet to be developed. Hence, the main purpose of this study is to develop an educational material to improve the risk perception of developing complications among patients with diabetes mellitus and to pilot the tool to assess the feasibility.

Methodology

This is a mixed method study carried out from September to October 2019 in the endocrinology department in a tertiary care centre of Puducherry. The department runs outpatient (OP) services twice in a week, and each day a minimum of 300 patients avail care during the OP time. Development and validation of the educational tool using Delphi's technique contributed to the qualitative part, and it was piloted among patients with diabetes mellitus (quantitative part).

A panel of eight members with more than 3 years' experience in the field of diabetes mellitus was selected from different disciplines for development of the tool. We restricted the sample size to fifty. Literature suggests that for a pilot trial, a sample size of thirty can be assumed [14]. This study was approved by the Institutional Ethics Committee, and informed consent was obtained from all the study participants. A thorough literature search was done to identify the traditional risk factors of diabetes-related complications. Risk factors for complications of diabetes mellitus were listed based on the odds ratio (OR), and the tool was finalised after three rounds of discussion among the panel (Fig. 1).

Development of the tool was followed by piloting among patients with diabetes mellitus. Socio-demographic variables, clinical characteristics of the study participants, behavioral factors and perception about risk of developing complications were enquired during the baseline assessment. While assessing clinical status of the patients, data like fasting blood sugar (FBS), systolic blood pressure, lipid profile and weight were extracted from their case sheets.

After the baseline assessment, ascertainment of the level of risk for each risk factor was done and marked on the tool to sensitise the patients about their status. The importance of following self-care practices for glycemic control was also discussed.

End line assessment was done using the same questionnaire; change in perception of developing complications and intention to adopt self-care practices was assessed. At the end of the study, feedback about the tool was obtained using a rating scale on the following domains: layout, understandability, actionability, recall, attractiveness and simplicity. Data entry was done in EpiData Entry Client version 4.0, and analysis was done using SPSS version 22. Data are presented as means, standard deviation and percentages.

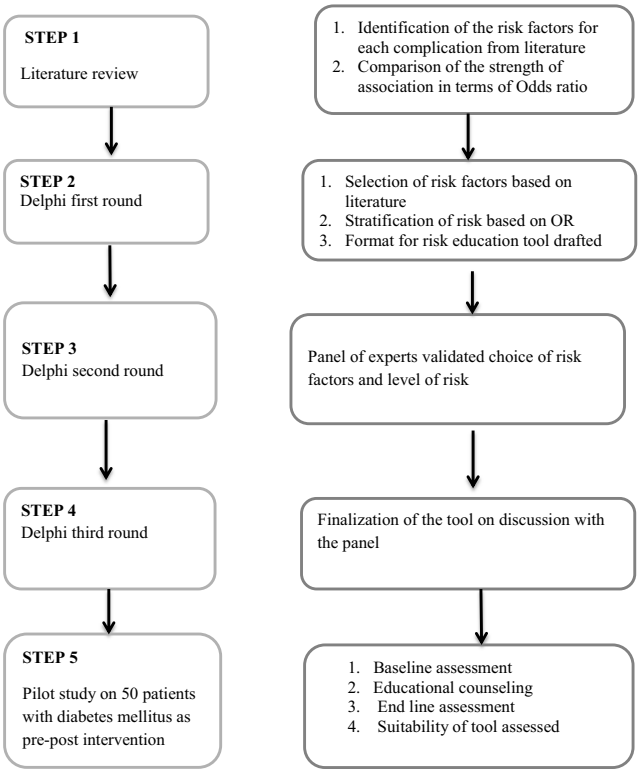

Fig. 1 Study procedure

Results

Around 55 articles were reviewed from both Indian and international journals published between 2000 and 2018. The first draft of 23 risk factors for complications of diabetes mellitus was submitted to the Delphi's panel. The suggestion given by panel on each risk factor was collected by the facilitator. Socio-economic status and gender from the risk factors were removed from the list due to poor strength of association. The number of risk factors was reduced to 12 at the end of the first round of Delphi. After the second round of Delphi, 11 important risk factors were included in the tool and categorised into low, moderate and high risk. Further, categories of family history and hyperlipidemia were corrected to include the risk strata. Medication adherence was defined based on standard tool (Morisky 4-item scale) as high, medium and low and used as low, moderate and high risk of complications.

The final round of Delphi's technique contributed to the colour coded format of the educational tool (low, green; moderate, yellow; and high, red), and risk factors were arranged into modifiable (risks 1–8) to non-modifiable (risks 9–11). Diabetes Complications Risk Educational Tool (DiREcT) thus developed had additional illustrations to depict complications as images and also translated into the local language. Content and construct validity of the developed tool were also assessed by the panel. Operational definitions and risk stratification procedures were defined and approved by the panel members.

A pilot study was done on 50 patients with diabetes mellitus with mean age of 52.9 years (range 35–75 years). More than half (54%) were females and education up to 10th class. Around half of them (46%) were from lower-middle class family. Half of the patients with diabetes mellitus were in the category of overweight and pre-hypertensive. Three-fifth of the patients had a family history of either DM or hypertension. Only 18% of patients reported high medication adherence, and one-fourth of the participants were doing physical activity more than 150 min/week (Table 1).

Table 2 shows the stratification of patients with diabetes mellitus in accordance with the categories of each risk factor. More than half of the participants constituted a high-risk category for sugar control and dietary pattern, but only one-third from medication adherence. Half of the study participants were in the moderate-risk category in the case of systolic BP control, BMI and age of onset; however, very less percentage contributed to the high-risk category from duration of disease and family history (18% and 16% respectively). After the counselling intervention, the patient's perception about risk of developing complications increased from 26 to 60%. At baseline, none of the participants believed that they are at risk of developing complications, but after the health counselling using the tool, 6% of them perceived their high-risk status (Figs. 2 and 3).

Table 3 shows changes in intention to do self-care practices before and after the intervention. Majority of the patients with diabetes mellitus were not using tobacco and alcohol, and they monitored blood sugar regularly. There was an increase of 26% regarding intended to do the physical activity of minimum 150 min/week. In case of intention to reduce body weight, an increase of 36% occurred after the intervention. Regarding feedback about the tool, majority of them reported good category for layout and actionability of the tool. More than half of the participants opined that it was very easy to understand and memorise the contents of the tool (Table 4).

Discussion

In this study, we developed a new educational tool about the risk stratification of diabetes-related complications using Delphi's technique. By piloting this tool among 50 patients with diabetes mellitus, we could assess the utility and effectiveness in improving risk perception.

The 11 risk factors were categorised in to high, moderate and low and colour coded as red, yellow and green considering the concept of health literacy of the patients. Many studies identified visual tools as a better health education aid to change health behaviors of the patients [15]. Similarly, few studies from India developed and applied educational tool such as colour coded graphical recording of HbA1C and colour coded HbA1C thermometer [16, 17]. In contrast to our study, they focussed on a single entity (control status of HbA1C) than other potential risk factors of diabetic complications [18]. But for the application of this tool, technical knowledge and trained manpower are needed. In contrast to this, the tool which we developed is simple to understand; thus, its application in primary care settings and community level will be easy.

The risk factors were arranged from modifiable to non-modifiable, and each one ranked based on their potency to cause complications of diabetes mellitus. Poor glycemic control and hypertension are important risk factors (OR 2.33 to 7.61) [19–21]. Poor glycemic control was the dominant risk factor for all types of complications as reported by Dagliati et al. [18] using machine learning. Similarly, among non-modifiable risk factors, duration of diabetes was ranked as a major risk factor (OR 2.20 to 6.50) [22–24]. Fowler [25] reported that glycemic control, duration of disease and hypertension were the main risk factors contributing to microvascular complications of diabetes mellitus, especially for diabetic retinopathy [25]. Duration of disease and BMI were also found to be more powerful risk factors for diabetic retinopathy and diabetic neuropathy, while hypertension had a major role in diabetic nephropathy [18].

In the baseline assessment, half of the study participants were aware about the complications. Among those who were

Table 1 Socio-demographic, clinical and behavioral characteristics of the patients with diabetes mellitus in Puducherry (2019) ($N = 50$)

Variables	Categories	*n*	%
Age (years)	35–45	13	26
	46–55	17	34
	56–65	16	32
	66–75	4	8
Educational status	No formal education	13	26
	Primary school (1–8)	31	62
	Secondary school (9–12)	3	6
	Graduation and above	3	6
Gender	Male	23	46
	Female	27	54
Occupation	Employed	24	48
	Unemployed	5	10
	Home maker	21	42
Socio-economic status*	Class 1 (< 1050)	6	12
	Class 2 (1051–2101)	23	46
	Class 3(2102–3503)	3	6
	Class 4 (3504–7007)	12	24
	Class 5 (≥ 7008)	6	12
Fasting blood sugar (mg/dL)	< 100	5	10
	101–125	12	24
	≥ 126	33	66
Systolic blood pressure (mmHg)	Normal (≤ 120)	22	44
	Prehypertension (140–159)	25	50
	Hypertension (≥ 160)	3	6
Lipid profile (mg/dl)	All four values normal	20	40
	Any two values abnormal + LDL normal	14	28
	Any three including LDL is abnormal	16	32
Duration of disease	< 5 years	27	54
	5–10 years	14	28
	> 10 years	9	18
BMI[a]	Underweight (< 18.5)	5	10
	Normal (18.5–22.99)	17	34
	Over weight (23–24.99)	25	50
	Obese (≥ 25)	3	6
Family history of either DM or HTN	Yes	30	60
	No	20	40
Co-morbidity	Hypertension	10	20
	Hyperlipidemia	14	28
	HTN and hyperlipidaemia	16	32
	CVD	1	2
	Others*	1	2
	Nil	8	16
Tobacco use	Yes	7	14
	No	43	86
Medication adherence[b]	High	9	18
	Medium	22	44
	Low	19	38
Physical activity[c]	≥ 150 min/week	13	26
	100–149 min/week	21	42
	< 100 min/week	16	32

*Modified BG Prasad scale, [a] Asian BMI criteria, [b] Morisky 4-item scale, [c] based on WHO criteria. Other co-morbidities—thyroid dysfunction, PCOD

aware, 18% were aware about the cardiovascular complications of diabetes. This could be due to the age, poor education and socio-economic status of the participants. Moreover, overcrowded clinics offer very little time for counselling and education regarding risks. Studies have reported that half of the general population and more than 70% diabetic population knew that diabetes could affect other organs [6]. But other studies conducted in different parts of India reported varying levels of awareness about diabetes and its related complications (25–40%) [12].

In our study, people who perceived that they were not at risk of developing complications at baseline were increased more than two times after using the tool. Of the participants, 60% of them reported change in perception regarding risk of developing complications after introducing the tool. This could be due to the educational content of the tool as well as the risk ascertainment interaction that happened in the process. Cooper et al. also reported statistically a significant ($p < 0.003$) change in awareness and perception regarding diabetes and its complications after educational counselling [26]. Malathy et al. also reported a significant change in knowledge, attitude and self-care practices after the health education [27].

Table 2 Distribution of study participants in different risk categories of diabetes-related complications based on the DiREcT tool in Puducherry (2019) ($N = 50$)

Variable	Low risk *n* (%)	Moderate risk *n* (%)	High risk *n* (%)
Glycemic control (FBS-mg/dl)	5 (10)	12 (24)	33 (66)
Systolic blood pressure (mmHg)	22 (44)	25 (50)	3 (6)
Lipid profile	20 (40)	14 (28)	16 (32)
BMI	22 (44)	25 (50)	3 (6)
Smoking status	32 (64)	13 (26)	5 (10)
Physical activity	13 (26)	21 (42)	16 (32)
Medication adherence	9 (18)	22 (44)	19 (38)
Dietary pattern	2 (4)	14 (28)	34 (68)
Duration of diabetes	27 (54)	14 (28)	9 (18)
Age of onset	6 (12)	26 (52)	18 (36)
Family history	20 (40)	22 (44)	8 (16)

RISK STRATIFICATION FOR DEVELOPMENT OF DIABETES RELATED COMPLICATIONS			
	LOW RISK	**MODERATE RISK**	**HIGH RISK**
1.Poor glycemic control (Fasting)	<100	101-125	≥ 126
2. Systolic BP (mmHg)	120 – 139	140- 159	>160
3.Hyperlipidemia (mg/dl)	*All four normal	Any two values higher +LDL normal	Any three including LDL is higher
4. Obesity	18.5-22.9	23-24.9	25-29.9
5. Smoking	Never smoker	Ex-smoker	Current smoker
6. Physical activity	≥150 min/week	100-149 min/week	<100 min/week
7. Dietary pattern	More vegetables& proteins+ less carbohydrate	Less proteins& vegetables+ more carbohydrate	Only carbohydrate
8.Medication adherence	High adherence	Medium adherence	Low adherence
9. Duration of diabetes	<5 years	5-11 years	>10 years
10. Family history	1st degree relative*- no DM complication and HTN	1st degree relative-H/O either one	1st degree relative-H/O both
11. Age of onset	≥ 60 years	41-60 years	20- 40 years

Fig. 2 Model of the Diabetes Complications Risk Educational Tool (DiREcT). All four values = LDL, HDL, VLDL and triglyceride; 1st degree relative = parents, children and siblings; ex-smoker—stopped smoking since 1 month. Medication adherence assessed by 4 questions such as ever forgotten, not careful about taking, stop taking if feel better and stop taking if feel worse based on a scale where 1 = yes and 0 = no

However, as this study was done in a single time point, only intention to adopt lifestyle practices was recorded. One-third of the participants reported willingness to increase physical activity and reduce body weight. This is comparable to similar interventions based on health belief model. However, further follow-up of these patients is required to measure adherence to self-care practices to observe better outcomes like improved BP/glycemic control and reduction of diabetes-related complications. Periodic reassessments by the patients themselves may also be helpful to reinforce healthy behavior.

Our study found that the colour coded tool was effective in improving the awareness and perception regarding risk of developing diabetes-related complications. This might be due to several reasons. First, this tool allows for easy assessment of patient status for each risk factor into low-, moderate- and high-risk categories. This will enable risk perception among patients with high risk to adopt self-care practices and at the same time encourages the patients with low risk to maintain their status. Secondly, the tool provides a holistic picture of the patient's risk status, without focussing on glycemic control alone. So it can be used by healthcare providers at all levels to monitor the high-risk patients by using this tool and counsel regarding the early suitable interventions accordingly.

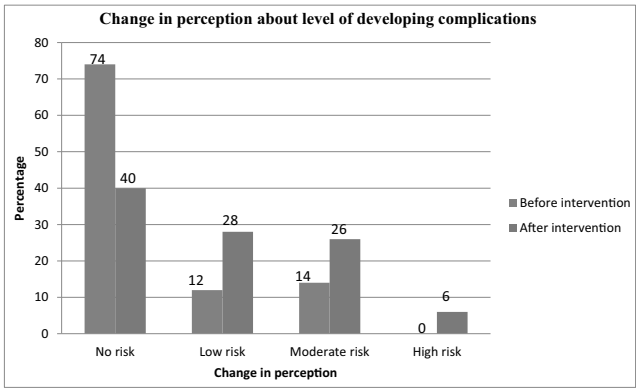

Fig. 3 Perception about level of developing complication among patients with diabetes mellitus before and after the intervention in a tertiary care hospital from September to October 2019 ($N = 50$)

The importance of enabling the environment to improve physical activities thus improving the health status and preventing various non-communicable diseases was mentioned by Sook et al. in a study to find out the associated factors of level of physical activities [28]. At the same time, Lee et al. reported the importance of assessing factors associated with gestational diabetes and its role to cause neonatal respiratory distress [29]. Considering all these findings, we can say that the DiREcT tool may also be used in other settings such as pregnancy and elderly groups and other concurrent risk factors for cardiovascular disease such as physical inactivity and hypertension, which can also be used in daily OPD services, education sessions and community programmes.

Many studies have been conducted to find out the awareness level and prevalence of diabetic complications. Awareness about the complications of diabetes is influenced by many other factors like education, socio-economic status, health literacy and quality of the care provided by the health

Table 3 Intention to do self-care practices among patients with diabetes mellitus before and after the educational counselling using DiREcT tool in Puducherry (2019) ($N = 50$)

Variable	Before *n* (%)			After *n* (%)		
Intention to	Agree	Neutral	Disagree	Agree	Neutral	Disagree
Do exercise at least 150 min per week	33 (66)	10 (20)	7 (14)	46 (92)	0	4 (8)
Reduce body weight	27 (54)	14 (28)	9 (18)	45 (90)	4 (8)	1 (2)
Avoid starch rich food	37 (74)	6 (12)	7 (14)	49 (96)	2 (4)	0
Avoid sugar	44 (88)	4 (8)	2(4)	48 (96)	2 (4)	0
Check blood sugar regularly	48 (96)	1 (2)	1 (2)	50 (100)	0	0
Stop tobacco use	48 (96)	2 (4)	0	50 (100)	0	0
Stop alcohol use	49 (98)	1 (2)	0	50 (100)	0	0

system. So awareness along with the perception about risk of developing complications and practicing of lifestyle modifications will play a major role in decreasing various types of diabetes complications.

Currently, to predict the risk of diabetes mellitus, tools like ADA diabetes risk score and IDRS are available. These tools were developed after conducting cohort studies and validated by independent population survey. Further research using the DiREcT tool on a larger sample of patients with complications of diabetes mellitus can be done to validate the tool. Identification of the patients with high risk of developing complications forms the pillar of both secondary and tertiary levels of prevention that include disease treatment to reduce burden due to complications and disabilities.

Conclusion

This study concludes that the risk stratification tool for complications of diabetes mellitus is effective in improving risk perception and intention to adopt lifestyle. This is a low cost and non-invasive process to identify the patients with high-risk status. It can be used by the healthcare provider at the primary healthcare level for both educating and monitoring of patients with high risk.

Table 4 Feedback about DiREcT tool by patients with diabetes mellitus attending OPD in a tertiary care hospital Puducherry (2019) ($N = 50$)

Variables	Very good *n* (%)	Good *n* (%)	Average *n* (%)	Below average *n* (%)
Layout	6 (12)	39 (78)	4 (8)	1 (2)
Easy to understand	37 (74)	13 (26)	0	0
Easy to memorise	31 (62)	16 (32)	1 (2)	2 (4)
Attractive	42 (84)	5 (19)	1 (2)	2 (4)
Actionable	14 (28)	33 (66)	2 (4)	1 (2)
Simplicity	42 (84)	7 (14)	1 (2)	0

Compliance with ethical standards

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

Ethics approval This study was approved by the Institutional Ethics Committee (reference number JIP/IEC/2019/0311).

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

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):511–517
https://doi.org/10.1007/s13410-020-00908-2

ORIGINAL ARTICLE

Risk of LGA in pregnant women with different GDM status and risk profiles

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Received: 26 September 2020 / Accepted: 22 December 2020 / Published online: 2 January 2021

Abstract

Objectives To compare risk of large-for-gestational age (LGA) infants and pregnancy outcomes between pregnant women with different gestational diabetes (GDM) status.

Methods GDM screening was offered to 1510 women during 24–28 weeks of gestation and during first visits for those with GDM risks. Women were categorized into 3 groups: Group1: women without GDM; group2: women at-risk but did not have GDM; and group3: women with GDM. Rate of LGA and other outcomes were compared and associated factors were evaluated.

Results A total of 408 women were in group1 (without GDM), 893 were in group2 (at-risk but without GDM), and 209 had GDM (group3). GDM women were significantly more likely to be older, multiparous, overweight/obese, have previous GDM, have less gestational weight gain, and have preeclampsia. Rates of LGA were 11.3% in group 1, 17.2% in group 2, and 22% in group 3 ($p = 0.001$). Independent risk of LGA was 2nd trimester weight gain > 7 kg in group1 (adjusted OR 2.65, 95%CI 1.36–5.15). In group 2, independent associated factors were overweight/obesity (adjusted OR 2.04, 95%CI 1.38–3.01) and 2nd trimester weight gain > 7 kg (adjusted OR 1.59, 95%CI 1.1–2.28). In group 3, independent associated factors were 2nd trimester weight gain > 7 kg (adjusted OR 2.3, 95%CI 1.07–4.98) and suboptimal glycemic control (adjusted OR 3.12, 95%CI 1.3–7.69).

Conclusion Women with different GDM status had different characteristics and outcomes. Second trimester weight gain > 7 kg was the common independent risk of LGA and suboptimal glycemic control increased LGA risk in GDM women.

Keywords Gestational diabetes · LGA · Weight gain · Risks

Introduction

Gestational diabetes mellitus (GDM) is among the common complications during pregnancy that affects an increasing number of pregnant women globally [1–3]. Associated maternal and perinatal complications include preeclampsia, cesarean delivery rate, large for gestational age (LGA) fetus, fetal macrosomia, and future risk for diabetes mellitus [1–3]. To date, there is still no global consensus on the most appropriate strategy for GDM screening and diagnosis. A 2-step approach with a 50-g glucose challenge test (GCT) and 100-g oral glucose tolerance test (OGTT) is still recommended by some international organizations [1, 2, 4]. Universal screening during 24–28 weeks of gestation is recommended and early screening is also suggested among women at high-risk for GDM [1, 2, 4].

Although GDM is associated with various adverse pregnancy outcomes, at-risk women who do not have GDM could also be at increased risk for adverse outcomes as well. Previous studies have reported that GDM-related outcomes also developed among women without GDM who had positive GCT (false-positive GCT) as well [5–7]. Nonetheless, the previous studies have variations in population characteristics, GDM risks, screening and diagnostic strategies, and screening and diagnostic test used.

Currently, only women who are diagnosed with GDM are treated, but not those who do not have GDM, regardless of GCT results of GDM risks. These women are managed similar to other normal pregnant women. However, pregnant women with different glycemic and GDM profiles might have different degree of glucose intolerance and different risks of adverse pregnancy outcomes. In addition, there is also an evidence that treatment of mild maternal hyperglycemia could help reducing the risks of fetal overgrowth, shoulder dystocia, cesarean

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delivery, and hypertensive disorders [8]. Therefore, understanding the relationships between different GDM profiles and adverse outcomes could lead to better care of the women.

The primary objective of this study was to compare risk of LGA and other pregnancy outcomes between pregnant women with different GDM status and risk profiles, i.e., without GDM, at-risk but did not have GDM, and GDM. In addition, associated factors for LGA in each GDM status and risk profiles were evaluated.

Materials and methods

After approval from the Siriraj Institutional Review Board, a total of 1510 pregnant women attending antenatal clinic before 24 weeks of gestation were included in this retrospective cohort study. Women with pre-existing diabetes and those with severe fetal anomalies or fetal death were excluded. A 50-g GCT was used as a screening test with 140 mg/dL cutoff value and a 100-g OGTT was used to diagnose GDM, using Carpenter and Coustan criteria [9]. Screening was performed on all women during 24–28 weeks. Additional early screening was also performed during first antenatal visit among women at high risk for GDM and the tests were repeated during 24–28 weeks if the initial tests were normal. Women were considered at-risk for GDM if they had any of the following characteristics: age ≥ 30 years, DM in family, pre-pregnancy BMI ≥ 25 kg/m^2, previous GDM, previous macrosomia, previous congenital anomaly or intrauterine fetal death, and hypertension [10].

In this study, the women were categorized into 3 groups according to their GDM status and risk profiles. Group 1 were women without GDM; group 2 were women with GDM risks but did not have GDM; and group 3 were women with GDM. All women diagnosed with GDM initially received dietary counseling, nutritional therapy, and glucose monitoring, either by self-monitoring or intermittent plasma glucose testing during antenatal care visits. Pharmacological therapy with insulin was initiated as necessary. A 2-h postprandial plasma glucose of < 120 mg/dL and/or fasting plasma glucose of < 95 mg/dL was set as glycemic target. Optimal glycemic control was defined as more than 80% of plasma glucose monitoring results were within glycemic targets.

Data were extracted from medical records, including baseline, obstetric and antenatal care data, GDM risks, diagnosis of GDM, and pregnancy outcomes. The Institute of Medicine (IOM) recommendation is used to classify pre-pregnancy BMI and gestational weight gain (GWG) [11]. Weight gain during 2nd trimester was estimated from weight differences between 14 and 16 and 26–28 weeks of gestation. LGA was defined as neonatal birth weight of ≥ 90th percentile for gestational age using the WHO weight percentiles calculator [12].

Various characteristics were described using descriptive statistics as appropriate. Baseline, obstetric, GDM-related characteristics, and pregnancy outcomes were compared among the 3 groups using chi-square test and one-way analysis of variance (ANOVA) with Bonferroni test for post hoc comparison as appropriate. In each group, risk factors for LGA were initially evaluated by univariate analysis and logistic regression analysis was further performed to determine independent associated factors for LGA, adjusted for potential confounders. A p value of < 0.05 was considered statistical significant.

Results

A total of 1510 women were included and baseline characteristics are shown in Table 1. Majority of women had at least 1 GDM risks (73%) and mean GA at screening and diagnosis of GDM were 10.7 and 16.1 weeks of gestation. Prevalence of GDM was 13.8%. All the women were categorized into 3 groups according to GDM status and risk profiles. Group 1 consisted of 408 women without GDM (27%), group 2 consisted of 893 women with GDM risks but did not have

Table 1 Baseline characteristics of participants (n = 1510)

Characteristics	N (%)
Mean age ± SD (years)	30.8 ± 6.1
Mean BMI ± SD (kg/m^2)	22.3 ± 4.3
Nulliparous	847 (56.1)
BMI category	
Underweight	266 (17.6)
Normal	927 (61.4)
Overweight/obese	317 (21)
GDM risks	
Age ≥ 30 years	925 (61.3)
DM in family	286 (18.9)
BMI ≥ 25 kg/m^2	317 (21)
Previous GDM	20 (1.3%)
Previous macrosomia	3 (0.2)
Previous fetal death or anomaly	7 (0.5)
Hypertension	35 (2.3)
Number of GDM risks	
No risk	408 (27)
1 risk	693 (45.9)
≥ 2 risks	409 (27.1)
GDM characteristics	
GA at diagnosis ± SD (weeks)	16.1 ± 8.6
GDM status and risk profiles	
Group1: no GDM	408 (27)
Group 2: at risk, no GDM	893 (59.1)
Group3: GDM	209 (13.8)

GDM (59.1%), and group 3 consisted of 209 women with GDM (13.8%).

Various characteristics were compared among the 3 groups and the results are demonstrated in Table 2. Women in group 1 were significantly more likely to be younger, underweight, and nulliparous. In terms of GDM risks, age ≥ 30 years, BMI of ≥ 25 kg/m^2, previous GDM, and hypertension were significantly more common among those diagnosed with GDM (group 3).

Pregnancy outcomes were compared among the 3 groups and the results are demonstrated in Table 3. Women with GDM were significantly less likely to gain weight greater than recommendation and less likely to have 2nd trimester weight gain of > 7 kg and had lower GA at delivery than the other 2 groups. Rate of LGA was 22% in GDM compared to 17.2% in group 2 and 11.3% in group 1, with a significant linear trend ($p = 0.001$). Preeclampsia was significantly more common in GDM than the other 2 groups ($p = 0.002$).

Univariate analysis of risk factors for LGA was performed and the results are shown in Table 4. In group 1 (no GDM risk), significant risks were gestational weight gain greater than recommendation (RR 2.6, 95%CI 1.3–5.0, $p = 0.003$) and 2nd trimester weight gain of > 7 kg (RR 2.5, 95%CI 1.4–4.4, $p = 0.001$). In group 2 (at risk but without GDM), significant risks were overweight/obesity (RR 1.6, 95%CI 1.2–2.1, $p = 0.003$), gestational weight gain greater than recommendation (RR 2.0, 95%CI 1.4–2.8, $p < 0.001$), and 2nd trimester weight gain of > 7 kg (RR 1.4, 95%CI 1.1–1.9, $p = 0.02$). In group 3 (GDM), significant risks were overweight/obesity (RR 1.7, 95%CI 1.02–2.8, $p = 0.039$), hypertension (RR 2.3, 95%CI 1.2–4.3, $p = 0.03$), 2nd trimester weight gain of > 7 kg (RR 2.1, 95%CI 1.3–3.5, $p = 0.006$), and suboptimal glycemic control (RR 2.1, 95%CI 1.2–3.3, $p = 0.012$).

Logistic regression analysis was performed to determine independent associated factors for LGA in each group, adjusting for potential confounders, and the results are shown in Table 5. Among those in group 1 (no GDM), the only significant risk was 2nd trimester weight gain of > 7 kg (adjusted OR 2.65, 95%CI 1.36–5.15, $p = 0.004$). Among those in group 2 (at risk but without GDM), significant risks were overweight/obesity (adjusted OR 2.04, 95%CI 1.38–3.01, $p < 0.001$), and 2nd trimester weight gain of > 7 kg (adjusted OR 1.59, 95%CI 1.1–1.28, $p = 0.013$). Among those in group 3 (GDM), significant risks were 2nd trimester weight gain of > 7 kg (adjusted OR 2.3, 95%CI 1.07–4.98, $p = 0.034$) and suboptimal glycemic control significantly decreased the risk (adjusted OR 3.12, 95%CI 1.3–7.69, $p = 0.011$).

Discussion

Prevalence of GDM in this study was 13.8% and majority of the women were at risk but did not have GDM (59.1%). The 3

Table 2 Comparison of various characteristics between 3 GDM profiles

	Group 1 No GDM $N = 408$	Group 2 At risk, no GDM $N = 893$	Group 3 GDM $N = 209$	p value
Mean age ± SD (years)	24.4 ± 3.6	32.8 ± 5.0	35.1 ± 4.4	< 0.001[a]
Mean BMI ± SD (kg/m^2)	19.9 ± 2.6	22.9 ± 4.4	24.1 ± 4.7	< 0.001[a]
Nulliparous	279 (68.4)	476 (53.3)	92 (44)	< 0.001
BMI category				< 0.001
Underweight	130 (31.9)	121 (13.5)	15 (7.2)	
Normal	278 (68.1)	527 (59)	122 (58.4)	
Overweight/obese	0 (0)	245 (27.4)	72 (34.4)	
GDM risks				
Age ≥ 30 years	NA	732 (82)	193 (92.3)	< 0.001
DM in family	NA	222 (24.9)	64 (30.6)	0.087
BMI ≥ 25 kg/m^2	NA	245 (27.4)	72 (34.4)	0.044
Previous GDM	NA	7 (0.8)	13 (6.2)	< 0.001
Previous macrosomia	NA	2 (0.2)	1 (0.5)	0.468[b]
Previous fetal death or anomaly	NA	7 (0.8)	0 (0)	0.199[b]
Hypertension	NA	22 (2.5)	13 (6.2)	0.005
Number of GDM risks				< 0.001
1 risk	NA	594 (66.5)	99 (47.4)	
≥ 2 risks	NA	299 (33.5)	110 (42.6)	

[a] Analysis of variance, Differences were significant statistically between each group ($p < 0.001$)

[b] Fisher exact test

Table 3 Comparison of various pregnancy outcomes between 3 GDM profiles

Pregnancy outcomes	Group 1 No GDM $N = 408$	Group 2 At risk, no GDM $N = 893$	Group 3 GDM $N = 209$	p value
Mean GA at delivery ± SD (weeks)	38.2 ± 1.6	38.1 ± 1.5	37.7 ± 1.7 [c]	< 0.001[a]
Mean birth weight ± SD (kg)	2999.1 ± 429.9	3082.2 ± 423.5 [c]	3075.0 ± 513.2	0.006[a]
Mean GWG ± SD (kg)	14.9 ± 5.2	13.9 ± 5.2	11.8 ± 4.8 [c]	< 0.001
Mean 2nd trimester WG ± SD (kg)	6.7 ± 2.4	6.2 ± 2.5	5.3 ± 2.6 [c]	< 0.001
GWG category				< 0.001
Less than recommended	106 (26)	201 (22.5)	72 (34.4)	
As recommended	152 (37.3)	327 (36.6)	83 (39.7)	
Greater than recommended	150 (36.8)	365 (40.9)	54 (25.8)	
2nd trimester WG > 7 kg	179 (43.9)	327 (36.6)	52 (24.9)	< 0.001
Route of delivery				< 0.001
Vaginal delivery	262 (64.2)	374 (41.9)	73 (34.9)	
Primary CS	114 (27.9)	335 (37.5)	88 (42.1)	
Repeat CS	32 (7.8)	184 (20.6)	48 (23)	
Preeclampsia	15 (3.7)	37 (4.1)	20 (9.6)	0.002
Neonatal outcomes				
LGA	46 (11.3)	154 (17.2)	46 (22)	0.001[b]
Macrosomia	9 (2.2)	19 (2.1)	5 (2.4)	0.972
Apgar at 1 min < 7	22 (5.4)	35 (3.9)	15 (7.2)	0.109
Apgar at 5 min < 7	2 (0.5)	6 (0.7)	1 (0.5)	0.899
Neonatal hypoglycemia	10 (2.5)	34 (3.8)	12 (5.7)	0.119
NICU admission	6 (1.5)	13 (1.5)	8 (3.8)	0.057

[a] Analysis of variance

[b] Significant linear trend, $p = 0.001$

[c] Significant difference from the other 2 groups ($p < 0.01$)

groups of women had different characteristics and outcomes. As expected, GDM women were more likely to be older, multiparous, overweight, and obese. However, they gained significantly less weight than the other 2 groups, possibly due to the results of the treatment provided. LGA risk increased significantly with increasing abnormality of glycemic profiles in a linear fashion, from 11.2% in those without GDM to 22% in GDM women. At-risk women who did not have GDM were also at increased risk of LGA, up to 17.2%. This could reflect that there might be different degrees of glucose intolerance between different glycemic and GDM profiles. The results were similar to a previous study that reported risk of LGA of 15.6% and 13.1% in women without GDM with normal and false-positive GCT, respectively [13]. Other previous studies have also reported that women without GDM, but with positive GCT, were at increased risk of various adverse outcomes, including LGA, macrosomia, shoulder dystocia, and cesarean delivery [5–7].

Mild maternal hyperglycemia in women without GDM has been reported to associate with adverse pregnancy outcomes, probably due to some degree of glucose intolerance. An evidence suggested that treatment of mild maternal hyperglycemia could reduce the risks of fetal overgrowth, shoulder dystocia, cesarean delivery, and hypertensive disorders [8]. Therefore, greater attention should be paid on these at-risk women even though they did not have GDM. A more intensive counseling on nutrition and behavioral changes should also be provided similar to those diagnosed with GDM to minimize the risk of adverse outcomes. This is of greater importance since this group of women contributed to almost 60% of all women.

Subgroup analysis revealed that factors associated with LGA differed between women with different GDM profiles. However, 2nd trimester weight gain of > 7 kg was the common independent associated risk factor in every group of women. Previous studies from the same institute have reported the importance of 2nd trimester weight gain and its effect on adverse outcomes. Second trimester weight gain of > 7 kg significantly increased the risk of LGA in both women without GDM who had normal and false-positive GCT [13] and also significantly increased the risk of GDM after normal first trimester screening [14]. Since weight gain is modifiable, pregnant women should control their weight gain to minimize the risk of LGA, regardless of GDM diagnosis and glycemic profiles.

Table 4 Univariate analysis of risk of LGA according to 3 GDM profiles

LGA risks	Group 1: No GDM		Group 2: At risk, No GDM		Group 3: GDM	
	RR (95%CI)	*p* value	RR (95%CI)	*p* value	RR (95%CI)	*p* value
Age ≥ 30	NA		0.9 (0.6–1.4)	0.776	0.5 (0.3–1.1)	0.126
Multiparous	1.4 (0.8–2.4)	0.245	1.3 (0.9–1.7)	0.107	1.5 (0.8–2.5)	0.153
BMI						
Overweight/obesity	NA	NA	1.6 (1.2–2.1)	0.003	1.7 (1.02–2.8)	0.039
Underweight	0.5 (0.3–1.05)	0.057	0.8 (0.5–1.4)	0.435	NA	0.065
Normal	1.0		1.0		1.0	
DM in family	NA	NA	1.1 (0.8–1.5)	0.725	0.9 (0.5–1.6)	0.695
Previous GDM	NA	NA	1.7 (0.5–5.4)	0.347	1.4 (0.6–3.4)	0.489
Previous macrosomia	NA	NA	2.9 (0.7–11.7)	0.315	4.6 (3.6–6.0)	0.220
Hypertension	NA	NA	1.3 (0.6–2.9)	0.565	2.3 (1.2–4.3)	0.030
GWG category						
> recommended	2.6 (1.3–5.0)	0.003	2.0 (1.4–2.8)	< 0.001	1.7 (0.9–3.0)	0.056
< recommended	0.9 (0.4–2.3)	0.844	0.7 (0.4–1.2)	0.171	0.7 (0.3–1.4)	0.280
As recommended	1.0		1.0		1.0	
2nd trimester WG > 7 kg	2.5 (1.4–4.4)	0.001	1.4 (1.1–1.9)	0.02	2.1 (1.3–3.5)	0.006
Suboptimal glycemic control	NA	NA	NA	NA	2.1 (1.2–3.3)	0.012

NA not available

As optimal glycemic control is key in GDM management, [1, 2] the results of this study showed that optimal glycemic control is an independent factor related to reduction of LGA risk among GDM women. Similar association has been previously reported. Suboptimal glycemic control during pregnancy is associated with adverse maternal and fetal outcomes, both in GDM and those with preexisting diabetes [15]. A more recent study reported that poor glycemic control was associated with perinatal complications including LGA and respiratory disorders [16]. Another study also reported that glycemic control at delivery was predictive of LGA in women with type 2 DM [17].

Table 5 Independent risk factors for LGA according to 3 GDM profiles, adjusted for potential confounders

Risk factors	Adjusted OR	95% CI	*p* value
Group1: no GDM[a]			
2nd trimester weight gain > 7 kg	2.65	1.36–5.15	0.004
Group 2: at-risk, no GDM[b]			
BMI ≥ 25 kg/m^2	2.04	1.38–3.01	< 0.001
2nd trimester weight gain > 7 kg	1.59	1.1–2.28	0.013
Group 3: GDM[b]			
2nd trimester weight gain > 7 kg	2.3	1.07–4.98	0.034
Suboptimal glycemic control	3.12	1.3–7.69	0.011

[a] Adjusted for age, parity, and BMI

[b] Adjusted for age, parity, BMI, and GDM risks

The results of this study also showed that preeclampsia was significantly increased among GDM. This was in consistent with previous studies. GDM and preeclampsia share similar risk factors, including advanced maternal age, nulliparity, and pre-pregnancy obesity [18]. Women with GDM had higher maternal age and were more likely to be overweight or obese that might have partly contributed to the increase in preeclampsia, as obesity has been associated with a 2–3-fold increased incidence of preeclampsia and also appears to be dose-related [19, 20]. A previous study also reported that GDM diagnosis prior to 20 weeks of gestation and poor glycemic control were the most significant risk factors for preeclampsia [21]. However, conflicting results have been reported from a recent study that did not find a significant association between GDM and the development of preeclampsia [22]. Further studies are needed to explore and understand the relationship and risk of preeclampsia among women with GDM.

The strengths of this study may include the relatively large sample of pregnant women and all the women received similar GDM screening and diagnostic procedures according to institutional guideline. Moreover, the determination of LGA

was based on the data from the same population. However, there are some limitations to mention. GDM screening and diagnostic strategies that vary between studies could limit generalizability of the results and comparison of the results between studies would be less valid. Although a recent survey showed that majority of the countries in Asia-Pacific region use a 1-step approach for GDM screening [23], a 2-step approach similar to current study is still used in Thailand and some other counties. The results may still be useful and applied to those using similar approach. Misclassification of 2nd trimester weight gain group could occur but the errors should be minimal due to relatively uniform antenatal care schedule with minimal variations and it should not significantly alter the results.

In conclusion, women with different GDM profiles had different characteristics and pregnancy outcomes, suggesting that they might be different groups of women that possessed different risks of adverse outcomes. Pregnant women with different glycemic profiles might require different approaches and management. As weight gain is the common factor associated with LGA and it is modifiable, appropriate dietary counseling and close weight gain monitoring can be beneficial to all women regardless of glycemic and GDM profiles in order to reduce the risk of LGA. In addition, a more intensive surveillance of fetal growth should be considered among pregnant women who gain weight rapidly. Nonetheless, further studies are still needed to evaluate and explore in more details the differences between women with different glycemic and GDM profiles. In addition, the most appropriate and effective management of these women to minimize the risk of adverse perinatal outcomes are to be investigated and implemented.

Authors' contributions D.B., C.P., and T.W. plan and design the study together. C.P. and T.W. were responsible for data collection. D.B. analyzed the data and interpreted the results. C.P. and T.W. drafted the manuscript and D.B. revised the manuscript. All the authors approved and agreed with this final version of the manuscript.

Data availability Not applicable.

Compliance with ethical standards

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

Ethics approval The study was approved by the Siriraj Institutional Review Board.

Consent to participate Not applicable.

Consent for publication Not applicable.

Code availability Not applicable.

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International Journal of Diabetes in Developing Countries (July–September 2021) 41(3):518–521
https://doi.org/10.1007/s13410-020-00913-5

CASE REPORT

Rare neurological complications associated with critically ill pediatric diabetic ketoacidosis—a report of two cases

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Received: 11 August 2020 / Accepted: 23 December 2020 / Published online: 7 January 2021

Abstract

Introduction This case series highlights rare reversible neurological complications encountered in two children who presented with diabetic ketoacidosis (DKA) and were critically ill.

Case reports The first child was a 5-month-old baby with transient neonatal diabetes who had prolonged coma, atypical findings of diffusion restriction on neuroimaging and later quadriplegia due to rhabdomyolysis when he presented with DKA. The second child was a 16-year-old boy with classical type 1 diabetes mellitus who developed quadriplegia due to rhabdomyolysis and polyneuromyopathy. Both the children were critically ill with hemodynamic compromise, renal impairment and dyselectrolytemia. Both had full recovery on follow-up at the end of 1 month and 10 months, respectively.

Conclusion The severity of DKA at presentation with altered sensorium and/or hemodynamic compromise with renal involvement and dyselectrolytemia and a coexistent infection may have more propensity for such complications. Strict implementation of DKA management guidelines and a multidisciplinary approach to deal with the critically ill child is needed to optimize the outcome in such situations.

Keywords Case report · Diabetic ketoacidosis · Diffusion restriction · Rhabdomyolysis · Polyneuromyopathy · Critical illness myopathy and neuropathy

Introduction

Cerebral edema, the associated raised intracranial pressure and coma are the most dreaded neurological complications of diabetic ketoacidosis (DKA). However, other complications like arterial ischemic and haemorrhagic strokes, venous sinus thrombosis, pontine and extrapontine myelinolysis, permanent cognitive impairment and reversible blindness have also been reported [1–4].We aim to describe two critically ill children with two different causes of childhood diabetes who presented with rare neurological complications associated with severe DKA.

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Case presentation

Case 1 A 5-month-old male baby presented with 5 days history of fever, lethargy, vomiting and breathing difficulty. The child was diagnosed with DKA, and due to worsening respiratory distress, the child was ventilated and transferred. On admission, the child had Glasgow coma scale (GCS) of 4 and was febrile, tachycardic, tachypnoeic and hypotensive with signs of bronchopneumonia. There were no signs of meningeal irritation or papilledema. There was hypotonia, quadriparesis and diffuse hyporeflexia. Initial investigations confirmed DKA with mild renal impairment with negative blood and urine cultures. A provisional diagnosis of neonatal diabetes (NDM) was made, and he was initiated on treatment for DKA as per International Society for Adolescent and Pediatric Diabetes (ISPAD) protocol. The child remained comatosed for the next 72 h. CT brain did not show cerebral edema. The child had deep vein thrombosis involving left lower limb veins. As sensorium did not show the expected

improvement even after DKA resolved, MRI brain was taken which revealed diffusion restriction in bilateral basal ganglia, occipital lobes and around central sulcus (Fig. 1). CSF and EEG were noncontributory. Sensorium improved after 72 h, but quadriplegia persisted with hypotonia and hyporeflexia. Subsequent CPK done was 16,395 U/L suggestive of rhabdomyolysis, but urine myoglobulin was negative. Intravenous immunoglobulins and mitochondrial cocktail were given considering the multiaxial neurological involvement. Muscle biopsy, NCV and TMS and GCMS for inborn errors of metabolism were normal. The CPK progressively declined over the next 10 days and gradually movement of all 4 limbs improved. The child was discharged with GCS of 15 on insulin, oral anticoagulants and mitochondrial cocktail. Genetic testing revealed a heterozygous missense mutation in ABCC8, pArg1183Trp confirming the diagnosis of transient NDM. The child was switched over to glibenclamide which was tapered gradually and stopped after 9 months when HbA1c improved to 5.1%. MRI brain after 1 year showed complete resolution of the lesions (Fig. 1). The child had normal developmental milestones and is now 5 years old.

Case 2 A 16-year-old boy presented with abdominal pain and fever with progressive lethargy, tachypnoea and altered sensorium. He was diagnosed with DKA with renal dysfunction and referred. He was initiated on treatment for DKA as per ISPAD protocol. In view of anuria and severe acidosis, hemodialysis was initiated. He gradually improved and was switched over to subcutaneous insulin when he developed a gluteal abscess. On the tenth day of onset of illness, he was noted to have flaccid quadriparesis with areflexia. EMG was suggestive of myopathy with muscle irritability consistent with possibility of diffuse myositis. CPK was 7805 U/L. NCV was suggestive of a diffuse sensorimotor axonal neuropathy. Muscle biopsy was normal. Rhabdomyolysis with a parainfectious or metabolically mediated polyneuromyopathy was considered one of the differentials, and he was subjected to two sessions of plasmapheresis with which muscle power

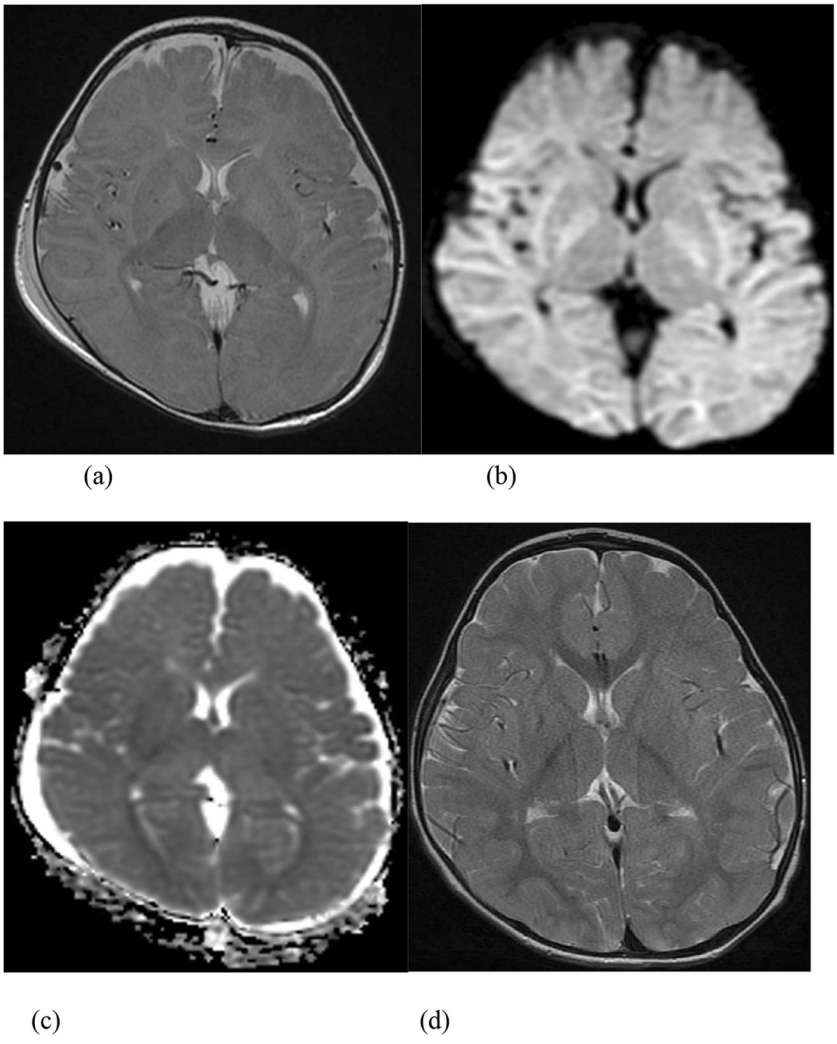

Fig. 1 MRI brain of case 1. **a** T2W image shows hyperintensities in bilateral basal ganglia and white matter tracts. **b**, **c** Diffusion weighted and ADC images reveal cytotoxic oedema. **d** T2W image on follow-up at 1 year shows complete resolution of the lesion

improved. Renal function also improved with time. The neurological deficit completely recovered in 10 months with physiotherapy without any recurrences. C peptide was 0.455 ng/ml and anti-insulin antibodies were positive confirming a diagnosis of type 1 diabetes.

The biochemical parameters at presentation and follow-up of the two cases are given in Table 1.

Discussion

This case series highlights rare neurological complications associated with DKA in two children who were critically ill with two different etiologies for DKA—NDM and type 1 DM, respectively. These included cerebral cytotoxic edema with diffusion restriction on MRI in patient 1, probable rhabdomyolysis with elevated CPK in patients 1 and 2 and acute polyneuromyopathy in patient 2.

T2 hyperintensities in basal ganglia with restricted diffusion found in patient 1 during the acute phase completely resolved on follow-up without any residual sequelae. Convergence of multiple reversible predisposing factors like hyperglycemia, hypernatremia, hyperosmolarity and parainfectious immunological derangements leading to osmotic swelling of axons and myelin along with the breakdown of the blood-brain barrier might have contributed to these findings [5].

Acute neuromuscular complications in the setting of DKA is relatively rare and is more commonly seen in a combined DKA and hyperglycemic hyperosmolar state(HHS) when it is associated with multiple metabolic derangements like high glucose, urea, creatinine, sodium, and osmolarity along with low pH, phosphorous, and potassium. These may lead to increased intracellular calcium which can activate the proteases leading to muscle cell damage [6, 7]. Both patients 1 and 2 who developed rhabdomyolysis with markedly elevated CPK levels more than 10 times the upper limit of normal had high serum sodium, osmolarity and blood glucose at presentation in addition to hypophosphatemia and hypokalemia. Polyneuromyopathy during a critical illness is well described and can result from microcirculatory dysfunction, cellular hyperglycemia, acidosis and hypoalbuminemia which can enhance endoneural edema and contribute to bioenergetic failure leading to primary axonal degeneration [8]. Patient 2 was noted to have a flaccid paralysis on the tenth day of illness when there was an associated infection, suggesting that there might be the possibility of an underlying immune-mediated inflammatory response also contributing to the pathophysiology of the clinical presentation, and hence immunotherapy was given. But the full recovery and absence of relapse rule out a chronic immunological disease.

Critical illness polyneuropathy (CIP) and critical illness myopathy (CIM) are close differential diagnosis in both these cases, but CIP and CIM are used as umbrella terms to describe the final clinical presentation of diverse pathophysiological processes affecting the neuromuscular axis during a critical illness and are diagnosis of exclusion. In the classical CIM, CPK levels are only minimally elevated, and levels above 10 times the upper limit of normal as in our cases may suggest rhabdomyolysis or necrotizing myositis. By this case report, we would like to highlight this association between rhabdomyolysis, DKA and hyperosmolar state although it may be difficult to differentiate it from CIM/CIP.

Table 1 Biochemical parameters at admission

On admission	Case 1	Case 2
Random blood glucose (mg/dl)	385	360
HbA1c	16.5% (5.1% LFU)	15% (9.2% LFU)
Urine ketone	3 +	1 +
pH(venous)	7	6.95
Bicarbonate	11	3.05
Blood urea (mg/dl)	87	52
Serum creatinine (mg/dl)	1.5 (0.3LFU)	4.96 (0.6 LFU)
Serum osmolarity (mOsm/kg)	346	319
Serum sodium	147	140
Serum potassium	2.7	2.9
Serum calcium (mg/dl)	7.6	9.4
Serum phosphorus (mg/dl)	3.7	0.75 (3.2 LFU)
CPK IU/ml	16,895 (13,651, 10,156, 6561, 1885, 609, 243, 51) over 10 days	7805 (4795, 1457, 920, 616, 220) over 6 days

LFU last follow-up

Common factors in both these cases were the severity of DKA at presentation with altered sensorium, hemodynamic compromise, renal impairment and/or dyselectrolytemia and a precipitating infection. The pathophysiology may be multifactorial with the metabolic derangements, underlying infections and the critical illness all playing a part. Strict implementation of DKA management guidelines with diligent monitoring of fluid and electrolyte balance and a high clinical suspicion and early detection of these neurological deficits with a coordinated multidisciplinary team approach will help in achieving a better long-term outcome.

Acknowledgement Exeter Clinical Laboratory International- University of Exeter for the genetic testing of neonatal diabetes mellitus in Case 1.

Authors' contributions SRC, NB, and VKP wrote the manuscript and collected data. PVP and SN helped manage the patients, gave critical inputs and supported the research.

Data availability Available.

Compliance with ethical standards

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

Ethical approval Institutional Ethics committee approval obtained IRB-AIMS-2019-105.

Patient consent Taken.

Code availability Not applicable.

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VISION STATEMENT

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TRAINEE GRANTS (Up to 10 grants)

Research Grants upto INR 200000 to support outstanding thesis/ research work by first year MD/DNB/ PHD students/Research fellows from India.

Eligibility Criteria
All Postgraduates in First year MD, DM /DNB from any of the institutions in the country are eligible to apply

How to apply?
Send in your Research proposals by email to the RSSDI Secy/ Chairman research committee by email/ apply directly on web site.

Research proposal should have following proofs-

1. A supporting letter from your guide/ head of department stating that this is a bonafide project for your thesis and also mentioning the dates of you joining the program and expected date of graduation. The guide must also state that he/she will stand guarantee for the work done
2. A detailed budget
3. Thesis proposal approved by the department/appropriate institutional authority
4. Approval by the ethics committee

Selection Process
Proposals will be reviewed by the research committee of the RSSDI.

Disbursement of Grant
A minimum of 50% of the grant amount will be disbursed initially. Further disbursement will be done annually based on submission of progress reports on the work done and utilisation of sanctioned amount. These reports must be filed to the secretary of the RSSDI

Responsibility:
All grant awardees are expected to present their work at RSSDI Annual Conference during research presentation's session. Failure to file progress reports annually and when requested by the RSSDI and failure to present progress at RSSDI Annual conf may result in the forfeiture of the grant.

All awardees are expected to follow the tenets of responsible and ethical conduct of research. Unethical or fraudulent use of RSSDI research funds will warrant adverse action from the society including

forfeiture of grant, black listing in the society's databases and other legal recourses that are available to the society.

Publication
The RSSDI expects that the grant source be acknowledged in all publications and submissions made with regards to the research done with the grant.

All awardees are encouraged to submit their work to the RSDDI Journal IJDDC

CALL for RESEARCH PROPOSALS for GRANTS (up to 5 lacs)

Research proposals are invited from Indian scientists, who are members of RSSDI interested in conducting research in the field of Diabetes, Endocrinology& Metabolism, for funding by RSSDI

The proposals may of clinical or translational research importance. A maximum grant amount of INR 5 Lakhs will be sanctioned. All grants will be reviewed by the research committee.

The detailed proposals should include the following:
Title, names of principal and co investigators, summary, introduction/ background, review of literature, aims, methodology, study design and detailed plan of work & bibliography.

Brief biodata of principal investigator and other co-investigators.

Importance of work
Detailed Budget sought along with full justification/ proposed utilization, of funding sought from RSSDI

Whether the project is being partly funded from any other source? If yes, please mention the source and the amount received.

Ethics Committee clearance of the Institution or other bonafide body.

How to apply
All applications should be addressed to:
1. The Secretary , RSSDI
2. Soft copy of the research proposal should be sent to Secretary, RSSDI

When to apply
Proposals will be accepted Twice a year. Once between 1st Jan - 31st April & then July 1st to 30th Nov.

All research proposals will be reviewed by Research committee over a period of 4-6 weeks & approved proposals will be provided Research Grant after fulfilling all documentation by 30th June & then 31st December of each year.

MAJOR RESEARCH GRANT PROPOSALS- usually not more than one at a given time.

Above 10 Lacs upto a total amount of 50 Lacs will be Granted to RSSDI initiated, owned, multi-centric, clinical or translational research, having long term application of scientific and clinical findings, which can translate into strategies for improving health-care delivery, patient outcomes, and community health in India.

Such research proposals will be carried out in only centres with research capabilities across India.

TRAVEL GRANTS FOR YOUNG DIABETES RESEARCHERS TO ATTEND INTERNATIONAL CONFERENCES

Criteria for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential "Advanced Certificate Course in Diabetology". This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has

List of RSSDI Accredited Centres

Sl. No	Institute Name	Institute Location
1.	Diacon Hospital	Bangalore, Karnataka
2.	North Delhi Diabetes Centre	New Delhi, Delhi
3.	Prithvi Hospital	Tumkur, Karnataka
4.	Total Diabetes Hormone Institute	Indore, Madhya Pradesh
5.	Dia Care - A Complete Diabetes Care Centre	Ahemdabad, Gujarat
6.	Sonal Diabetes Hospital	Surat, Gujarat
7.	Jothydev's Diabetes and Research Center	Trivandrum, Kerala
8.	Advanced Endocrine & Diabetes Hospital	Hyderabad, Telangana
9.	Sunil's Diabetes Care N' Research Centre	Nagpur, Maharashtra
10.	Marwari Hospital and Research Centre	Guwahati, Assam
11.	Down Town Hospital	Guwahati, Assam
12.	St.Theresa's Hospital	Hyderabad, Telangana
13.	Aegle Clinic	Pune, Maharashtra
14.	Lilavati Hospital & Research Centre	Bandra West, Mumbai
15.	Srajan Hospital	Udaipur, Rajasthan
16.	Endeavour Clinics & Dr. Sambit's Centre of Diabetes and Endocrinology	Bhubaneswar, Odisha
17.	ILS Hospital, Salt Lake	Salt Lake City, Kolkata
18.	Belle Vue Clinic	Dr. U N Brahmacahri Sreet, Kolkata
19.	Arthur Asirvatham Hospital	Mdurai, Tamil Nadu
20.	M V Hospital for Diabetes	Chennai, Tamilnadu
21.	Sarvodaya Hospital and Research Centre	Faridabad, Uttar Pradesh
22.	Galaxy Speciality Centre	Sodala, Jaipur

carefully looked into all aspects of this course & has accredited & recognized 22 centres across India at present and more centers are being inspected for accreditation. National Faculties and experts of RSSDI chosen from Academia visit these centers from time to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

COURSE DETAILS

Name of the Course: Advanced Certificate Course in Diabetology

Duration: 2 Years – Post MBBS & 1 Year - Post MD / DNB (Gen - Medicine)* (Full Time) Educational.

Qualification: A candidate must possess MBBS degree from ANY of the recognized university approved by Medical Council of India (*The duration of the course is 1 Year for those with MD/ DNB in Internal Medicine. Candidates having MD degree in other specialties will have to do the course over 2 Years).

Number of seats: 2 seats per year for every eligible teacher as per rules of Medical Council of India (MCI).

Selection of Candidates: Selection for the Certificate course is through a performance evaluation by Theory test for 90 marks (90 minutes duration) which is conducted at all accredited centres. The result is displayed WITHIN 3 days on the Web site of JNU and RSSDI. Post MD (Internal Medicine) will be given !

COURSE FEES:

- Rs 30000/- (for post MD/DNB (internal medicine), 1 year program)
- Rs. 50000/- (for post MBBS, MD in other branches, 2 years program)

Session: Two sessions are run annually, in January and in July. Prospectus details are available on the RSSDI website. All applications must be sent to Jaipur National University.

ANNOUNCEMENTS

Dear Member,

Please update your Membership details like Complete Postal Address, Email Id, Pan No. & Mobile no. after log in your membership area on our website www.rssdi.in under sub heading Membership corner, so that we can send you RSSDI Newsletter & Journals.

49th Annual Conference of RSSDI-RSSDI 2021

Date: 11th - 14th November 2021

Registered with Registrar of News Papers, India vide no. RNI-43019/85

International Journal of Diabetes in Developing Countries

Volume 41 | Issue 3 | July–September 2021

Springer