ISSN 0973-3930

Volume 39 | Issue 3 | July–September 2019

# International Journal of Diabetes in Developing Countries

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



Springer

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International Journal of Diabetes in Developing Countries is published 4 times a year. Volume 39 (4 issues) of will be published in 2019.

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

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# International Journal of Diabetes in Developing Countries

**Volume 39 · Number 3 · July–September 2019**

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):417–418
https://doi.org/10.1007/s13410-019-00763-w

EDITORIAL

# The clinical relevance of lipohypertrophy

S V Madhu[1]

Published online: 3 August 2019


Lipohypertrophy (LH) has been a well recognized complication of insulin therapy for a long time. However, the true magnitude of its consequences for management of patients with diabetes is being realized lately. Several recent studies have not only shown that it is far more common than was traditionally believed, but also that its presence can significantly compromise the success of insulin therapy. Therefore, it becomes critical that all physicians and paramedical personnel involved in diabetes care should be alert in detecting and addressing the issue promptly and appropriately to ensure continued efficacy of insulin treatment.

It has been estimated that the prevalence of LH in insulin-treated patients is 38% [1] based on a meta-analysis of 26 studies. Prevalence rates are higher among Asians compared with Europeans and also higher in type 2 diabetes mellitus patients (49%) than in type 1 (34%) [1]. The use of ultrasound has been shown by some to improve diagnostic sensitivity both in terms of the frequency and the extent of detection [2]. A recent audit from India [3] reported a much lower prevalence at 12.5% of all insulin users although this was a retrospective collection of cases based only on clinical examination and could be an underestimate while another survey [4] on injection technique found that based on a physical examination by nurses, one in five diabetic patients on insulin had LH. Two recent studies [5, 6] from India reported that more than 60% of the patients developed LH. Lipohypertrophy was found to be more prevalent in obese patients and those with hypoglycemia and higher HbA1C [7].

LH which appears as a rubbery swelling in the subcutaneous (SC) tissue at injecting sites is believed to develop secondary to the lipogenic action of insulin or to the trauma related to injections [5]. Various factors reported to be associated with a greater risk of LH in diabetic patients include longer duration of insulin use, failure to injection site rotation, needle reuse [4], longer needles, longer duration of diabetes [7, 8], low BMI, total daily dose of insulin, twice daily insulin regimen, the use of conventional insulins compared with analogues [5], and poor glycemic control [8, 9]. It has been shown that the risk of LH with insulin reuse is substantially higher than those who do not reuse. Seventy percent of those who reuse needles develop LH, and the risk has been shown to double when the needle has been reused 3–5 times [10].

In the current issue, Pahuja et al. [11] explore the frequency and factors associated with LH in a tertiary hospital from West India. The study confirms the high prevalence of LH (68%) reported in recent studies from India. They report that longer duration of diabetes and longer duration of insulin use and the total daily dose of insulin were important risk factors. The study also highlights the gap between patient's awareness and management of LH reinforcing the need for increasing awareness about this important condition both among physicians and patients.

Several adverse consequences of LH on diabetes and its complications have been reported. Injection of insulin into areas of LH leads to its decreased and variable absorption that results in enhanced insulin requirements and glycemic variability, unexplained hypoglycemia, and more frequent diabetic ketoacidosis [4]. In fact, a sixfold higher occurrence of unexplained hypoglycemia and a sevenfold higher occurrence of glycemic variability have been reported in patients with LH compared with those without. The resultant poor glycemic control increases the risk of long-term diabetic complications such as cardiovascular disease, amputation, retinal diseases, and kidney disease [1]. Besides these, diabetic patients with LH require much higher doses of insulin leading to a greater financial burden.

Proper rotation of injection sites and avoiding needle reuse result in prevention or significant reduction of LH which has resulted in improved glycemic control [10]. In children with LH who received specific instructions from diabetic nurses to rotate injection sites and not to reuse needles, it was shown

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that in 3 months, 90% of LH lesions had resolved and glycemic control had significantly improved [4]. However, other workers reported no effect of avoiding reuse of needles [5]. Injection technique (IT) education, particularly with respect to use of shorter needles, has been shown to effectively prevent LH in prospective randomized controlled trials [12].

Achieving and sustaining good glycemic control in insulin-treated patients is a major challenge and early detection of LH and timely correction of injection technique are critical in attaining this goal. The FIT, India guidelines endorsed by Research Society for Study of Diabetes in India as well as the Endocrine Society of India, provides useful guidance on best practices in this regard [13]. Clinicians involved in diabetes care would do well to make special efforts to focus on various aspects of insulin injection technique among insulin users for successful glycemic management in their practice.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):419–425
https://doi.org/10.1007/s13410-018-0710-6

REVIEW ARTICLE

# Importance of the Madras Diabetes Research Foundation-Indian Diabetes Risk Score (MDRF-IDRS) for mass screening of type 2 diabetes and its complications at primary health care centers of North India

Mohammad Mustufa Khan[1] · Gyanendra Kumar Sonkar[2] · Sangeeta Singh[2] · Satyendra Kumar Sonkar[3]

Received: 30 July 2018 / Accepted: 13 December 2018 / Published online: 4 January 2019


**Abstract**
Diabetes in combination with abdominal obesity and hypertension is the leading cause of cardiovascular diseases (CVD) and death. Type 2 diabetes (T2DM) increases CVD risk in two- to fourfold. The prevalence of CVD is constantly rising in India and is higher in urban areas. In addition, more than 50% of diabetic patients are still undiagnosed in the general population. The Madras Diabetes Research Foundation-Indian Diabetes Risk Score (MDRF-IDRS) is a cost-effective and very simple tool, which can be used at a primary health care center routinely. It is observed that people with increasing age, sedentary lifestyle, greater abdominal obesity, and family history of diabetes are at a high risk for diabetes and its complications. MDRF-IDRS is helpful for mass screening of diabetes and its complications in North Indian population to prevent the incidence of diabetes and with lifestyle interventions, knowledge, and awareness, it helps to reduce and revert the complications of diabetes. It is suggested that regular practice of MDRF-IDRS-based screening at primary health care center may also reduce the burden at the critical care center in North India.

**Keywords** Diabetes · Cardiovascular disease · MDRF-IDRS · Life style intervention · Primary health care center · Critical care center

## Introduction

India has the highest global burden of diabetes after China. In 2015, India has 69.2 million diabetic patients and the prevalence of diabetes was 8.7% [1]. However, a recent study in the 15 states of India reported that the overall prevalence of diabetes is 7.3% in India, but there are large differences in diabetes prevalence from state to state in India. The prevalence of diabetes varied from 4.3% in Bihar to 10.0% in Punjab. This also varied from 11.2% in urban areas to 5.2% in rural areas [2]. In addition, more than 50% of diabetic patients are still undiagnosed in the general population [3].

Conditions of diabetes and its complications are worsened in the low socio-economic society of both urban and rural population, because of the delayed diagnosis of disease and lack of strict monitoring and proper treatment [2]. Mohan et al. [4] suggested that epidemiology of diabetes in India may be due to specific Asian Indian phenotypic characteristics, which included greater insulin resistance, the greater degree of abdominal obesity at lower body mass index (BMI), and genetic predisposition of diabetes.

The prevalence of cardiovascular diseases (CVD) is constantly rising in India and is higher in urban areas. A systematic review of studies on CVD in Asian Indians has documented that the prevalence in urban areas is 2.5–12.6% and in rural areas, it is between 1.4–4.6% [5]. The overall prevalence of CVD in south Indian population is estimated to be 11%, a tenfold increase as compared to the prevalence in urban India in the 1970s [6].

Type 2 diabetes (T2DM) increases the risk of CVD in around two- to fourfold. It is expected that almost 30% of cases may have a 5-year coronary heart disease (CHD) risk

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similar to the general population. Bertoluci and Rocha [7] suggested that lifetime risk seems to be consistently high in almost all patients with diabetes. T2DM patients who are above 40 years age, diabetes duration of more than 10 years, and the presence of a first-degree family history with premature CHD, chronic hyperglycemia, and severe hypoglycemia are factors that increase CVD risk [7].

Diabetes in combination with abdominal obesity and hypertension is the leading cause of CVD and death [8]. Sharma et al. [9] reported that diabetes and its combinations are contributing huge burden on the critical care center, increase excess health expenditures, and lost productivity among individuals. Diabetes is a lifestyle-based disease and can be prevented at a very initial stage. It can be also reverted with proper diagnosis and treatment. Innovative solutions are needed to cope with diabetes and its associated cost burdens in India [9].

## MDRF-IDRS

MDRF-IDRS is a cost-effective and very simple tool, which can be used at a primary health care center routinely to reduce the incidence of diabetes and its complications. In this tool, a combination of age, family history, and two modifiable risk factors (waist circumference (WC) and physical activity (PA)) included and are given simple score (< 30 at lower risk, 30–50 at moderate risk, and ≥ 60 at high risk of diabetes, respectively) [4, 10]. Several regional studies have validated this tool and found this very effective, simple, and low cost in individual population. In South India, 43% in Chennai [11], 19% in urban Pondicherry [12], 31.2% in rural Tamil Nadu [13], and 70.1% in Bangalore [14] are at high risk of diabetes. In West India, 36.6% in Pune [15] and 53.4% in Nagpur [16] are at high risk of diabetes. In Central India, 19.6% in Gwalior ([17]) and 68.8% in Bhopal [18] are at a high risk of diabetes. Similarly, in North India, 67.2% in Lucknow [19] are at high risk of diabetes, while 94.5% of the Delhi population are at moderate or high risk of diabetes [20], and 54% in Haryana at high risk of diabetes [21]. In North-West India, 69% in Shimla [22] and 70.6% in Jammu [23] are found to be at high risk of diabetes. Also, In East India, 31.5% in West Bengal [24] are reported to be at high risk of diabetes (Fig. 1).

MDRF-IDRS also has a significant impact on the screening of CVD and CAD in individual population. Mohan et al. [25] have also found that subjects with diabetes had a higher risk of developing diabetic complications [25]. The prevalence of CAD in the high-risk MDRF-IDRS group was significantly higher (2.2%) compared with the medium-risk MDRF-IDRS group (0.8%) and the low-risk MDRF-IDRS group (0.06%) [4].

## Role of IDRS contents in diabetes and its complications

### Age

Prevalence of T2DM and central obesity have been reported to be 30.42% and 64.04% in the elderly population, respectively, and out of which, 80% of patients are associated with hypertension (HTN). This kind of deadly combination badly affects the lifespan and quality of life with the increasing age [26]. Palta et al. [27] reported that the risk of all-cause, CVD, and cancer mortality significantly appeared to increase with diabetes (≥ 8.0% HbA1c) among older adults. Males aged over 55 years who are at risk of CVD have poorer knowledge and awareness of risk factors for diabetes and CVD [28]. Dhana et al. [29] suggested that preventive interventions for targeting cardiometabolic risk factors should be considered in the elderly population with and without weight status. Disease duration also increases with the increasing age in individuals. Srinivasan et al. [30] strongly suggested that it is needed to target the intense therapeutic intervention within 0–5 years of T2DM because the irreversible CVD damage has already occurred between 5 and 10 years of T2DM and there is no scope for interventions beyond 10 years.

### Family history of diabetes

The presence of diabetes in the family enhances prodiabetic genetic burden and severity of diabetes with/without the existence of genes, which might increase the risk of T2DM and CVD. Based on the genetic score by using 36 gene variants, genetic predisposition of T2DM has been significantly associated with an increased risk of CVD in diabetic patients [31].

In a comparative study of Iraqi and Swede population, the family history of diabetes (FHD) was higher in Iraqis and the incidence of diabetes was also much higher at the younger age; this might be due to the high family predisposition of diabetes. This suggests that family environment, eating habit, and genetic background influence the incidence of T2DM and CVD risk in T2DM patients [32]. In a large observational study, FHD was associated with non-calcified plaque in diabetic patients, which increased CVD risk. However, in normal individuals and prediabetic, having FHD was not associated with coronary atherosclerosis [33]. In addition, a study demonstrated that diabetes individuals with FHD have increased the risk of atherosclerotic CVD as compared to without FHD. This suggests that genetic factors which are associated with the incidence of familial diabetes may increase the risk of atherosclerotic CVD [34].

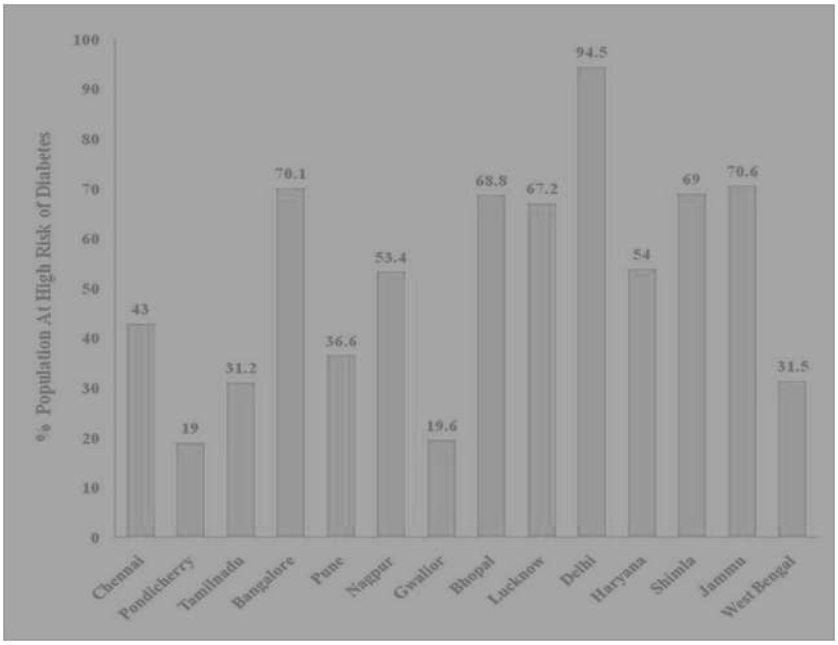


**Fig. 1** Population at high risk of diabetes

Hariri et al. [35] suggested that FHD is not only a risk factor for a disease but is also positively associated with risk awareness and risk-reducing behaviors. It may provide a useful screening tool for the detection and prevention of diabetes. However, Zhao et al. [36] suggested that risk of T2DM was collectively affected by FHD and dietary habits. Prevalence of T2DM may decrease in Chinese with FHD by nutrition educational intervention program [36]. This kind of intervention program may also play a major role to decrease the incidence of T2DM and its complications in the Indian population who have FHD.

## Waist circumference

Individuals who have deep subcutaneous abdominal adipose tissue (deep SCAT) have more metabolic activity and its continued expansion may unfavorably affect insulin resistance and CVD risk [37]. Generalized obesity is measured by body mass index (BMI), while abdominal obesity is measured by waist circumference (WC) and waist-to-hip ratio (WHR). Asian Indians have approximately twofold higher hepatic fat accumulation as compared to other ethnic groups and associated with greater insulin resistance [38]. Similarly, South Asians have a higher intra-abdominal visceral tissue, especially who have diabetes as compared to white Caucasians [39, 40]. For the Caucasian population, a BMI $\geq$ 30 kg/m$^2$ or waist sizes larger than 88 cm for women and 102 cm for men is recommended. Deepa et al. [41] suggested that for Asian Indians, the generalized obesity as BMI $\geq$23 kg/m$^2$ and the abdominal obesity sizes as WC $\geq$90 cm for men and $\geq$80 cm for women were defined using WHO Asia Pacific guidelines [42, 43]. A multiregional study reported wide differences between the prevalence of generalized obesity, abdominal obesity, and combined obesity and also within rural and urban Indian population [44]. In addition, the prevalence of abdominal obesity was found approximately twofold higher than generalized obesity in north Indian population [19]. The increased risk of morbidity and mortality is associated with generalized as well as abdominal obesity [45]. The main cause of obesity-related death is CVD, for which abdominal obesity is a predisposing factor. Abdominal obesity is the increased visceral adipose tissue, which is associated with the range of metabolic abnormalities, including reduced insulin sensitivity, decreased glucose tolerance, adverse lipid profile, and increased insulin resistance which are the main risk factors for T2DM and CVD [46]. In addition, abdominal obesity predicted the prevalence of atherosclerotic CVD and its risk factors in the elderly Chinese population, while not with BMI [45]. However, overweight and general obesity and abdominal obesity are equally associated with increased risk of CVD and heart failure [47, 48]. The dietary pattern is also one of the most influencing factors for metabolic abnormalities due to rapidly changing lifestyle and is contributing to increasing numbers of obese, T2DM, CVD, and premature mortality in India. Epigenetic modifications are also responsible for regulating gene expression and phenotypic variations (obesity) without alteration in DNA sequence among South Asians [49].

## Physical activity

Anjana et al. [50] reported that a large percentage of people in India are inactive and have the sedentary lifestyle and less than 10% engaging in recreational physical activity [50]. The people who have a sedentary lifestyle are more prone to become obese and diabetic in the future. The long-term impact of this sedentary lifestyle makes them a victim of CVD and myocardial infarction (MI) also. According to the studies conducted by Mohan et al. [11] and Gupta et al. [13], it was found that people with sedentary and moderate physical activity had a high risk for diabetes. Inadequate physical activity (PA) is one of the leading risk factors for death worldwide. Inadequate PA is a key risk factor for non-communicable diseases (NCDs) such as T2DM, CVD, and cancer. PA has significant health benefits and contributes to preventing NCDs [51, 52]. PA was associated with reduced risk of CVD, CVD-associated death, and total mortality in men with T2DM. Walking and walking pace were associated with reduced total mortality [53]. Wahid et al. [54] recommended that PA levels (150 min of moderate aerobic exercise per week) were associated with lower risk of CVD mortality by 23%, CVD occurrence by 17%, and T2DM occurrence by 26%, respectively, after adjustment for body weight.

Deepa et al. [55] recommended that nearly 80% of T2DM cases of Asian Indian population can potentially be prevented by changing their lifestyle and dietary habits. The incidence of T2DM due to unhealthy diet alone 30.1%, unhealthy diet in the combination of PA 51.7%, and the combination of unhealthy diet and PA with obesity went up to 73.2%. Hamasaki [56] suggested that daily PA, which may be moderate to vigorous exercise, improves the outcomes of metabolic disease and reduces CVD risk and mortality. Hence, daily PA is important for the prevention and management of T2DM to reduce/delay its complications.

Elevated BMI and reduced PA play a significant role in the risk of hypertension in Chinese middle-aged and older population. The risk of hypertension associated with overweight and obesity and which can be reduced significantly by increased PA [57]. The study reported that regular and consistent exercise reduces abdominal obesity which promoted favorable changes in body composition. Exercise should be prescribed as medicine mainly for lifestyle-based diseases [58].

## Lifestyle interventions and deduction of burden at the critical care center

Several studies have estimated annual and cumulative economic costs of diabetes complications over time [59, 60]. These studies found that macrovascular disease, mainly CVD and stroke, accounted for more than 85% of the costs of complications associated with diabetes and that these conditions are a significant determinant of costs at an earlier time during the course of the disease than microvascular complications [59]. On the other side, this complicates the treatment of inpatients in the critical care center, because hyperglycemia is a potentially harmful and correctable abnormality in critically ill patients and is directly associated with mortality. The recovery of patients and the depth of hyperglycemia-related injury varied at the time of admission and diagnosis, which depended on medical conditions and the primary disease states of patients [61]. Maniarasu and Muthunarayanan [62] suggested that diabetic patients coming at the primary care center with a high prevalence of undiagnosed microvascular complications can be detected by routine screening for diabetes complications. By creating awareness and usefulness of early detection and prevention, progression and incidence of microvascular complications can be decreased. Mokta et al. [63] also suggested that uncontrolled blood sugar is the main culprit for the high prevalence of CVD risk factors and this should be controlled by improving disease management system and public awareness campaigning in the different regions of the country. In addition, Pradeepa et al. [44] suggested that economic burden of diabetes and its complications can be reduced by providing universal health care coverage, access of medicines, and early detection and treatment of the disorder. In this concern, Tripathy et al. [64] also indicated the need for systemic screening and awareness program to identify the undiagnosed diabetics in the adult population of the community to offer early treatment and regular follow-up.

Joshi [65] realized that cost-effective measures of diabetes care are necessary to put into practice to fight with current diabetes epidemic conditions in India. This can be possible by patient education, updating the medical facility and various disease management strategies, because the high cost of disease screening, diagnosis, monitoring, and management are the main hindrance in our country.

Decode study group [66] stated that diabetes shares many risk factors with other NCDs. These are age, physical inactivity, waist circumference, insulin resistance, dyslipidemia, and high blood pressure. Diabetes is an equivalent risk factor for CVD. Mohan and Anbalagan [67] recommended that this MDRF-IDRS tool is also useful for screening of CVD and other lifestyle-related NCDs at an early stage to reduce the severity of these diseases. Targeting the high risk of diabetes groups with educational information and knowledge of risk factors may improve their understanding of diabetes and its complications [28].

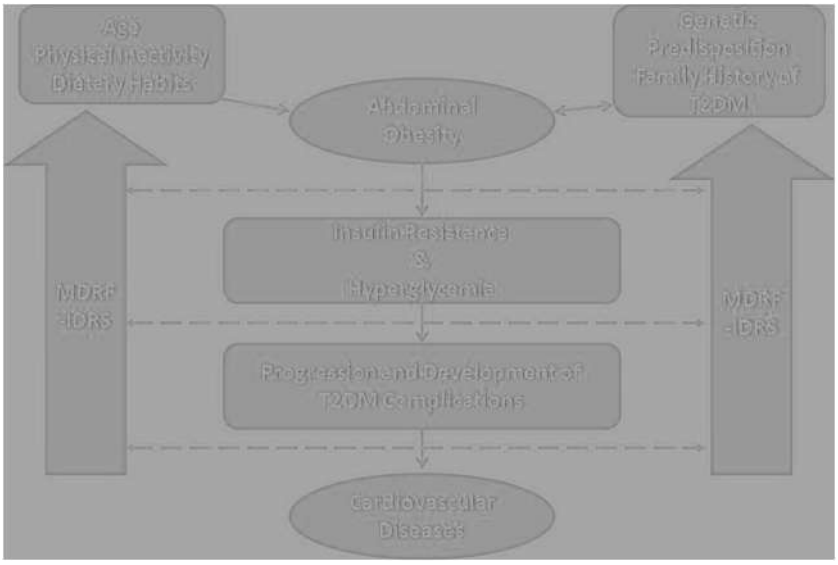


**Fig. 2** A lifestyle intervention study

A lifestyle intervention study has shown a cost-effective and significant way to prevent the incidence of diabetes and its complications (Fig. 2). It showed favorable changes in subclinical biomarkers, adipokines, cytokines, and gut hormones in overweight/obese Asian Indians with prediabetes not only during the intervention period, these changes constantly appeared after 12 months of intervention [68]. A longitudinal study suggested that moderate physical activity can significantly improve the fasting and postprandial blood sugar in T2DM patients [69].

In this regard, MDRF-IDRS suggested two modifiable risk factors (Waist circumference and physical activity). For lifestyle intervention, these two modifiable risk factors (WC and PA) are antagonistic. PA was inversely associated with the incidence of general obesity (BMI) as well as abdominal obesity (WC) [70]. A demographic surveillance–based study in rural areas of West Bengal, India, suggested that overweight/obese people and people with low level of physical activity are more likely to be diagnosed with diabetes [71]. In addition, the authors also emphasized that a lifestyle intervention/modification-focused strategy should be used to promote physical activity and traditional dietary intake in rural India to reduce the increasing burden of diabetes [71].

Lifestyle intervention, mainly PA, increases the body's metabolic rate, calorie consumption of calorie, activates the skeletal muscle, sensitizes insulin, and increases glucose uptake [72]. PA regulates the energy expenditure by increasing the secretion of various adipokine (like adiponectin) which reduces the insulin resistance [73, 74] and by decreasing the inflammatory marker (like TNF-alpha) which reduces the inflammation of adipose tissue in T2DM patients [75]. Adiponectin and TNF-alpha are sharing the same structural protein, but in an antagonistic action [76].

## Conclusion

This review promises that MDRF-IDRS might be helpful to screen and detect individuals at high risk for diabetes in North Indian population including primary health care centers. It may prevent the incidence of diabetes by lifestyle intervention and awareness and may also reduce the burden at the critical care centers and prevent premature mortality.



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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):426–431
https://doi.org/10.1007/s13410-019-00735-0

ORIGINAL ARTICLE

# Exploring the factors associated with lipohypertrophy in insulin-treated type 2 diabetes patients in a tertiary care hospital in Mumbai, India

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Received: 10 July 2018 / Accepted: 27 February 2019 / Published online: 30 March 2019


## Abstract

**Background** This exploratory study reports the prevalence of lipohypertrophy (LH) in insulin-treated type 2 diabetes patients attending a private tertiary hospital in Mumbai, India, and explores the factors associated with this condition.

**Methods** A total of 96 patients attending the outpatient department, completed an interviewer-administered questionnaire followed by an examination of insulin injection sites for the presence of LH by a trained diabetes nurse educator.

**Results** Nearly 68% of the respondents had LH. Most patients (82%) had LH on at least two injection sites, with the frontal areas of the right and left side of the abdomen being the most common sites. Despite a high prevalence of LH, the majority of patients were unaware that they had the condition (77%) and could not identify the causes (74%). Half of the patients reported awareness about the different sites for injecting insulin, injection techniques, and the need for rotating injection sites. Only 26% always rotated injection sites, while 16% changed needles more than half of the time in the week prior to the interview. Bivariate analysis found higher number of years with diabetes and on insulin and a higher insulin dose per day as significant factors ($p$ value < 0.05). Logistic regression showed a strong relationship between the presence of LH and the number of years on insulin ($p$ value < 0.05). The number of years on insulin for patients with and without LH averaged 8.2 and 3.7, respectively.

**Conclusion** Findings highlight a gap in patients' knowledge and management of LH, and call for a hospital-wide intervention encompassing patient education and provider sensitization.

**Keywords** Lipohypertrophy · Insulin complication · Type 2 diabetes · Urban · India

## Introduction

As of 2017, 72 million people in India were estimated to have diabetes, with the majority affected by type 2 diabetes [1]. This is expected to rise to 366 million by 2030 [2] signaling an impending health crisis. Individuals with type 2 diabetes often take insulin injections when oral medications and lifestyle interventions fail to control blood sugar levels [1, 3]. In 2010, it was estimated that only 3.2 million diabetes in India injected insulin [4]; this was due to inadequate access to insulin due to low availability and high prices [5]. However, the increasing disease burden is expected to lead to a corresponding rise in insulin intake. Insulin therapy comes with its own set of complications, and lipohypertrophy is one of the most common concerns [6, 7].

Lipohypertrophy (LH), a build-up of fat cells below the surface of the skin, is characterized by soft or firm swellings at sites repeatedly injected with insulin [8]. Injecting insulin in sites with LH may lead to erratic absorption of insulin [9] with the potential for poor glycemic control and unpredictable hypoglycemia [6, 10]. Suboptimal glycemic control subsequently increases the risk of adverse outcomes such as cardiovascular disease [10], amputation [11], retinal diseases [12], and kidney disease [13]. Apart from the health complications, LH also increases the economic burden on families as patients with LH consume more insulin [14]. Observational studies

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with diabetic patients and systematic reviews have estimated global prevalence of LH from 1.9 to 73.4% [15–17]. Prevalence data for LH among diabetic patients in India is scarce, with a few studies reporting the occurrence of LH anywhere between 11 and 41% [18–20].

Prevention and treatment of LH in diabetes patients requires an understanding of its occurrence and associated risk factors in the Indian setting. This study aims to fill a gap in the literature by exploring the factors associated with the prevalence of LH in insulin-treated type 2 diabetes patients in an urban city in India.

## Methods

This exploratory, cross-sectional study was conducted with insulin-treated type 2 diabetes patients attending the outpatient department at a private tertiary care hospital with 240 beds, located in suburban Mumbai. Ninety-six patients with a prior diagnosis of diabetes were purposively sampled from among a population of adults attending the outpatient department (OPD) of the hospital. This sample size was calculated based on Green's [21] recommendations emerging from a comprehensive overview of the procedures used to determine regression sample sizes. A trained interviewer, who is also a diabetes nurse educator fluent in English and Hindi, approached type 2 diabetes patients in the OPD, identified and included only those taking insulin, and asked for their consent to participate in the study. The interviewer read out a printed consent form verbatim in English or Hindi based on the language preferred by the patient, explaining the aims, procedure, and implications of the study as well as the confidential and voluntary nature of participation. After patients provided their written consent, the interviewer conducted interviews in a private room in the OPD. The trained interviewer also examined the patient for LH through observation and palpation of insulin injection sites. Data collection was conducted over a 5-week period. The questionnaire used in this study consisted of three sections: (i) sociodemographic and patient health details, (ii) insulin injection knowledge and practices, (iii) physical examination of insulin injection sites for LH.

Data was entered in MS-Excel 2007 and then analyzed using the SPSS software version 16.0. The presence of LH was the dependent variable. First, descriptive frequencies were generated for all variables. Bivariate analysis was conducted wherein nominal and continuous independent variables were tested with the dependent variable. Finally, independent variables significant at $p < 0.05$ level in the bivariate analysis were included in a logistic regression model with the dependent variable.

## Results

### Sociodemographic profile of respondents

Of the 96 insulin-treated type-2 diabetes patients interviewed, slightly more than three (62%) out of five were male; majority were married (98%), and 78% lived in their own home (Table 1). The mean and median age of the sample were 65.51 and 67 years respectively, with nearly 50% aged between 51 and 69 years. Half of the patients were college graduates or postgraduates, one third had completed 10th to 12th grade, while 15% reported level of schooling less than 10 years. Slightly more than half of the respondents were retired with a few who reported as being unable to work due to a disability; 26% reported as homemakers and another 21% worked fulltime or part-time. Around 2 (42%) out of 5 respondents reported a personal monthly income between INR 25,000 ($367) and INR 50,000 ($734), followed by 34% who reported an income of more than INR 50,000.

### Insulin injection practices

Patients had lived with diabetes for an average of 19.82 years and the reported mean HbAIC (%) and RBS values were 8.2 and 189.36 respectively. The mean and median period of being on insulin treatment was 6.8 and 4 years respectively. The insulin pen (77%) was most commonly used by patients to inject insulin while 23% used a syringe. An average, patients took 40.72 units of insulin per day (median of 39 units), with a minimum of 7 units and a maximum of 104 units. Half of the patients (51%) reported a premix insulin regimen followed by 24% who were on a basal and bolus regimen; 13% and 12% were on short/rapid acting and basal regimens respectively. Most patients (44%) took insulin injections twice a day while a quarter (25%) reported taking four injections, i.e., pre-meals and at bedtime. Nearly all (97%) patients were aware of the current daily insulin dose; only half reported awareness about the different sites for injecting insulin and injection techniques. While 84% of the patients reported rotating injection sites, around 58% did this sometimes and 26% always rotated sites. Similarly, while eight (81%) in ten patients had changed their needle for insulin injections in the week prior to the interview, around 43% changed the needle less than half of the time, 19% reported changing the needle about half of the time, 16% did this more than half the time, while a minor 4% always changed their needles each time.

### Lipohypertrophy

Almost two-thirds (68%, $n = 65$) of all respondents had lipohypertrophy (LH); of these 41 (63%) had swellings that were both visible and palpable, while 32% had only palpable swellings (Table 2). About 63% of respondents with LH had

**Table 1** Description of sample and bivariate analysis of independent variables with lipohypertrophy in Type 2 Diabetes patients on insulin ($n = 96$)

| Independent variables | | *N* (%) | Do not have LH | Have LH | *p* value |
|---|---|---|---|---|---|
| *Sociodemographic profile* | | | | | |
| Age (mean: 65.51, median: 67) | | | 31 (63.8) | 65 (66.3) | 0.322 |
| Age | Below 50 yrs | 10 (10.4) | 5 (50.0) | 5 (50.0) | 0.447 |
| | 51–69 yrs | 47 (49.0) | 14 (29.8) | 33 (70.2) | |
| | 70 and above | 39 (40.6) | 12 (30.8) | 27 (69.2) | |
| Sex | Male | 60(62.5) | 18 (30.0) | 42 (70.0) | 0.535 |
| | Female | 36(37.5) | 13 (36.1) | 23 (63.9) | |
| Marital status | Married | 94 (97.9) | 30 (31.9) | 64 (68.1) | 0.588 |
| | Never married | 2 (2.1) | 1 (50.0) | 1 (50.0) | |
| Residence | Own home | 75 (78.1) | 27 (36.0) | 48 (64.0) | 0.142 |
| | Others | 21 (21.9) | 4 (19.1) | 17 (80.9) | |
| Education | No school | 6 (6.3) | 3 (50.0) | 3 (50.0) | 0.534 |
| | Schooling up to 9th grade | 15 (15.6) | 3 (20.0) | 12 (80.0) | |
| | 10th to 12th grade | 30 (31.3) | 11 (36.7) | 19 (63.3) | |
| | Graduate/post graduate | 45 (46.9) | 14 (31.1) | 31 (68.9) | |
| Current employment | Employed | 20 (20.8) | 6 (30.0) | 14 (70.0) | 0.339 |
| | Homemaker | 25 (26.1) | 11 (44.0) | 14 (56.0) | |
| | Retired/disabled | 51 (53.1) | 14 (27.5) | 37 (72.5) | |
| Income | Less than INR 25,000 | 23 (24.0) | 6 (26.1) | 17 (73.9) | 0.412 |
| | INR 25,000-INR 50,000 | 40 (41.7) | 14 (35.0) | 26 (65.0) | |
| | More than INR 50,000 | 33(34.3) | 11 (37.9) | 18 (62.1) | |
| Insulin injection practices | | | | | |
| Years with diabetes (mean: 19.82, median:18) | | | 31 (15.7) | 65 (21.7) | 0.006 |
| Years on insulin (mean: 6.8083, median: 4) | | | 31(3.7) | 65(8.2) | 0.002 |
| HBAIC (mean: 8.2417, median: 7.9) | | | 31 (7.9) | 65(8.3) | 0.236 |
| HBAIC | Less than 7 | 16 (16.6) | 8 (50) | 8 (50) | 0.251 |
| | 7 to 8 | 55 (57.2) | 16 (29.1) | 39 (70.9) | |
| | Above 9 | 25 (26) | 7 (28) | 18 (72) | |
| RBS (mean: 189.36, median: 189.50) | | | 31 (181.81) | 65(192.97) | 0.416 |
| Injection device | Pen | 74 (77.1) | 27 (36.5) | 47 (63.5) | 0.107 |
| | Syringe | 22(22.9) | 4 (18.2) | 18 (81.8) | |
| Insulin dosage (mean: 40.72, median: 39) | | | 29 (33.55) | 64 (43.97) | 0.022 |
| Patient's insulin regimen | Basal | 11 (11.5) | 5 (45.5) | 6 (54.5) | 0.731 |
| | Basal & bolus | 23 (24.0) | 6 (26.1) | 17 (73.9) | |
| | Premix | 49 (51.0) | 16 (32.6) | 33 (67.4) | |
| | Short/rapid acting | 13 (13.5) | 4 (30.8) | 9 (69.2) | |
| Insulin injection practices | | | | | |
| No. of insulin injections per day | Once a day | 14 (14.6) | 7 (50.0) | 7 (50.0) | 0.234 |
| | Pre-meals | 16 (16.7) | 7 (43.8) | 9 (56.2) | |
| | Twice a day | 42 (43.8) | 11 (26.2) | 31 (73.8) | |
| | Pre-meals & bedtime | 24 (25.0) | 6 (25.0) | 18 (75.0) | |
| Aware of current daily insulin dose | No | 3 (3.1) | 2 (66.7) | 1 (33.3) | 0.196 |
| | Yes | 93 (96.9) | 29 (31.2) | 64 (68.8) | |
| Aware of different site for injecting insulin | No/not sure | 48 (50.0) | 15 (31.3) | 33 (68.7) | 0.827 |
| | Yes | 48 (50.0) | 16 (33.3) | 32 (66.7) | |
| Aware of injection technique | No/not sure | 47 (49.0) | 17 (36.2) | 30 (63.8) | 0.426 |
| | Yes | 49 (51.0) | 14 (28.6) | 35 (71.4) | |
| Aware of rotating the site of injection | No/not sure | 54 (56.3) | 18 (33.3) | 36 (66.7) | 0.805 |
| | Yes | 42 (43.7) | 13 (30.9) | 29 (69.1) | |
| Past 1 week how often patient changed the needle | Changed the needle | 78 (81.3) | 26 (33.3) | 52 (66.7) | 0.650 |
| | Never | 18 (18.8) | 5 (27.8) | 13 (72.2) | |
| Patient rotates the injection sites with each injection | Never | 13 (15.5) | 3 (23.1) | 10 (76.9) | 0.394 |
| | Yes | 71 (84.5) | 25 (35.2) | 46 (64.8) | |

swellings on two insulin injection sites, while a smaller proportion of 6% and 3% had LH in three and four sites respectively. The most common insulin injection sites with LH were the frontal areas of the right side of the abdomen (52%) and the left side of the abdomen (46%). Three (59%) out of five respondents were unaware of the term lipohypertrophy. Slightly less than four (77%) in five respondents with LH were not aware or unsure that they had this condition. Only 26% of all patients knew the causes of LH. When asked if they would inject the LH site, more than two (45%) in five responded in the affirmative.

A bivariate analysis with the presence of LH as the dependent variable and various independent variables in Table 1 revealed that respondents living with diabetes for

**Table 2** Lipohypertrophy among patients ($n = 96$)

| Descriptive variable | | Number | Percent |
|---|---|---|---|
| Presence of LH | No | 31 | 32.3 |
| | Yes | 65 | 67.7 |
| Total number of sites with LH | 1 | 18 | 27.7 |
| | 2 | 41 | 63.1 |
| | 3 | 2 | 3.1 |
| | 4 | 4 | 6.2 |
| Sites with LH (only affirmative responses presented) | Right arm-front | 14 | 21.5 |
| | Right arm-back | 2 | 3.1 |
| | Left arm-front | 13 | 20 |
| | Left arm-back | 2 | 3.1 |
| | Right abdomen-front | 34 | 52.3 |
| | Right abdomen-back | 0 | 0 |
| | Left abdomen-front | 30 | 46.2 |
| | Left abdomen-back | 0 | 0 |
| | Right thigh-front | 13 | 20 |
| | Right thigh-back | 0 | 0 |
| | Left thigh-front | 14 | 21.5 |
| | Left thigh-back | 0 | 0 |
| LH visible or palpable ($N = 65$) | Visible | 3 | 4.6 |
| | Palpable | 21 | 32.3 |
| | Both | 41 | 63.1 |
| Aware of LH | No/not sure | 54 | 58.7 |
| | Yes | 38 | 41.3 |
| Aware that they have LH | No/not sure | 50 | 76.9 |
| | Yes | 15 | 23.1 |
| Aware of causes of LH | No/not sure | 70 | 73.7 |
| | Yes | 25 | 26.3 |
| Intent to inject in LH site | Never | 32 | 55.2 |
| | Yes | 26 | 44.8 |

a higher number of years (mean, 21.7 years), taking insulin injections for a higher number of years (mean, 8.2 years), and with a higher insulin dose per day (mean, 43.97 units) were more likely to have LH ($p$ value < 0.05). A logistic regression was run with the dependent variable and the three aforementioned independent variables that were statistically significant in the bivariate analysis. None of the variables in this logistic regression model were found to be statistically significant. Since rotation of injection sites has been reported as a significant risk factor for LH in other studies, we included this variable into the logistic regression model along with the three aforementioned variables that were significant in the bivariate analysis. This analysis showed that taking insulin for a higher number of years (mean = 8.2 years) was significantly associated with having LH, and that these patients were 1.12 times more likely to have LH than those taking insulin for a lesser number of years ($p$ value < 0.05) (Table 3).

**Table 3** Summary of logistic regression analysis

| No | Factor | $p$ value | Exp (B) |
|---|---|---|---|
| 1 | Number of years with diabetes | 0.492 | 1.022 |
| 2 | Number of years on insulin | 0.043 | 1.120 |
| 3 | Insulin dose per day | 0.170 | 1.019 |
| 4 | Rotation of injection sites | 0.328 | 2.121 |

## Discussion

This cross-sectional study examined LH in insulin-treated type 2 diabetes patients in Mumbai and found that nearly 68% had LH. This is higher than other reported studies in India [19, 20]. The study found a strong association between the presence of LH and longer duration of insulin treatment; that is, patients taking insulin injections for a higher number of years were more likely to have LH. This finding is supported by other studies [8, 22]. Most patients with LH had observed palpable swellings on at least two insulin injection sites, with the frontal areas of the abdomen being the most common sites.

The growth-inducing character of insulin could have a multiplying effect on the fat tissue [23, 24]. Furthermore, pain sensations diminish in LH sites, which is one of the reasons why diabetic patients tend to always administer their injections in the same site [22]. This finding about the amount of time on insulin as a risk factor can be particularly useful for further research on the onset of LH and development of protocols for examination and diagnosis for diabetes care physicians and nursing staff. While insulin injection is a long-term therapy that cannot be avoided, early diagnosis and management of LH become critical. Only half of the respondents in this study, all type 2 diabetes patients on insulin, reported complete knowledge about insulin injection practices.

Respondents living with diabetes for a higher number of years and those taking a higher insulin dose per day were more likely to have LH in the bivariate analysis; although not significant in the multivariate logistic model. Factors such as higher number of injections per day, non-rotation of injection sites, needle reuse were not found to be associated with occurrence of LH in this study. Other studies, however, indicate that these risk factors do affect the development of this condition [6, 19, 20, 24–26].

Thus, the immediate implication of this study is the need for establishing a comprehensive hospital-wide intervention that encompasses both patient education programs and health providers' sensitization and training on Lipohypertrophy. Both physicians and nursing staff across all levels will need to be trained to recognize LH, periodically examine insulin-treated patients for this condition, and help patients to plan a management approach. As part of a patient education program for prevention and management of LH, patients will need training on needle use [27], injection techniques [26, 28], rotation of

injection sites [29], regular examination of injection sites for swelling [8], and avoidance of injecting in LH sites [9]. Mobile phones can be used to supplement face-to-face training on correct insulin injection practices; reminders to examine injection sites regularly could be sent as mobile phone texts and social media applications such as WhatsApp, which are popular in India, can be used for audio and video messages.

This study had some limitations. A skin ultrasound scan is the gold standard for detecting LH [30]; however, in our study, it was not possible to conduct an ultra-scan. We addressed this limitation by ensuring that an experienced and trained diabetes nurse educator conducted observations and clinical examination to identify and confirm the presence of the condition in the patients. Respondents for this study were recruited from among patients attending the outpatient department of a private tertiary care hospital in Mumbai, and they may have different characteristics compared to those attending inpatient departments and public health facilities, limiting the generalizability of the results. Future research must assess the prevalence and risk factors for LH in a larger sample from varied healthcare facilities serving patients from different socioeconomic groups, to understand the wider epidemiological situation. Qualitative interviews with patients can also help to further explain the factors associated with LH.

## Conclusion

Most respondents were unaware they had Lipohypertrophy and did not know about its causes; the majority also lacked knowledge about correct insulin injection techniques, highlighting a major knowledge gap. The presence of LH in many patients, especially those with a longer duration of insulin treatment, also indicates that work has to be done on the provider side. As LH is associated with suboptimal glucose control that could potentially lead to several health complications, it is imperative that diabetes health education interventions help patients develop the required competencies and achieve self-reliance in identifying and managing this condition.

**Acknowledgements** We also thank the management of Dr. L.H. Hiranandani Hospital, Powai for permission to conduct the study and the doctors, nurses, and research staff for their support in the data collection process in the inpatient wards. Thanks also go to Shirlly Koshy for data management. Most importantly, we are grateful to the patients who freely gave their time and inputs.

### Compliance with ethical standards

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

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors. The study was approved by the Ethics Committee of Dr. L.H. Hiranandani Hospital, Mumbai.

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

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):432–436
https://doi.org/10.1007/s13410-018-0696-0

ORIGINAL ARTICLE

# The maternal and offsprings' characteristics associated with HOMA-IR in young adults: a prospective cohort study

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Received: 10 August 2018 / Accepted: 10 October 2018 / Published online: 25 October 2018


**Abstract**
Diabetes is an important chronic disease. Many studies from Western countries found that both maternal environment during pregnancy and early offspring life's factors can lead to diabetes and insulin resistance of offspring during adult life (Barker's hypothesis). However, this data was limited in Asia. Therefore, this study was conducted to determine the predictor of diabetes in Thailand by using the surrogate markers: the homeostasis model assessment-estimated insulin resistance (HOMA-IR). The design was a prospective cohort study. First, pregnant women were recruited at first attendance at the antenatal care clinics (ANC) in our centers during 1989–1990. Various predictors were collected at subsequent ANC visits, during delivery, and 1 year after delivery. Then, in 2010, we followed up both mothers and offspring again. The characteristics of mothers and offspring which had $p$ value $< 0.2$ in the univariate analysis were selected to be analyzed by multivariate analysis. There were 592 offsprings (272 males and 320 females). HOMA-IR is statistically found to be associated with the increasing of BMI, both of mother and offspring, as well as the elevated plasma triglyceride and the history of diabetes of the offspring. Interestingly, in contrast with Western reports, in-utero environment in this study in Thais did not have any significant association with HOMA-IR. Perhaps this association might be confounded by ethnic factors. Our novel finding implies that any offspring who have high BMI mother have a high chance to develop insulin resistance and perhaps diabetes in future, so they should be aware and prevent any modifiable risk factors.

**Keywords** Diabetes · HOMA-IR · Maternal · Offspring · Insulin resistance

## Introduction

Diabetes is a chronic disease which has many complications. There are many reports showing increasing prevalence of diabetic patients worldwide [1], in particular, there has been a rapid increase in the prevalence of type 2 diabetes, and also complications of the disease in Asian population in recent years [2, 3]. This data was correlated with the data from Thai National Health Examination Survey. In 2014, the prevalence of diabetes in Thai people aged 15 years and older was 8.9%, which was higher than 6.9% in 2009 [4, 5]. In addition is the rising of obesity rates. Both the number of obesity cases and the prevalence of early onset type 2 diabetes mellitus have been increasing over the past few decades [6–8]. In childhood and adolescence, this has become a serious health problem [9]. The impact of diabetes in childhood and adolescence is different from adults. The management for this group of patient is very complicated in terms of physical development, cognitive development, and self-care skill. This care is a burden and the families are more involved. Early-onset type 2 diabetes mellitus is one of the risk factors of a negative impact on a child's growth, development, and psychosocial issues such as reductions in growth velocity, low self-esteem, and eating disorder [10–14].

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With the various complications, many undesired consequences of diseases occurred, along with physical, economic, and social burdens. Severe complications of the disease, including cardiovascular diseases, kidney diseases, and retinopathy, are major causes of mortality in patients, mainly by cardiovascular disorders [15]. Apart from medical complications, another considered importance of diabetes is the high expense of the disease. Recent studies revealed that insulin resistance can be managed with diet and changes in lifestyle to delay the development of type 2 diabetes. Early recognition and early intervention of patients at risk for diabetes mellitus, like insulin resistance period, is very beneficial [16]. Therefore, the earlier the stage of insulin resistance period is detected, the better.

Insulin resistance (IR) occurs when peripheral tissues reduce their response to normal hormone insulin level. IR can induce many clinical symptoms, such as glucose intolerance, diabetes, and metabolic syndrome. It can be measured by HOMA-IR, homeostasis model assessment-estimated insulin resistance, calculated by using fasting blood glucose and fasting blood insulin level [17]. There are many factors increasing HOMA-IR scores, such as age, sex, body mass index (BMI), alcohol consumption, triglyceride level, dysglycemia, and C-reactive protein level [18–23]. In addition, maternal factors seem to be important, including maternal fasting insulin level, maternal gestational hypertension, maternal nutritional status during pregnancy, and maternal concentration of 25-hydroxyvitamin D (25-OHD) during pregnancy [24–31]. Breastfeeding and maternal nutritional status during breastfeeding can also affect an individual HOMA-IR score [32].

From Manios et al. [21] study, it can be interpreted that children with obesity tend to have higher fasting insulin levels and HOMA-IR scores than the others. Moreover, there is a positive association between birth weight and later obesity [33]. In conclusion, children with high birth weight have a higher risk to develop diabetes mellitus.

There is a positive relationship between maternal and offspring BMI, as well as its birth weight and insulin resistance. Maternal BMI not only during pregnancy but also before pregnant period is also critical [27, 34, 35]. In contrast, Maftei et al study demonstrated that maternal BMI affected their child, but gaining BMI during pregnancy is not associated [27].

Many studies from Western countries found that both maternal environment during pregnancy and early offspring life's factors can lead to diabetes and insulin resistance of offspring during adult life (Barker's hypothesis) [36, 37]. However, this data was limited in Asia. Therefore, this study was conducted to determine the predictors of progression of diabetes mellitus in Thailand by using the surrogate markers: the homeostasis model assessment-estimated insulin resistance (HOMA-IR).

## Materials and methods

The design was a prospective cohort study with a 20-year follow-up, with women recruited at first attendance at the antenatal care (ANC) clinics of two centers, and followed up through their routine attendance to delivery. Socio-demographic and other background data were collected at recruitment retrospectively, using structured and pre-tested questionnaires. Further information was collected at subsequent visits and after delivery using additional pre-tested questionnaires.

The study was carried out by a consortium consisting of the Research Institute for Health Sciences (RIHES), and a number of faculties of Chiang Mai University, and the Health Promotion Center Region 10. The sample size of the study population was planned as approximately 592 pregnant women. First, pregnant women were recruited at first attendance at the antenatal care clinics (ANC) in our centers during 1989–1990. Various potential predictors were collected at every ANC visits, during delivery and 1 year after delivery (for offspring). Then, in 2010, we followed up both mothers and offspring again. The HOMA-IR in offspring was measured (HOMA-IR = fasting plasma glucose × fasting plasma insulin level/22.5).

Inclusion criteria are pregnant women who attended antenatal clinics and having gestational age less than or equal to 24 weeks. Exclusion criteria are pregnant women recruited in the study who did not give birth at either of the two selected study centers. Twin deliveries, stillbirths, and abortions were also excluded.

Nutritional information was collected by two methods: the 24-h food recall method and the food frequency method, using pre-tested questionnaire forms. Because the inclusion criteria allowed recruitment up to 24 weeks gestation, the first interview was carried out when the first visit was in the second trimester in some cases.

Blood drawing of 15 ml whole blood was done by venepuncture of each pregnant woman who consented at ANC visits in the ranges of gestational age, 10–12 weeks, 13–24 weeks, and 25–36 weeks for the first, second, and third trimesters. After the birth, all specimens from mothers of low birth weight infants were selected for the study of blood biochemistry factors. All assays were carried out in the biochemistry and hematology laboratories at RIHES.

Baseline characteristics were described by gender of the offspring. Pearson correlation coefficient of HOMA-IR was calculated for each characteristic of mothers and offspring. Kruskal-Wallis test or Mann-Whitney *U* Test was tested for non-continuous data. The characteristics, which has $p$ value < 0.2, were calculated for the univariate and multivariate analysis of log-HOMA-IR. Significance was defined as a $p$ value of less than 0.05. All statistical

**Table 1** Characteristics of participants at birth and age 20 years (592 cases)

| Characteristics | Mean value (95% confidence interval) | |
|---|---|---|
| | Female offspring (320 cases) | Male offspring (272 cases) |
| Offspring data | | |
| Birth weight (gram) | 2939.09 (2892.79–2985.40) | 3040.63 (2987.44–3093.81) |
| Body mass index | 20.92 (20.43–21.40) | 21.79 (21.30–22.29) |
| Waist circumference (cm) | 73.87 (72.65–75.08) | 79.16 (77.86–80.45) |
| Plasma cholesterol (mg/dl) | 169.69 (165.97–173.41) | 167.53 (163.30–171.75) |
| Plasma high-density lipoprotein (mg/dl) | 59.04 (57.41–60.68) | 53.29 (51.72–54.87) |
| Plasma triglyceride (mg/dl) | 69.70 (66.04–73.56)[a] | 82.76 (77.97–87.84)[a] |
| Systolic blood pressure (mmHg) | 108.44 (107.27–109.61) | 121.99 (120.67–123.31) |
| Diastolic blood pressure (mmHg) | 71.88 (70.73–73.02) | 76.26 (74.96–77.56) |
| Smoking history, *n* (%) | 14 (4.38) | 89 (32.72) |
| Maternal data | | |
| Age at 1990 (years) | 26.47 (25.96–26.99) | 25.93 (25.40–26.46) |
| Body mass index at 1990 | 21.37 (21.09–21.64) | 21.34 (21.04–21.65) |

[a] Geometric mean (95% CI)

calculations were performed with the STATA for Windows version 13.0, version13.0. This research was submitted and approved by Research Ethics Committee, Faculty of Medicine, Chiang Mai University.

## Results

A total of 592 of mothers and offspring were enrolled. The baseline characteristics are presented in Table 1. There are 272

**Table 2** The univariate and multivariate analytic of log-HOMA-IR and characteristics of mothers and offspring

| Baseline characteristics | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | Correlation coefficient | 95% CI | *p* value | Correlation coefficient | 95% CI | *p* value |
| Maternal characteristics | | | | | | |
| Body mass index 2010 | 0.0683 | − 0.012–0.098 | 0.1274 | 0.0191 | 0.001–0.037 | 0.044* |
| Fasting plasma glucose 2010 | − 0.0202 | − 0.011–0.007 | 0.6551 | −0.0003 | − 0.007–0.001 | 0.189 |
| Plasma glucose tolerance 2010 | 0.0850 | − 0.000–0.006 | 0.0680 | 0.0010 | − 0.000–0.002 | 0.164 |
| Plasma triglyceride 2010 | 0.0652 | − 0.001–0.006 | 0.1441 | | | |
| Systolic blood pressure 2010 | 0.0724 | − 0.002–0.019 | 0.1054 | | | |
| Gestational age at delivery period 1990 | 0.0741 | − 0.014–0.174 | 0.0941 | 0.0223 | − 0.010–0.055 | 0.179 |
| Fat intake at 3rd trimester | 0.0788 | − 0.001–0.010 | 0.0937 | | | |
| Offspring characteristics | | | | | | |
| Age 2010 | − 0.0862 | − 0.060–0.001 | 0.0436 | | | |
| Body mass index 2010 | 0.3342 | 0.119–0.191 | 0.0000 | 0.0485 | 0.034–0.063 | 0.000* |
| Waist circumference 2010 | 0.3243 | 0.044–0.071 | 0.0000 | | | |
| Plasma glucose tolerance 2010 | 0.2373 | 0.012–0.026 | 0.0000 | | | |
| Plasma cholesterol 2010 | 0.1023 | 0.001–0.010 | 0.0140 | 0.0012 | − 0.000–0.003 | 0.166 |
| Plasma high-density lipoprotein 2010 | − 0.1095 | − 0.027-0.004 | 0.0085 | | | |
| Plasma triglyceride 2010 | 0.3362 | 0.010–0.016 | 0.000 0 | 0.0029 | 0.002–0.004 | 0.000* |
| Systolic blood pressure 2010 | 0.1086 | 0.004–0.029 | 0.0091 | | | |
| Diastolic blood pressure 2010 | 0.1139 | 0.006–0.036 | 0.0062 | | | |
| Mean intimal media thickness 2010 | 0.1040 | 1.322–12.077 | 0.0147 | | | |
| Diabetes history 2010 | 11.4596 | 8.853–14.066 | 0.0000 | 1.7449 | 0.870–2.619 | 0.000* |
| History of hypertension 2010 | 8.5147 | 5.835–11.194 | 0.0000 | | | |

* $p$ value < 0.05 was considered statistically significant

cases for male offspring and 320 cases female offspring. Pearson correlation coefficient of HOMA-IR and characteristics of mothers and offsprings, Kruskal-Wallis test or Mann-Whitney U Test of HOMA-IR and characteristics of mothers or offsprings, and correlation coefficients of HOMA-IR by nutrient intakes are calculated. However, based on literature, seven maternal characteristics and 12 offspring's characteristics are selected and calculated by univariate and multivariate analysis as demonstrated in Table 2.

There was no statistically significant association between predictors in 1989–1990 with HOMA-IR, whereas body mass index (BMI) of mother (95% CI = 0.001–0.037, $p = 0.044$), BMI of offspring (95% CI = 0.034–0.063, $p < 0.001$), plasma triglyceride of offspring (95% CI = 0.002–0.004, $p < 0.001$), and diabetes history of offspring's in 2010 visit (95% CI = 0.870–2.619, $p < 0.001$) were positively associated with log-HOMA-IR. However, 95% CI of diabetes history of offspring in 2010 visit does not show statistically significant.

## Discussion

Increasing of HOMA-IR is associated with risk of diabetes in later life. In this study, we attempted to find predictor of diabetes in the early life of the offspring by the surrogate marker which is HOMA-IR. Results reveal that body mass index in 2010 of mother, body mass index in 2010 of offspring, plasma triglyceride in 2010 of offspring, and diabetes history of offsprings in 2010 visit showed statistically significant association with positive relations with HOMA-IR. However, other maternal and offsprings' characteristics are not statistically significant to associate with HOMA-IR. In fact, all factors, except BMI of mother, were previously reported for the association in several studies [18–22, 38–40]. According to the association with BMI of mothers, the investigators hypothesize that obesity (high BMI) was considered as a genetic disease [41, 42]. Whitaker and colleagues revealed there is a strong association between parental weight status and risk of childhood obesity, which is significantly stronger for maternal weight. Besides from the genetic factor, this association may be affected by maternal uterine environment on offspring adiposity and maternal feeding strategies [43].

Moreover, obesity is a risk factor for diabetes and insulin resistance [44]. Therefore, offspring of their high BMI mothers who inherited those defects could develop insulin resistance. Interestingly, in contrast with Western reports, in-utero environment in this study in Thais did not have any significant association with HOMA-IR. Perhaps this association might be confounded by ethnic factors.

The limitation of this study is the few number of offspring that are at risk of getting diabetes. As a result, only some predictors are shown for a statistically significant relationship with HOMA-IR. However, we aimed to find predictors of insulin resistance in the early life of the offspring at the beginning. Our novel finding implied that any offspring who has a high BMI mother has a high chance to develop insulin resistance and perhaps diabetes in the future. BMI of mother and plasma triglyceride of offspring are not risk factors of screening for type 2 diabetes and pre-diabetes from both the American Diabetes Association (ADA) 2016 Guidelines and Clinical Practice Guidelines for Diabetes 2017 by Thai Diabetes association.

Both guidelines consider screening for all children beginning at age 10 who are overweight and have two or more of the following risk factors, which include family history of type 2 diabetes, ethnic factors and signs of insulin resistance, maternal history of GDM, and hypertension. Our novel finding can be used to optimize screening for DM in pediatric populations [45, 46], so they should be aware and prevent any modifiable risk factors. This may also benefit doctors to conduct community interventions to prevent the disease.

## Conclusion

In conclusion, HOMA-IR is statistically found to be associated with the increasing of body mass index, both of mother and offspring, as well as the elevated plasma triglyceride and the history of diabetes of the offspring. Further studies will shed more light on this field.

**Acknowledgments** The authors would like to thank Pien Chiowanich, MD, from the Department of Pediatrics, Faculty of Medicine, Chiang Mai University, and Ms. Prakaydao Kaima from the Research Institute for Health Sciences together with the Health Promotion Center Region 10. Finally, our accomplishment would be impossible without all participants.

**Funding** This study was financially supported by Chiang Mai University.

### Compliance with ethical standards

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

**Ethical approval** The Local Research Ethics Committee approved the study (Project Number 17/52) and participants gave written consent and participant anonymity was preserve.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):437–443
https://doi.org/10.1007/s13410-018-0668-4

ORIGINAL ARTICLE

# Association of metabolic risk factor clustering and all-cause mortality in adults with non-metabolic syndrome: the rural Chinese cohort study

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Received: 28 March 2018 / Accepted: 25 June 2018 / Published online: 2 August 2018


**Abstract**
Studies demonstrated an increased risk of death with aggregation of metabolic syndrome (MetS) components in the general population; however, the association is unclear in people with non-MetS. We aimed to explore the effect of aggregated metabolic risk factors on all-cause mortality in the non-MetS population. Questionnaire interviews and physical and blood biochemical factors for 13,749 adult participants were analyzed at baseline between July–August 2007 and July–August 2008. We followed up 11,604 participants (84.4%) between July–August 2013 and July–October 2014. Cox proportional-hazards regression produced multivariate adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) to evaluate metabolic risk factor clustering at baseline for all-cause mortality at follow-up. During a mean of 5.93 years of follow-up, 742 deaths (508 males) occurred. With aggregated metabolic risk factors, mortality rate increased overall for male and female participants ($p < 0.001$). For all participants, adjusted HRs for death increased from 1.29 to 1.91 with increased number of metabolic risk factors; risk of death was increased with elevated blood pressure (BP), low high-density lipoprotein cholesterol (HDL-C) level, and high glucose level (HR 1.43 [95% CI 1.23–1.67], 1.17 [1.01–1.37], and 1.24 [1.06–1.45], respectively). A similar association was observed in males but not females, except for elevated BP. Mortality risk increases with aggregated metabolic risk factors except for females, and might be attributed to high BP, low HDL-C levels, and high glucose levels in the non-MetS Chinese population.

**Keywords** Cohort study · Metabolic risk factor · Metabolic syndrome · Mortality

## Introduction

Metabolic syndrome (MetS) represents a constellation of several metabolic abnormalities, including central obesity, high levels of triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C), elevated serum levels of fasting glucose, and elevated blood pressure (BP) [1]. MetS significantly increases the risk of cardiovascular disease (CVD) [2–9] and all-cause mortality [3–6, 8, 10–14]. A prospective cohort study based on 11 European cohorts found that MetS significantly contributed to the increased CVD and all-cause mortality in the non-diabetic population [15]. Two cohort studies reported a positive association of aggregation of MetS components and CVD mortality in Chinese [5] and Japanese [16]. However, in the non-MetS population, the predictive value of aggregated metabolic risk factors for mortality is unknown.

Several MetS definitions have been proposed by different organizations over the years [17]. The identification of people at high risk of CVD and diabetes according to the International Diabetes Federation (IDF) definition is useful for clinicians and patients [1]. Overall, 42.6% of urban and 45.0% of rural area deaths were attributed to CVD according to a report on CVD in China (2016) [18]. Therefore, we used the IDF definition to define MetS and non-MetS participants in the present study.

Most epidemiological studies have targeted participants living in urban areas or cities, so studies in rural areas are limited. Moreover, the rural population in China accounts for the great proportion. Therefore, in this 6-year cohort study

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of a rural adult Chinese population, we evaluated the relationship between aggregated metabolic risk factors and all-cause mortality in non-MetS participants.

## Methods

### Study design and subjects

This study was a population-based, prospective cohort study, and participants were selected by cluster randomization from eligible candidates listed in the residential registration record from Xin'an County, Henan Province, China, during July to August of 2007 and July to August of 2008. We selected Cijian and Tiemen towns from Xinan County, and then 64 villages of which were randomly selected. Participants had no severe psychological disorders, physical disabilities, Alzheimer's disease, dementia, tuberculosis, acquired immune deficiency syndrome, or other infectious diseases. We excluded participants with (1) MetS ($n = 6388$) and (2) who were unable to complete anthropometric or blood pressure or blood sampling measurements (waist circumference WC [$n = 5$] or BP [$n = 1$] or levels of TG [$n = 27$], HDL-C [$n = 15$], or fasting glucose [$n = 9$]). We established a dataset of 13,749 non-MetS adult participants. Of the 13,749 participants, 11,604 (84.4%) were followed up. In the study, all participants were interviewed and examined by trained and dedicated study staff. All the participants gave their written informed consent, and the study protocol was approved by the Medical Ethics Committee of Shenzhen University Health Sciences Center.

### Anthropometric parameters

At the baseline examination, we collected information about socio-demographic characteristics (gender, age, education level, marital status, and income), smoking and drinking status, physical activity, and medical history for all participants using a self-administered questionnaire in an in-person interview. A cigarette smoker was defined as having smoked at least 100 cigarettes during the lifetime [19]. An alcohol drinker was defined as having consumed alcohol 12 or more times in the past year. The physical activity level was categorized as high, moderate, or low according to the International Physical Activity Questionnaire scoring protocol [www.ipaq.ki.se]. WC was analyzed as the mean of two measurements to the nearest 0.1 cm at the 1-cm surface level above the navel with participants standing.

### BP parameter

An electronic sphygmomanometer (HEM-770A Fuzzy, Omron, Japan) was used to record BP three times at the right arm with participants in the seated position, following approximately 5 min of rest, and at 30-s intervals. We used the mean of three measurements as the BP level of participants.

### Biochemical parameters

Venous blood sampling was performed by local physicians after at least 8-h overnight fasting. Serum was separated by centrifugation at 3000 rpm and 4 °C for 10 min. We transferred plasma to an EP tube for storage in a low-temperature freezer for blood examination. The lipid profile (TG and HDL-C) and fasting glucose level were determined using the Hitachi 7060 automatic biochemical analyzer (Japan) with standardized procedures.

### MetS ascertainment

MetS was defined as central obesity (WC ≥ 90 cm for males or ≥ 80 cm for females) plus any two of the following four metabolic disorders: high TG level (TG ≥ 1.7 mmol/L), low HDL-C level (HDL-C < 1.03 mmol/L for males or < 1.29 mmol/L for females), increased BP (systolic BP [SBP] ≥ 130 mmHg or diastolic BP [DBP] ≥ 85 mmHg or use of antihypertensive drugs), and high glucose level (≥ 5.6 mmol/L or drug treatment for elevated glucose) [1]; otherwise, non-MetS was defined. The aggregated number of metabolic risk factors (0, 1, 2, 3, 4) was based on the number of the above five metabolic disorder components.

### Outcome variable

Death information was collected by face-to-face interview with relatives, local village physicians, or other health care providers by a self-administered questionnaire. Dedicated study staff checked and confirmed the death information with vital registration data from the local Center for Disease Control and Prevention.

### Statistical analyses

Data are presented as the median (interquartile range) for non-normally distributed continuous variables, and a number (%) for categorical variables. Wilcoxon rank sum and chi-square tests were used to evaluate differences in continuous and categorical variables, respectively. Mortality rate was calculated by dividing the number of deaths by person-years of follow-up for the study participants. The trend of mortality with increased metabolic risk factors was assessed by the Cochran-Mantel-Haenszel test. Cox proportional-hazards regression analysis was used to estimate the association between metabolic disorder clustering and deaths adjusted for gender (except for the gender-specific model), age, education, marital status, mean individual monthly income, tobacco and alcohol

consumption, and physical activity. Simultaneously, we analyzed mortality by specific metabolic risk factors in the above-adjusted model plus general metabolic risk factors. All statistical analyses involved the use of SAS 9.1 (SAS Inst., Cary, NC); a two-tailed $p < 0.05$ was considered statistically significant.

## Results

During a mean of 5.93 years' follow-up (68,749 person-years), 742 deaths (508 males) occurred among 11,604 participants. The number of deaths was 87 (63 males), 254 (190 males), 277 (171 males), 94 (67 males), and 30 (17 males) for participants with 0, 1, 2, 3, and 4 metabolic risk factors, respectively. Table 1 presents the baseline characteristics of study participants by gender. Compared with female participants, males were older, had a higher level of education and mean individual income (by month), and higher rates of smoking and drinking, lower rates of physical activity, and greater WC, and higher SBP, DBP, TG level, and fasting glucose ($p < 0.001$). Male participants were less often married or cohabiting, with lower HDL-C levels, and had less metabolic risk factor clustering than females ($p < 0.001$).

With aggregated metabolic risk factors, the mortality rate was substantially increased overall ($\chi^2 = 62.632$, $p < 0.001$), for males ($\chi^2 = 57.374$, $p < 0.001$) and for females ($\chi^2 = 24.959$; $p < 0.001$), with 0 to 4 metabolic risk factors. Moreover, mortality with 0, 1, 2, 3, and 4 metabolic risk factors was significantly higher for males than females (7.97 vs. 4.36, 15.79 vs. 4.92, 18.37 vs. 7.34, 25.70 vs. 10.03, and 30.14 vs. 18.44/1000 person-years, respectively; all $p < 0.05$) (Fig. 1).

Table 2 summarizes hazard ratios (HRs) and 95% confidence intervals (CIs) for death by number of metabolic risk factors. As compared with no metabolic risk factors, the HR for death increased overall with 1, 2, 3, and 4 metabolic risk

**Table 1** Baseline characteristics of study participants by gender

| Variables | Overall ($n = 11,604$) | Male ($n = 5504$) | Female ($n = 6100$) | $p$ value |
|---|---|---|---|---|
| Age (years) | 50 (40–60) | 54 (43–63) | 46 (38–56) | < 0.001 |
| Education | | | | < 0.001 |
| ≤ Primary school | 5189 (44.72) | 2060 (37.43) | 3129 (51.30) | |
| ≥ Middle school | 6415 (55.28) | 3444 (62.57) | 2971 (48.70) | |
| Married/cohabitating | 10,367 (89.36) | 4841 (88.00) | 5526 (90.59) | < 0.001 |
| Mean individual income (monthly) | | | | < 0.002 |
| < 500 RMB | 10,804 (93.35) | 5080 (92.48) | 5724 (94.13) | |
| 500–1000 RMB | 617 (5.33) | 331 (6.03) | 286 (4.70) | |
| ≥ 1000 RMB | 153 (1.32) | 82 (1.49) | 71 (1.17) | |
| Cigarette smoker | 3805 (32.79) | 3785 (68.77) | 20 (0.33) | < 0.001 |
| Alcohol consumption | 1498 (12.91) | 1452 (26.38) | 46 (0.75) | < 0.001 |
| Physical activity | | | | < 0.001 |
| Low | 5797 (49.96) | 3131 (56.89) | 2666 (43.70) | |
| Moderate | 2347 (20.23) | 967 (17.57) | 1380 (22.62) | |
| High | 3460 (29.82) | 1406 (25.55) | 2054 (33.67) | |
| WC (cm) | 77.50 (72.10–83.60) | 79.50 (73.75–85.50) | 76.15 (71.00–80.83) | < 0.001 |
| SBP (mmHg) | 118.67 (109.00–130.67) | 121.00 (111.67–133.00) | 116.00 (106.67–128.00) | < 0.001 |
| DBP (mmHg) | 74.67 (68.67–81.67) | 75.67 (69.33–82.67) | 74.00 (68.33–81.00) | < 0.001 |
| TG (mmol/L) | 1.16 (0.86–1.58) | 1.19 (0.88–1.65) | 1.14 (0.84–1.51) | < 0.001 |
| HDL-C (mmol/L) | 1.18 (1.02–1.37) | 1.12 (0.97–1.29) | 1.25 (1.07–1.43) | < 0.001 |
| FPG (mmol/L) | 5.23 (4.91–5.57) | 5.26 (4.92–5.61) | 5.21 (4.91–5.52) | < 0.001 |
| No. of metabolic risk factors | | | | < 0.001 |
| 0 | 2199 (18.95) | 1290 (23.44) | 909 (14.90) | |
| 1 | 4202 (36.21) | 2040 (37.06) | 2162 (35.44) | |
| 2 | 4035 (34.77) | 1605 (29.16) | 2430 (39.84) | |
| 3 | 937 (8.07) | 468 (8.50) | 469 (7.69) | |
| 4 | 231 (1.99) | 101 (1.84) | 130 (2.13) | |

Data are the median (interquartile range) for continuous variables or number (percentage) for categorical variables

*DBP*, diastolic blood pressure; *FPG*, fasting plasma glucose; *HDL-C*, high-density lipoprotein cholesterol; *SBP*, systolic blood pressure; *TG*, triglycerides; *WC*, waist circumference

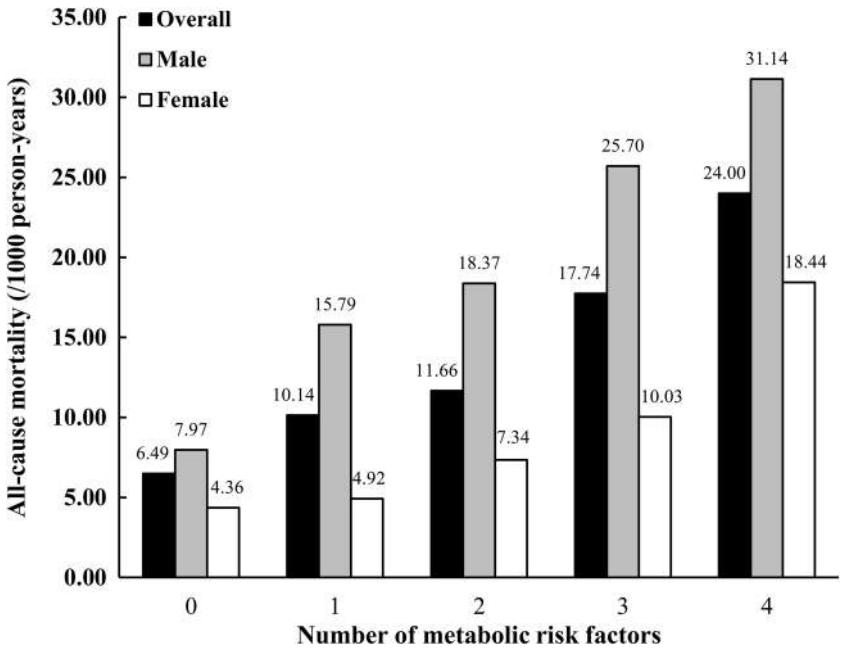


**Fig. 1** All-cause mortality rate for the non-metabolic syndrome participants by the number of metabolic risk factors

factors (1.57 [95% CI 1.23–2.01], 1.85 [1.45–2.36], 2.84 [2.12–3.81], and 3.96 [2.61–6.00], respectively; $p < 0.001$), for males (1.97 [1.48–2.63], 2.36 [1.77–3.16], 3.32 [2.35–4.69], and 4.08 [2.39–6.98]; $p < 0.001$) and for females (1.16 [0.86–1.56], 1.77 [1.13–2.78], 2.46 [1.41–4.30], and 4.72 [2.39–9.32]; $p < 0.001$). After adjustment for gender (except for the gender-specific model), age, education level, marital status, mean individual monthly income, tobacco and alcohol consumption, and physical activity, the association was consistent overall (HR 1.29 [1.01–1.66], 1.44 [1.13–1.84], 1.60 [1.19–2.15], and 1.91 [1.26–2.91], respectively; $p < 0.001$); for males the association was 1.43 [1.08–1.91], 1.50 [1.12–2.01], 1.96 [1.39–2.78], and 2.09 [1.22–3.59], respectively; $p < 0.001$. However, for females, the HR trend for all-cause deaths was not significantly associated with an increasing number of metabolic risk factors ($p = 0.214$).

Females showed lower mortality (1/1000 person-years) than males among those with increased WC (4.85 vs. 11.36), high BP (12.94 vs. 27.86), high TG levels (7.66 vs. 12.65), low HDL-C levels (5.89 vs. 17.39), and high glucose levels (10.30 vs. 21.43) (all $p < 0.05$) (Table 3). Of note, risk of death was decreased with increasing WC for males and females (HR 0.70 [95% CI 0.49–1.00] and 0.68 [0.50–0.94]) with high TG levels for males (0.77 [0.61–0.96]) in an unadjusted model. After multivariable adjustment, risk of death increased with high BP overall (HR 1.43 [95% CI 1.23–1.67]), for males (1.48 [1.23–1.78]) and for females (1.34 [1.02–1.78]). Risk of death also increased and with low HDL-C levels and high glucose levels overall (HR 1.17 [95% CI 1.01–1.37] and 1.24 [1.04–1.65]), for males (1.26 [1.05–1.51] and 1.22 [1.01–1.47]) but not females (0.99 [0.76–1.30] and 1.30 [0.98–1.73]) (Table 3).

**Table 2** Cox proportion-hazards regression for all-cause deaths by number of metabolic risk factors

| No. of metabolic risk factors | Overall | | Male | | Female | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | HR (95% CI)† | HR (95% CI) | HR (95% CI)† | HR (95% CI) | HR (95% CI)† |
| 0 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 1 | 1.57 (1.23–2.01)* | 1.29 (1.01–1.66)* | 1.97 (1.48–2.63)* | 1.43 (1.08–1.91)* | 1.16 (0.72–1.87) | 1.16 (0.86–1.56) |
| 2 | 1.85 (1.45–2.36)* | 1.44 (1.13–1.84)* | 2.36 (1.77–3.16)* | 1.50 (1.12–2.01)* | 1.77 (1.13–2.78)* | 1.18 (0.75–1.87) |
| 3 | 2.84 (2.12–3.81)* | 1.60 (1.19–2.15)* | 3.32 (2.35–4.69)* | 1.96 (1.39–2.78)* | 2.46 (1.41–4.30)* | 0.98 (0.56–1.73) |
| 4 | 3.96 (2.61–6.00)* | 1.91 (1.26–2.91)* | 4.08 (2.39–6.98)* | 2.09 (1.22–3.59)* | 4.72 (2.39–9.32)* | 1.48 (0.74–2.94) |
| *p* for trend | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.214 |

*CI*, confidence interval; *HR*, hazard ratio

† Adjusted for gender (except for gender-specific model), age, education, marital status, mean individual monthly income, tobacco and alcohol consumption, and physical activity

*$p < 0.05$

**Table 3** Cox proportional-hazards regression for all-cause mortality by specific metabolic risk factor

| Study participants | Metabolic risk factors | | | | |
|---|---|---|---|---|---|
| | Increased WC‡ | High BP§ | High TG level\| | Low HDL-C level¶ | High glucose** |
| Overall | | | | | |
| No. of participants | 2151 | 3555 | 2276 | 5285 | 2740 |
| No. of deaths | 82 | 424 | 139 | 315 | 250 |
| Person-years of follow-up | 13,001 | 20,264 | 13,309 | 31,476 | 15,394 |
| Crude mortality, /1000 person-years | 6.31 | 20.92 | 10.44 | 10.01 | 16.24 |
| HR (95% CI) | 0.53 (0.42–0.67)* | 3.26 (2.82–3.77)* | 0.97 (0.81–1.17) | 0.87 (0.75–1.01) | 1.82 (1.56–2.12)* |
| HR (95% CI)† | 0.89 (0.70–1.14) | 1.43 (1.23–1.67)* | 0.86 (0.71–1.05) | 1.17 (1.01–1.37)* | 1.24 (1.06–1.45)* |
| Male | | | | | |
| No. of participants | 482 | 1922 | 1268 | 1916 | 1470 |
| No. of deaths | 33 | 302 | 94 | 196 | 176 |
| Person-years of follow-up | 2905 | 10,840 | 7433 | 11,268 | 8212 |
| Crude mortality, /1000 person-years | 11.36 | 27.86 | 12.65 | 17.39 | 21.43 |
| HR (95% CI) | 0.70 (0.49–1.00) | 2.98 (2.50–3.56)* | 0.77 (0.61–0.96)* | 1.18 (0.99–1.41) | 1.61 (1.34–1.93)* |
| HR (95% CI)† | 0.86 (0.59–1.24) | 1.48 (1.23–1.78)* | 0.90 (0.71–1.14) | 1.26 (1.05–1.51)* | 1.22 (1.01–1.47)* |
| Female | | | | | |
| No. of participants | 1669 | 1633 | 1008 | 3369 | 1270 |
| No. of deaths | 49 | 122 | 45 | 119 | 74 |
| Person-years of follow-up | 10,096 | 9425 | 5877 | 20,208 | 7182 |
| Crude mortality, /1000 person-years | 4.85 | 12.94 | 7.66 | 5.89 | 10.30 |
| HR (95% CI) | 0.68 (0.50–0.94)* | 3.19 (2.64–4.12)* | 1.25 (0.91–1.73) | 0.82 (0.64–1.06) | 1.96 (1.48–2.58)* |
| HR (95%CI)† | 0.87 (0.62–1.23) | 1.34 (1.02–1.78)* | 0.84 (0.60–1.18) | 0.99 (0.76–1.30) | 1.30 (0.98–1.73) |

*BP*, blood pressure; *CI*, confidence interval; *HDL-C*, high-density lipoprotein cholesterol; *HR*, hazard ratio; *TG*, triglycerides; *WC*, waist circumference

†Adjusted for gender (except for gender-specific model), age, education, marital status, mean individual monthly income, tobacco and alcohol consumption, physical activity, and metabolic risk factors

‡ Systolic BP [SBP], diastolic BP [DBP], TG, HDL, and fasting glucose levels

§ WC, TG, HDL, and fasting glucose levels

| WC, SBP, DBP, HDL, and fasting glucose levels

¶ WC, SBP, DBP, TG, and fasting glucose levels

**WC, SBP, DBP, TG, and HDL levels)

*$p < 0.05$

## Discussion

This prospective analysis of 11,604 rural Chinese adults shows that the all-cause mortality rate increased linearly with aggregated metabolic risk factors overall and for both male and female participants, but with lower mortality for females than males. We observed some positive associations between metabolic risk factor clustering and elevated mortality risk overall, and for both male and female participants on univariate analysis. After adjusting for multiple covariates, the association was consistent overall and for male, but not female, participants. Significant independent risk factors for mortality were high BP, low HDL-C levels, and high glucose level overall and for males, but only high BP for females.

One study found that the mortality rate was markedly higher for males than for females, and MetS was found to increase the all-cause mortality risk for males but not females in the general Chinese older population [5], which is similar to our results. Risk of death was decreased with increasing WC (waist circumference) or with high TG levels but the association was not observed in an adjusted model. It might be a reasonable explanation that confounding factors distort the real result. The gender difference might be explained by the higher median WC, SBP, DBP, TG, and fasting glucose levels and lower HDL-C levels found in males (Table 1), because disorders of these factors were found to be metabolic risk factors in the current study. People with good health awareness are more likely to have a healthy behavior lifestyle,

which might delay the occurrence of death. Therefore, different health awareness by sex may be an explanation for the different findings [20]. Overall, people with multiple metabolic factors have a higher CVD mortality risk [6, 15, 16, 21]. A specific combination of MetS components was the best predictor for all-cause mortality after adjusting for multiple covariates [10, 22]. We found risk of death to be greater for those participants with rather than those without an increased number of metabolic risk factors.

All our findings revealed that aggregated metabolic risk factors play an important role in elevated mortality risk for non-MetS participants, but whether all metabolic risk factors contributed significantly to the effect was unknown. Therefore, we stratified participants by specific metabolic risk factors and found the risk of death increased among non-MetS people with high BP for both sexes, and low HDL-C levels and high glucose for males, which further explains the gender differences between metabolic risk factor clustering and mortality. Conversely, high glucose levels and low HDL-C levels have previously been found to be associated with elevated all-cause mortality for women, but not men [23], and increased risk of death to be significantly related to impaired fasting glucose for both sexes and with high BP for males and borderline high BP for females (HR 1.49 [95% CI 0.99–2.24]) [5]. The CVD risk of death has been found to increase by 2.07 times among those with high BP and be borderline significant with high blood glucose levels (HR 1.45, 95% CI 0.99–2.14) [16]. One meta-analysis found a 1.5-fold risk of all-cause death for participants with MetS [13]; the fact that our study only consists of a non-MetS population might be a better explanation for the difference between our findings and previous studies [5, 23]. Regarding the somewhat inconclusive consistency among studies, further research is essential to confirm the findings.

The advantages of our study included its novel evaluation of the association between metabolic risk factor clustering and death according to number of metabolic risk factors in a relatively large non-MetS cohort population. All study participants were from communities, which is more suitable for extrapolation to the general population. However, participants were from rural areas, which might decrease the representativeness. We analyzed the association of metabolic factors and deaths taking into account only the baseline information on metabolic factors because no metabolic factor information was available at follow-up for the death cases; however, the status of metabolic factors could have changed during follow-up. This means the relation between metabolic parameters and death might be over- or underestimated even after adjustment for gender, age, education level, marital status, income, tobacco and alcohol consumption, and physical activity. We had a rate of lost to follow-up at 15.6%, which might introduce bias because of significant differences in baseline gender, age, education level, marital status, income and smoking by response status.. Consequently, our findings should be carefully interpreted and extrapolated.

In conclusion, the risk of mortality is related to the number of metabolic risk factors overall and for male non-MetS participants. The increased mortality might be due to high BP, low HDL-C levels, and high glucose levels. The finding suggests that precautionary and appropriate preventive measures should be taken for non-MetS patients with metabolic risk factor clustering, especially males with high BP, low HDL-C levels, or high glucose levels.

**Acknowledgments** The authors thank all the participants of the survey and all the survey staff.

**Funding information** This study was supported by the National Natural Science Foundation of China (no. 81673260) and the Science and Technology Development Foundation of Shenzhen (nos. JCYJ2016030715570 and JCY20170412110537191).

## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):444–450
https://doi.org/10.1007/s13410-018-0663-9

ORIGINAL ARTICLE

# Prevalence of metabolic syndrome in beta thalassemia major adolescents in southern Iran: a cross-sectional study

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Received: 13 January 2018 / Accepted: 11 June 2018 / Published online: 5 July 2018


**Abstract**
Even though beta thalassemia major (β-TM) patients' survival has improved, there are growing concerns about long-term complications and its related therapeutic side effects. We aimed to investigate the prevalence of metabolic syndrome in the β-TM patients in southern Iran. This cross-sectional study was conducted on 100 beta thalassemia major patients, aged 12–40 years and their age- and gender-matched controls. Weight, height, blood pressure, and waist circumference of the patients were evaluated by a single trained physician. Serum triglyceride, total cholesterol, high-density lipoprotein, low-density lipoprotein, and fasting plasma glucose were assessed. World Health Organization (WHO) body mass index (BMI) curves and International Diabetes Federation (IDF) metabolic syndrome criteria were used as a reference. Statistical Package for Social Sciences (SPSS) software version 18.0 was used for analysis. Prevalence of underweight, overweight, and obesity in our patients was 28, 5, and 1%, respectively. In patients with thalassemia (β-TM), prevalence of metabolic syndrome was 22% and was associated with BMI ($p = 0.001$). It was more prevalent in females (26.3% in females vs. 8.3% in males) ($p = 0.045$). Serum ferritin was associated with abdominal obesity ($p = 0.028$) and hypertriglyceridemia ($p = 0.017$). This study revealed that 22% of β-TM patients had IDF criteria of metabolic syndrome and low HDL was the most frequent positive criterion. Future studies should be conducted to find out the relevant factors to reduce the cardiovascular morbidities associated with metabolic syndrome in β-TM patients.

**Keywords** Metabolic syndrome · Beta thalassemia major · Obesity · Iran

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## Introduction

Beta thalassemia major (β-TM) is a hereditary anemia resulting from the absence or severe deficiency of β-globin chain production, which leads to chronic hemolytic anemia [1]. These patients are in need of regular blood transfusion to improve oxygen carrying capacity and their overall wellbeing. However, such treatment might lead to iron overload and its related morbidities, such as cardiac, liver, and endocrine system damages [2]. In recent decades, combination of blood transfusion and iron-chelating therapies has improved the life expectancy and quality of life of β-TM patients [3].

While β-TM patients' survival has improved, there are growing concerns about long-term complications of this disease and its related therapies [2]. One important complication can be metabolic syndrome, which is accompanied by hyperglycemia, dyslipidemia, abdominal obesity, and hypertension.

Metabolic syndrome can increase cardiovascular atherosclerotic risks and diabetes mellitus [4]. Hyperinsulinemia and insulin resistance are well documented in β-TM patients [1, 4–7]; however, its association with metabolic syndrome has not been investigated. Furthermore, there are some sporadic reports about hypertension [8], hypertriglyceridemia [9, 10], and obesity [11]. To the best of our knowledge, there has been no report about the prevalence of metabolic syndrome in β-TM patients. Consequently, we aimed to investigate the prevalence of metabolic syndrome in β-TM patients in southern Iran.

## Material and methods

The present cross-sectional study was conducted in thalassemia clinics affiliated to Shiraz University of Medical Sciences, Fars Province, Iran, in 2015. All patients were registered in these clinics after the clinical diagnosis and peripheral blood evaluation and hemoglobin electrophoresis. We enrolled 100 patients with beta thalassemia major, selected through simple randomized sampling. Diabetes mellitus and secondary hypertension were our exclusion criteria. Also, adolescents, who had any type of disability that would prevent us from measuring their weight, height, and waist circumference, and pregnant women were excluded from the study. Healthy age- and gender-matched adolescents were enrolled in the control group. Controls were randomly selected from a cohort study in Kavar, located in south of Fars Province, which include 478 normal population checked for metabolic syndrome.

The patients were transfusion-dependent before the age of 2. All patients were treated with deferoxamine as an iron-chelating therapy. This study was approved by both the local ethics committee and vice-chancellor of research at Shiraz University of Medical Sciences. All patients signed a written informed consent.

### Anthropometric data and blood pressure

Weight and height of patients were assessed by a single trained physician. While the patient was standing without shoes; height was measured using an Altura Exata portable stadiometer and the number was rounded to the nearest 0.5 cm. Weight was evaluated using a standard scale (Seca, Germany), while the patient wore light clothes, which was rounded to the nearest 0.1 kg. Body mass index (BMI) was calculated through the standard formula:

$$\mathrm{BMI}\ \left(\mathrm{kg/m^2}\right) = \mathrm{Weight}\ (\mathrm{kg})/[\mathrm{height}\ (\mathrm{m})]^2$$

We classified the patients according to the World Health Organization (WHO) BMI curves specified by age and gender into four categories [12]: underweight, BMI $Z$ score $< -2$; normal, $-2 \geq Z < +1$; overweight, $+1 \geq Z < +2$; and obese adolescents, BMI $Z$ score more than + 2.

Waist circumference (WC) was measured with a Sanny inelastic measuring tape at the midpoint between the last rib and the iliac crest, while the patient was standing relaxed.

Blood pressure (BP) was assessed with a mercury sphygmomanometer (Riester, Germany) using a standard method [13]. The recorded blood pressure was the mean of two BP readings with a 5-min interval in a sitting position.

### Biochemical measurements

Serum triglyceride (TG), total cholesterol (Chol), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and fasting plasma glucose (FPG) were assessed on a Dirui-T-240 auto-analyzer with an enzymatic method (New and high Tec, Jilin, China), in Shiraz endocrinology and metabolism research center.

### Metabolic syndrome definition

We used the International Diabetes Federation (IDF) consensus worldwide definition to define metabolic syndrome [14]. According to these criteria, presence of at least three of the below components was classified as metabolic syndrome:

(1) Central obesity (< 16 years: WC ≤ 90th percentile and ≤ 16 years: WC ≤ 94 cm in males and ≤ 80 cm in females), which is defined for Eastern Mediterranean and Middle East population.
(2) Raised TG level: ≤ 150 mg/dL
(3) Reduced HDL cholesterol: < 40 mg/dL in males and < 50 mg/dL in females
(4) Raised blood pressure: systolic BP ≤ 130 mmHg or diastolic BP ≤ 85 mmHg
(5) Raised FPG: FPG ≤ 100 mg/dL

### Statistics

Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) software version 18.0. Numerical data was mentioned as mean ± SD. $p$ value less than 0.05 was considered as significant. Normality of data distribution was evaluated using Kolmogorov-Smirnov test. We compare normally distributed data by Student's $t$ test and not-normal ones by Mann-Whitney test. Qualitative data comparisons were carried out using chi-square test.

## Results

One hundred patients with beta thalassemia major aged 23.7 ± 5.9 years and their age- and gender-matched controls were enrolled in this study (Table 1). Patients with thalassemia included 24 males and 76 females, and controls included 36 males and 64 females ($p$ value = 0.07). General characteristics and biochemical studies of our patients were defined by gender shown in Table 2. Male patients had higher diastolic blood pressure ($p = 0.018$), higher serum ferritin level ($p = 0.002$), and lower HDL level ($p = 0.024$). Prevalence of underweight, overweight, and obesity in our patients was 28, 5, and 1%, respectively. BMI categories according to WHO classification in both genders of our patients and controls are shown in Table 3. BMI of thalassemia patients is lower than that of the controls ($p < 0.001$). We also did not observe any significant difference between prevalence of overweight or obesity among both genders ($p = 0.23$).

Prevalence of hyperglycemia, abdominal obesity, low HDL, hypertriglyceridemia, and hypertension in patients with thalassemia was 32, 32, 90, 23, and 4%, respectively. Figure 1 and Table 4 reveal that metabolic syndrome was more prevalent in thalassemia patients ($p < 0.001$). Table 5 shows that there was no significant difference between the prevalence of these criteria in males and females with thalassemia major, except for abdominal obesity, which was more prevalent in females ($p < 0.001$). Data revealed that 22% of our patients had metabolic syndrome, which was more prevalent in females (26.3% in females vs. 8.3% in males), ($p = 0.045$). Metabolic syndrome in our patients was associated with BMI ($p = 0.001$). However, it was not associated with age ($p = 0.217$), ferritin ($p = 0.702$), hemoglobin ($p = 0.734$), and transfusion intervals ($p = 0.215$). Serum ferritin was associated with abdominal obesity ($p = 0.028$) and hypertriglyceridemia ($p = 0.017$), but it was not associated with hyperglycemia ($p = 0.381$), hypertension ($p = 0.33$), and low HDL ($p = 0.073$).

**Table 1** General characteristics and the biochemical studies of beta thalassemia major patients and their healthy controls (mean ± SD)

| Variable | Case | Control | $p$ value |
|---|---|---|---|
| Age (years) | 23.7 ± 5.9 | 22.6 ± 5.8 | 0.175 |
| Gender (male/female) | 24/76 | 36/64 | 0.07 |
| Height (cm) | 155.8 ± 9.2 | 166 ± 10.6 | < 0.001 |
| Weight (kg) | 49.6 ± 8.6 | 61.6 ± 13.8 | < 0.001 |
| BMI (kg/m$^2$) | 20.3 ± 2.9 | 22.1 ± 3.7 | < 0.001 |
| Systolic BP (mmHg) | 101 ± 10.1 | 108 ± 13.4 | < 0.001 |
| Diastolic BP (mmHg) | 62 ± 10.6 | 70.6 ± 9.6 | < 0.001 |
| FBS (mg/100) | 98.7 ± 23.6 | 74.7 ± 11.3 | < 0.001 |
| Triglyceride (mg/dL) | 131 ± 87 | 77.1 ± 55.3 | < 0.001 |
| Cholesterol (mg/dL) | 118 ± 41 | 151 ± 31 | < 0.001 |
| HDL (mg/dL) | 33.2 ± 11.6 | 44.2 ± 12.4 | < 0.001 |
| LDL (mg/dL) | 57.6 ± 25.4 | 92.2 ± 27.4 | < 0.001 |
| HbA2 (%) | 2.7 ± 1.3 | 1.2 ± 0.7 | 0.041 |
| Hbf (%) | 92.9 ± 4.6 | 1.1 ± 0.9 | < 0.001 |
| MCV (fL) | 70.5 ± 8.3 | 85 ± 9.7 | < 0.001 |
| Hb (g/dL) | 9.4 ± 1.1 | 13.4 ± 2.9 | < 0.001 |
| HTC (%) | 27.1 ± 2.6 | 46 ± 5.4 | < 0.001 |
| Ferritin (ng/mL) | 2643 ± 1157 | 77 ± 37.5 | < 0.001 |

*BMI*, body mass index; *BP*, blood pressure; *FBS*, fasting blood sugar; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *Hb*, hemoglobin; *HTC*, hematocrit

**Table 2** General characteristics and the biochemical studies of beta thalassemia major patients in both sexes

| Variable | Male | Female | $p$ value |
|---|---|---|---|
| Age (years) | 22.3 ± 6.5 | 24.2 ± 5.7 | 0.183 |
| Gender | 24 | 76 | – |
| Height (cm) | 158.6 ± 10.3 | 154.9 ± 8.6 | 0.086 |
| Weight (kg) | 49.5 ± 10.3 | 49.7 ± 8.1 | 0.918 |
| BMI (kg/m$^2$) | 19.5 ± 2.5 | 20.6 ± 3.1 | 0.09 |
| Systolic BP (mmHg) | 103.7 ± 8.5 | 100.6 ± 10.5 | 0.18 |
| Diastolic BP (mmHg) | 67 ± 8.9 | 61 ± 10.8 | 0.018 |
| Waist circumference (cm) | 78 ± 7.2 | 77.5 ± 7.4 | 0.789 |
| FBS (mg/100) | 99.7 ± 15.3 | 98.4 ± 25.7 | 0.812 |
| Hemoglobin (g/dL) | 9.6 ± 1.1 | 9.4 ± 1.1 | 0.493 |
| Ferritin (ng/mL) | 3693 ± 2173 | 2293 ± 1760 | 0.002 |
| Triglyceride (mg/dL) | 140 ± 10.9 | 128 ± 96 | 0.559 |
| Cholesterol (mg/dL) | 115 ± 37 | 119.6 ± 42 | 0.667 |
| HDL (mg/dL) | 28.5 ± 8.1 | 34.7 ± 12.3 | 0.024 |
| LDL (mg/dL) | 60.7 ± 28.8 | 56.6 ± 24.3 | 0.494 |
| Transfusion interval | | | |
| Every 2 weeks | 12 (50%) | 29 (38%) | 0.182 |
| Every 3 weeks | 11 (46%) | 36 (47%) | |
| Every 4 weeks | 1 (4%) | 11 (15%) | |

*BMI*, body mass index; *BP*, blood pressure; *FBS*, fasting blood sugar; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein

## Discussion

The present study revealed that 6% of our thalassemic patients were either overweight or obese. Also, it was shown that 22% of our β-TM patients had metabolic syndrome, and low HDL was the most frequent positive criterion. The present study showed that metabolic syndrome was more prevalent in females. Furthermore, it was shown that metabolic syndrome was associated with BMI; whereas, abdominal obesity and hypertriglyceridemia were associated with serum ferritin.

**Table 3** WHO BMI categories in the beta thalassemia major patients, in both sexes

| BMI categories* | Total** | | Male | | Female | |
|---|---|---|---|---|---|---|
| | Patients | Controls | Patients | Controls | Patients | Controls |
| Underweight | 28% | 16% | 37.5% | 5.6 | 25.3% | 21.9 |
| Normal BMI | 66% | 62% | 62.5% | 69.4 | 66.7% | 57.8 |
| Overweight | 5% | 18% | 0% | 19.4 | 6.7% | 17.2 |
| Obese | 1% | 4% | 0% | 5.6 | 1.3% | 3.1 |

*BMI*, body mass index

*There is no significant difference between BMI categories of both sexes in beta thalassemia patients ($p$ value = 0.23)

**BMI of thalassemia patients is lower than that of the controls ($p < 0.001$)

Prevalence of metabolic syndrome in adults is 26–29% (16–20% in males and 30–37% in females) in Iran [13, 15–20], and abdominal obesity is the most prevalent positive criterion. We showed that 2% of healthy adolescents (aged 22.6 ± 5.8 years) in southern Iran have metabolic syndrome, and also low HDL was the most common criterion. However, there is no validated data about the prevalence of metabolic syndrome in β-TM patients. Hence, it was revealed that 8.3% of males and 26.3% of females with β-TM have metabolic syndrome, which is in a similar range of the normal population. However, low HDL was the most prevalent criterion in β-TM patients. Gozashti et al. revealed that prevalence of metabolic syndrome in minor β-TM patients was lower than that in the normal population, and they proposed that minor thalassemia might act as a protective factor for metabolic syndrome [21].

Abnormal glucose tolerance and insulin resistance were previously reported in multi-transfused β-TM patients [5–7, 22]. Also, it was shown that insulin resistance was associated with metabolic syndrome [19, 20]. In β-TM patients, hyperglycemia might be due to early impairment of β cell function and insulin resistance. Iron overload and chronic hepatitis C (HCV) could play a significant role in this regard [6]. Some previous studies reported the relationship between serum

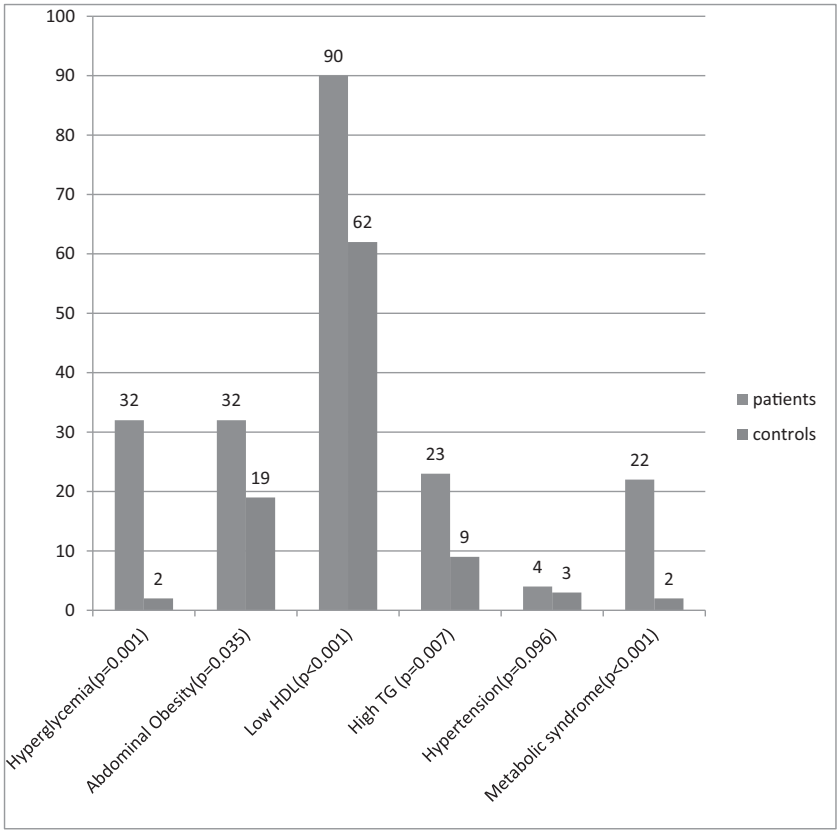


**Fig. 1** Frequency of positive IDF criteria of metabolic syndrome comparisons between beta thalassemia patients and their controls

**Table 4** Frequency of positive IDF criteria of metabolic syndrome in controls compared to beta thalassemia major patients

| Criteria | Case (%) | Control (%) | *p* value |
|---|---|---|---|
| Hyperglycemia | 32 (32) | 2 (2) | < 0.001 |
| Abdominal obesity | 32 (32) | 19 (19) | < 0.035 |
| Low HDL | 90 (90) | 62 (62) | < 0.001 |
| High TG | 23 (23) | 9 (9) | < 0.007 |
| Hypertension | 4 (4) | 3 (3) | < 0.096 |
| Metabolic syndrome | 22 (22) | 2 (2) | < 0.001 |

*HDL*, high-density lipoprotein; *TG*, triglyceride

ferritin levels and insulin resistance in non-diabetic women [23], in over weight and obese population [24], and in Korean men [25]. These results suggest that iron overload is associated with insulin resistance even in non-thalassemic populations [25]. Our result revealed that there is a high prevalence of hyperglycemia (32%) in major thalassemic patients; however, it was not associated with serum ferritin. Also, Kirim et al. revealed that there was no association between β-TM minor and metabolic syndrome criteria, regardless of insulin resistance [26]. Other possible explanation could be liver damage or HCV infection that might play an important role in hyperglycemia of β-TM patient that needs to be investigated in future studies.

Fung et al. reported that 26.5% of non-transfused thalassemia in Canada had BMI of more than 25. This was more than that of non-transfused thalassemia, and females had higher body fat index in comparison to men [11]. It was shown that 6% of our β-TM patients had BMI of more than 25, while, 32% of β-TM patients had abdominal obesity, which was significantly higher in women. The differences between the Fung et al. study and our report could be attributed to the differences in culture and lifestyle [27]. They also revealed that abdominal obesity was associated with serum ferritin. Fung et al. suggested that hypogonadism and growth hormone deficiency secondary to iron overload in β-TM patient played an important role in abdominal obesity [11].

**Table 5** Frequency of positive IDF criteria of metabolic syndrome in beta thalassemia major patients classified by sex

| Criteria | Total (%) | Male (%) | Female (%) | *p* value |
|---|---|---|---|---|
| Hyperglycemia | 32 (32) | 8 (33.3) | 24 (31.6) | 0.872 |
| Abdominal obesity | 32 (32) | 0 (0) | 32 (42.1) | < 0.001 |
| Low HDL | 90 (90) | 23 (96) | 67 (88.2) | 0.275 |
| High TG | 23 (23) | 9 (37.5) | 14 (18.4) | 0.053 |
| Hypertension | 4 (4) | 1 (4.2) | 3 (4) | 0.971 |
| Metabolic syndrome | 22 (22) | 2 (8.3) | 20 (26.3) | 0.045 |

*HDL*, high-density lipoprotein; *TG*, triglyceride

Low level of total cholesterol accompanied with low serum HDL, low serum LDL, and hypertriglyceridemia was well shown in β-TM patients [10, 28–32]. This might be due to increased erythropoiesis that could result in increased cholesterol requirement for cell membrane formation in these patients [29, 33]. Some studies have found a link between serum ferritin and changes in lipid profile of β-TM patients [34, 35]. Khera et al. also reported on a syndrome called hypertriglyceridemia thalassemia in four β-TM patients [9]. Even though pathogenesis of such syndrome is still unclear, it might be associated with high risk of developing atherosclerosis and acute pancreatitis at a young age [9]. Bordbar et al. revealed a genotype-phenotype correlation between lipid profile and different β-globin gene mutations [10]. The most severe gene mutation (β°/β°) in thalassemia patients is accompanied with high reduction in serum lipids [10]. In the present study, we found a high prevalence of low HDL (90%). Also, 23% of our β-TM patients had hypertriglyceridemia, associated with their serum ferritin.

Prevalence of hypertension in β-TM patients was previously reported at 6.7% [36]. In our study, 4% of our β-TM patients had hypertension, which was close to the prevalence in the normal population [13]. However, heart rate variability was reduced in β-TM patients due to early autonomic neuropathy in their hearts [37]. Similar to our results, Tabatabaie et al. revealed that serum ferritin, hemoglobin, and transfusion intervals did not affect the abnormal changes in blood pressure [36].

Although there are several novelties in the present research, such as being the first to evaluate the metabolic syndrome prevalence in β-TM patients, it was also the first to evaluate the prevalence of abdominal obesity and hypertension in β-TM patients of southern Iran. However, there were some limitations. We did not check the fasting insulin level to estimate the Homeostatic Model Assessment (HOMA) index. We suggest considering the evaluation of the HOMA index in β-TM patients in future researches to find out a more accurate estimation of the metabolic syndrome in β-TM patients and its association with insulin resistance.

## Conclusion

This study revealed that 22% of β-TM patients had IDF criteria of metabolic syndrome and low HDL was the most frequent positive criterion. Metabolic syndrome was more prevalent in female patients. Abdominal obesity and hypertriglyceridemia were associated with serum ferritin. Further studies should be performed to find the related factors, to reduce the cardiovascular morbidities associated with metabolic syndrome in β-TM patients.

**Acknowledgments** The authors wish to thank Mr. H. Argasi at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for his invaluable assistance in editing this manuscript.

## Compliance with ethical standards

The study was approved by Shiraz University of Medical Sciences (SUMS) local Ethics Committee and vice-chancellor of research at SUMS. Written informed consent form was signed by all participants and their parents.

**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 2019) 39(3):451–457
https://doi.org/10.1007/s13410-019-00715-4

ORIGINAL ARTICLE

# First trimester zonulin levels and adiposity as predictive indices of gestational diabetes mellitus

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Received: 31 July 2018 / Accepted: 13 January 2019 / Published online: 31 January 2019


## Abstract

**Background** This study was aimed at determining the levels of serum zonulin during the first trimester in pregnant women and to examine the relationship between zonulin and obesity in the development of gestational diabetes mellitus (GDM). Available evidence suggests that the permeability of the gut may be associated with obesity and insulin resistance both of which are characteristics of GDM.

**Methods** This was a prospective longitudinal study in which a cohort of 314 pregnant women was monitored from first trimester at the Volta Regional Hospital, Ho, Ghana. Maternal serum zonulin and lipids were analyzed during the first trimester, and body mass index (BMI) was calculated for each participant. Glucose challenge tests (GCT) and oral glucose tolerance tests (OGTT) were done between 24 and 28 weeks of pregnancy, and diagnosis of GDM was made in accordance with the American Diabetes Association (ADA) criteria.

**Results** Women who developed GDM had elevated serum zonulin levels with sensitivity, specificity, and cutoff points of 80.95%, 80.41%, and > 47.5 ng/mL respectively in predicting GDM. With positive predictive value (PPV) and negative predictive value (NPV) of 0.708 and 0.986, respectively, zonulin has been shown by this study to be a good predictor of GDM. After adjusting for maternal age and BMI, obese pregnant women with elevated plasma zonulin were 109 times likelier to develop GDM as compared to those with normal BMIs.

**Conclusions** Zonulin levels are increased significantly during the first trimester of pregnancy in women with GDM, and these increases precede the onset of GDM.

**Keywords** Zonulin · Obesity · Dyslipidemia · Gestational diabetes mellitus

## Introduction

Zonulin, discovered almost two decades ago, is the only physiological mediator that is known to regulate gut permeability of tight junctions (TJs); consequently, increases in its concentration are associated with increase in intestinal permeability [1]. The increased permeability of the TJs has been found to be associated with upregulation of inflammatory markers [2] and may play a role in the pathogenesis of polycystic ovary syndrome [3]. Zonulin has also been shown to have positive correlation with insulin resistance in non-pregnant women [3, 4]. Other studies have established that zonulin levels are considerably elevated in obesity and type 2 diabetes and that it shows positive correlation with tumor necrosis factor α (TNF-α)and interleukin 6 (IL-6) [5, 6].

In the second trimester of pregnancy, insulin resistance usually develops and thus causes about 1.5–2.5 times more insulin levels in normal pregnant women compared to non-pregnant women. This phenomenon is largely attributable to the antagonistic effect exerted on insulin by human placental lactogen, which is produced by the syncytiotrophoblast [7]. In

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a study done at Turku University Hospital in Finland, serum zonulin concentration was found to be associated with higher odds of developing GDM during the second trimester [8]. Normal pregnancy is also associated with insulin resistance and dyslipidemia with a rise in triglycerides (TGs) and small low-density lipoprotein (LDL) particles especially after 20 weeks of gestation. These changes are associated with hormonal changes such as estradiol, progesterone, cortisol, and human chorionic somatomammotropin (hCS) [9]. Several risk factors have been associated with GDM including advanced maternal age, ethnic background, being overweight, family history of type 2 diabetes mellitus, and previous diagnosis of GDM [10]. In a study aimed at determining the risk factors for developing GDM, higher BMI was demonstrated as a strong risk factor for GDM [11]. In another study involving Korean women, the investigators concluded that high pre-gestational BMI was a significant risk factor of GDM and suggested that pre-gestational weight control may be an important tool in the prevention of GDM [12]. Similarly, total cholesterol (TC), LDL cholesterol, very low-density lipoprotein (VLDL) cholesterol, and TGs have been found to be elevated in women with GDM while high-density lipoprotein (HDL) cholesterol has been found to be reduced among women with GDM [13].

Available data indicates that GDM is associated with increased zonulin levels [8]: zonulin concentration is known to regulate gut permeability reversibly by disassembling intestinal tight junctions [14, 15]. Zonulin regulates the trafficking of macromolecules and digested food nutrients across gastro intestinal tract; therefore, different geographical regions of the world may be affected differently by its actions due differences in environmental factors. To date, no relationship between zonulin levels and GDM has been documented among Ghanaian pregnant women. The aim of this study therefore was to investigate the relationship between zonulin levels and GDM among Ghanaians in the Ho Municipality.

## Methods

The participants were selected from a longitudinal study of 314 pregnant women for pregnancy associated complications at the Volta Regional Hospital, Ho, Ghana. The participants were examined from 11 to 13 weeks of gestation till term. Height was measured of participants not wearing shoes using a stadiometer to the nearest 0.5 cm with the study participants standing upright and heels put together and the head in the horizontal plane. Weight was measured in kilograms using the Bioimpedance analyzer (BIA) (BSD01, Pure Pleasure, a division of the Stingray Group, Cape Town, South Africa). All participants wore light clothing at the time the weight was taken. The BMI was determined using the BIA according to the manufacturer's instruction. Blood was collected during the first trimester between 7:00 am and 8:00 am after an overnight fast lasting between 10 and 16 h from each participant. Five milliliters of blood was collected from each participant and 3 ml put into fluoride anticoagulant tubes while the remaining 2 ml of blood was put into serum separator tubes. The samples were then centrifuged at 3000 rpm for 5 min at 4 °C and plasma and sera separated. The plasma samples were used for glucose estimation, while the sera were used for the estimation of zonulin and lipid profile. All participants were screened for GDM where the women's plasma glucose was tested after a 50 g oral glucose load between 24 and 28 weeks of gestation. When the plasma glucose measured after 1 hour was $\geq 7.8$ mmol/l, the woman was referred to do OGTT. Diagnosis of GDM was made after 24 weeks of gestation using the results of the OGTT based on the criteria of the American Diabetes Association [16].

Serum lipid panel analysis was performed with the VITROS$^{(R)}$ dry-chemistry analyzer; (Ortho-Clinical Diagnostics, Johnson & Johnson, 50-100 Holmers Farm Way, High Wycombe, Buckinghamshire, HP124DP, United Kingdom) while serum zonulin levels were determined in duplicates using human zonulin ELISA Kit (Immundiagnostik AG, Bensheim, Germany).

## Power and statistical analysis

All data analyses were performed using the SPSS software (version 20.0 systat, Inc. Germany) and GraphPad Prism, (version 5.0, San Diego California, USA). Data was presented as mean ± SD. In all the statistical analysis, a value of $p < 0.05$ was considered to be significant and at a 95% confidence interval. The area under the receiver operating characteristic (ROC) curve (AUC) is generally considered as a measure of the accuracy of a test/marker. Hence, if the AUC is 50% or less, it is established that the result can be seen as a random guessing and therefore not significant. This is represented by diagonal line in the ROC plot [17]. We decided to test if zonulin had some accuracy (AUC ~ 60%). Therefore, we determined minimum power of 80% with alpha 5%. Every pregnant woman in the hospital is screened for GDM between 24 and 28 weeks of gestation for GDM. The prevalence of GDM in our previous study in the Volta Region of Ghana was about 7% [18]; therefore, we assumed that 7% of the study population would develop GDM. Consequently, the total sample was calculated to be 314 out of which 6.7% (21 women) developed the GDM. The sample size and power calculation were performed using SAS® %ROCPOWER macro [19].

## Results

A total of 21 out of 314 developed GDM representing 6.7% with data on 2 participants missing. Those who developed

**Table 1** Anthropometric and biochemical characteristics of women with GDM and without GDM

| Variables | With GDM ($N = 21$) | Without GDM ($N = 291$) | $p$ value |
|---|---|---|---|
| Age (years) | 33.7 ± 6.0 | 29.6 ± 6.0 | 0.003* |
| BMI | 34.2 ± 4.1 | 26.1 ± 3.9 | < 0.001* |
| Gestational age (weeks) | 11.5 ± 0.7 | 11.7 0.8 | 0.423 |
| Biochemical markers | | | |
| TG (mmol L-1) | 2.27 ± 0.07 | 1.77 ± 0.80 | < 0.001* |
| TC (mmol L-1) | 7.24 ± 0.16 | 5.77 ± 0.17 | < 0.001* |
| HDL (mmol L-1) | 1.20 ± 0.7 | 1.44 ± 0.8 | 0.024* |
| LDL (mmol L-1) | 4.4 ± 0.17 | 3.79 ± 0.16 | < 0.001* |
| VLDL (mmol L-1) | 1.13 ± 0.03 | 0.8 ± 0.04 | < 0.001* |
| Zonulin (ng mL-1) | 59.50 ± 9.0 | 41.84 ± 7.21 | < 0.0001* |

Data is presented as mean ± SD

*TG* triglycerides, *TC* total cholesterol, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *VLDL* very low-density lipoprotein cholesterol

*Signifies $p < 0.05$. Data on two participants missing

GDM were significantly older than those without GDM. The BMI was significantly higher in the GDMs compared to those without GDM (Table 1). The triglycerides, total cholesterol, LDL cholesterol, and VLDL cholesterol were all significantly higher in the GDMs while the HDL cholesterol was significantly lower in the GDMs compared with control showing general dyslipidemia among those who subsequently developed gestational diabetes mellitus (Table 1).

First trimester zonulin concentration was significantly higher in those who subsequently developed GDM as compared to those who did not develop the GDM. The performance of zonulin in discriminating those who are likely to develop GDM is presented in Table 2 and Fig. 1. The receiver operator characteristic curve in Fig. 1 shows that the curve for zonulin indicates a large area under the curve away from the reference line showing its ability to positively predict GDM. In Fig. 1, the area under the curve is 0.901, 95% CI (0.987–1.000) with a $p$ value of < 0.0001. With the likelihood ratio of 4.1, and sensitivity and specificity of 80.95% and 80.41%, respectively, it is evident that the measurement of zonulin during the first trimester can help in determining pregnancies that are likely to result in gestational diabetes mellitus. The cutoff point of > 47.50 ng/mL is suggested from our results as the value which positively discriminates women who are likely to develop GDM (Table 2). This optimal cutoff point was chosen by calculating the ROC sensitivity and specificity pairs and choosing the pair with the minimal distance between them. With a PPV and a NPV of 0.708 and 0.986, respectively, this study has demonstrated that first trimester zonulin level can be used to effectively predict those who are likely to develop GDM (Table 2). When the predictive values were stratified according to the BMI categories, the PPV and NPV were not affected with regard to the normal weight and the obese groups;however, there was a drop in the PPV for the overweight category while the NPV remained very high (Table 2).

We decided to perform logistic regression analysis to adjust for confounders. Table 3 presents results for the goodness of fit statistic for the logistic regression for three models. Model 1 includes the predictors TG, TC, HDL, LDL, VLDL, and zonulin. Model 2 adjusts for potential confounding variable age with the predictors in model 1. Model 3 adjusts for potential confounding variables age and BMI with the predictors in model 1.

The −2log (likelihood) statistic measures how poorly the model predicts an event of interest, the smaller the statistic, the better the model. It can clearly be seen from Table 3 that model 3 has the small statistics which imply that adjusting for the confounding variable BMI made it a better model.

The Cox and Snell $R^2$ and Nagelkerke $R^2$ are coefficients of determination used to estimate the proportion of variance in the dependent variable which is explained by the independent variable. Interpreting the Nagelkerke $R^2$ which is an adjusted version of the Cox and Snell $R^2$, the independent variable explains 67% of the variation in the dependent variable (GDM). Model 2 explains 70% of the variation after adjusting for age, and model 3 explains 84.9% of the variation in the dependent variable. Adjusting for BMI increased the coefficient of determination significantly by 14.9% while age increased it by 3% which is an indication of the contribution of BMI to the model. The Akaike information criterion (AIC) is also an estimator of the relative quality of statistical models: the smaller the estimate, the better the model. Another useful measure to assess the utility of a logistic regression model is the correct classification rate (CCR). Results from the table suggest that adjusting for BMI increases the predictive ability of the model.

Table 4 presents results for the model parameter for the three models. In model 1, zonulin was the only statistically significant predictor in the model ($p$ value < 0.05). A unit increase in zonulin increases the odds of GDM by 1.25. Results from model 2 (age-adjusted) also revealed that zonulin was the only statistically significant predictor with approximately the same odds (1.26) as model 1. Age was found to be insignificant. All the other variables though insignificant had similar odds ratio for models 1 and 2 which suggests that age is not a significant confounder in predicting GDM. Results from model 3 (age- and BMI-adjusted) revealed that zonulin and BMI were statistically significant. The adjusted odds ratio increased to 1.42 for zonulin implying a unit increase in zonulin increases the odds of GDM by 1.42. Obese pregnant women are 108.9 times likelier to develop GDM as compared to those with normal BMIs. The adjusted odds ratios for the other variables in model 3 are quite different comparatively. This suggests evidence of confounding which is BMI in predicting GDM.

**Table 2** Diagnostic values for zonulin in the prediction of GDM

| Zonulin cutoff (ng/mL) | Sensitivity (%) | Specificity (%) | Likelihood ratio | PPV | NPV |
|---|---|---|---|---|---|
| > 47.50 | 80.95 | 80.41 | 4.133 | 0.708 | 0.986 |
| Positive and negative predictive values for zonulin stratified by BMI category | | | | | |
| BMI category | – | – | – | PPV | NPV |
| Normal weight | – | – | – | 1.000 | 1.000 |
| Overweight | – | – | – | 0.333 | 1.000 |
| Obese | – | – | – | 1.000 | 0.889 |

BMI categories: BMI ($Kg/m^2$) of less than 18.5 = underweight, 18.5–24.9 = normal weight, 25–29.9 = overweight, and ≥ 30 = obese

## Discussions

We examined the relationship between first trimester zonulin level and subsequent development of GDM in the course of the pregnancy. Our study showed that significantly higher serum zonulin concentration determined between 11 and 13 weeks of gestation existed in women who later developed GDM than those who remained normoglycemic throughout the pregnancy period. The results also revealed that adjusting for age as a confounding factor does not affect the ability of zonulin in predicting GDM. This finding is in consonance with a report suggesting an increase in zonulin levels among those with GDM [8] and type 2 diabetes mellitus [20] and possible involvement of this physiological modulator of intercellular tight junctions in the pathogenesis of GDM and can also be used as a predictive index of GDM. The likely mechanism involving zonulin in the development of gestational diabetes mellitus is by interfering in the action of insulin receptors and stimulation of inflammation [21] . Furthermore, when the intestinal barrier is bridged, infectious agents and dietary antigens to mucosal immune elements gain access to the body which may lead to increase in immune reactions and destruction of beta cells of pancreas and possible increase in cytokine production. It is therefore expected that an increase in zonulin levels will lead to rise in proinflamatory adipocytokines such as leptin. Leptin levels, in an earlier study, had been demonstrated to increase during the first trimester in women who later developed GDM [18]. The increase in leptin level is also thought to be due to increase in the levels of cytokines like tumor necrosis factor (TNF)-α and interleukin (IL)-6 which are known to upregulate leptin production [22], the consequent effect being an increase in insulin resistance [23]. Insulin resistance is associated with the development of GDM [24]; therefore, with the increased levels of zonulin demonstrated in this study among women with GDM, insulin resistance is probably linked to increase of circulating levels of serum zonulin among pregnant women. Previous studies have demonstrated the association between zonulin and insulin resistance [3, 6]. The results of this study have shown that obese pregnant women are about 109 times likelier to develop GDM than their normal weight counterparts and corroborate a previous study which linked high maternal weight to substantial risk of developing GDM [25]. Previous studies had established the relationship between zonulin, TNF- α, IL-6, and obesity all of which are risk factors of type 2 diabetes mellitus and GDM [5, 6]. It appears zonulin might be involved in promoting insulin resistance and consequently GDM. The proinflammatory cytokines like TNF-α and IL-6 are also elevated in obese women, [5, 6] and so, if a pregnant woman is obese and has increased plasma concentration of zonulin, the likelihood of developing GDM will increase considerably as demonstrated in this study. Obesity results in approximately threefold increase in the risk of developing GDM [10].

Among subjects who subsequently developed GDM, triglyceride levels were significantly higher than those who did not. The results of this study are in an agreement

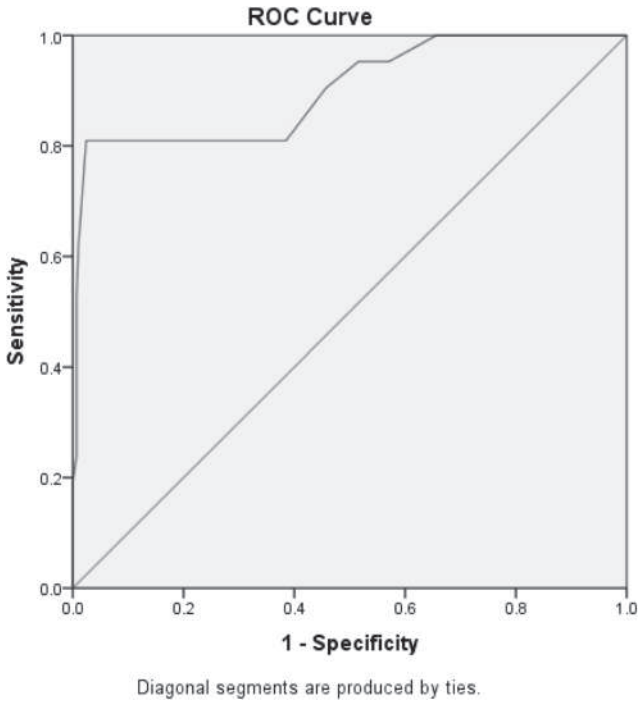


**Fig. 1** ROC curve for the mean zonulin levels in predicting GDM

**Table 3** Goodness of fit statistics (variable presence GDM)

| Models | −2log (likelihood) | $R^2$ (Cox and Snell) | $R^2$ (Nagelkerke) | AIC | CCR (%) |
|---|---|---|---|---|---|
| Model 1 | 59.623 | 0.261 | 0.670 | 73.623 | 98.08 |
| Model 2 | 54.542 | 0.273 | 0.700 | 70.542 | 98.08 |
| Model 3 | 28.633 | 0.331 | 0.849 | 48.633 | 98.40 |

Model 1 includes the predictors TG, TC, HDL, LDL, VLDL, and zonulin. Model 2 includes age with the predictors in model 1. Model 3 includes age and BMI with the predictors in model 1

*AIC* Akaike information criterion, *CCR* correct classification rate

with earlier reports which showed a significant upsurge in the level of triglycerides in pregnancies affected by glucose intolerance as compared to those with euglycemia during pregnancy [26, 27]. However, one report did not demonstrate significant increase in TGs in women with previous GDM cases as compared to those with normal glucose tolerance [28]. The inconsistencies might be due to variations in method and timing of sample collection. This is partly due to the fact that the GDM group of the participants studied in that research, comprised women with earlier GDM, who had been treated with either insulin or diet modifications and it is likely that the treatment as well as the time lapse between the GDM and sample collection time could have affected the lipid profile.

Pregnancy considerably adjusts cholesterol metabolism resulting in dyslipidemia which could play a part in the pathogenesis of GDM. This could be the reason why those with GDM had significant increase in total cholesterol as compared to those without GDM. This is in consonance with a report which showed significant difference in total cholesterol levels between GDMs and normal pregnancy [29]. A Pakistani study on the lipid profile and serum insulin levels in GDM reported that women with GDM had significantly higher cholesterol levels compared with the controls [27].

In this study, LDL cholesterol concentrations were significantly higher in those who developed GDM which corroborates previous reports [27, 29–33]. However, in other studies [34, 35], lower LDL concentrations were described in GDMs compared with the controls.

VLDL cholesterol concentrations for the GDMs were significantly raised as compared with that for the controls. This may be due to the high TG levels observed in this study. VLDL is formed from TG produced in the liver de novo or by re-esterification of free fatty acids. Therefore, VLDL concentration will rise when TG level rises. This is in consonance with earlier reports by various researchers [27, 36].

The increases in the concentrations of TGs, total cholesterol, and LDL cholesterols may be due to the fact that during pregnancy, fat storage surges [37] and progesterone which rises after the 20th week of gestation, acts in a manner to change the lipostat in the hypothalamus causing an increase in the lipid concentration in gestational diabetes mellitus [31]. It is likely that the increase in fat storage shown by some authors [37] might have started much earlier in the first

**Table 4** Regression analysis of factors associated with gestational diabetes mellitus

| Source | Model 1 | | | Model 2 | | | Model 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Value | Pr > Chi$^2$ | Crude odds ratio | Value | Pr > Chi$^2$ | Adjusted odds ratio 1 | Value | Pr > Chi$^2$ | Adjusted odds ratio 2 |
| Intercept | − 16.7 | < 0.001 | | 21.91 | < 0.001 | | − 32.03 | 0.001 | |
| AGE (years) | | | | 0.15 | 0.030 | 1.16 | 0.11 | 0.275 | 1.11 |
| TG (mmol L$^{-1}$) | − 0.13 | 0.903 | 0.88 | − 0.08 | 0.944 | 0.92 | 0.12 | 0.967 | 1.13 |
| TC (mmol L$^{-1}$) | − 0.19 | 0.673 | 0.82 | − 0.14 | 0.760 | 0.87 | − 0.68 | 0.377 | 0.51 |
| HDL (mmol L$^{-1}$) | − 0.37 | 0.456 | 0.69 | − 0.27 | 0.601 | 0.76 | 0.25 | 0.689 | 1.28 |
| LDL (mmol L$^{-1}$) | 0.43 | 0.323 | 1.54 | 0.34 | 0.446 | 1.41 | 0.78 | 0.259 | 2.18 |
| VLDL (mmol L$^{-1}$) | 3.53 | 0.151 | 34.03 | 3.47 | 0.198 | 32.09 | 4.20 | 0.331 | 66.69 |
| Zonulin (ng mL$^{-1}$) | 0.22 | < 0.001 | 1.25 | 0.23 | < 0.001 | 1.26 | 0.35 | 0.004 | 1.42 |
| BMI (normal weight) | | | | | | | 0.00 | | |
| BMI (overweight) | | | | | | | − 0.48 | 0.800 | 0.62 |
| BMI (obese) | | | | | | | 4.69 | 0.011 | 108.9 |

BMI categories: BMI (Kg/m$^2$) of less than 18.5 = underweight, 18.5–24.9 = normal weight, 25–29.9 = overweight, and ≥ 30 = obese

trimester as observed in this study where hyperlipidemia is demonstrated in the first trimester of GDM pregnancies.

Our study demonstrates significantly lower HDL cholesterol levels for the GDMs compared to those who did not develop GDM. This is, however, contrary to one report which did not find significant difference in HDL levels between GDM and normal pregnant women [38]. In this study, the samples were taken prior the occurrence and subsequent diagnoses of GDMs while their samples were taken after the inception of the disease. Conversely, our report is similar to other reports [27, 29], which showed significantly lower HDL concentrations in GDMs compared with those who did not develop the condition. Interestingly, some authors reported no association between HDL and GDM [39].

## Conclusion

This study has demonstrated significant rise in zonulin between 11 and 13 weeks of gestation among pregnant women with GDM, and this biomarker contributes significantly to the prediction of GDM in pregnant women irrespective of maternal age.

**Acknowledgments** The researchers express sincere gratitude to the maternity and laboratory departments of the Volta Regional Hospital, Ho, Ghana, for granting them permission to carry out the project in the facility.

### Compliance with ethical standards

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

**Ethical review** The study protocol was reviewed and approved by the joint committee on human research, publications, and ethics of the Kwame Nkrumah University of Science and Technology and the Komfo Anokye Teaching Hospital, Kumasi, Ghana. Protocol number: CHRPE/AP350/14.

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



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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):458–462
https://doi.org/10.1007/s13410-018-0698-y

ORIGINAL ARTICLE

# Noninvasive screening tool to detect undiagnosed diabetes among young and middle-aged people in Chinese community

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Received: 19 June 2018 / Accepted: 15 October 2018 / Published online: 27 October 2018


**Abstract**
To develop a noninvasive screening tool for diagnosing type 2 diabetes among young and middle-aged people in Chinese community. In total, 1432 participants without diabetes diagnosis were enrolled from Chinese communities. Diabetes was defined as fasting plasma glucose (FPG) ≥ 126 mg/dL (≥ 7.0 mmol/L) or glycated hemoglobin (HbA1c) ≥ 6.5%. The noninvasive diabetes screening model score was developed using the coefficients of the final multivariable logistic regression model. Undiagnosed diabetes was detected using a receiver-operating characteristic curve and the area under the curve (AUC). Of the 1432 participants, 142 (9.9%) were newly diagnosed with diabetes through FPG or HbA1c, 67 (4.7%) through FPG alone, and 121 (8.4%) through HbA1c alone. The noninvasive diabetes screening model was developed using significant risk factors, namely age, family history of diabetes, hypertension, waist circumference, body mass index, smoking, daily consumption of vegetables, and daily consumption of fruits. The cutoff score of 22.5 was the optimum to detect undiagnosed diabetes with an AUC of 0.758 (95% confidence interval 0.714–0.803), sensitivity of 83.1%, and specificity of 60.0%. We developed a practical and effective noninvasive screening tool for detecting undiagnosed diabetes among young and middle-aged people in Chinese community.

**Keywords** Diabetes screening · Undiagnosed · Noninvasive · Young and middle-aged people · China

## Introduction

Diabetes, which has emerged as a major cause of morbidity, premature mortality, and increasing health-care costs over the past several decades, was estimated to affect 425 million adults aged 20–79 years worldwide in 2017 [1]. China has 20% of the world population and more than 100 million people with diabetes, with the most rapid increase in disease prevalence in the young to middle-aged group [1]. Moreover, diabetes is an insidious disease, and a little fewer than half of those with diabetes worldwide remain unaware until symptoms or complications develop some years later [2]. Early diabetes management may prevent or delay diabetes progression and complications. Thus, early detection is crucial to control the disease.

Screening for diabetes among asymptomatic individuals has been shown to be potentially cost-effective and is associated with a reduction in mortality and cardiovascular disease [3–5]. However, in China, invasive methods (i.e., fasting plasma glucose [FPG], oral glucose tolerance tests, and glycated hemoglobin [HbA1c]) are used currently for diabetes screening, which would not be cost-effective or convenient, especially for large populations such as that of China.

Noninvasive risk scores are used to identify asymptomatic or unaware individuals who are more likely to have diabetes, which reduces the number of people who should receive further diagnostic testing. Several diabetes risk score (DRS) models have been developed to detect undiagnosed diabetes [6–9]. These DRS models performed well in their study populations, but could not always be generalized from one population to another [10, 11], and Asian models might be more suitable for Asian populations compared with non-Asian models [11]. China lacks an effective and accepted noninvasive tool to screen for diabetes. This study was designed to develop a noninvasive screening tool for undiagnosed type 2 diabetes mellitus (T2DM) among young and middle-aged people in Chinese community.

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## Methods

### Study participants

Data were obtained through a cross-sectional survey conducted from July to August 2015 in Longzihu District, Bengbu, China. The participants, who were selected by simple random sampling from seven resident communities, met the following criteria: (1) adults aged 18–60 years; (2) no diabetes diagnosis; (3) living in the community for more than 6 months; and (4) ability to complete the survey.

In total, 1602 participants completed the survey, of which 170 participants had missing values on key variables and extreme abnormal values (FPG < 3.0 mmol/L or > 50 mmol/L, HbA1c < 3.0, weight < 30 kg, and waist circumference < 30 cm). Therefore, the remaining 1432 participants (558 males and 874 females) were included in this study.

### Primary variables measure

Every participant was advised to fast for at least 8 h before a personal face-to-face questionnaire, anthropometric measurement, and laboratory biochemical tests. A venous blood specimen was drawn and analyzed for glucose concentration using the glucose oxidase immobilized oxygen electrode method. HbA1c was determined with high-performance liquid chromatography. Height and weight were measured. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference was measured with a piece of non-elastic soft tape attached at the mid-level between the lowest rib and the iliac crest. We assessed daily fruit consumption by asking a simple question: "how much fruit did you consume per day in the past week?" Response categories included "less than 300 g" and "300 g or more." Furthermore, daily vegetable consumption data were obtained by asking a similar question: "how much vegetable did you consume per day in the past week?" Response categories included "less than 400 g" and "400 g or more."

### Diabetes definition

Diabetes was defined as FPG ≥ 126 mg/dL (≥ 7.0 mmol/L) or HbA1c ≥ 6.5%.

### Statistical analysis

Statistical analysis was performed using the software package SPSS 17.0 for Windows. Multivariable logistic regression was applied to estimate the odds ratio (OR) for having undiagnosed diabetes. The noninvasive diabetes screening model score was developed using the coefficients of the final multivariable logistic regression model. Each coefficient was multiplied by a factor of 10, and the score number was rounded to the nearest integer. A sum score was calculated for each subject by adding the score for each variable in a noninvasive diabetes screening model. The receiver-operating characteristic curve (ROC) and the area under the curve (AUC) were produced to detect undiagnosed diabetes according to the diabetes noninvasive screening model. The sensitivities were plotted against the *y*-axis, and the false-positive rates (one-specificity) were plotted against the *x*-axis, then the ROC curve was plotted. The optimal cut-points were located at the peak of the curve where the sum of sensitivity and specificity is maximal. A $p$ value of < 0.05 was considered significant.

## Results

Of the 1432 participants without diagnosed diabetes, 142 (9.9%) were newly diagnosed with diabetes through FPG or HbA1c, 67 (4.7%) through FPG alone, and 121 (8.4%) through HbA1c alone. Those with newly diagnosed diabetes were more likely to be male, older, overweight, obese, current smokers, have a family history of diabetes, have hypertension, consume < 400 g of vegetables daily, and consume < 300 g of fruits daily, compared with the nondiabetic group. However, alcohol was not associated with diabetes status in this study.

The noninvasive diabetes screening model was developed using significant risk factors, namely age, family history of diabetes, hypertension, waist circumference, BMI, smoking habit, daily consumption of vegetables, and daily consumption of fruits. These variables, together with the β-coefficients of the multivariable logistic regression model, the ORs, and 95% confidence interval (CI), are shown in Table 1. The total noninvasive diabetes screening score ranged from 0 to 62. The cutoff score of 22.5 was optimum to detect undiagnosed diabetes with an AUC of 0.758 (95% CI 0.714–0.803), a sensitivity of 83.1%, and a specificity of 60.0%. Figure 1 provides a visual presentation of the ROC curves based on the sum of the scores. We divided the scores into five groups (group 1 < 10; group 2 10–20; group 3 20–30; group 4 30–40; and group 5 ≥ 40). The incidences of undiagnosed diabetes associated with groups 1–5 were 2.6%, 3.0%, 8.9%, 13.6%, and 44.1%, respectively ($p < 0.001$; Fig. 2).

## Discussion

In the present study, we developed a practical and effective noninvasive screening tool for detecting undiagnosed diabetes among young and middle-aged people in Chinese community. The cut-point was 22.5 with an AUC of 0.758, a sensitivity of 83.1%, and a specificity of 60.0%. In group 1, the incidence of diabetes was 2.6%, and it was 44.1% in group 5. Higher noninvasive screening score was clearly associated with higher diabetes incidence.

**Table 1** The noninvasive diabetes screening model score points defined from the coefficients of the multivariable regression analysis

| Independent variable | β | OR (95% CI) | *p* value | Risk score allocated |
|---|---|---|---|---|
| Age (years) | | | | |
| < 45 | | 1.00 | | 0 |
| ≥ 45 | 1.06 | 2.87 (1.73–4.77) | < 0.001 | 11 |
| Family history of diabetes | | | | |
| No | | 1.00 | | 0 |
| Yes | 0.86 | 2.36 (1.49–3.76) | < 0.001 | 9 |
| Hypertension | | | | |
| No | | 1.00 | | 0 |
| Yes | 0.47 | 1.60 (1.04–2.44) | 0.031 | 5 |
| Current smoker | | | | |
| No | | 1.00 | | 0 |
| Yes | 0.77 | 2.16 (1.44–3.24) | < 0.001 | 8 |
| Waist circumference (cm) | | | | |
| Men, < 85;women, < 80 | | 1.00 | | 0 |
| Men, ≥ 85;women, ≥ 80 | 0.74 | 2.09 (1.23–3.57) | 0.007 | 7 |
| BMI (kg/m$^2$) | | | | |
| < 24 | | 1.00 | | 0 |
| ~ 24 | 0.22 | 1.25 (0.77–2.02) | 0.377 | 2 |
| ≥ 28 | 0.75 | 2.12 (1.21–3.73) | 0.009 | 8 |
| Daily consumption of vegetables (g) | | | | |
| ≥ 400 | | 1.00 | | 0 |
| < 400 | 0.76 | 2.13 (1.15–3.95) | 0.016 | 8 |
| Daily consumption of fruits (g) | | | | |
| ≥ 300 | | 1.00 | | 0 |
| < 300 | 0.61 | 1.85 (1.08–3.16) | 0.026 | 6 |

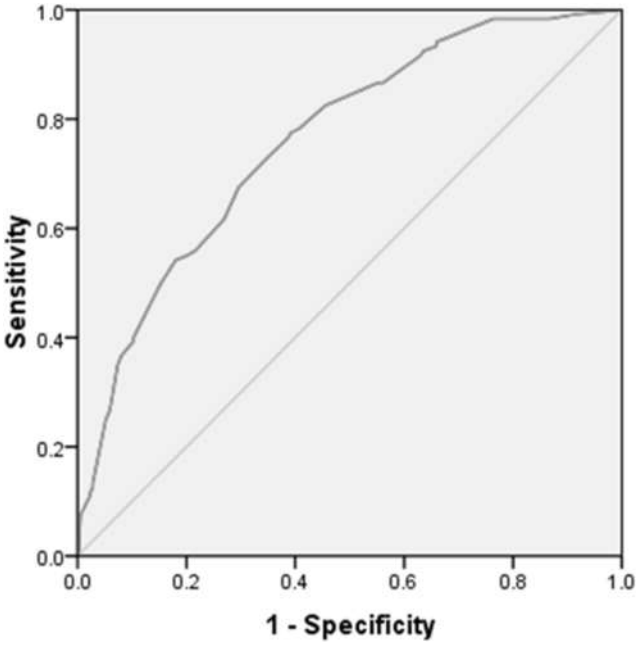


**Fig. 1** ROC curves for detecting undiagnosed diabetes among young and middle-aged people. The area under the ROC curve for the noninvasive diabetes screening scores was 0.758 (95% CI 0.714–0.803).The optimal cutoff point was 22.5 with sensitivity 83.1% and specificity 60.0%

In China, a free health examination program has been established for people aged older than 60 years, which includes diabetes screening through FPG. Diabetes screening for young and middle-aged people often occurs when they decide to get a health examination done or see a doctor due to some other ailment or disease. In fact, it is a type of "opportunistic" screening for young and middle-aged populations. Typically, diabetes is screened after the occurrence of classical symptoms or complications. Therefore, the need of a simple and practical screening tool for young and middle-aged people is urgent. A number of DRS models have been developed to predict T2DM risk [12–14]. However, many DRS models include laboratory test data including blood glucose, triglycerides, and cholesterol. The inclusion of these invasive blood tests might make it impractical to screen diabetes in a large population, although it could efficiently identify the undiagnosed diabetes. Some DRS models without laboratory data have been performing well in Germany [15], Finland [6], and Oman [9]. However, He et al. assessed the validity of different DRS models in a Chinese population [11], and they achieved an AUC of 0.590–0.683. Thus, DRS models could not always be popularized for other populations. In this study, our results provide a useful tool to screen for diabetes in Chinese communities.

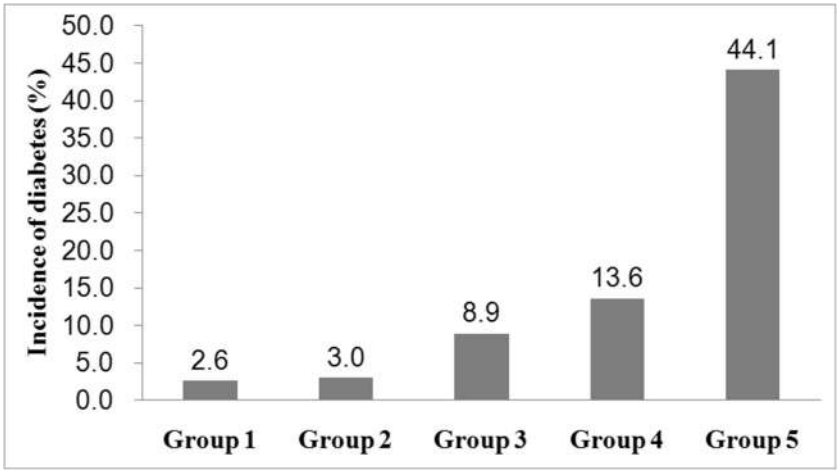


**Fig. 2** Incidence of T2DM according to the scores in noninvasive diabetes screening model. We divided the scores into five groups (i.e., group 1 < 10; group 2: 10–20; group 3: 20–30; group 4: 30–40; and group 5: ≥ 40). The incidences of undiagnosed diabetes associated with groups 1–5 were 2.6%, 3.0%, 8.9%, 13.6%, and 44.1%, respectively ($p < 0.001$)

The noninvasive diabetes screening tool comprised of eight items, namely age, family history of diabetes, hypertension, BMI, waist circumference, smoking habit, daily consumption of vegetables, and daily consumption of fruits. Factors included in the tool represent two types of established risk factors. One aspect is the nonmodifiable risk factors such as age, family history of diabetes, and hypertension. These factors are important components to assess an individual's risk and have previously been widely used in diabetes screening models [16, 17]. Conversely, it has been noted that most of these factors are modifiable, such as smoking habit [18], BMI [19], waist circumference [20], and daily consumption of vegetables and fruits [21], and they are substantially associated with T2DM risk. Previous studies on DRS models have mentioned dietary factors which are important and closely associated with the risk of diabetes [22]. In this study, insufficient intake of vegetables or fruits corresponded to a significant increase in diabetes. Adding insufficient intake of vegetables and fruits to the diabetes screening model led to an increase in AUC from 7.400 to 7.586. These modifiable factors included in the diabetes screening tool, especially dietary factors, could provide guidance information for diabetes prevention among the screened population.

This diabetes screening tool is attractive because it was based on a series of predictors that were measurable and convenient with noninvasive methods. Thus, it is practical for a community health worker as well as for a layperson to perform this noninvasive diabetes risk assessment. When an individual finishes the survey of diabetes screening tool and scores more than 22.5, a blood test is recommended for diabetes diagnosis. The noninvasive diabetes screening tool has a positive predictive value of 18.61%, a negative predictive value of 96.99%, and a diagnostic efficiency of 62.29%. Although 16.9% of undiagnosed diabetes cases were not detected and 40.0% of nondiabetes cases were detected as diabetes, the screening enabled 56.0% of the population to avoid blood tests and identified 83.1% of undiagnosed diabetes cases. Further research is needed to perform cost-benefit analysis about diabetes population screening using this tool.

This study has several limitations. First, this diabetes screening model was established on a cross-sectional study, but not a prospective study. It is beneficial to screen undiagnosed diabetes in large populations, but not suitable for predicting future diabetes. Second, we defined diabetes on the basis of FPG or HbA1c tests, but not on an oral glucose tolerance test. Some individuals had diabetes that was undetectable with FPG or HbA1c. Third, some potential noninvasive and measurable indexes were not included in our study (e.g., glucose in urine and nonclassical symptoms that could enhance the ability to identify undiagnosed diabetes).

In conclusion, the present study developed a noninvasive diabetes screening tool that can detect diabetes among young and middle-aged people in Chinese communities.

**Acknowledgments** This manuscript was edited by Wallace Academic Editing. We thank and express our deep appreciation to those who participated in our study.

**Funding information** The work was supported by the National Natural Science Foundation of China (81703227) and key program of natural science foundation for colleges in Anhui province (KJ2017A231, KJ2017A229).

## Compliance with ethical standards

The study was approved by the Ethics Committee of Bengbu medical college, and informed consent was obtained from all participants.

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

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

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

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):463–470
https://doi.org/10.1007/s13410-018-0667-5

ORIGINAL ARTICLE

# Lower quality of life, lower limb pain with neuropathic characteristics, female sex, and ineffective metabolic control are predictors of depressive symptoms in patients with type 2 diabetes mellitus treated in primary care

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Received: 13 February 2018 / Accepted: 25 June 2018 / Published online: 8 July 2018


**Abstract**
The primary objective of this study was to identify if lower limb pain with neuropathic characteristics is predictive of depressive symptoms in patients with type 2 diabetes mellitus (T2DM) treated in primary care in Brazil. It was investigated if diabetic and non-diabetic related variables could influence depressive symptoms. A quantitative and cross-sectional study was carried out in two Basic Health Units with users of the Brazilian Public Healthcare System, who were evaluated for depressive symptoms (BDI), quality of life (QoL, SF6D), pain intensity, neuropathy (loss of plantar sensitivity—LOPPS), body composition (DEXA), biochemical tests, sociodemographic variables, and comorbidities. The patients were stratified into four groups: $N^+P^+$, $N^+P^-$, $N^-P^+$, and $N^-P^-$ (N = neuropathy and P = pain). One hundred twenty-one diabetics were selected by random sampling between August 2016 and June 2017. Neuropathy affected 53.8% of these individuals, and 59.5% reported intense pain. Overall depressive symptoms scores showed a positive correlation with pain intensity and a negative correlation with QoL. Depressive symptoms were reported by 66.9% of the sample, mostly female, with better levels of cholesterol, HDL and LDL; comorbidities; worse QoL; greater intensity of pain; impaired sleep; and painful neuropathy ($N^+P^+$). The predictive factors for depressive symptoms were lower QoL, pain with neuropathic characteristics, female sex, obesity, and ineffective glycemic control. These data may contribute to the understanding of the complexity of patients with T2DM who are treated in primary care and to public policies planning of care directed at the needs of this population.

**Keywords** Depression · Diabetes mellitus · Type 2 · Pain · Diabetic neuropathies · Diabetes complications

## Introduction

The association between depression and diabetes mellitus (DM) has been strongly demonstrated, with a prevalence of depression between 2.6 and 70% reported in the population with type 2 diabetes mellitus (T2DM) [1–7].

Therefore, depression is a condition to be observed in patients with T2DM; this condition affects 5 to 8% of the

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population in some period of life and is twice as prevalent in women and three times more prevalent in patients with DM [3]. It is estimated that at least one third of people with DM suffer from clinically relevant depression [8, 9].

Depression is defined by the World Health Organization (WHO) as a mental illness that may be accompanied by anxiety and that presents with a depressed mood, loss of interest and pleasure, and changes in sleep and appetite [10].

High rates of depression have mainly been attributed to lifestyle, behaviors, and complications related to T2DM. Among these complications, it is observed that the relationship between depressive symptoms and diabetic neuropathy (ND) is characterized by symptoms, such as intense pain, discomfort, and/or numbness in the limbs, especially in the feet and/or calves [9, 11–21].

In Brazil, a study conducted with data from the National Health Survey (which consists of a home-based epidemiological survey, where a single adult from each selected household responds to the themes) observed the presence of depression in diabetics as being more prevalent in women, in young adults (between 30 and 59 years), and in those with physical limitations and both microvascular (neuropathies) and macrovascular complications [12].

As predictive factors of depression in patients with T2DM, complications of diabetes, such as painful diabetic neuropathy, female sex, comorbidities, low educational level, low socioeconomic level, and ineffective metabolic control, among others, have been previously described [11, 18, 19, 21, 22].

However, in Brazil, there is a lack of studies that investigate the prevalence of depressive symptoms and predictive factors in the T2DM population which is attended in primary care, especially the relationship to neuropathic pain.

The primary objective of this study was to identify if lower limb pain with neuropathic characteristics is predictive of depressive symptoms in patients with T2DM treated in primary care in Ceilândia—the city of the Federal District with the highest urban density, Central-West Region of Brazil. It was investigated as well that diabetic- and non-diabetic-related variables previously described in the literature as having a relation to depressive symptoms and T2DM.

## Materials and method

A cross-sectional, descriptive study was carried out in two Basic Health Units (UBS) of Ceilândia, Federal District, Central-West Region, Brazil. This study is part of a larger project in which several aspects of T2DM are studied in primary care. The study participants were users of the Brazilian Public Healthcare System and enrolled in the Diabetes Program. The sample was randomized, and the patients who met the following inclusion criteria participated in the lottery: age greater than 18 years, diagnosis of T2DM for more than 6 months, and verified in an electronic medical record. Pregnant women and patients with metal implants, which would make it impossible to perform the DEXA (Dual-Energy X-ray Absorptiometry) examination, were excluded from the study. According to the sample calculation, 121 participants were selected considering a margin of error of 5%. The data collection period was from August 2016 to June 2017.

A structured instrument was used to determine the sociodemographic variables (sex, age, schooling, racial group, and familiar income), presence of comorbidities, impaired sleep, insulin use, and diagnosis time. The anthropometric variables, weight and height, were obtained from Lohman's techniques [23] to obtain the body mass index (BMI). The percentage of body fat was identified through the DEXA (Prodigy Advance, GE Medical Systems Lunar, USA) examination, which was carried out at the Biophysics Laboratory from the School of Ceilândia (FCE), University of Brasília (UnB). The patients were placed in the dorsal decubitus position, and a large scanning arm passed over their body. Additionally, the DEXA machine was always regulated and operated by a technically trained professional.

Plasma triglyceride levels, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, fasting blood glucose, and glycated hemoglobin (HbA1c) were analyzed in the Clinical Analysis Laboratory of FCE/UnB. The patients were asked to fast for 12 h for the collection of blood. Hypercholesterolemia was defined as total serum cholesterol $\leq 160$ mmol/L, HDL $\leq 40$ mmol/L, LDL $\leq 160$ mmol/L, and hypertriglyceridemia when the values were $\leq 150$ mmol/L [24]. Fasting blood glucose $\geq 126$ mg/dl and HbA1c (%) 7.0 were used for adults and 7.5 to 8.5 for the elderly [25].

The neurological assessment of feet followed the indicated parameters for the screening of diabetic neuropathy and loss of plantar protective sensation (LOPPS) from the Latin American Association of Diabetes [26], Brazilian Society of Diabetes [25], and American Diabetes Association [27]. Investigation of the LOPPS was performed with the 10-g monofilament and neurological tests: toothpick (deep pain sensitivity), vibration sensitivity (128-Hz tuning fork), and Aquileu reflex (hammer); temperature was evaluated by immersion of the tuning fork in 70% alcohol and was tested at cold temperature. LOPPS was considered when one or more of the neurological tests presented altered results. The plantar protective sensation was considered absent in the face of two inaccurate responses of the three test applications, also valid for each point [28]. The presence of pain in the feet and/or calves was evaluated using the Numeric Scale (0 to 10 points) and considered pain with neuropathic characteristics.

Depressive symptoms was evaluated by applying the Beck Depression Inventory (BDI) [29], translated and validated to Brazilian Portuguese language [30] and diabetic population [7, 31]. In addition, it has been used in studies with the adult

and elderly population of diabetics in Brazil [8, 32–34]. BDI has 21 items, which describe cognitive, affective, and somatic behavioral manifestations of depression, with a total possible score from 0 to 63 points, categorized in severity levels such as the following: (1) No depression to mild depression, ≤ 9 points; (2) Mild to moderate depression, 10 to 18 points; (3) Moderate to severe depression, 19 to 29 points; and (4) Severe depression, 30 to 63 points.

To evaluate quality of life (QoL), the Short-Form 6 dimensions (SF-6D) was used in the Brazilian Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36). The SF-6D has a single score ranging from 0 to 1 point, where 0 represents the worst health condition and 1 is the best. This questionnaire is divided into six dimensions: functional capacity, global limitation, social aspects, pain, mental health, and vitality [35].

The data were analyzed with the software Statistical Package for the Social Sciences (SPSS) version 20.0, and the simple frequencies and dispersion measurements (mean and standard deviation) were initially calculated. The Kolmogorov-Smirnov test was applied to test the normality of the variables. Student's *t* test was used to verify differences in means between the two groups. The chi-square test ($\chi^2$) was used to compare variables expressed by frequencies and, finally, Pearson's correlation was used to analyze the correlation between the rates of depression, quality of life, and pain intensity. Stepwise multiple logistic regression analysis was performed to determine the predictor variables of depressive symptoms. The significance level considered was $p < 0.05$.

## Results

A total of 121 subjects participated in the study, and four profiles were found after identification of signs and symptoms of diabetic neuropathy: patients with pain in the feet and/or calves associated with LOPPS, indicating likely diabetic neuropathy (painful diabetic neuropathy—$N^+P^+$, $n = 44$, 36.4%); patients with pain in the feet and/or calf and the absence of LOPPS, indicating unlikely diabetic neuropathy detectable by the performed tests, despite the painful symptoms (unlikely diabetic neuropathy, with pain—$N^-P^+$, $n = 28$, 23.1%); patients with no complaints of pain in the feet and/or calves and with LOPPS, indicating probable painless diabetic neuropathy (painless diabetic neuropathy—$N^+P^-$, $n = 21$, 17.4%); and patients without complaints of pain in the feet and/or calves and without LOPPS, indicating unlikely diabetic neuropathy (no diabetic neuropathy and no pain—$N^-P^-$; $n = 28$; 23.1%).

The analysis of sociodemographic and clinical data showed that 79.3% were female, mean age of 64 years (SD ± 9.7); average schooling of 6.7 years (SD ± 3.8); self-identified as brown (45.4%); familiar income ≤ 1 minimum wage (46.3%); average time of T2DM diagnosis of 10.2 years (SD ± 7.7) (Table 1). Regardless of detectable neuropathy, severe pain in the feet and/or calves was reported by 72 patients (59.5%, 7.2 ± 2.1, mean ± SD, $N^+P^+$ and $N^-P^+$ profiles), and LOPPS was present in 65 patients (53.8%, $N^+P^+$ and $N^+P^-$ profiles).

Mild to severe depressive symptoms (BDI 17.1 ± 5.2) were present in 81 subjects (66.9%) and were significantly associated with female sex; better cholesterol levels, HDL and LDL; presence of comorbidities; worse QoL; impaired sleep; greater intensity of pain in the feet and/or calves, and painful diabetic neuropathy ($N^+P^+$) (Table 1). On the other hand, the absence of depressive symptoms was significantly associated with non-painful diabetic neuropathy ($N^+P^-$) (Table 1). A small part of the sample, 6.6% ($n = 8$), reported the use of antidepressant medication prescribed with the purpose of treating pain by five patients and treating depression by three patients. Also, depressive symptoms were found in three patients and pain with neuropathic characteristics in all subjects (data not show).

The general depression scores presented a negative correlation with quality of life ($r = -0.453$; $p = 0.000$); in other words, those individuals with higher depression scores reported lower quality of life indexes (Fig. 1a). On the other hand, a positive correlation ($r = 0{,}307$, $p = 0{,}001$) was found between depression and pain intensity in the feet and/or calves; higher depression scores were present in those individuals who also had higher pain intensity (Fig. 1b).

Multiple logistic regression was performed to analyze which variables studied can predict the risk of depressive symptoms. Individuals from female sex, with higher BMI, HbA1c, glycemia, and pain intensity in the feet and/or calves values, presented a risk of developing depressive symptoms 3.45; 1.121; 0.576; 1.017; and 0.826 times, respectively (Table 2). The clinical profiles $N^+P^+$ and $N^-P^+$, i.e., those individuals who have painful symptoms in common, presented risks 4516 and 4896 higher, respectively, of depressive symptoms (Table 2). These data suggest that in addition to factors related to female sex and uncontrolled metabolic, the presence of pain with neuropathic characteristics is a predictive factor for depression. Patients who reported lower quality of life scores are 8382 times at risk, demonstrating that lower quality of life scores can strongly predict the presence of depressive symptoms (Table 2).

## Discussion

The sample in this study presented as a majority of women, elderly, basic education or less, who self-declared as belonging to the brown racial group, low family income (probably near or at the poverty line), and with a decade of living with T2DM. More than half reported depressive symptoms and

**Table 1** Sociodemographic, biochemical, and clinical characteristics of the study subjects ($n = 121$), with or without depressive symptoms. Brasília, 2016–2017

| Variables | Depressive symptoms | | Total | |
|---|---|---|---|---|
| | Yes ($n = 81$/66.9%) | No. ($n = 40$/33.1%) | ($n = 121$) | $p$ |
| Sex (female/male) (%) | 85.5/14.5 | 65.8/34.2 | 79.3/20.7 | 0.013* |
| Age (years) | 63.3 ± 10.0 | 65.2 ± 8.5 | 64.0 ± 9.7 | 0.152** |
| Schooling (years, mean ± SD) | 7.3 ± 3.7 | 6.0 ± 3.8 | 6.7 ± 3.8 | 0.098** |
| Racial group[#] (*n*/%) | | | | 0.383* |
| White | 31/38.3 | 11/27.5 | 39 (32.3) | |
| Brown | 35/43.2 | 18/45.0 | 55 (45.4) | |
| Black | 15/18.5 | 11/27.5 | 27 (22.3) | |
| Yellow | 0/0 | 0/0 | 0/0 | |
| Indigenous | 0/0 | 0/0 | 0/0 | |
| Familiar income (n/%) | | | | 0.552** |
| ≤ 1 minimum wage | 40/49.4 | 16/40.0 | 56 (46.3) | |
| 2 and 3 minimum wages | 27/33.3 | 14/35.0 | 41 (33.9) | |
| 4 and 5 minimum wages | 9/11.1 | 8/20.0 | 17 (14.0) | |
| ≥ 6 minimum wages | 5/6.2 | 2/5.0 | 7 (5.8) | |
| DM time (years ± SD) | 9.6 ± 7.3 | 11.3 ± 8.6 | 10.2 ± 7.7 | 0.054** |
| BMI (kg/m$^2$) (mean ± SD) | 30.8 ± 5.6 | 31.2 ± 5.6 | 30.7 ± 5.7 | 0.696** |
| PBF (% ± SD) | 40.6 ± 8.2 | 41.0 ± 8.2 | 40.8 ± 8.6 | 0.843** |
| Total cholesterol (mean ± SD) | 182.5 ± 33.6 | 189.7 ± 48.3 | 186.9 ± 41.3 | 0.022** |
| Triglycerides (mean ± SD) | 147.2 ± 71.5 | 163.5 ± 91.6 | 158.2 ± 91.2 | 0.142** |
| HDL (mean ± SD) | 47.3 ± 7.2 | 44.9 ± 11.8 | 46.4 ± 9.0 | 0.035** |
| LDL (mean ± SD) | 105.5 ± 30.8 | 107.8 ± 45.4 | 107.0 ± 37.3 | 0.008** |
| HbA1c (mean ± SD) | 6.9 ± 1.7 | 6.9 ± 1.8 | 7.0 ± 1.8 | 0.911** |
| Fasting glycemia (mean ± SD) | 144.6 ± 61.6 | 157.7 ± 72.6 | 149.8 ± 66.6 | 0.176** |
| Comorbidities (%) | 45.8 | 26.3 | 48 (39.7) | 0.032* |
| Insulin use (%) | 22.9 | 28.9 | 30 (24.8) | 0.309* |
| QoL SF6D | 0.75 ± 0.07 | 0.81 ± 0.07 | 0.571 ± 0.269 | 0.000** |
| Impaired sleep (%) | 53 | 31.6 | 56 (46.3) | 0.022* |
| Pain intensity | 5.5 ± 3.7 | 2.6 ± 3.7 | 7.2 (2.1) | 0.000** |
| $N^+P^+$ | 42.2 | 23.7 | 44 (36.4) | 0.038* |
| $N^-P^+$ | 25.3 | 18.4 | 28 (23.1) | 0.278* |
| $N^+P^-$ | 12 | 28.9 | 21 (17.4) | 0.024* |
| $N^-P^-$ | 20.5 | 28.9 | 28 (23.1) | 0.212* |
| BDI (mean/± SD) | 17.1/5.2 | 7.3/2.0 | 14.1 ± 6.37 | 0.000** |
| Depressive symptoms (*n*, %) | | | | |
| No/mild | – | 40/100 | 40 (33.1) | |
| Mild/moderate | 57/70.4 | – | 57 (47.1) | |
| Moderate/severe | 20/24.7 | – | 20 (16.5) | |
| Severe | 4/4.9 | – | 4 (3.3) | |

*SD* standard deviation, [#] As defined in Brazil, self-identified; *T2DM time* type 2 diabetes mellitus time; *BMI* body mass index; *PBF* percent body fat; *HDL* high-density lipoproteins; *LDL* low density lipoproteins; *HbA1c* Glycated hemoglobin; *QoLSF6D overall score* quality of life SF6D; Pain intensity in feet and/or calves: $N^+ P^+$ painful diabetic neuropathy; $N^- P^+$ unlikely diabetic neuropathy, with pain; $N^+ P^-$ painless diabetic neuropathy; $N^- P^-$ no diabetic neuropathy and no pain; *BDI* Beck Depression Inventory (BDI). *$\chi^2$; **$t$

presented, on clinical examination, pain with neuropathic characteristics. In this study, obesity, poor glycemic control, female sex, feet and/or calf pain, and worse QoL rates were found predictors for depressive symptoms. These findings show that there are signs predicting the evolution and complications of T2DM, expressed by depressive symptoms and painful diabetic neuropathy, besides impairments in QoL [25, 27, 28, 31]. Within Brazil, Central-West Region and Federal District, as in other countries around the world, there is a wide disparity concerning sociodemographic aspects and

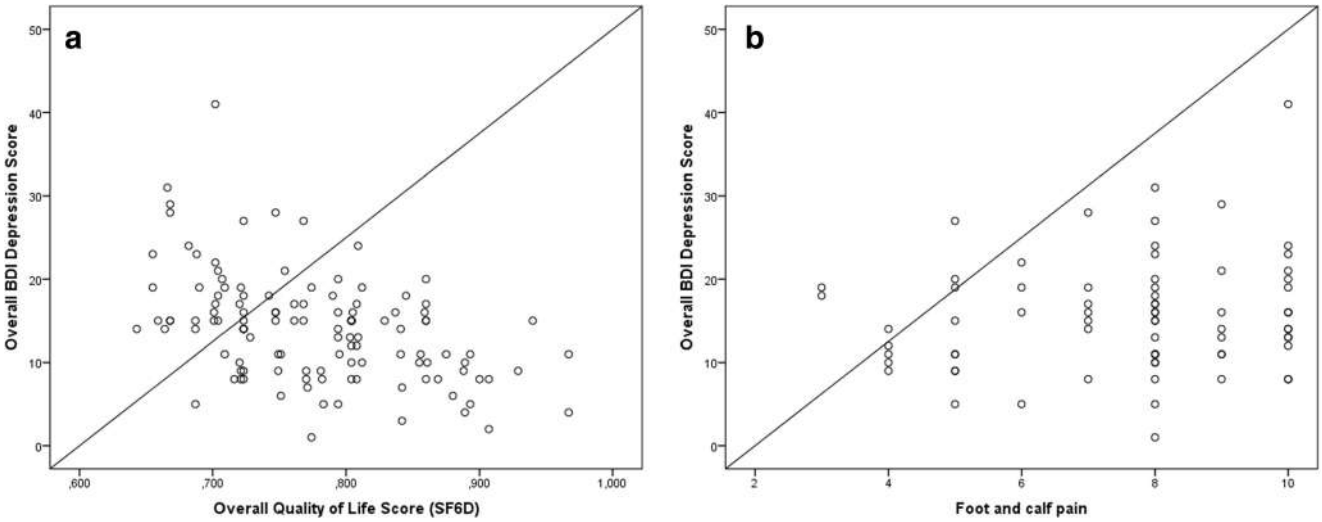


**Fig. 1** Correlation between depressive symptoms and quality of life (panel **a**, $n = 121$) or pain intensity (numerical scale) in feet and/or calves (panel **b**, $n = 72$) of patients with T2DM. Brasília, 2016–2017

population health. In fact, risk factors associated with the presence of depression in patients with DM include female gender, no spouse, poor social support, low educational level, low socioeconomic status, poor glycemic control, neuropathy, comorbidities, physical impairment, painful diabetic neuropathy, and history of depression [11, 13, 14, 17, 19, 21].

The depressive symptoms reported in this study were classified, for the most part, as mild to moderate, a result also found in other studies [18, 32–34, 36]. Concerning its prevalence in T2DM population, there is a variation being 12.5–70.7% in international studies [4–6] and 18.6–62.8% in the Brazilian population [8, 33, 37, 38]. Possible explanations may include the use of different instruments, different cutoff points for the same instruments, and characteristics of the sample, especially health care level of complexity in which it was evaluated (household surveys, outpatient, primary, secondary or tertiary care). In this sense, the most used instrument in Brazilian studies is the BDI, in diabetes outpatient care services [6, 8, 32–34]. Regarding the characteristics of the sample, the present study found the same ones described in a household survey carried out in the Southern Region as predictors of depressive symptoms in older Brazilians with diabetes: women, younger elderly, less education, and less income [37].

QoL in this study was found the strongest predictor for depression. Low QoL scores have been observed in patients with diabetes and depression [13, 16, 39], corroborating our study results. It is known that a worse QoL can significantly impair the treatment of patients with chronic diseases, since these patients have a negative perception of their lives, in addition to a lower treatment adherence [13]. In a study with 437 patients from Uganda, a prevalence of 34.8% of depression and a worse QoL for these individuals were observed [36].

The prevalence of diabetic neuropathy range from 13.8 to 50% [11, 21, 40, 41], and intense chronic pain is one of its clinical manifestations described as being found in one in five people living with DM [21]. Approximately 25–55% of DM patients develop painful diabetic neuropathy [42–45]. Similar values to those found in our study were observed in a multicenter study in China and in England, which found intense neuropathic pain in 55% of the sample from the community [42]. In this sense, chronic pain has been described as the cause of emotional changes such as depression, anxiety, lack of pleasure, impairments in sleep, social life, and incapacitation [21, 22, 27, 46]. Painful diabetic neuropathy affects psychosocial functioning, expressed by advanced levels of anxiety and depression.

In this study, depressive symptoms were associated with the presence of pain with neuropathic characteristics and female sex. Similar results were found in a study in which painful diabetic polyneuropathy and being women were predictors of depression [11]. In fact, female sex is a non-modifiable demographic factor strongly associated with DM, painful diabetic neuropathy, and depression [1–3, 6, 11, 45]. Differences in biology, culture, lifestyle, environment, and socioeconomic status can influence predisposition, development, and clinical presentation of these conditions [3, 45]. Research indicates that T2DM is a risk factor for the development of depression, since blood glucose is a potent regulator of mood states [2, 18, 20, 47]. A study with 4283 elderly in Japan investigated the association between depression and diabetic complications and concluded that glycated hemoglobin was considered a predictor for depression [9]. In this

**Table 2** Predictors of depressive symptoms in a multiple logistic regression analysis: odds ratio and confidence interval (95% CI) of study subjects ($n = 121$), Brasília, 2016–2017

| Variables | Odds ratio | 95% IC | *p* |
|---|---|---|---|
| Sex (female) | 3.450 | 1.290–9.227 | 0.014 |
| Age | 1.003 | 0.952–1.057 | 0.909 |
| Schooling | 0.994 | 0.883–1.18 | 0.917 |
| Racial group[#] (Brown) | 0.348 | 0.096–1.257 | 0.107 |
| Familiar income (≤ 1 minimum wage) | 0.406 | 0.047–3.488 | 0.411 |
| T2DM time | 1003 | 0.997–1.009 | 0.280 |
| BMI | 1121 | 1.011–1.241 | 0.029 |
| PBF | 1091 | 0.993–1.198 | 0.069 |
| Total cholesterol | 1001 | 0.989–1.012 | 0.911 |
| Triglycerides | 1001 | 0.995–1.007 | 0.691 |
| HDL | 0.954 | 0.886–1.027 | 0.212 |
| LDL | 0.984 | 0.946–1.022 | 0.398 |
| HbA1c | 0.576 | 0.341–0.972 | 0.039 |
| Fasting glycemia | 1017 | 1.002–1.031 | 0.024 |
| Comorbidities | 0.904 | 0.313–2.608 | 0.851 |
| Insulin use (%) | 2642 | 0.823–8.481 | 0.103 |
| QoL SF6D | 8382 | 2.202–31.915 | 0.002 |
| Impaired sleep | 2260 | 0.882–5.786 | 0.089 |
| Pain intensity | 0.826 | 0.731–0.933 | 0.002 |
| $N^+P^+$ | 4516 | 1.215–16.788 | 0.024 |
| $N^-P^+$ | 4896 | 1.124–21.374 | 0.034 |
| $N^+P^-$ | 0.616 | 0.144–2.636 | 0.514 |
| $N^-P^-$ | 0.586 | 0.140–2.442 | 0.463 |

[#] As defined in Brazil, self-identified; *T2DM time* type 2 diabetes mellitus time; *BMI* body mass index; *PBF* percent body fat (PBF); *HDL* high-density lipoproteins; *LDL* low-density lipoproteins; *HbA1c* glycated hemoglobin; *QoLSF6D overall score* quality of life SF6D; Pain intensity in feet and/or calves: $N^+ P^+$ painful diabetic neuropathy; $N^- P^+$ unlikely diabetic neuropathy, with pain; $N^+ P^-$ painless diabetic neuropathy; $N^- P^-$ no diabetic neuropathy and no pain

context, in India, a case-control study identified a prevalence of depression of 42.2% in diabetics and 4.39% in individuals without DM. It was observed that 19% of patients with diabetic neuropathy and depression presented worse glycemic levels [48]. Another finding was a positive correlation between glycemic indexes and depression scores in the diabetic elderly in Romania. The authors emphasized that depression can lead to worsening of glycemic control, since the depressive patient does not satisfactorily engage in nutritional control, physical activity, foot care, and frequent glycemic monitoring [49].

In addition to glycemic control, body mass index is a significant predictor of the development of depression in patients with T2DM, corroborating the results of a survey of elderly people in Romania, in which 71.2% of the patients presented with depression. It was observed that for each increase in BMI of 1 kg/m$^2$, the probability of depression increases by 10.1% [48]. In Palestine, the results from a survey of 294 patients showed that 40.2% had depression and there was an association between high rates of depression (BDI) and obesity (BMI ≥ 30 kg/m$^2$) [50].

In this study, we used specific instruments and protocols that are widely reliable and recommended in the evaluation of depressive symptoms, QoL, and pain. The neurological assessment of feet for LOPPS tracking and subsequent classification of risk is recommended and used in primary care in Brazil. It is necessary to better understand and characterize the four profiles found in this sample, especially to understand the pain in the lower limbs that arises before alterations in the examination of the feet can be detected, in the case of the $N^-P^+$ group, considering that pain is demonstrated to be a predictor of depressive symptoms.

## Conclusion

Our data suggest that the population living with T2DM and treated in primary care presents a complex framework of the evolution of complications associated with T2DM, mainly painful neuropathy, which generates negative effects, often resulting from pain associated with changes in sleep, mood, and QoL, as one of the consequences of glycemic control. The results of this study may assist in more effective programs for managing depression and other complications of T2DM, such as neuropathic pain and metabolic control in the primary care setting.

**Acknowledgments** We would like to offer thanks to all patients who accepted to participate in this study. We also thank the director and the team of the Health Units of Ceilândia; the Foundation of Support to Research of the Federal District (FAP-DF) for financing this research; the Department of Health (SES-DF) for authorizing the development of this research; and the members of the Health Care and Aging Research Group-GEPSEN/UnB, professors, and students (PhD, master's degree, scientific initiation, and volunteers) for the help in the development of this work.

**Author contribution** LRL, MIF, and MMS worked on study design, data collection and analysis, interpretation, drafting, and editing of the manuscript. CRGV, TCMSBR, WSS, and SSF collaborated on interpretation, data analysis, editing, and review of the manuscript data. All authors read and approved the final manuscript.

## Compliance with ethical standards

After the draw, the patient was invited to participate in the study. The data collection occurred in the Basic Health Units, after the approval of the ethics committee and signature of the participant in the free and informed consent term. Participants received a description of the study and were informed about the purpose, risks, and benefits. This research was approved by the Ethics Committee of the Health Department of the Federal District (SES/DF) number 1355211/2015 and CAAE (50367215.5.0000.5553).

**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 2019) 39(3):471–477
https://doi.org/10.1007/s13410-018-0707-1

ORIGINAL ARTICLE

# Effect of prenatal zinc supplementation on adipose tissue-derived hormones and neonatal weight, height and head circumference in women with impaired glucose tolerance test: randomized clinical controlled trial

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Received: 7 May 2018 / Accepted: 4 December 2018 / Published online: 3 January 2019


## Abstract

**Background** It is well known that normal pregnancy exposes mothers to a diabetogenic state. The important role of adipose tissue in the regulation of insulin resistance has been repeatedly proven. This organ carries out the regulation of insulin resistance by producing adipocytokines involved in the pathogenesis of gestational diabetes mellitus (GDM). The present study aims to evaluate the effects of zinc supplementation on serum leptin, visfatin, tumor necrosis factor-α (TNF-α), adiponectin and zinc-α2-glycoprotein (ZAG) levels in pregnant women with impaired glucose tolerance test (IGTT) results.

**Methods** In this randomized, placebo-controlled, double-blind clinical trial, 46 pregnant women with impaired glucose tolerance test results were randomly distributed into zinc (n = 23) and placebo (n = 23) groups and received 30 mg zinc supplement per day in the form of zinc gluconate and placebo, respectively, for 8 consecutive weeks. The study was conducted in the Shabestar district of northwestern Iran.

**Results** Supplementation after adjusting for confounding variables resulted in a significant reduction in plasma leptin (p = 0.035) and TNF-α (p = 0.027) levels in the zinc group compared with the placebo group. Serum visfatin levels were significantly increased, and serum ZAG levels were significantly decreased in both groups. However, the changes in adiponectin concentration were not significant in either group after intervention nor were the anthropometric parameters in fetuses whose mothers received the zinc supplement.

**Conclusions** It seems that zinc supplementation may be considered as a complementary supplement together with the medical management of patients with IGT or GDM. However, further studies are needed before definite conclusions can be drawn.

**Keywords** Zinc · Leptin · Visfatin · Zinc-–$\alpha_2$-glycoprotein · Pregnancy

## Introduction

Gestational diabetes mellitus (GDM) is one of the most common complications of pregnancy that causes adverse outcomes in both mother and fetus [1]. Its prevalence is rapidly increasing worldwide. Insulin resistance and β cell dysfunction are related to the pathogenesis of GDM [2].

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Adipose tissue was previously considered to be a long-term energy storage organ, but it is now appreciated as an active endocrine organ with alternative specimen for the pathogenesis of insulin resistance and GDM [3]. This function of adipose tissue is mediated by its ability to secrete adipokines [4].

Leptin as an adipokine is produced by both white adipose tissue and the placenta [5]. Maternal leptin levels increase during the first and secondary trimesters of pregnancy and reach an extreme in the late second or early third trimester [6]. Therefore, leptin may have an important functional role during pregnancy [5].

Visfatin is regarded as a pro-inflammatory cytokine [7]. It has shown insulin-mimetic effects in vivo and in vitro. It is expressed by fetal membranes and secreted from amniotic epithelial cells during pregnancy [8]. In GDM, the concentration of visfatin has been reported to be either increased [9, 10] or decreased [11, 12].

Zinc-alpha-2-glycoprotein (ZAG) is a novel adipokine that is initially isolated from human plasma but is widely distributed in many body fluids such as saliva, milk, and urine [13]. The biological actions of ZAG have not been fully understood, but it has been shown to be an identical lipid-mobilizing factor (LMF) [14]. Also, it can increase pancreatic insulin content and improve glucose tolerance test results [15]. Despite evidence from animal and human studies, little data exists about the role of ZAG in GDM women.

Adiponectin is an adipocytokine that performs an important role in the regulation of glucose and lipid metabolism [16]. It has been shown to have insulin-sensitizing, anti-inflammatory, and antidiabetic properties [17]. In pregnancy, adiponectin is reduced physiologically by increasing concentrations of estrogens and β-human chorionic gonadotropin [18].

TNF-α is an inflammatory mediator produced by monocytes and macrophages in adipose tissue [19]. Most studies show that increased levels of TNF-α in obesity and GDM can have multiple effects on insulin sensitivity in muscles, liver, or beta cells of the pancreas, ultimately leading to insulin resistance.

Zinc plays an important role as a trace element and is known to have insulin mimetic action [20]. Molecular and cellular studies have demonstrated that zinc supplementation can be protective against type 2 diabetes [21]. However, there are a few reports in the literature regarding the effect of zinc supplementation on adipocytokines levels in pregnant women with impaired glucose tolerance (IGT). Because women with IGT, i.e., suffering high blood glucose levels, are at high risk of perinatal complications, although they lack GDM [22, 23], the effects of zinc supplementation on serum leptin, visfatin, TNF-α, adiponectin, and ZAG levels in pregnant women with IGT were evaluated.

## Subjects and methods

### Participants

In this randomized, double-blind, placebo- controlled clinical trial (allocation ratio 1:1), those pregnant women who were invited to take screening gestational diabetes mellitus in their 24–28 weeks of pregnancy have been selected as study subjects. Rohzendeh health and therapeutic center in Shabestar district, North West of Iran, has been chosen for this research project. The study took place between December 2012 and April 2013. The professional physician visited all mothers and prescribed oral glucose challenge test for each person individually. For this test, each subject was given 50 g of glucose orally, and blood glucose was measured after 1 h. If blood glucose levels were ≥ 130 mg/dL, the OGTT (oral glucose tolerance test) was performed as follows in order to excluding whom that had GDM [24].

- FBS (fasting blood sugar) ≥ 92 mg/dL
- 1 h blood sugar ≥ 180 mg/dL following a 75-g oral glucose load
- 2 h blood sugar ≥ 153 mg/dL following a 75-g oral glucose load [24].

The exclusion criteria comprised the history of diagnosis diabetes or chronic disease and specific infections, alcohol consumption, and smoking at recruitment.

### Study design

Sample size was determined based on prior data that was concluded from a study performed by Kim et al. for the leptin variable with 5% significance and 80% power [25]. Total sample size was 40 individuals plus 6 persons to allow for withdrawals; i.e., 46 subjects were required for this study. The randomized block procedure was used to allocate subjects (46 pregnant women) to one of two treatment orders (A or B) by computer-generated allocation schedule (Random Allocation Software) in which A was the zinc supplementation group and B was the placebo group. Due to the scarcity of an appropriate evidence-based dosage for zinc in IGT patients, the doses suggested in a previous study on pregnancy were used in this study [26]. The zinc group received a 30-mg zinc tablet (in the form of zinc gluconate, NatureMed, USA) daily for eight consecutive weeks between meals and not together with any other vitamin or mineral supplement. The second group received a placebo (starch) in the same manner. The placebo tablets were supplied by the School of Pharmacy, Tabriz University of Medical Sciences, Iran, and were similar to the zinc gluconate tablets in color and size. The study drugs were double blinded to both investigator and participants. No important changes were seen in the trial methods after the start

of the study. A specific dietary plan was assigned to each participant using her gestational status. A member of the in-charge staff made regular weekly phone calls to all participants to ensure they had the appropriate pills.

### Assessment of variables

All subjects were observed via a monthly visit during the trial. The researcher calculated the participants' BMI using its formula which is weight in kilograms divided by height in meters squared based on pre-pregnancy weight. For all pregnant women, gestational age was calculated by 1st trimester ultrasonography.

Five-milliliter fasting blood sample was taken before and after of intervention to measure the levels of serum leptin, visfatin, TNF-α, adiponectin, and ZAG levels. Visfatin, ZAG, adiponectin, and TNF-α were measured by human ELISA kit (BioVendor, Germany). Leptin was measured by human ELISA kit (DIAsource, Belgium).

Infants' length was measured from crown-to-heel with a ruled board that has an attached piece of wood at one end and a movable piece at the other end. Weight measurements using standard scales with accurately 0.1 kg and head circumference were measured with a tape measure.

### Statistical analysis

Data was presented using mean (SD) for numeric variables and frequency (%) for categorical variables. Distribution of the data was illustrated via Kolmogorov-Smirnov goodness of fit test. Results were expressed as mean (SD) for normal and median (interquartile range) for non-normal data. After Log transformation, data would be normally distributed. Paired *t* test was used to compare before and after scores. Independent *t* test was used to compare the results. An analysis of covariance test (ANCOVA) was used to adjust the effects of confounding factors. $p$ value $< 0.05$ was considered statistically significant. For data entry and analysis, the SPSS software version 21 (SPSS Inc. IL, Chicago, USA) was put into work.

## Results

The number of the participants who met the criteria of the study was 46 pregnant women with IGT. In the zinc group, one subject did not receive allocated intervention due to need for insulin therapy. In the placebo group, one subject discontinued intervention due to premature delivery (Fig. 1). Thus, 44 subjects (zinc group = 22; placebo group = 22) completed the study with the mean age of 29.45 ± 4.21 years in the zinc group and 29.82 ± 5.41 years in the placebo group (gestational age was 24–28 weeks in both group). No side effects were reported following the zinc consumption or placebo in IGT women throughout the study. As it is illustrated in Table 1, baseline traits of the participants in groups of the study did not have any significant statistical difference.

Serum levels of leptin, visfatin, ZAG, adiponectin, and TNF-α before and after supplementation are presented in Table 2. After adjusting for baseline values, a significant reduction in leptin ($p = 0.03$) and TNF-α ($p = 0.02$) levels and a significant rise in zinc ($p = 0.04$) level were seen in treatment group compared to placebo (Table 2). We did not find any significant effect of zinc supplementation on serum visfatin, ZAG, and adiponectin concentration compared with placebo. In addition, within-group comparisons also showed that the level of ZAG significantly decreased and the mean concentration of visfatin significantly increased post-intervention in both groups ($p < 0.05$).

Mean of weight (3219 vs. 3221 g, $p = 0.98$), height (49.93 vs. 49.75 cm, $p = 0.70$), and head circumference of newborns (34.80 vs. 34.78 cm, $p = 1.00$) have no significant differences between zinc and placebo groups, respectively.

## Discussion

The WHO has identified zinc deficiency as one of the 10 major factors contributing to chronic disease in developing countries such as Iran [27]. Using 30 mg of zinc gluconate daily for 8 weeks led to a significant reduction in plasma leptin and TNF-α level in the women with IGT in the zinc group. Interestingly, serum levels of visfatin and ZAG were notably increased and decreased, respectively, in both groups. Nevertheless, adiponectin concentration changes after intervention were not significant in either group.

The pathogenesis of both IGT and GDM involves very similar and complex metabolic pathways. Despite much progress, to date, no clear evidence on underlying functions by which some factors are involved in the pathophysiology of GDM is available. This remains an interesting subject for research. Recently, the vital function of adipose tissue-derived hormones in the pathophysiology of GDM was proven.

It has been proven that oxidative stress plays a key role in the pathogenesis of diabetes mellitus. Surprisingly, zinc with its antioxidant trait [28] guards insulin and pancreatic beta cells against free radicals [29]. The effect of zinc has repeatedly been approved in the insulin function, which includes its synthesis, storage, and secretion [28]. Moreover, it improves insulin function by stimulating insulin tyrosine kinase receptors and raising the phosphorylation of tyrosine kinase enzyme [20]. Furthermore, the stability of the insulin hexamer structure is related to zinc [28].

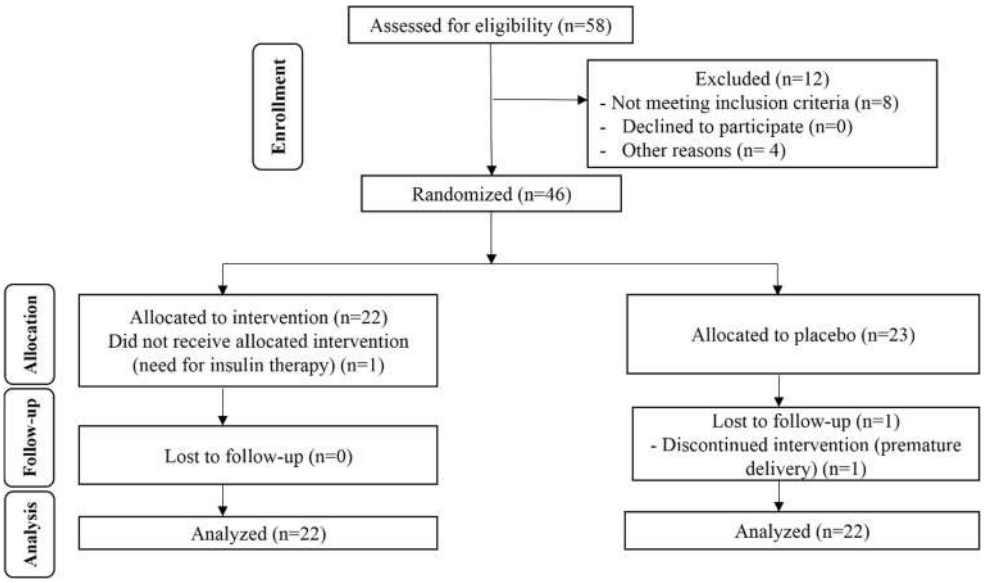


**Fig. 1** Patients' flow diagram of randomized controlled trial

Maternal leptin serum levels rise constantly during the first and second trimesters of pregnancy, and they intensify late in the second or early in the third trimester [6, 30]. In the current study, serum leptin levels in both groups decreased close to the time of delivery, but after adjusting for confounding factors, this decrease was significant in the zinc group only. In contrast to the current findings, increased [31] or unchanged [25, 32] levels of serum leptin have been reported. Decrease in leptin levels observed in this study has been reported in some previous studies as well [33].

The relationship between zinc and leptin can be attributed to the effect of ZAG on leptin. ZAG is a novel adipokine which acts in adipocyte metabolism. In obesity and insulin resistance, due to the inflammatory processes, deregulated expression of ZAG gene encoding is in line with low levels of adiponectin and heightened levels of leptin [34, 35]. In addition, insulin is a stimulator of leptin secretion [36, 37]. Insulin resistance and hyperinsulinemia can cause increased secretion of leptin in pregnant women with GDM and IGT. The authors have previously shown that this supplementation caused a slight decrease in insulin levels in the zinc group. This reduction in insulin levels can cause a significant reduction in leptin levels in those receiving the supplement [38].

**Table 1** Baseline characteristics of study subjects

| Variables | Zinc ($n = 22$) Mean ± SD | Placebo ($n = 22$) Mean ± SD | $p^a$ |
|---|---|---|---|
| Age (year) | 29.45 ± 4.21 | 29.82 ± 5.41 | 0.805 |
| Weight (kg) | 70.05 ± 11.23 | 68.43 ± 11.33 | 0.638 |
| Height (m) | 1.58 ± 0.05 | 1.60 ± 0.03 | 0.057 |
| BMI (kg/m$^2$) | 28.34 ± 4.17 | 26.82 ± 3.73 | 0.210 |

*SD* standard deviation, *BMI* body mass index

[a] Independent sample *t* test

The important role of ZAG in metabolic disorders such as insulin resistance-derived diseases has recently been approved [39]. There has been less focus on ZAG levels in pregnant women, but heterogeneous reports have shown low levels [40, 41], fixed [13], or intensified [42, 43] levels of it in obese subjects. Differences in the ethnic backgrounds and general characteristics of patients might explain these conflicting results. So far, most of the evidence supports the existence of an inverse relationship between ZAG and insulin resistance [40, 44, 45]. In this study, it was found that ZAG levels were significantly decreased in both study groups. It can be noted that treatment for IGT and insulin resistance with zinc gluconate in pregnant women can prevent further reductions in ZAG levels. Although the mechanism between zinc and ZAG is not clear, zinc may act as an agent for enhancing the expression of ZAG. For a definitive result, further research on the cellular basis is needed.

Recently, the metabolic influence of visfatin has been in the spotlight. In agreement with several studies [46–48], the visfatin concentration increased significantly in subjects of the current study due to their physiological conditions. An insulin-resistant state can cause increased visfatin ranges in GDM women [49]. Since zinc affects insulin action through the modulation of insulin receptor tyrosine kinase activity [20], it will be efficient through the action of insulin on the secretion of visfatin.

TNF-α as a potential mediator of inflammation can be a predictor of GDM and could play an important role in insulin resistance during pregnancy [50]. Recent studies have demonstrated that serum levels of TNF-α are increased in normal pregnancy as well as GDM [17, 51]. An in vitro experiment

**Table 2** The effect of zinc supplementation on biochemical parameters[a]

| Variable | | Zinc ($n = 22$) | Placebo ($n = 22$) | $p$[b] |
|---|---|---|---|---|
| Leptin (ng/mL) | Baseline | 14.14 (7.48–19.86) | 13.26 (6.90–21.31) | 0.686 |
| | End of trial | 13.13 (8.01–19.02) | 12.96 (7.55–18.91) | 0.035 |
| | $p$[c] | 0.735 | 0.509 | |
| Visfatin (ng/mL) | Baseline | 1.38 (0.82–2.11) | 0.99 (0.66–1.57) | 0.132 |
| | End of trial | 3.20 (1.33–4.28) | 2.53 (1.55–4.29) | 0.678 |
| | $p$[c] | 0.01 | 0.001 | |
| ZAG (μg/mL) | Baseline | 23.62 (19.15–75.53) | 23.37 (8.47–49.71) | 0.255 |
| | End of trial | 16 (10.28–28.25) | 8.65 (6.17–16.30) | 0.236 |
| | $p$[c] | 0.008 | 0.033 | |
| TNF-α (pg/mL) | Baseline | 117.12 (98.23–186.56) | 110.45 (93.23–145.45) | 0.962 |
| | End of trial | 106.51 (95.17–251.01) | 109.34 (98.23–153.78) | 0.027 |
| | $p$[c] | 0.565 | 0.267 | |
| Adiponectin (μg/mL) | Baseline | 13.41 (1.43) | 13.6 (1.22) | 0.499 |
| | End of trial | 14.01 (1.42) | 14.07 (1.33) | 0.982 |
| | $p$[c] | 0.082 | 0.369 | |
| Zinc (μg/dL) | Baseline | 77.77 (22.28) | 64.09 (18.44) | 0.032 |
| | End of trial | 87.54 (19.03) | 71.81 (19.36) | 0.041 |
| | $p$[c] | 0.012 | 0.069 | |

*ZAG* zinc $\alpha_2$-glicoprotein, *TNF-α* tumor necrosis factor-α

[a] Data are expressed as mean (SD) or median (interquartile range), as appropriate

[b] Obtained from ANCOVA, adjusted for baseline values

[c] Obtained from paired *t* test

showed that placentas from women with GDM released more TNF-α in response to glucose stimulus than placentas from normal pregnant women [52].

The current results are partly consistent with the results of a group of Turkish authors [53] who demonstrated a significant decrease in serum TNF-α levels after zinc supplementation. Nevertheless, Kara et al. [54] reported that TNF-α level was increased following zinc supplementation in young wrestlers. However, the evidence is in favor of reduced serum levels of TNF-α after supplementation with zinc [55]. Inconsistent results may be due to special physical conditions of study participants or different doses or periods of supplementation.

The role of adiponectin, which enhances insulin secretion and action, is opposite the effects of TNF-α [56]. Adiponectin is believed to be an insulin-sensitizing factor involved in the regulation of glucose [57]. There is a general consensus of opinion that decreased adiponectin serum levels are significantly associated with diabetes, obesity, and hypertension [58]. Despite hypoadiponectinemia in pregnancy, the placenta is a major source of expressed adiponectin, and there is a significant increase in cord plasma adiponectin with gestational age, especially in the late days of pregnancy [59]. Elevated serum levels of adiponectin in both groups of the current study can be explained with this reason. Similar to the results of the current study, Kim. et al. [25] found no change in serum adiponectin levels with zinc supplementation. No significant difference in adiponectin concentration was found among the participants of this study, but percentage changes in it during intervention tended to increase more in the zinc group than in the placebo group.

No significant differences were seen in the neonatal anthropometric indices which were measured after birth. No significant differences in birth weight, length, or head circumference were observed in the supplementation group. These results agree with those of previous studies conducted by Merialdi et al. [60] and Caulfield et al. [61]. Despite the important role of zinc for healthy pregnancy outcomes, the results of experimental studies of maternal zinc supplementation and birth weight are inconsistent [62, 63]. Generally, differences in the ethnic backgrounds and general characteristics of the participants might explain these conflicting results.

In brief, it seems that zinc supplementation has beneficial effects on adipokines, glycemic status, and insulin resistance [38] in pregnant women with IGT. However, further studies with larger sample sizes and longer intervention periods are needed to achieve a definite conclusion. The limitations of the current study include its small sample size and the fact that only IGT pregnant women were considered which restricts generalizability to a larger group. Furthermore, calcium and phosphorus intake, which could affect zinc supplementation, was not assessed.

## Conclusions

In conclusion, we demonstrate that zinc supplementation decreased significantly leptin and TNF-α level. These results support the positive effects of zinc supplementation in pregnant women with IGT. It is suggested that zinc may be considered as a complimentary supplement together with metformin and insulin treatment in patients with IGT and GDM.

**Acknowledgements** This work was supported by the Research Vice-chancellor and Nutrition Research Center of Tabriz University of Medical Sciences. The participation of all patients in this study is gratefully acknowledged.

**Funding/support** This work was supported by the Tabriz University of Medical Sciences [grant number: 91227].

**Authors' contribution** NR, ATE, NM, and MA performed the research and contributions to design of the study. NR prepared the primary draft. HM, NF, and MAJ: contribution to data analysis; ATE and MA: edited the final draft and final approval of the manuscript.

### Compliance with ethical standards

All the participants filled in the informed written consent and the researcher got the ethical permission for the study from the ethics committee of Tabriz University of Medical Sciences. The current study is registered in the Iranian Registry of Clinical Trials (IRCT registration number: IRCT 201212265670 N6) and full trial protocol can be accessed in IRCT website.

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

**Ethical approval** The study conformed to the 1964 Helsinki declaration and its later amendments. The study was approved by the ethics committee of Tabriz University of Medical Sciences.

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

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):478–485
https://doi.org/10.1007/s13410-018-0662-x

ORIGINAL ARTICLE

# A non-targeted metabolomics study on different glucose tolerance states

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Received: 25 March 2018 / Accepted: 4 June 2018 / Published online: 12 July 2018


**Abstract**
A non-targeted metabolomics method was employed to study metabolic characteristics in subjects with different glucose tolerance. Plasma samples of 120 participants with normal glucose tolerance (NGT), impaired glucose regulation (IGR), and type 2 diabetes (T2D) were collected. Gas chromatography/mass spectrometry (GC/MS) was used to profile and compare the plasma metabolome among the three groups. Through the use of multivariate statistical analysis, we found distinct metabolome change from NGT to IGR and to T2D. ANOVA found that the IGR and T2D groups had perturbations of monosaccharide and lipid metabolism, disorders of glucogenic amino acids, and branched-chain amino acid catabolism. Furthermore, we also found that the levels of 2-hydroxybutyrate and 2-ketoisocaproate were progressively increased with glucose tolerance severity. The results from this study help us better understand the relationship between plasma metabolism and glucose tolerance states and also suggest that 2-hydroxybutyrate and 2-ketoisocaproate may be closely associated with the development of T2D.

**Keywords** Type 2 diabetes · Impaired glucose regulation · Metabolomics · Gas chromatography/mass spectrometry

## Introduction

Diabetes is one of the most common metabolic disorders worldwide, seriously impacting human health, labor force, and economic status. As living standards improve, population aging, and increasing incidence of obesity, the prevalence of diabetes, especially type 2 diabetes (T2D), is rapidly accelerating year by year. Impaired glucose regulation (IGR), also known as pre-diabetes, is an abnormal intermediate state that exists between normal glucose tolerance (NGT) and T2D. According to the International Diabetes Federation and the American Diabetes Association, patients with T2D almost always undergo the period of IGR. Therefore, it is warranted to perform the proper investigations on the different states of glucose toleration, in order to help us penetrate into the disease progress process of diabetes and obtain insightful clues of early diagnosis and effective interventions for preventing or delaying the course of diabetes.

Metabolomics, profiling the global state of metabolites in biological fluids and tissues, is emerging as a field with tremendous promise in extending "omics" from the gene to the small molecule [1]. It measures the dynamic metabolic responses to pathophysiological stimuli or genetic modifications [2]. Metabolomics investigations combined with multivariate analysis serve in characterizing the offset of the body metabolism caused by physiological and/or pathophysiological changes through abundant endogenous information, which can easily reveal the differences in metabolism among various groups. In addition, metabolomics has begun to play a more important role in discovering and identifying potential biomarkers discriminating normal from abnormal states. Recognition of the differential metabolites can provide insight into the underlying molecular mechanism and is helpful in clinical diagnosis.

Since type 2 diabetes is a typical metabolic disease with a chronic dysfunction of metabolic system, many researchers have successfully used the platform of metabolomics to investigate metabolic alterations of pre-diabetes and/or diabetes in

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the last decade [3–7]. To date, published findings suggest that amino acid (branched-chain amino acids and glucogenic amino acids), lipid (phospholipids, sphingomyelins, free fatty acids and acylcarnitines), carbohydrate (glucose, mannose, galactose and fructose), and bile acid (cholate and deoxycholate) metabolism present the complex abnormalities in individuals with pre-diabetes and diabetes compared with control subjects. More recently, metabolomics research based on diabetes has mainly focused on the identifying novel predictive biomarkers associated with pre-diabetes and diabetes by using prospective study designs. Current evidence revealed the close correlation of branched-chain and aromatic amino acids with insulin resistance and future development of diabetes [8, 9]. The hexose sugars (fructose, mannose, galactose, and inositol) were strongly associated with higher risk of prediabetes and diabetes in these prospective studies [10, 11]. Lipidomics has also revealed that a number of lipids may be predictive of type 2 diabetes [12, 13], but inconsistent results have been reported in different studies [14]. Additionally, a few studies also identified some novel predictors of type 2 diabetes, including 2-aminoadipicacid [15], 2-ketoisocaproate [16], α-hydroxybutyrate [17], and so on. However, T2D is rarely a static condition, but rather one that evolves and changes over time during the lifespan of an individual [18]. Therefore, more attention should be paid to find the relationship between plasma metabolome and different states of glucose tolerance and further look for the metabolites that may be changed gradually with the glucose tolerance progression from NGT to IGR and to T2D.

In our present work, we performed a non-targeted metabolomics study based on GC/MS to illustrate the plasma metabolome change in different developing states of diabetes and find the progressively changed metabolites related with glucose tolerance. This study will help us profoundly realize the development process of diabetes and explore the underlying molecular mechanism.

## Material and methods

### Chemicals

Methoxyamine hydrochloride, MSTFA (*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide), pyridine, tridecanoic acid, and methyl laurate were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol was purchased from Tedia (USA).

### Sample collection

Plasma samples were collected by Datong NO.3 People's Hospital. Totally, 120 age-, gender- and body mass index-matched subjects were included for non-targeted metabolomic analysis. Thirty-nine subjects had NGT (fasting plasma glucose < 5.6 mmol $L^{-1}$ and 2-h glucose < 7.8 mmol $L^{-1}$), 40 subjects had IGR (fasting plasma glucose between 5.6–7.0 mmol $L^{-1}$ and/or 2-h glucose between 7.8 and 11.1 mmol $L^{-1}$), and 41 subjects had T2D (fasting plasma glucose > 7.0 mmol $L^{-1}$ and/or 2-h glucose > 11.1 mmol $L^{-1}$). All of the T2D participants were newly diagnosed without any anti-diabetic drugs. Plasma samples were collected after an overnight fasting in the standard protocol and immediately stored frozen at − 80 °C until use. The study was conducted in accordance with the principles of the Declaration of Helsinki.

### Sample preparation

Prior to metabolomic analysis, the plasma samples were thawed at 4 °C, and 20 μL tridecanoic acid (250 μg $mL^{-1}$ in methanol) was added to a 100-μL aliquot of sample as an internal standard. Subsequently, 400 μL of methanol was pipetted into the mixture for protein precipitation. After vortexing for 30 s and centrifuging at 15,000 rpm for 20 min, the supernatant (450 μL) was transferred to a glass sampler vial and lyophilized at 10 °C. The dried extract was oximated using 65 μL of methoxyamine hydrochloride (20 mg $mL^{-1}$ in pyridine) at 40 °C for 90 min, and then trimethylsilylated using 65 μL of MSTFA for 60 min at 40 °C. The final solution was spiked with 20 μL external standard solution (0.9 mg $mL^{-1}$ methyl laurate dissolved in pyridine).

### Metabolomic analysis

Metabolomic analysis was performed using an Agilent 7890/5975C-GC/MSD system (Agilent Co.,USA). Separation was achieved on a fused-silica capillary column (30 m × 0.25 mm i.d.) chemically bonded with 0.25 μm DB-5 stationary phase (J&W Scientific, Folsom, CA, USA). The injection temperature was 300 °C and the split ratio was 10:1. Helium was used as the carrier gas with a constant velocity of 1.0 mL $min^{-1}$. The column temperature was initially kept at 70 °C for 2 min, changed to 90 °C at 3 °C $min^{-1}$ and then increased to 200 °C at 2 °C $min^{-1}$, finally to 320 °C at a rate of 15 °C $min^{-1}$, and held for 5 min. The effluent was introduced into the electron ionization source. The following parameters were used: interface temperature, 280 °C; ion source temperature, 230 °C; and the detector voltage, 1.38 kV. Full scan mode was employed in the mass range of 33–500 amu at a rate of 3.1 spectra $s^{-1}$. The solvent delay time was set at 8.5 min.

### Data analysis

GC/MS raw data were exported in the NetCDF format, and then preprocessed by using the XCMS toolbox [19]. The

parameters of retention time correction and peak alignment were set to default values except for full width at half-maximum (FWHM = 4) and group (bw = 5). The area of each variable was normalized to the internal standard in the same chromatogram. The resulting data were then exported into SIMCA-P software version 11.0 (Umetrics, Umea, Sweden) for multivariate statistical analysis. Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were carried out to visualize the global metabolome change among the different glucose tolerance groups. Subsequently, ANOVA by the SPSS 13.0 software (SPSS, Chicago, IL) was used to find differential metabolites among the three groups. $p < 0.05$ was considered significant. Metabolites identification was performed by searching the NIST database installed in the equipment system, with a similarity threshold of 75%, with all of them verified by commercial standard samples.

## Results

### Clinical administration

The clinical practice data are shown in Table 1. Fasting plasma glucose and 2-h plasma glucose concentrations in T2D patients were significantly higher than those in both NGT and IGR subjects, fasting insulin, and C-reaction protein had similar changes although were not statistically significant. As for blood lipids, the levels of triglycerides, total cholesterol and LDL-c were all higher in T2D patients than those in the other two groups, while LDL-c level showed no statistical difference. Meanwhile, the concentration of HDL-c was lower in T2D patients than in the other two groups with statistical significance. The levels of fasting plasma glucose, 2-h plasma glucose, and triglycerides were also remarkably higher in IGR group than those in NGT group, as expected.

### Metabolomic analysis

The plasma metabolic profile was analyzed by GC/MS. Figure 1 gives the total ion chromatogram (TIC) of a representative plasma sample. To verify the reproducibility and reliability of the method including sample preparation and instrument performance, quality control samples derived from the equal pooling of all samples were prepared and analyzed accompanying with real samples in the whole analytical workflow. The relative standard deviations (RSDs) of the retention time and peak area of the main peaks were less than 1 and 15%, respectively, which reflected acceptable levels of variability for overall process. In addition, methyl laurate as the external standard was utilized to further evaluate the stability of the analytical platform. The RSDs of the retention time and the peak area of methyl laurate in the quality control samples were 0.04 and 5.6%, respectively, indicating that the instrument performance was perfectly stable during the whole analytical process.

To learn whether or not we can distinguish the three groups (NGT, IGR, and T2D) using the GC/MS data and understand the relationship between plasma metabolome and different states of glucose tolerance, we first performed a principal component analysis (PCA) model. The score plot (Fig. 2a) using two components ($R^2X = 0.66$) shows a separation tendency from NGT to IGR, and further to T2D group, suggesting that, from the perspective of metabolomics, the metabolome differences indeed existed among the three groups and IGR was the intermediate status. A partial least squares discriminant analysis (PLS-DA) model with two components ($R^2Y = 0.48$, $Q^2Y = 0.30$) was also constructed in order to visualize the cluster more clearly. It is evident from Fig. 2b that a complete separation between NGT and T2D groups was achieved, demonstrating that the metabolic characteristics of T2D was quite different with NGT. The data points from IGR group mainly located in the middle, with less overlap with NGT but more overlap with T2D, indicating that the metabolic states of some subjects with IGR were already approaching T2D. Furthermore, the results of permutation test (intercepts $R^2 = 0.106$, $Q^2 = -0.18$) suggested that there was no overfitting and the model was reliable.

To better explore the different glucose tolerance-related changes in the metabolites, ANOVA and multiple comparison were employed to select significantly changed metabolites among the groups. As shown in Table 2, a total of 23 metabolites were obviously different. In detail, compared to the NGT, the metabolic state of T2D resulted in increased levels of 2-hydroxybutyrate, branched-chain amino acids (isoleucine, leucine, valine), 2-ketoisocaprate, inositol, monosaccharide (fructose, galactose, glucose), glycerol, glycerate, and free fatty acids (FFA C16:0, FFA C18:1, FFA C18:0), as well as decreased levels of citrate, malate and glucogenic amino acids including alanine, glycine, serine, threonine, phenylalanine, glutamine, and asparagine. Additionally, the IGR group had striking higher concentrations of 2-hydroxybutyrate, 2-ketoisocaprate, leucine, isoleucine, glucose, and FFA C16:0 together with lower levels of citrate, glycine, and asparagine compared to those in NGT group. Finally, when compared with IGR group, T2D patients had remarkably higher levels of 2-hydroxybutyrate, 2-ketoisocaprate, valine, inositol, glycerate, glycerol, FFA C18:0, fructose, galactose as well as glucose, but lower levels of alanine, phenylalanine, and glutamine. In addition to glucose, 2-hydroxybutyrate and 2-ketoisocaproate also distinctly gradually increased from NGT to IGR and further to T2D. The results of the current study are well in-line with our previous preliminary investigation in another diabetic population (unpublished data). Box plots of the two metabolites are shown in Fig. 3 to visually present the distribution of variables among the three groups.

**Table 1** Biochemical parameters for NGT, IGR, and T2D groups

| | NGT ($n = 39$) | IGR ($n = 40$) | T2D ($n = 41$) |
|---|---|---|---|
| No. (male/female) | 20/19 | 19/21 | 20/21 |
| Age (year) | 60 ± 1[a] | 60 ± 1 | 60 ± 1 |
| Current smokers | 10 | 9 | 12 |
| Alcohol use | 7 | 8 | 8 |
| Body mass index (kg/m$^2$) | 24.9 ± 3.3 | 25.2 ± 2.3 | 26.0 ± 3.2 |
| Fasting plasma glucose (mmol/L) | 5.34 ± 0.56 | 5.93 ± 0.44[b] | 7.88 ± 1.67[c,d] |
| Fasting plasma insulin (μIU/mL) | 7.32 ± 5.65 | 7.44 ± 5.37 | 26.9 ± 99.1 |
| 2 h plasma glucose (mmol/L) | 6.16 ± 0.55 | 8.25 ± 1.41[b] | 14.9 ± 6.20[c,d] |
| Uric acid (μmol/L) | 269.4 ± 63.7 | 321.5 ± 115.8[b] | 301.1 ± 88.8 |
| Total cholesterol (mmol/L) | 4.75 ± 0.85 | 4.80 ± 0.95 | 6.33 ± 0.71[c,d] |
| Triglyceride (mmol/L) | 1.17 ± 0.62 | 1.62 ± 0.81[b] | 1.95 ± 0.89[c,d] |
| HDL cholesterol (mmol/L) | 1.42 ± 0.37 | 1.30 ± 0.24 | 0.95 ± 0.26[c,d] |
| LDL cholesterol (mmol/L) | 2.82 ± 0.67 | 2.96 ± 0.76 | 3.15 ± 0.68 |
| C-reaction protein (mg/L) | 2.88 ± 2.83 | 4.44 ± 5.29 | 6.15 ± 12.1 |

[a] Mean ± SD

[b] $p < 0.05$ for IGR vs. NGT

[c] $p < 0.05$ for T2D vs. IGR

[d] $p < 0.05$ for T2D vs. NGT

## Discussion

In order to investigate the complex perturbations of the metabolism related to the glucose tolerance states, we carried out a GC/MS-based non-targeted metabolomics study to investigate plasma samples from NGT, IGR, and T2D subjects. Our results showed a distinct metabonome change from NGT to IGR and to T2D. Moreover, differential metabolites revealed disturbances of various biological pathways in IGR and T2D groups. In addition to glucose, we found that the levels of 2-hydroxybutyrate and 2-ketoisocaproate progressively increased with impaired glucose tolerance severity. Although blood glucose level is routinely used to screen and assess diabetes, our results suggested that glucose measurement alone is not comprehensive and the metabolites associated with the development of T2D should be considered.

In carbohydrate metabolism, we observed the elevation of glucose, fructose, and galactose in IGR and/or T2D groups

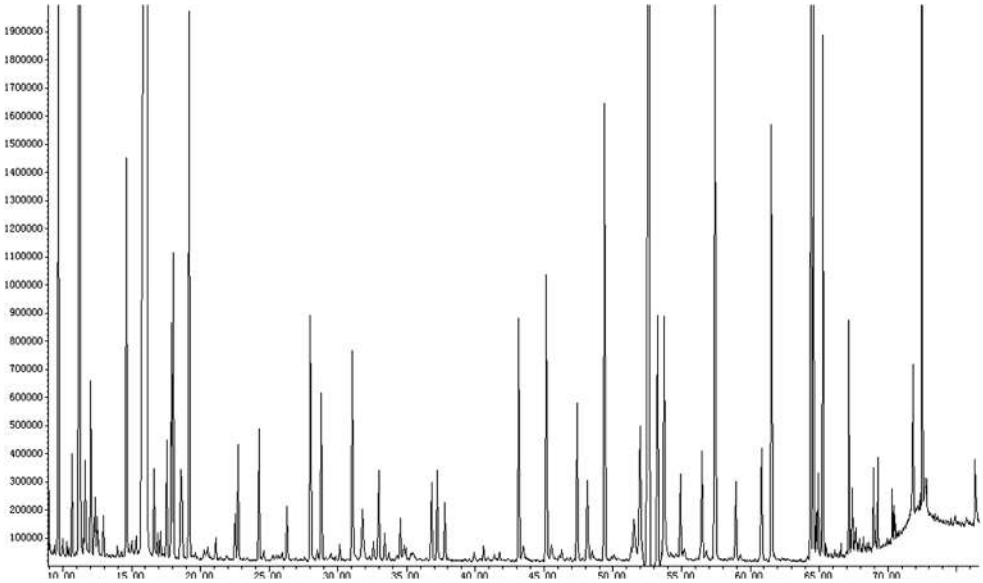

**Fig. 1** Representative total ion chromatograms (TIC) of plasma sample from a participant

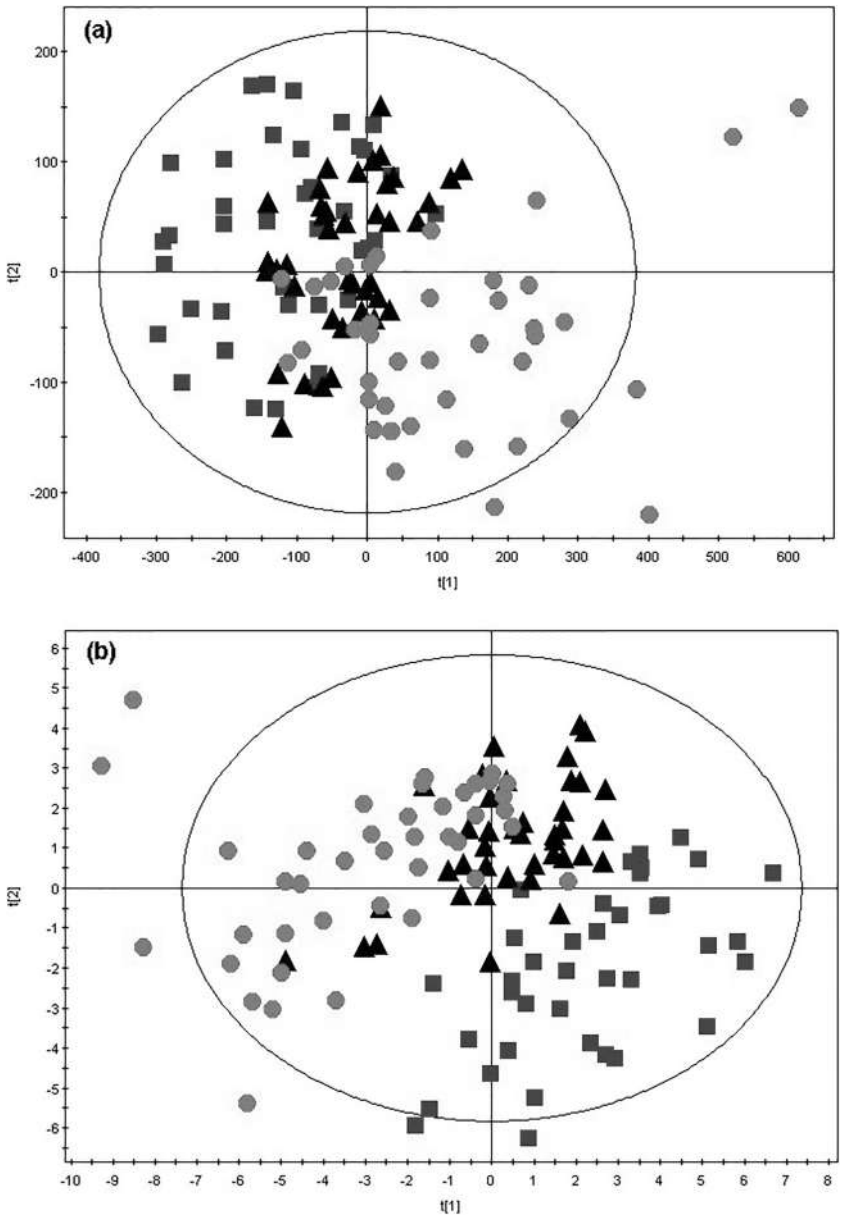


**Fig. 2** **a** PCA and **b** PLS-DA score plots for NGT (blue squares), IGR (black triangles), and T2D (red dots) groups

compared to the NGT group. These data suggested that the perturbations of glycolysis in IGR and T2D groups results in the accumulation of monosaccharide, which causes harm to the metabolism homeostasis. It has been noted that long-term high level of fructose can stimulate lipogenesis and induce hepatic insulin resistance [20, 21], and galactose is associated with retinopathy [22, 23].

In addition, those with IGR and/or T2D had elevated levels of branched-chain amino acids and reduced glucogenic amino acids, when compared to NGT. In the states of IGR and T2D, the rate of utilization of glucose is reduced relatively, resulting in the disturbance of energy metabolism. Therefore, the body needs other fuel molecules to enter TCA cycle to supply sufficient amount of energy. The reduced levels of glucogenic amino acids indicate that a large number of amino acids are catabolized and ultimately form many intermediates of TCA cycle. The levels of branched-chain amino acids, in contrast, increased in IGR and/or T2D groups. It has been documented that the activity of branched-chain α-keto acid dehydrogenase, an enzyme in branched-chain amino acids catabolism, is downregulated in diabetes mellitus [24, 25]. Therefore, under the control of this key enzyme, the catabolism of branched-chain amino acids in patients is blocked, and consequently, the concentrations of branched-chain amino acids in blood increased.

Furthermore, higher concentration of free fatty acids as well as glycerol in IGR and/or T2D patients was observed. It is due to the effects of antilipolytic and promoting fat storage

**Table 2** Identified significantly changed metabolites in NGT, IGR, and T2D groups

| Metabolite | Fold change[a] In IGR(vs. NGT)/ *p* value[b] | Fold change In T2D(vs. IGR)/ *p* value | Fold change In T2D(vs. NGT)/ *p* value | Biological pathways |
|---|---|---|---|---|
| Valine | 1.1↑/0.323 | 1.3↑/0.031 | 1.4↑/0.001 | Valine metabolism |
| Leucine | 1.2↑/0.025 | 1.0/0.511 | 1.2↑/0.023 | Leucine metabolism |
| Isoleucine | 1.1↑/0.037 | 1.1↑/0.414 | 1.2↑/0.010 | Isoleucine metabolism |
| 2-ketoisocaprate | 1.2↑/0.009 | 1.2↑/0.015 | $1.4↑/1.3 \times 10^{-4}$ | Isoleucine metabolism |
| 2-hydroxybutyrate | 1.3↑/0.001 | 1.2↑/0.026 | $1.5↑/1.6 \times 10^{-4}$ | Propanoate metabolism |
| Alanine | 1.0/0.412 | 0.8↓/0.025 | 0.8↓/0.013 | Alanine metabolism |
| Glycine | 0.7↓/0.003 | 1.0/0.627 | 0.7↓/0.009 | Glycine metabolism |
| Serine | 0.9↓/0.067 | 0.9↓/0.236 | 0.8↓/0.008 | Serine metabolism |
| Threonine | 0.9↓/0.295 | 0.9↓/0.291 | 0.8↓/0.031 | Threonine metabolism |
| Phenylalanine | 1.0/0.355 | 0.7↓/0.002 | 0.7↓/0.003 | Phenylalanine metabolism |
| Glutamine | 0.9↓/0.203 | 0.8↓/0.036 | 0.7↓/0.001 | Glutamine metabolism |
| Asparagine | $0.7↓/1.1 \times 10^{-4}$ | 1.0/0.632 | $0.7↓/3.9 \times 10^{-5}$ | Asparagine metabolism |
| Malate | 0.8↓/0.072 | 0.9↓/0.132 | 0.7↓/0.014 | TCA cycle |
| Citrate | 0.8↓/0.022 | 1.0/0.279 | 0.8↓/0.030 | TCA cycle |
| Inositol | 1.1↑/0.329 | 1.1↑/0.041 | 1.2↑/0.035 | Inositol phosphate metabolism |
| Fructose | 1.1↑/0.336 | 1.3↑/0.002 | 1.4↑/0.001 | Fructose and mannose metabolism |
| Galactose | 1.1↑/0.224 | 1.2↑/0.010 | 1.3↑/0.002 | Galactose metabolism |
| Glucose | 1.1↑/0.030 | $1.4↑/2.5 \times 10^{-5}$ | $1.5↑/5.1 \times 10^{-6}$ | Glucose metabolism |
| Glycerate | 1.2↑/0.374 | 1.2↑/0.035 | 1.4↑/0.001 | Pentose phosphate metabolism |
| Glycerol | 1.1↑/0.505 | 1.3↑/0.019 | 1.4↑/0.002 | Lipid metabolism |
| FFA C16:0 | 1.1↑/0.043 | 1.1↑/0.150 | 1.2↑/0.014 | Lipid metabolism |
| FFA C18:0 | 1.1↑/0.241 | 1.2↑/0.034 | 1.3↑/0.001 | Lipid metabolism |
| FFA C18:1 | 1.1↑/0.378 | 1.1↑/0.092 | 1.2↑/0.025 | Lipid metabolism |

(↑) upregulated, (↓) downregulated

[a] Fold change was calculated from the mean values of each group

[b] *p* value was calculated from multiple comparison. (↑) upregulated, (↓) downregulated

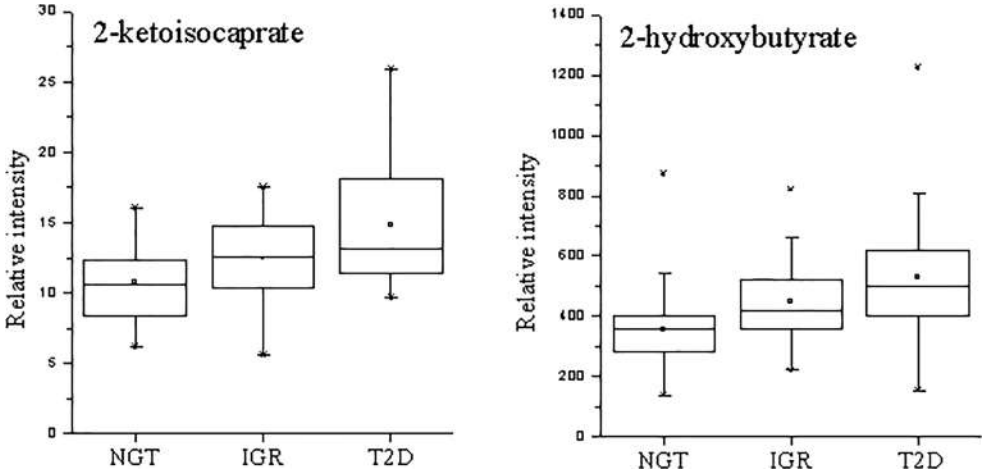


**Fig. 3** Box plots of 2-ketoisocaprate and 2-hydroxybutyrate among the three groups

are weakened in patients with the impaired islet function [26, 27]. High levels of circulating fatty acids may, in turn, induce or exacerbate insulin resistance, thereby accelerating the onset of T2D [28, 29].

On the basis of the results, we can understand the metabolic dysregulations in the states of IGR and T2D. Reduction of glycolysis induces the manifold utilization of glucogenic amino acids, while in the meantime, the burden of glucose could be aggravated as a result of glucogenic amino acids transforming into glucose through gluconeogenesis. An abnormal high level of glucose can decrease the sensitivity of insulin and contribute to hyperlipidemia. Conversely, hyperlipidemia is the basis for the pathophysiology of insulin resistance and consequently exacerbates blood glucose, both as cause and effect, a vicious circle. Ultimately, TCA cycle as the center of metabolism is also disturbed, manifested as decreased levels of malate and citrate in our study.

Moreover, we also found the progressively changed metabolites related with different glucose tolerance besides glucose. Distinctly, the levels of 2-ketoisocaprate and 2-hydroxybutyrate were increased in IGR and T2D groups compared with those in the NGT group, and their concentrations in the T2D group were also higher than those in the IGR group. 2-Ketoisocaprate, derived from the deamination of isoleucine, is the substrate of branched-chain $\alpha$-keto acid dehydrogenase. The gradual accumulation of 2-ketoisocaprate from NGT to IGR and to T2D indicates that isoleucine metabolism, particularly 2-ketoisocaprate, may be correlated with the pathological process of T2D. 2-Hydroxybutyrate is an organic acid and is produced during the pathway of threonine catabolism or glutathione anabolism. The accumulation of 2-hydroxybutyrate may be due to the disorders of upstream metabolism. Firstly, many researches have demonstrated that oxidative stress plays a critical role in the pathogenesis of diabetes mellitus [30–32]. Under dramatically increased oxidative stress states in IGR and T2D, large amounts of cystine are converted to cysteine to form glutathione [33, 34]; meanwhile, more 2-hydroxybutyrate is released as a by-product during this process. Secondly, 2-hydroxybutyrate is also formed from threonine catalyzed by serine-threonine dehydratase. In our study, the decrease of threonine level together with the increase of 2-hydroxybutyrate concentration in IGR and T2D patients suggest that the enhancement of threonine catabolism may be another reason for the elevation of 2-hydroxybutyrate. In other prospective studies, 2-ketoisocaprate [16] and 2-hydroxybutyrate [17] were found as the predictor of diabetes, respectively. Overall, from the data obtained in our study and others, we speculate that the two metabolites including 2-ketoisocaprate and 2-hydroxybutyrate may be not only good predictors for diabetes but also closely associated with the dynamic development of diabetes. Certainly, a lot of experiments are needed to verify the hypothesis and further explore the underlying molecular mechanisms of the two metabolites in future.

In the current study, a non-targeted metabolomics approach based on the combination of GC/MS and statistical analysis was employed to study the plasma metabolic patterns of subjects with NGT, IGR, and T2D. The metabolic changes in IGR and T2D groups included perturbations of monosaccharide and lipid metabolism, disorders of glucogenic amino acids, and branched-chain amino acid catabolism. At the same time, metabolites associated with the development of diabetes were also successfully obtained, manifested by 2-ketoisocaprate and 2-hydroxybutyrate as highlighted above. In conclusion, the metabolome alterations are the mirror image of different glucose tolerance states, and recognition of the relationships of 2-ketoisocaprate and 2-hydroxybutyrate with diabetes will help us deeply comprehend the pathological process of diabetes.

**Acknowledgments** We are indebted to all the subjects who participated in this study.

**Funding** This manuscript was supported by the doctoral start-up grant (No.2013-25) from Shanxi Datong University of China.

## Compliance with ethical standards

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

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

**Patient consent** All the procedures were approved by institutional ethics review board of Datong NO.3 People's Hospital, and written informed consent was obtained from each participant.



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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):486–498
https://doi.org/10.1007/s13410-018-0680-8

ORIGINAL ARTICLE

# Muscle-derived IL-6 improved insulin resistance of C2C12 cells through activating AMPK and inhibiting p38MAPK signal pathway in vitro

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Received: 24 January 2018 / Accepted: 18 July 2018 / Published online: 22 August 2018


## Abstract

This paper was to probe into whether IL-6 improved insulin resistance by AMPK and p38MAPK signal pathway. The C2C12 cell lines were purchased from ATCC. The cells of control group were cultured in high-glucose DMEM culture medium. $CaCl_2$ with different concentrations was respectively used to culture C2C12 cells; then, glucose concentrations in the nutrient solution following 24 h were measured in the preliminary experiment. Firstly, cells of A group (control group) were normally cultured C2C12 cells, cells of B, C, and D group were respectively cultured with palmitic acid 48 h, palmitic acid 24 h, and IL-6 24 h, and IL-6-shRNA 48 h, glucose concentrations, IL-6 mRNA and GLUT4 mRNA levels, p-p38MAPK, p-AMPK, p-IRS-1, and p-PI-3K protein contents of each group in the nutrient solution following 48 h were measured. Secondly, cells of A group (control group) were palmitic acid-cultured C2C12 cells, cells of B and C group were respectively cultured with palmitic acid 24 h and CaCl2 24 h, and palmitic acid 24 h CaCl2 24 h, and IL-6-shRNA 24 h, and the same indexes were measured with the first experiment. The preliminary experimental result showed that CaCl2 feed can induce C2C12 cell contraction, and CaCl2 with different concentrations was able to decrease significantly glucose concentrations in the nutrient solution following 24 h ($p < 0.05$). The result of the first experiment showed that, compared with B group, p-AMPK, p-IRS-1, and p-PI-3K protein contents, GLUT4 mRNA level, and glucose ingestion capacity of C group significantly increased ($p < 0.05$), and p-p38MAPK protein content of it significantly decreased ($p < 0.05$). Compared with A group, those of D group significantly decreased ($p < 0.05$), and p-p38MAPK protein contents of it significantly increased ($p < 0.05$). The result of the second experiment showed that, compared with A group, those of B group significantly increased ($p < 0.05$), and p-p38MAPK protein content significantly decreased ($p < 0.05$). Compared with B group, those of C group significantly decreased ($p < 0.05$), and p-p38MAPK protein contents significantly increased ($p < 0.05$). This experiment firstly discovered that exogenous calcium-cultured can induce contraction of C2C12 cells. Furthermore, muscle-derived IL-6 improved insulin resistance of C2C12 cells through activating AMPK and inhibiting p38MAPK signal pathway.

**Keywords** IL-6 · Insulin resistance · C2C12 cells · AMPK · p38MAPK

## Introduction

It is well-known that exercise can improve insulin resistance (IR). However, its mechanism has not been clarified completely. In recent years, studies about "myokine" played important roles on understanding the mechanism. Myokine was a kind of hormone-like substance, which was secreted by exercise organ (skeletal muscle), while human body exercised, and regulated substance metabolism and energy metabolism [1]. The first myokine was IL-6 [2], and lately discovered myokine was irisin [3], Metrnl [4], and so on.

Exercise can significantly increase systemic plasma IL-6; furthermore, research results had showed that increase of IL-6 was derived from skeletal muscle [5]. Muscle-derived IL-6 can increase glucose ingestion of skeletal muscle, glucose output of liver, mobilization, and utilization of free fatty acid [6, 7]. The research results of Wallenius et al. showed that the

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carbohydrates and lipid metabolisms in IL-6 gene knockout mice disordered, onset obesity, and significant IR symptom appeared, compared with wild-type mice. Furthermore, low-dose IL-6 injection can improve the symptom [8]. Above research results demonstrated that muscle-derived IL-6 has vital roles for the improvement of IR. However, up to now, the mechanism which muscle-derived Il-6 improved insulin resistance had not clarified completely.

As sensor of energy metabolism, AMPK played direct roles on improving IR. One scholar thought that the emerging IR symptom in IL-6 gene knockout mice was possibly related to the decrease of AMPK activity in skeletal muscle and adipose tissue [9]. Other research showed that IL-6 promoted insulin-induced glucose utilization and fatty acid oxidation by activating AMPK [10]. The first purpose of this manuscript is to probe into whether muscle-derived IL-6 improved IR by activating AMPK signal pathway.

As stress-activated protein kinase, p38MAPK was able to be activated by LPS, TNF-α, heat shock, ultraviolet rays, $H_2O_2$, and hypertonic conditions. Research results showed that PRDX-1 can induce liver IR through activating p38MAPK [11]. Zhen reported that reseveratrol can reduce glucose and improve insulin resistance by inhibiting TLR4 downstream factors, including p38MAPK signal pathway [12]. Other researches discovered that both high glucose and high fat can activate p38MAPK and develop IR [13, 14]. Above research results demonstrated that the activation of p38MAPK signal pathway plays important roles on the development of IR. However, whether exercise activates p38MAPK is in dispute. Researches reported that different muscle contractions activated p38MAPK signal pathway [15, 16]; however, other study results showed that p38MAPK protein contents and activities significantly decreased in normal rats and human skeletal muscle following endurance exercise [17, 18]. Therefore, the second purpose of this manuscript was to probe into whether muscle-derived IL-6 improved IR by p38MAPK signal pathway.

## Methods

### Cell culture

The C2C12 cell lines were purchased from ATCC. All the experimental procedures were performed according to the institutional guidelines of Hunan University of Science and Technology. The cells are cultured in high-glucose DMEM culture medium, 37 °C, 5% $CO_2$ incubator, including 10% fetal calf serum. When cell fusion is to 80%, cells are sent to passage with 0.25% pancreatin trypsinization and then are inoculated in new culture plates. Cells are used in follow-up experiments when cell fusion is to 70%.

### Cell grouping

Each group inoculates five hopes, and each hope inoculates 100 μL. When cells arised to adherence following 12 h culture, The former culture solutions were abandoned, and 100 μL culture solutions were added to each cell group respectively. CaCl2 with different concentrations was respectively used to culture C2C12 cells in the preliminary experiment. Firstly, cells of A group (control group) were normally cultured C2C12 cells, cells of B, C, and D group were respectively cultured with palmitic acid 48 h, palmitic acid 24 h and IL-6 24 h, and IL-6-shRNA 48 h. Secondly, cells of A group (control group) were palmitic acid-cultured C2C12 cells, cells of B and C group were respectively cultured with palmitic acid 24 h and CaCl2 24 h, and palmitic acid 24 h, CaCl2 24 h, and IL-6-shRNA 24 h.

**Reagents** Recombination IL-6 and $CaCl_2$ were purchased from Sigma (Sigma-Aldrich Corp., St. Louis, MO, USA). TRIzol was purchased from Invitrogen (Invitrogen, Carlsbad, CA, USA, #15596-026), RevertAid H Minus First Strand cDNA Synthesis Kit was purchased from Fermentas (MBI Fermentas, Vilnius, Lithuania, #K1631), DNase I was purchased from Fermentas (MBI Fermentas, Vilnius, Lithuania, #EN0521), RiboLock Ribonuclease Inhibitor was purchased from Fermentas (MBI Fermentas, Vilnius, Lithuania, #EO0381), and SYBR GreenPCR Master Mix was purchased from ABI (4309155).

**Primary antibodies** Rabbit p-p38 antibody (38kD) was purchased from Abcam (Abcam, London, England, ab7952), mouse p-PI-3K antibody (85kD) was purchased from Abcam (ab22653), mouse p-AMPKα antibody (62kD) was purchased from CST (2793), rabbit p-IRS-1 antibody (160kD) was purchased from SANTA (Santa Cruz, CA, USA, SC-559), and mouse GAPDH antibody (37kD) was purchased from ProMab (2005079).

**Second antibodies** Goat anti-rabbit IgG/HRP was purchased from SCBT (sc-2030), and goat anti-mouse IgG/HRP was purchased from ZYMED (#62-64201). Total protein extraction kit was purchased from ProMab (SJ-200501).

### Western blotting

p-p38MAPK, p-AMPKα, p-IRS-1, and p-PI-3K protein contents were determined by West blotting as early report [19]. Total protein from the C2C12 cells was isolated in RIPA lysis buffer. Equal protein extracts from each group were run on SDS-PAGE and then transferred to a polyvinylidene fluoride membrane. This was followed by blocking with 5% non-fat milk and incubation at 25 °C for 1 h. Thereafter, the membranes were incubated with a specific primary antibody at 4 °C overnight. This was followed by washing in buffer and incubation for 1 h with the suitable secondary antibody. The protein bands

of interest were visualized under a confocal microscope (LSM 700, Carl Zeiss Inc., Oberkochen, Germany).

### Real-time PCR

IL-6 mRNA and GLUT4 mRNA levels were determined by real-time PCR as previously described [20]. Total RNAs of C2C12 myotubes were isolated with TRIzol (Invitrogen, Carlsbad, CA, USA, #15596-026) and purified with the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instruction. cDNA was generated with reverse transcription, which was performed with 1 μg of total RNA and random primers by SuperScript II reverse transcriptase. All reactions were performed in duplicate, and the relative mRNA expression level of each target gene was normalized with that of GAPDH as an internal control. Primers are designed with primer3.0 software and synthesized by ProMab. IL-6-F:GGTGACAACCACGGCCTTCCC,IL-6-R:AAGCCTCCGACTTGTGAAGTGGT;GLUT4-F:TCCCACCAGACCCGCCCTTT,GLUT4-R:ACTCGCTGCCGAGGGGGTTC;GAPDH-F:AACTTTGGCATTGTGGAAGG,GAPDH-R:GGATGCAGGGATGATGTTCT.

### Statistical analysis

All analyses were performed with Sigma Plot 10.0 software. Data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with repeated measures and Bonferroni post hoc independent unpaired *t* tests were used to determine differences. Data were considered statistically significant at $p < 0.05$.

## Results

### Calcium-cultured C2C12 cell and induced contraction

The cells of control group were cultured in high-glucose DMEM culture medium. CaCl2 with different concentrations was respectively used to culture C2C12 cells; then, glucose concentrations in the nutrient solution following 24 h were measured in the preliminary experiment. As shown in Fig. 1a, the cell contraction can be discovered distinctly when cells are cultured with 100 μM $CaCl_2$. Certainly, other concentrations $CaCl_2$ also induced cell contraction. However, the

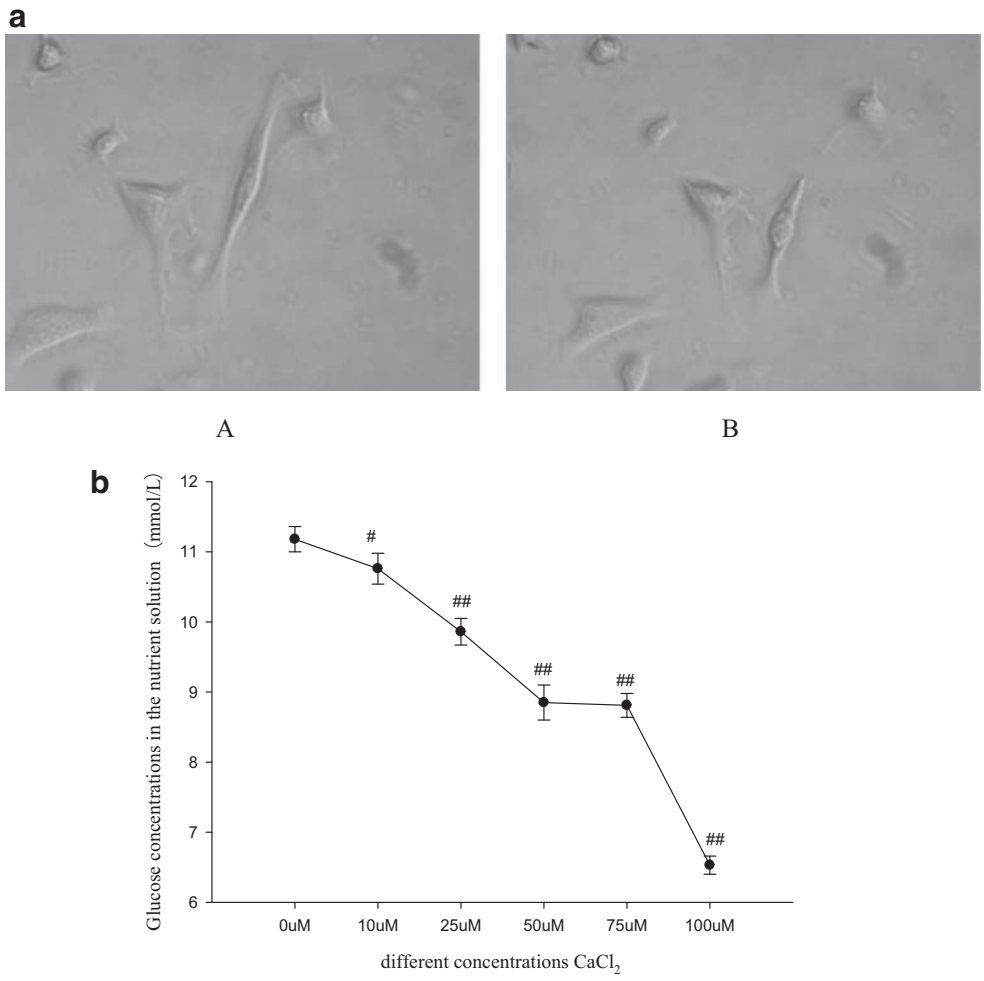



**Fig. 1** **a** C2C12 cells loaded with 100 μM $CaCl_2$ (A before $CaCl_2$—load, B after $CaCl_2$—load). **b** Glucose concentrations in the nutrient solution following 24 h different concentrations $CaCl_2$ culture (#respectively compared with that of 0 μM $CaCl_2$, $p < 0.05$; ##respectively compared with that of 0 μM I $CaCl_2$, $p < 0.01$)

effects were not so distinct. As shown in Fig. 1b, compared with 0 μM $CaCl_2$, the glucose concentrations in the nutrient solution significantly decreased following 24 h 10, 25, 50, 75, or 100 μM $CaCl_2$ culture ($p < 0.01$). By reason of the main energy of cell contraction that came from glucose ingestion in the culture solution, this result demonstrated that different concentration $CaCl_2$ cultures can induce cell contraction. To induce cell contraction, 100 μM $CaCl_2$ was selected to culture C2C12 cells.

At present, the main method to stimulate C2C12 cell contraction was low-frequency electrical stimulation [21]. Based on the fundamental principle that the trigger of skeletal muscle contraction was output of $Ca^{2+}$ from sarcoplasmic reticulum and basic fact that exogenous $Ca^{2+}$ can inflow into cytoplasm by voltage-dependent calcium channel [22], this preliminary experiment was to build up a model that exogenous calcium load induces cell contraction.

## IL-6-cultured insulin resistance C2C12 cell lines and IL-6shRNA-cultured C2C12 cells.

As shown in Figs. 2, 3, 4, and 5, following 48 h different culture, the glucose concentrations in the nutrient solution of D group (1000 ng/mL IL-6-cultured insulin resistance C2C12 cells induced by palmitic acid) significantly decreased ($p < 0.01$), p-AMPK, p-IRS-1, and p-PI-3K protein contents and GLUT4 mRNA level in C2C12 cells of C group significantly increased ($p < 0.01$); and p38MAPK protein content in C2C12

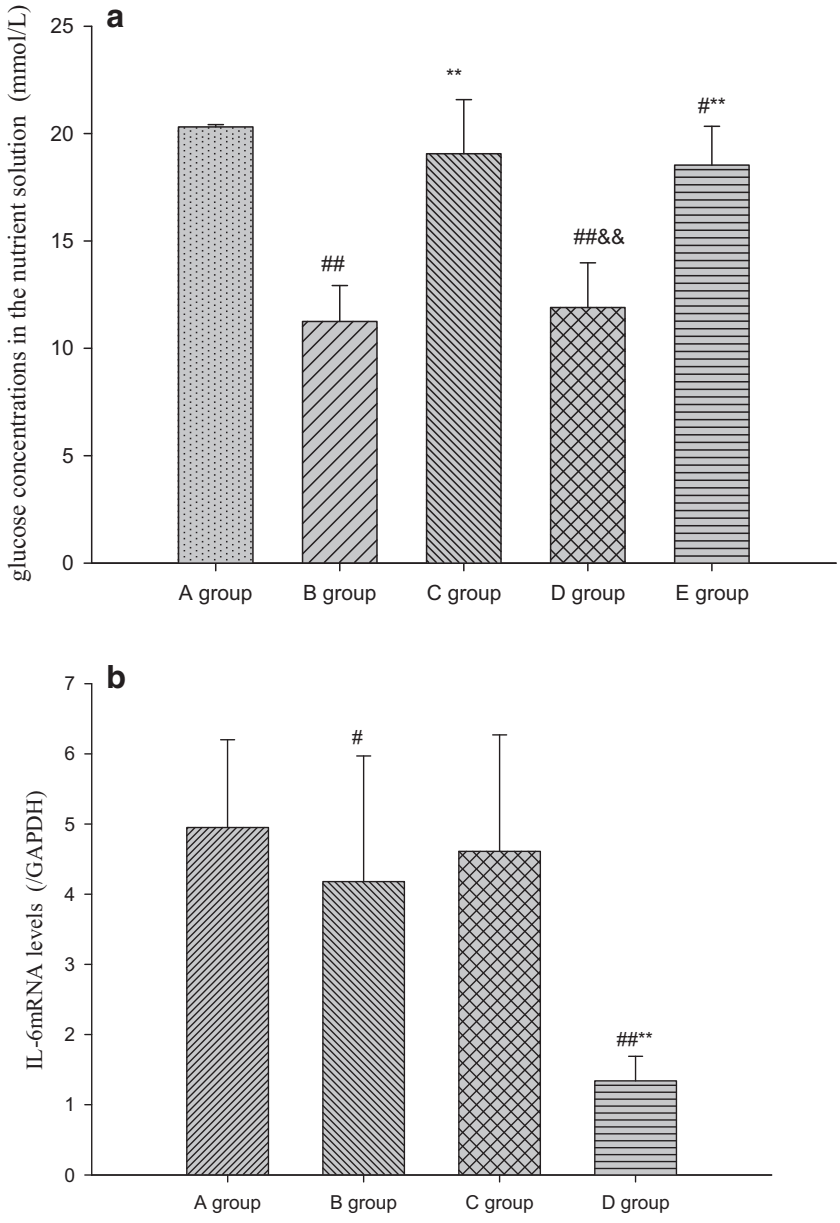



**Fig. 2** **a** Glucose concentrations of each group in the nutrient solution following 48 h culture (A group: normal nutrient solution; B group: normal C2C12 cell; C group: C2C12 cell+ palmitic acid; D group: C2C12 cell+ palmitic acid + IL-6; E group: C2C12 cell+ IL-6-shRNA; #glucose concentrations of each group are respectively compared with those of A group, $p < 0.05$; ##glucose concentrations of each group are respectively compared with those of A group, $p < 0.01$; **glucose concentrations of C, D, and E groups are respectively compared with those of B group, $p < 0.01$; &&glucose concentrations of D and E groups are respectively compared with those of C group, $p < 0.01$). **b** IL-6 mRNA levels of each group in the cells following 48 h culture (A group: normal C2C12 cell; B group: C2C12 cell+ palmitic acid 48 h; C group: C2C12 cell+ palmitic acid 24 h + IL-6 24 h; D group: C2C12 cell+ IL-6-shRNA 48 h; #IL-6 mRNA levels of each group are respectively compared with those of A group, $p < 0.05$; ##IL-6 mRNA levels of each group are respectively compared with those of A group, $p < 0.01$; **IL-6 mRNA levels of C and D groups are respectively compared with those of B group, $p < 0.01$)

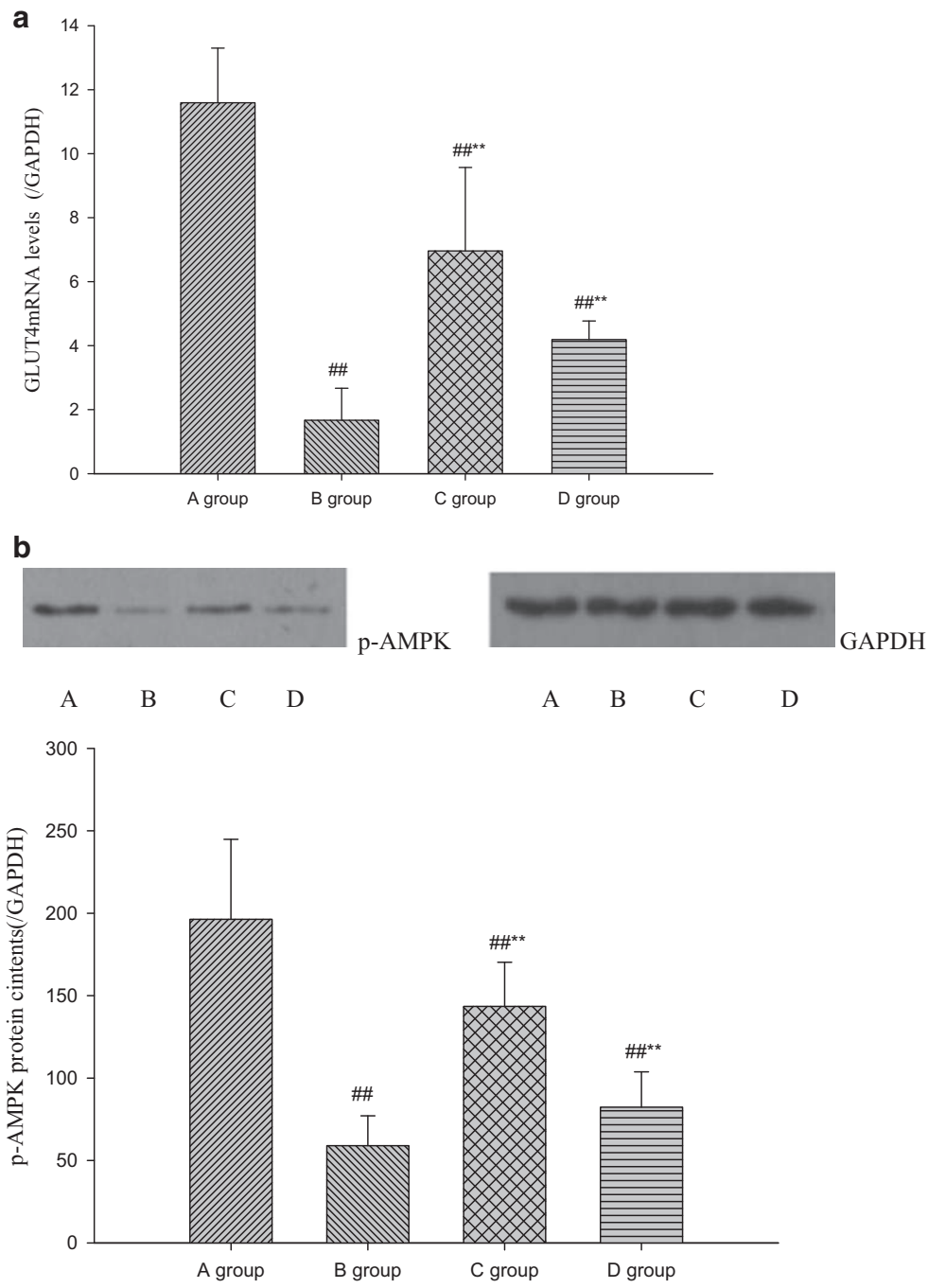



**Fig. 3** **a** GLUT4 mRNA levels of each group in the cells following 48 h culture (A group: normal C2C12 cell; B group: C2C12 cell+ palmitic acid 48 h; C group: C2C12 cell+ palmitic acid 24 h + IL-6 24 h; D group: C2C12 cell+ IL-6-shRNA 48 h; ##GLUT4 mRNA levels of each group are respectively compared with those of A group, $p < 0.01$; **GLUT4 mRNA levels of C and D groups are respectively compared with those of B group, $p < 0.01$). **b** p-AMPK protein content levels of each group in the cells following 48 h culture (A group: normal C2C12 cell; B group: C2C12 cell+ palmitic acid 48 h; C group: C2C12 cell+ palmitic acid 24 h + IL-6 24 h; D group: C2C12 cell+ IL-6-shRNA 48 h; ##p-AMPK protein contents of each group are respectively compared with those of A group, $p < 0.01$; **p-AMPK protein contents of C and D groups are respectively compared with those of B group, $p < 0.01$)

cells of D group significantly decreased ($p < 0.01$), respectively, compared with that in C group (insulin resistance C2C12 cells induced by palmitic acid). The results showed that IL-6-load activated AMPK and inhibits p38MAPK signal pathway, increased glucose transport and ingestion, activated insulin signal pathway, and enhanced insulin sensitivities in insulin resistance C2C12 cells.

As shown in Figs. 2, 3, 4, and 5, following 48 h different culture, the glucose concentrations in the nutrient solution of E group (IL-6shRNA-cultured C2C12 cells) significantly increased ($p < 0.01$), p-AMPK, p-IRS-1, p-PI-3K protein contents, and IL-6, GLUT4 mRNA level in C2C12 cells of C group significantly decreased ($p < 0.01$), and p38MAPK protein content in C2C12 cells of E group significantly increased ($p < 0.01$), respectively, compared with that in B group (normal C2C12 cells). The results showed that IL-6 gene silence inhibited AMPK and activated p38MAPK signal pathway, decreased glucose transport and ingestion, suppressed insulin signal pathway, and decreased insulin sensitivities in normal C2C12 cells.

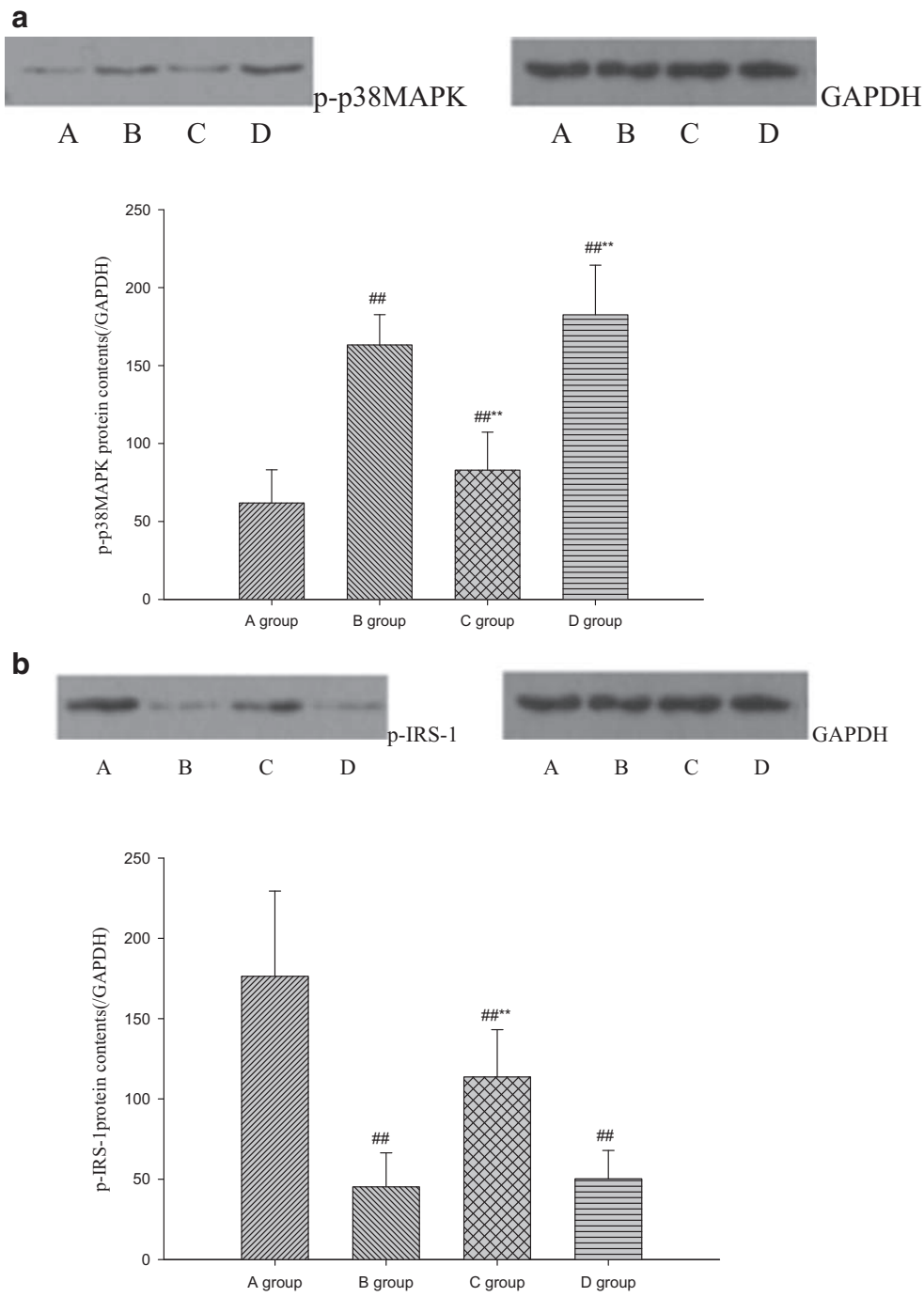



**Fig. 4** **a** p-p38MAPK protein content levels of each group in the cells following 48 h culture (A group: normal C2C12 cell; B group: C2C12 cell+ palmitic acid 48 h; C group: C2C12 cell+ palmitic acid 24 h + IL-6 24 h; D group: C2C12 cell+ IL-6-shRNA 48 h; ##p-p38MAPK protein contents of each group are respectively compared with those of A group, $p < 0.01$; **p-p38MAPK protein contents of C and D groups are respectively compared with those of B group, $p < 0.01$). **b** p-IRS-1 protein content levels of each group in the cells following 48 h culture (A group: normal C2C12 cell; B group: C2C12 cell+ palmitic acid 48 h; C group: C2C12 cell+ palmitic acid 24 h + IL-6 24 h; D group: C2C12 cell+ IL-6-shRNA 48 h; ##p-IRS-1 protein contents of each group are respectively compared with those of A group, $p < 0.01$; **p-IRS-1 protein contents of C and D groups are respectively compared with those of B group, $p < 0.01$)

Based on above analysis, the conclusion summarized by this experiment is that IL-6 was able to improve insulin resistance by activation of AMPK and inhibiting p38MAPK signal pathway. However, this experiment cannot demonstrate that exercise-induced IL-6 secreted from skeletal muscle had similar effects. So, our research group would probe into the effect and mechanism of skeletal muscle-derived IL-6 by calcium-load C2C12 cells to simulate muscle contraction in the second experiment.

## Calcium-cultured insulin resistance C2C12 cell lines and IL-6shRNA-cultured calcium-fed insulin resistance C2C12 cells

As shown in Figs. 6, 7, and 8, following 48 h different culture, p-AMPK, p-IRS-1, p-PI-3K protein contents and GLUT4 mRNA level in C2C12 cells of B group (100 μM $CaCl_2$-load insulin resistance C2C12 cells cultured by 0.6 mM palmitic acid) significantly increased ($p < 0.01$), and p38MAPK

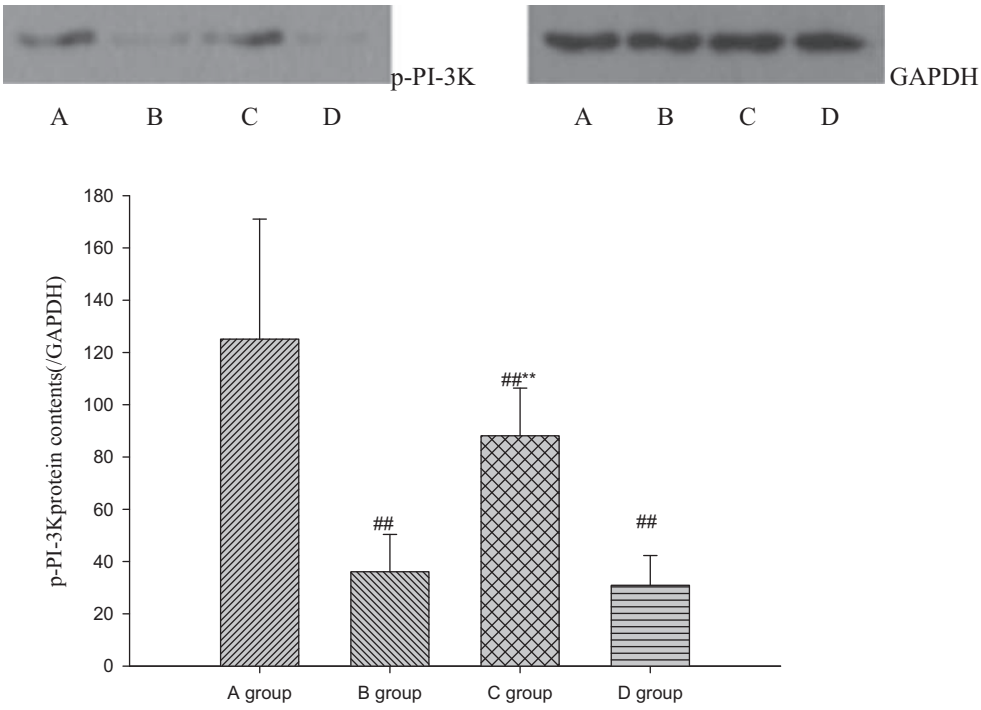



**Fig. 5** p-PI-3K protein content levels of each group in the cells following 48 h culture (A group: normal C2C12 cell; B group: C2C12 cell+ palmitic acid 48 h; C group: C2C12 cell+ palmitic acid 24 h + IL-6 24 h; D group: C2C12 cell+ IL-6-shRNA 48 h; ##p-PI-3K protein contents of each group are respectively compared with those of A group, $p < 0.01$; **p-PI-3K protein contents of C and D groups are respectively compared with those of B group, $p < 0.01$)

protein content in C2C12 cells of B group significantly decreased ($p < 0.01$), respectively, compared with those of A group (insulin resistance C2C12 cells cultured by 0.6 mM palmitic acid). The results showed that skeletal muscle contraction induced by $CaCl_2$-load activated AMPK and suppressed p38MAPK signal pathway, decreased glucose transport and ingestion, activated insulin signal pathway, and increased insulin sensitivities in insulin resistance C2C12 cells.

As shown in Figs. 6, 7, and 8, following 48 h different culture, p-AMPK, p-IRS-1, p-PI-3K protein contents and GLUT4 mRNA level in C2C12 cells of C group (IL-6shRNA-induced 100 μM $CaCl_2$-fed insulin resistance C2C12 cells cultured by 0.6 mM palmitic acid) significantly decreased ($p < 0.01$), and p38MAPK protein content in C2C12 cells of C group significantly decreased ($p < 0.01$), respectively, compared with those of B group (100 μM $CaCl_2$-fed insulin resistance C2C12 cells cultured by 0.6 mM palmitic acid). The results showed that the increase effect of insulin sensibilities induced by $CaCl_2$-fed in insulin resistance C2C12 cells significantly decreased, AMPK signal suppressed, and p38MAPK signal activated when IL-6 genes were in silence.

## Discussion

Through insulin resistance induced by high-fat or high-energy diets and streptozotocin (STZ) developed in vivo [23–25], however, it was induced by palmitic acid treatment in vitro [19, 26]. To discover the optimum concentration of palmitic acid to develop insulin resistance, this paper adopted palmitic acid treatment to develop insulin resistance based on previous reports. The preliminary experiment result showed that when the palmitic acid concentration reached 0.6 mM, the decrease of glucose concentration in culture solution became smooth and steady. So, in the next experiment, we adopted 0.6 mM concentration palmitic acid to develop insulin resistance.

As the first definite myokine, IL-6 can regulate glucose and lipid metabolism, increase glucose ingestion and utilization of skeletal muscle, and had important roles on the improvement of insulin sensitivities in skeletal muscle [6, 7]. The experiment of IL-6 gene knockout had demonstrated the effects. The research of Wallenius et al. showed that the carbohydrates and lipid metabolisms in IL-6 gene knockout mice disordered, onset obesity, and significant IR symptom appeared, compared with wild type mice. Furthermore, low-dose IL-6 injection can improve the symptom [8]. Bagdade et al. reported that the serum TG had not changed significantly in seven health male subjects injected recombination human IL-6 [27]. The research result of Wolsk et al. showed that recombination human IL-6 injection can selectively stimulate fat metabolism of skeletal muscle [28]. A few researches about culturing cells in vitro by IL-6 had reported. Meszaros et al. cultured human gristle cell lines C28/I2 and T/C28a2 by rh-IL-6 [29]. The research result of McGee et al. showed that rh-IL-6 treatment abdomen subcutaneous fat cells significantly increased the protein level of visfatin [30]. However, it was a pity that few reports about rh-IL-6 treatment myocytes can be discovered. We discovered that different concentration IL-6 treatment C2C12 cells significantly increased glucose ingestion, and the

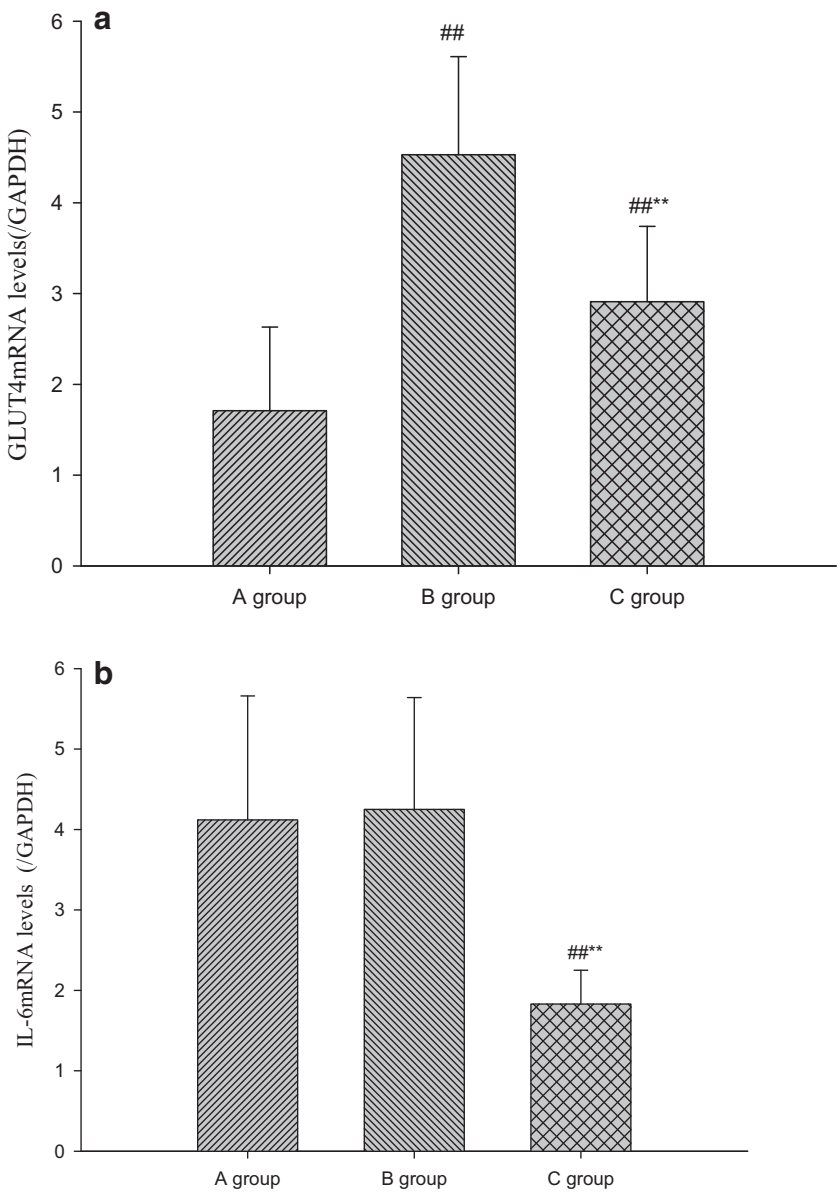



**Fig. 6** **a** GLUT4 mRNA levels of each group in the cells following respective period culture (A group: palmitic acid + C2C12 cell 24 h; B group: palmitic acid 24 h + $CaCl_2$ 24 h + C2C12 cell; C group: palmitic acid 24 h + $CaCl_2$ 24h + IL-6-shRNA 24 h + C2C12 cell; ##GLUT4 mRNA levels of each group are respectively compared with those of A group, $p < 0.01$; **GLUT4 mRNA level of C group is compared with that of B group, $p < 0.01$). **b** IL-6 mRNA levels of each group in the cells following respective period culture (A group: palmitic acid + C2C12 cell 24 h; B group: palmitic acid 24 h + $CaCl_2$ 24 h + C2C12 cell; C group: palmitic acid 24 h + $CaCl_2$ 24h + IL-6-shRNA 24 h + C2C12 cell; ##IL-6RNA levels of each group are respectively compared with those of A group, $p < 0.01$; **IL-6 mRNA level of C group is compared with that of B group, $p < 0.01$)

effect of 1000 ng/mL IL-6 is optimum. Based on previous reports and our experiment result, this paper adopted 1000 ng/mL IL-6 to culture C2C12 cells in the next experiment.

The result of research in vivo showed that exercise-induced release of IL-6 from skeletal muscle enhanced glucose ingestion by autocrine [5]. However, there were no demonstrations of studies in vitro. Thus, how to stimulate myocyte contraction and induce the release of IL-6 became the vital issue for our research. At present, the main method to stimulate myocyte contraction in vitro was low-frequency electrical stimulation [21, 31]. However, the trigger condition of skeletal muscle contraction in vivo was that nervous excitation reaches neuromuscular junction and caused the release of $Ca^{2+}$ from sarcoplasmic reticulum. So, it did not correspond with physiological laws for myocyte to contract by electrical stimulation directly. When cells excited, intracellular concentration of free $Ca^{2+}$ increases, and the source was $Ca^{2+}$ storage release and extracellular $Ca^{2+}$ inflow [22]. The trigger of skeletal muscle and cardiac muscle contraction was mainly due to the release of $Ca^{2+}$ from sarcoplasmic reticulum and extracellular $Ca^{2+}$ inflow in vivo, respectively. In myocyte, extracellular $Ca^{2+}$ inflow mainly depended on voltage-dependent calcium channel (VDCC). VDCC primarily was L type in skeletal muscle, and its characteristics were long opening time and slow inactivation. VDCC was activated by various medium, such as c-nucleotide, lipid ramification, and $Ca^{2+}$ itself. These mediums activated $Ca^{2+}$ channels or adjusted the activities of $Ca^{2+}$ channel following excitability electrical

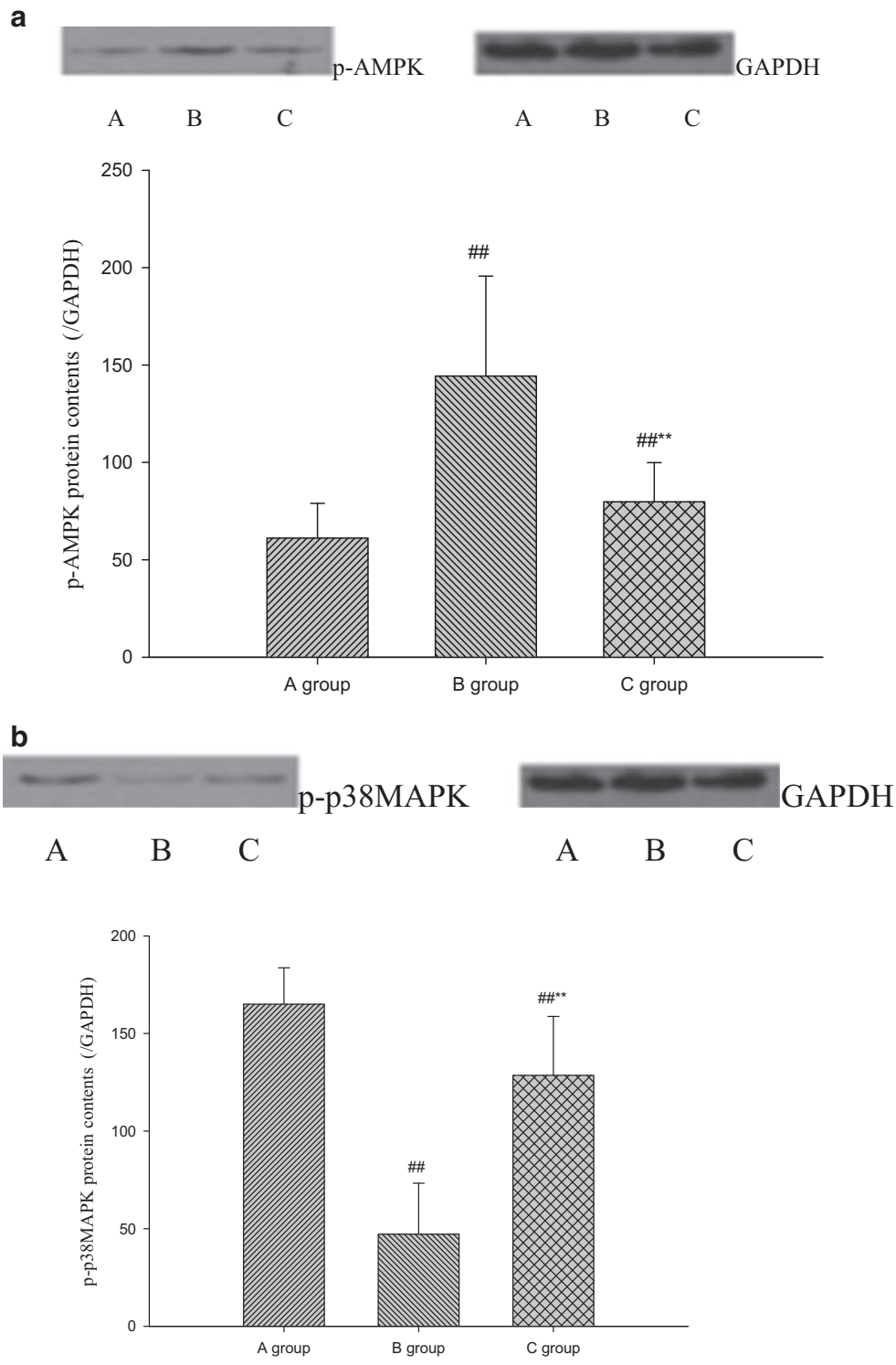
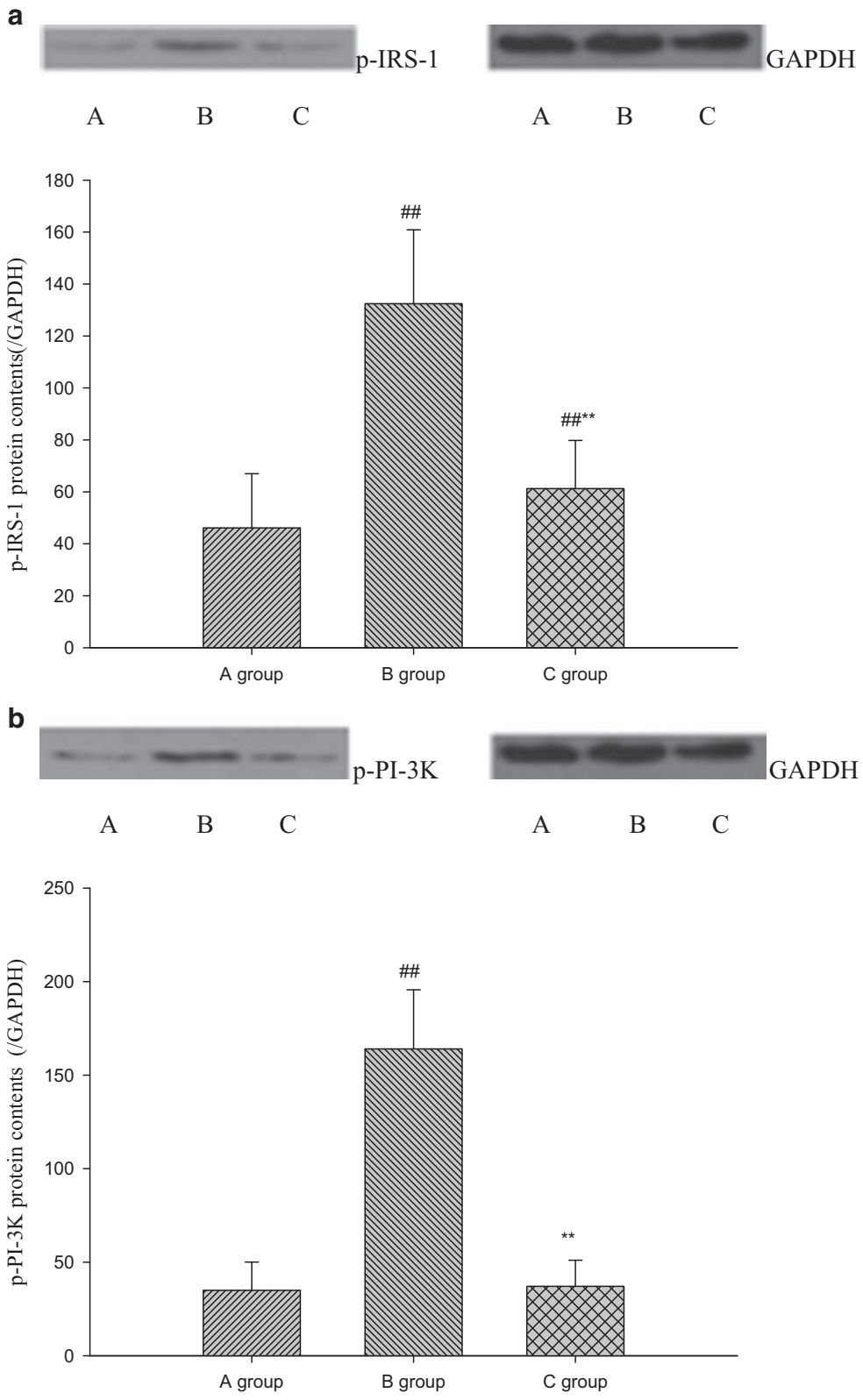



**Fig. 7** **a** p-AMPK protein content levels of each group in the cells following respective period culture (A group: palmitic acid + C2C12 cell 24 h; B group: palmitic acid 24 h + $CaCl_2$ 24 h + C2C12 cell; C group: palmitic acid 24 h + $CaCl_2$ 24 h + IL-6-shRNA 24 h + C2C12 cell; ##p-AMPK protein contents of each group are respectively compared with those of A group, $p < 0.01$; **p-AMPK protein content of C group is respectively compared with that of B group, $p < 0.01$). **b** p-p38MAPK protein content levels of each group in the cells following respective period culture (A group: palmitic acid + C2C12 cell 24 h; B group: palmitic acid 24 h + $CaCl_2$ 24 h + C2C12 cell; C group: palmitic acid 24 h + $CaCl_2$ 24 h + IL-6-shRNA 24 h + C2C12 cell; ##p-p38MAPK protein contents of each group are respectively compared with those of A group, $p < 0.01$; **p-p38MAPK protein contents of C group are compared with those of B group, $p < 0.01$)

stimulation [32, 33]. Therefore, the principle of $Ca^{2+}$-load and electrical stimulation might be similar. Based on the preliminary experiment result, C2C12 cell contraction can be discovered clearly under the microscope, and glucose concentration in 100 μM $Ca^{2+}$-load culture solution significantly decreased, compared with that in 0 μM $Ca^{2+}$-load culture solution. So, this manuscript firstly reported that 100 μM $Ca^{2+}$-load can stimulate contraction of myocyte. In the next experiment, we selected 100 μM $Ca^{2+}$-load to stimulate C2C12 cell contraction.

Previous report of our project team had showed that IL-6shRNA suppressed IL-6 gene expression in vivo [7]. Leslie




et al. discovered that mammary epithelial cells transformed by IL-6shRNA transfection Ha-rasV12 can silence IL-6 gene [34]. The research result of Chen et al. showed that IL-6siRNA was able to cause the IL-6 gene silence [35]. Thus, previous study results demonstrated that IL-6shRNA can suppress IL-6 gene expression in vivo, or in vitro.

PI-3K was a classics pathway for insulin signal. Insulin firstly binded with insulin receptor in cell surface and

◀ **Fig. 8** **a** p-IRS-1 protein content levels of each group in the cells following respective period culture (A group: palmitic acid + C2C12 cell 24 h; B group: palmitic acid 24 h + $CaCl_2$ 24 h + C2C12 cell; C group: palmitic acid 24 h + $CaCl_2$ 24 h + IL-6-shRNA 24 h + C2C12 cell; ##p-IRS-1 protein contents of each group are respectively compared with those of A group, $p < 0.01$; **p-IRS-1 protein content of C group is compared with that of B group, $p < 0.01$). **b** p-PI-3K protein contents levels of each group in the cells following respective period culture (A group: palmitic acid + C2C12 cell 24 h; B group: palmitic acid 24 h + $CaCl_2$ 24 h + C2C12 cell; C group: palmitic acid 24 h + $CaCl_2$ 24 h + IL-6-shRNA 24 h + C2C12 cell; ##p-PI-3K protein contents of each group are respectively compared with those of A group, $p < 0.01$; **p-PI-3K protein content of C group is compared with that of B group, $p < 0.01$)

activated its protein tyrosine kinase of β subunit. As a kind of anchoring protein, IRS combined with signal molecule including src homology 2 domain (SH2) and activated PI-3K/Akt signal pathway. Furthermore, the insulin sensitivity of skeletal muscle was ultimately related to the content of GLUT4. When the symptom of insulin resistance appeared, the phosphorylation PI-3K and IRS in various tissues [36, 37], the GLUT4 gene expression, and glucose ingestion of skeletal muscle [38] significantly decreased. Research results showed that improving insulin resistance for exercise or physical activity was related with activating IRS-1/PI-3K/Akt signal pathway [39, 40] and enhancing GLUT4 expression [41]. Previous research result of our project team had shown that exercise activated IRS-1/PI-3K/Ak signal pathway and GLUT4 gene expression; however, if IL-6 gene was silent by IL-6shRNA, the activation effect of exercise became lower [7].

The result of the first part showed that when palmitic acid-induced insulin resistance emerges, the phosphorylation PI-3K and IRS, the GLUT4 gene expression, and glucose ingestion of skeletal muscle significantly decreased. On the contrary, when insulin resistance C2C12 cells were treated with IL-6, it significantly increased. Furthermore, when normal C2C12 cells were cultured with IL-6shRNA, it significantly decreased. So, the results demonstrated that IL-6 activated IRS-1/PI-3K signal pathway, increased GLUT4 gene expression and glucose ingestion, enhanced the insulin sensitivity, and improved insulin resistance of skeletal muscle in vitro. However, the previous results were unable to explain the source of IL-6. In the next part, we used $Ca^{2+}$ feed to stimulate myocyte contraction, and we discovered that C2C12 cell contraction significantly activated IRS-1/PI-3K signal pathway and GLUT4 gene expression; however, after treating contractive C2C12 cells 48 h, the activated effect significantly decreased. Therefore, the results demonstrated that skeletal muscle contraction activated IRS-1/PI-3K signal pathway, increased GLUT4 gene expression and glucose ingestion, enhanced the insulin sensitivity, and improved insulin resistance of skeletal muscle by exercise-induced IL-6.

AMPK had intimate relation with insulin resistance. Research result indicated that AMPK phosphorylated No. 789 serine of IRS-1, which reinforced signal transduction of insulin [42]. Activation of AMPK improved the function of β cell by decreasing lipotoxicity of β cell and resisting β cell apoptosis [43]. Furthermore, activation of AMPK increased glucose ingestion of skeletal muscle, reduced glucose production and output of liver, and enhanced oxidation of fatty acid [44]. Another research result showed that activation of AMPK persistently activated IRS-1/PI-3K/Akt signal pathway [45]. When human body exercised, AMPK was activated by intracellular $Ca^{2+}$ increase and AMP/ATP ratio enhancement following $Ca^{2+}$ release from sarcoplasmic reticulum and energy consumption. Previous results had demonstrated that muscle-derived IL-6 activated IRS-1/PI-3K signal pathway, increased GLUT4 gene expression and glucose ingestion, enhanced the insulin sensitivity, and improved insulin resistance of skeletal muscle. Therefore, this paper was to probe into the effect of AMPK signal pathway.

The result showed that p-AMPK and insulin sensitivity significantly decreased, following C2C12 cells treated 24 h with palmitic acid or cultured by IL-6shRNA. Furthermore, following insulin resistance, C2C12 cells cultured with IL-6 24 h, p-AMPK, and insulin sensitivity significantly increased. The result also indicated that $Ca^{2+}$-fed insulin resistance C2C12 cells significantly enhanced p-AMPK and insulin sensitivity; however, following IL-6shRNa cultured with $Ca^{2+}$-cultured insulin resistance, C2C12 cells 48 h, p-AMPK, and insulin sensitivity significantly decreased. Therefore, this paper firstly discovered that skeletal muscle-derived IL-6 enhanced the insulin sensitivity and improved insulin resistance of skeletal muscle by activating AMPK in vitro.

As a kind of stress-activated protein kinase, p38MAPK participated in various physiological and pathological processes, such as inflammation, stress, cell cycle, and apoptosis. p38MAPK had important relation with insulin resistance. High glucose activated p38MAPK; however, the inhibitor of PKC was unable to prevent high glucose-induced increase of p38MAPK [46]. PRDX-1 induced insulin resistance by activating p38MAPK, which demonstrated that the activation of p38MAPK was connected with development of insulin resistance [11]. The research result of Yu et al. had showed that in the process of obese developed by high-fat diet, eNOS uncoupling induced by arginase II was through activating p38MAPK [47]. Therefore, the activation of p38MAPK became one of the key factors to develop insulin resistance. At present, as a target spot for curing complication of diabetes, p38MAPK caused major interest for medical researchers [48, 49].

Whether exercise activates p38MAPK was controversial. A few research results showed that different type exercises activated p38MAPK signal pathway [15, 16]; however, other research results indicated that p38MPAK protein content and

activities in skeletal muscle of normal rats and human body significantly decreased post-exercise 48 h [17, 18]. Previous research results of our project team had shown that high fat diet-induced insulin resistance was related to activation of p38MAPK, and exercise-induced IL-6 suppressed the activation of p38MAPK.

The result of first part showed that p-p38MAPK significantly increased, and insulin sensitivity significantly decreased, following treated 24 h with palmitic acid or cultured by IL-6shRNA. Furthermore, following insulin cultured with IL-6 24 h, p-p38MAPK significantly decreased, and insulin sensitivity significantly increased. The result indicated that $Ca^{2+}$-fed insulin resistance C2C12 cells significantly decreased p-p38MAPK and enhanced insulin sensitivity; however, following cultured with $Ca^{2+}$-fed, p-p38MAPK significantly increased, and insulin sensitivity significantly decreased. Therefore, this paper firstly discovered that skeletal muscle-derived IL-6 can enhance the insulin sensitivity and improve insulin resistance of skeletal muscle by suppressing p38MAPK in vitro.

**Funding information** This study was funded by the National Natural Science Foundation of China (grant number 31250004) and Youth Project of Educating Department of Hunan Province (grant number 13B025).

## Compliance with ethical standards

The experimental scheme accorded with regulation of Ethics Committee of Hunan University of Science and Technology.

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

**Human and animal rights and informed consent** This article does not contain any studies with human participants or animals performed by any of the authors.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):499–505
https://doi.org/10.1007/s13410-018-0695-1

ORIGINAL ARTICLE

# Pentraxin 3 and epicardial fat thickness are independently associated with diabetic retinopathy in diabetic patients

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Received: 10 May 2018 / Accepted: 24 September 2018 / Published online: 10 October 2018


**Abstract**
Previous studies have shown that diabetic retinopathy and inflammation are closely related. Pentraxin 3 (PTX3), high-sensitive C-reactive protein (HsCRP), and epicardial fat thickness (EFT) are parameters associated with inflammation. The aim of this cross-sectional study, was to determine if the levels of HsCRP, PTX3, and EFT were predictors or markers for diabetic retinopathy (DR). Cross-sectional study, 40 normal, nondiabetic subjects (group1), 50 type 2 diabetic patients without DR (group2), and 110 type 2 diabetic patients with DR (group3) were included. PTX3 and HsCRP concentrations were measured and transthoracic echocardiography was used to measure EFT. ANOVA and the post hoc multiple comparisons showed that differences among group 1 and group 2 and also among group 2 and group 3 for PTX3 ($p < 0.01$ and $p = 0.018$, respectively), for HsCRP ($p < 0.01$ and $p = 0.042$, respectively), and for EFT ($p < 0.01$ and $p = 0.021$, respectively) were statistically significant. The difference in serum creatinine was statistically significant only among group 2 and group 3 ($p < 0.01$). Diabetes duration (OR = 1.116, $p < 0.01$), PTX3 (OR = 8.516, $p = 0.039$), EFT (OR = 1.444, $p = 0.02$), and serum creatinine level (OR = 15.45, $p = 0.015$) have independent association with DR. Besides, the well-known factors such as diabetes duration and serum creatinine, PTX3, as a marker of inflammation, and EFT, as a marker of inflammation and vascular damage, were independently associated with DR in diabetic patients. Clinical trial number: 20.03.2015/30

**Keywords** Diabetic retinopathy · Pentraxin 3 · HsCRP · Epicardial fat tissue

## Introduction

The chronic complications of diabetes mellitus (DM) include retinopathy, nephropathy, neuropathy, and the cardiovascular disease. Because of the devastating consequences of these complications, early detection of the risks of the development of diabetic complications may aid in the prevention of these complications.

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Diabetic retinopathy (DR), one of the common complications of diabetes, causes low vision and blindness in adults in working age group around the World [1]. Furthermore, the presence of retinopathy may be a predictor of the occurrence of other diabetic complications and may reveal patients at high risk. The prevalence of diabetic retinopathy increases with the duration of diabetes and the patients' age. Also, the other risk factors are systemic atherosclerosis, renal impairment, pregnancy, and anemia [2].

C-reactive protein (CRP) is a useful marker in clinical practice for the diagnosis of inflammatory conditions. Some studies have shown that type 2 DM is related with increased plasma concentrations of CRP, which is involved in the native immune reaction and inflammation [3–5]. DR is one of the microvascular complications of DM caused by neurovascular inflammation. The relationship between CRP and DR is controversial. There are data suggesting that CRP is associated with DR [6], however, there are opposite conclusions too [7].

Pentraxin 3 (PTX3): a newly identified acute-phase glycoprotein and a soluble receptor acting as an opsonin and it resembles CRP structurally and functionally. PTX3 protein

is produced in vascular endothelial cells, macrophages, dendritic cells, neutrophils, and fibroblasts [8]. Its level directly elevates the inflammatory status of the vasculature [9], associated with systemic inflammation or sepsis [10], acute myocardial infarction [11, 12], unstable angina pectoris, heart failure, and psoriasis [10, 13–16].

Epicardial fat thickness (EFT) shows visceral adipose tissue around the heart. This adipose tissue is located between the pericardial and myocardial layer. It is metabolically active and it acts as an endocrine organ. It produces some cytokines causing endothelial dysfunction, oxidative stress, inflammation, and atherosclerosis [17, 18]. EFT is measured with different imaging techniques. These are echocardiography, magnetic resonance imaging, and multidetector computer tomography [19]. Echocardiography is an easily accessible and relatively cheap method and the echocardiographic measurements of EFT show good correlation with magnetic resonance imaging [20].

We aimed to investigate the relation of serum high-sensitive C-reactive protein (HsCRP), PTX3, and EFT measurement with DR.

## Method

### Sampling

It was a cross-sectional study. One hundred sixty diabetic patients and 40 healthy control subjects were included in the study. The clinical research was performed between March 2015 and June 2016 at the Departments of Endocrinology, Ophthalmology, and Cardiology. Screening for the presence or absence of diabetic retinopathy was performed by ophthalmologists using fundoscopy in mydriasis. Retinal examination by binocular biomicroscopy was performed in the Bozok University Ophthalmology clinic. Diabetic patients who were referred from the Department of Endocrinology were enrolled in this study after the retinal examination in the Department of Ophthalmology. Forty normal, non-diabetic subjects (Group 1), 50 diabetic patients without DR (Group 2), and 110 diabetic patients with DR (Group 3) were enrolled. Medical histories, age, and gender were recorded. Weights and heights of participants were measured. Body mass index (BMI) was calculated by (weight in kg)/(height in meters)$^2$. HbA1C, serum glucose, lipids, creatinine, PTX3, and high-sensitivity CRP (HsCRP) were studied for all patients. The blood sampling was taken after fasting for 10 to 12 h. Blood samples were centrifuged at room temperature for 5 min at 5000 RPM. The extracted serum was kept in ice bags and put in deep freezers at − 80 °C. An enzyme-linked immunosorbent assay (ELISA) kit was used for measuring PTX3 levels and also a highly sensitive ELISA kit was used to measure HsCRP levels.

### Echocardiography

EFT was evaluated by transthoracic echocardiography using a Philips Logic Affiniti 50G machine (Philips, Amsterdam, Netherlands) and a broadband transducer by the same cardiologist. EFT was measured from the parasternal long-axis view on the right ventricle's free wall at the end-diastole during three cardiac cycles. In the parasternal long-axis window, the hypoechoic space on the right ventricular free wall was defined as EFT.

Exclusion criteria were known cardiovascular disease or events, end-stage renal failure, active infection, and known rheumatologic and inflammatory diseases.

All statistical analyses were calculated with the Statistical Package for the Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA). $p$ values $\leq 0.05$ were considered statistically significant. One-way analysis of variance test was used to compare groups. Parameters with non-normal distribution were evaluated by using Kruskal Wallis. Logistic regression analysis was used for multivariate analysis.

## Results

### Patient characteristics

Groups 1 to 3 included 40, 50, and 110 patients, respectively. There was statistically significant difference among the groups for age (group 1: 47.18 ± 10.25 years; group 2: 56.44 ± 8.81 years; group 3: 60.13 ± 8.51 years; $p < 0.01$), gender (male/female) (group 1: 8/32; group 2: 14/36; group 3: 50/60; $p < 0.01$), and BMI (group 1: 29.81 ± 5.86; group 2: 34.36 ± 4.67; group 3: 33.45 ± 6.45, $p < 0.01$) (Table 1). The post hoc multiple comparison method showed no differences among group 2 and group 3 for gender and BMI ($p = 0.079$ and $p = 0.64$, respectively).

### Laboratory results

There was statistically significant difference among the groups for PTX3 (ng/mL) (group 1: 1.681 ± 0.282; group 2: 1.949 ± 0.235; group 3: 2.079 ± 0.318; $p < 0.01$), HsCRP (mg/dL) (group 1: 0.901 ± 0.193; group 2: 1.070 ± 0.177; group 3: 1.144 ± 0.218; $p < 0.01$), HbA1C (%) (group 1: 5.59 ± 0.33; group 2: 8.34 ± 2.00; group 3: 9.16 ± 2.16, $p < 0.01$), fasting glucose (mg/dL) (group 1: 96.2 ± 8.49; group 2: 178.48 ± 85.38; group 3: 227.60 ± 104.49; $p < 0.01$), and serum creatinine (mg/dl) (group 1: 0.75 ± 0.11; group 2: 0.81 ± 0.15; group 3: 0.93 ± 0.24; $p < 0.01$). The post hoc multiple comparison method showed that differences among group 1 and group 2 and also among group 2 and group 3 for PTX3 ($p < 0.01$ and $p = 0.018$, respectively), for HsCRP ($p < 0.01$ and $p = 0.042$, respectively) (Fig. 1), for HbA1C ($p < 0.01$

**Table 1** Demographic and clinical data of the participants

| | Group 1 DM (−) | Group 2 DM (+), DR (−) | Group 3 DM (+), DR (+) | $p < 0.05$ |
|---|---|---|---|---|
| Age | 47.18 ± 10.25 | 56.44 ± 8.81 | 60.13 ± 8.51 | $p < 0.01$ |
| Gender (male/female) | 8/32 | 14/36 | 50/60 | $p < 0.01$ |
| Height (cm) | 162 ± 6.67 | 159.78 ± 8.90 | 161.62 ± 9.49 | $p = 0.39$ |
| Weight (kg) | 77.21 ± 15.39 | 87.36 ± 11.35 | 86.34 ± 14.36 | $p < 0.01$ |
| Body mass index (kg/m$^2$) | 29.81 ± 5.86 | 34.36 ± 4.67 | 33.45 ± 6.45 | $p < 0.01$ |
| DM duration (year) | – | 9.88 ± 5.75 | 13.71 ± 6.60 | $p < 0.01$ |
| PTX3 (ng/mL) | 1.681 ± 0.282 | 1.949 ± 0.235 | 2.079 ± 0.318 | $p < 0.01$ |
| HsCRP (mg/dL) | 0.901 ± 0.193 | 1.070 ± 0.177 | 1.144 ± 0.218 | $p < 0.01$ |
| Epicardial fat thickness (mm) | 4.27 ± 0.91 | 5.91 ± 1.38 | 6.53 ± 1.38 | $p < 0.01$ |
| HbA1C (%) | 5.59 ± 0.33 | 8.34 ± 2.00 | 9.16 ± 2.16 | $p < 0.01$ |
| Fasting glucose (mg/dL) | 96.2 ± 8.49 | 178.48 ± 85.38 | 227.60 ± 104.49 | $p < 0.01$ |
| Creatinine (mg/dL) | 0.75 ± 0.11 | 0.81 ± 0.15 | 0.93 ± 0.24 | $p < 0.01$ |
| Total cholesterol (mg/dL) | 194.45 ± 46.02 | 210.54 ± 45.08 | 206.98 ± 41.82 | $p = 0.21$ |
| Triglyceride (mg/dL) | 142.38 ± 84.21 | 217.80 ± 156.26 | 196.00 ± 116.95 | $p < 0.01$ |
| HDL(mg/dL) | 48.81 ± 9.36 | 46.68 ± 10.39 | 44.41 ± 9.99 | $p = 0.03$ |
| LDL (mg/dL) | 121.54 ± 36.36 | 121.57 ± 37.61 | 124.01 ± 34.28 | $p = 0.88$ |

Continuous variables are presented as X ± SD, and the categorical variables as *N* (%)

*DM (+)*, participants with diabetes mellitus; *DM (−)*, participants without diabetes mellitus; *DR (+)*, participants with diabetic retinopathy; *DR(−)*, participants without diabetic retinopathy; *HsCRP*, high-sensitive C-reactive protein; *PTX3*, pentraxin 3

and $p = 0.023$, respectively), and for fasting glucose ($p < 0.01$ and $p < 0.01$, respectively) were statistically significant. The difference in serum creatinine was statistically significant only among group 2 and group 3 ($p < 0.01$) (Fig. 1). There was no statistically significant difference in lipid parameters among group 2 and group 3 ($p \geq 0.05$).

### Echocardiographic examination

There was statistically significant difference among the groups for EFT (mm) (group 1: 4.27 ± 0.91; group 2: 5.91 ± 1.38; group 3: 6.53 ± 1.38; $p < 0.01$). The differences among group 1 and group 2 and also among group 2 and group 3 for EFT ($p < 0.01$ and $p = 0.021$, respectively) were statistically significant (Fig. 1). EFT was positively correlated with PTX3 ($r = 0.226$, $p < 0.01$), HsCRP ($r = 0.220$, $p < 0.01$) levels, and the duration of diabetes ($r = 0.326$, $p < 0.01$).

### Analysis of factors contributing to DR development in diabetic patients

We evaluated the correlation of study variables and DR in diabetic patients (group 2 and group 3, total 160 patients). Age ($r = 0.196$, $p = 0.013$), DM duration ($r = 0.271$, $p < 0.01$), fasting glucose ($r = 0.226$, $p < 0.01$), HbA1C ($r = 0.178$, $p = 0.024$), serum creatinine ($r = 0.238$, $p < 0.01$), PTX3 ($r = 0.201$, $p = 0.011$), HsCRP ($r = 0.164$, $p = 0.038$), and EFT ($r = 0.207$, $p < 0.01$) were correlated with DR in diabetic patients.

We also performed a logistic regression analysis to determine the variables that were independently associated with diabetic retinopathy. When diabetic retinopathy was taken as the dependent variable and the other variables—including age, gender, diabetes duration, PTX3, HsCRP, HbA1c, glucose, and serum creatinine levels—were taken as the independent variables, we found that diabetes duration (OR = 1.116, $p < 0.01$), PTX3 (OR = 8.516, $p = 0.039$), EFT (OR = 1.444, $p = 0.02$), and serum creatinine level (OR = 15.45, $p = 0.015$) have independent association with diabetic retinopathy.

## Discussion

In the present study, we investigated whether echocardiographic EFT measurement, HsCRP and PTX3 may be used as diabetic retinopathy predictors in patients with type 2 diabetes mellitus, in addition to well-known predictors of retinopathy such as poor glycemic control, age of the patient, and duration of diabetes. We found that beside diabetes duration and serum creatinine level, PTX3 and EFT were independently associated with diabetic retinopathy in diabetic patients.

Serum PTX3 has come out as a novel marker and is specific to vascular inflammation than other proteins in the pentraxin family such as CRP [21]. Diabetes mellitus plays a

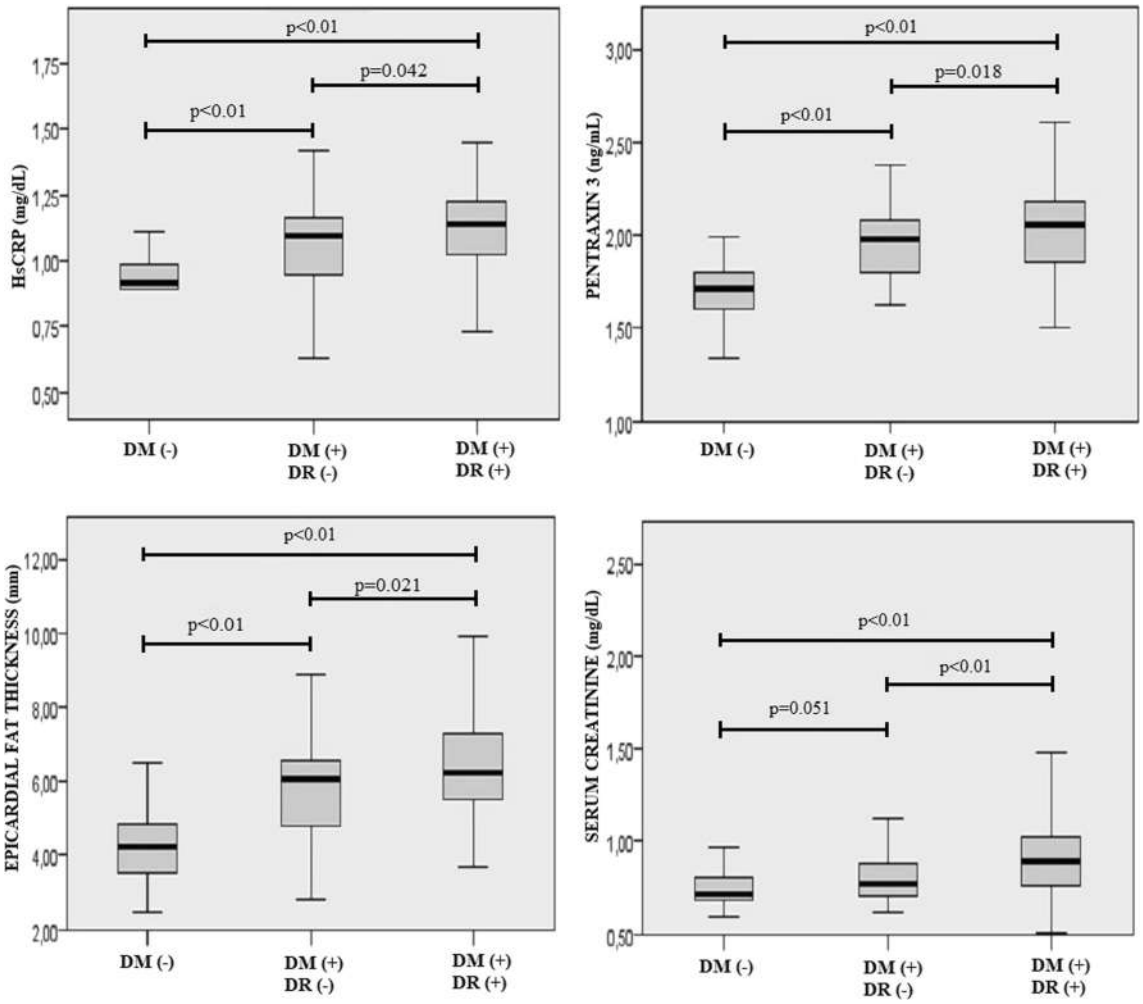


**Fig. 1** HsCRP, PTX3, EFT, and serum creatinine levels in three different groups. The values of the columns represent the mean and standard deviation of high-sensitive C-reactive protein, pentraxin 3, epicardial fat thickness, and serum creatinine in different groups. ANOVA and the post hoc multiple comparison tests were performed in three groups, and *p* values representing the difference between groups were reported. DM (+), participants with diabetes mellitus; DM (−), participants without diabetes mellitus; DR (+), participants with diabetic retinopathy; DR (−), participants without diabetic retinopathy; HsCRP, high-sensitive C-reactive protein; PTX3, pentraxin 3; EFT, epicardial fat thickness

certain role in intravascular inflammation, causes endothelial dysfunction, and accelerated progression of atherosclerosis. CRP and PTX3 have a role in the inflammatory response, endothelial dysfunction, and atherosclerosis [22, 23]. The increase in PTX3 and HsCRP levels in diabetic patients is thought to be precious as prognostic factors for vascular complications like diabetic retinopathy [24, 25]. In 2014, Yang et al. demonstrated that plasma PTX3 is positively associated with diabetic retinopathy development and progression and may be a more accurate predictor of diabetic retinopathy development than HsCRP [26]. The other study showed that serum and vitreous PTX3 concentrations are associated with presence of diabetic retinopathy [27]. In this study, in accordance with the previous data [24–27], PTX3 and HsCRP levels were higher in diabetic patients with retinopathy compared with both healthy controls and diabetic patients without retinopathy. PTX3 and HsCRP were strongly correlated with diabetic retinopathy in diabetic patients. This data suggests that diabetic retinopathy is closely related with systemic inflammation in diabetic patients. Notwithstanding, in logistic regression, PTX3 showed an independent association with diabetic retinopathy while HsCRP did not. These results are coherent with the results of the study by Yang et al. [26]. In this context, compared with HsCRP, PTX3 level may be a better marker to predict DR in diabetic patients. PTX3 levels are generally low in healthy subjects with a concentration of ≤ 2 ng/ml [28]. In our study, with the influence of exclusion criteria, the mean PTX3 level of the entire study population was $1.967 \pm 0.329$ and was compatible with this study. As a result of these data, another issue that needs to be emphasized is the ability of PTX3 to predict retinopathy with slight changes, even at these relatively low levels. These slight raises of

PTX3 may be related with the low-grade inflammation in diabetic patients. PTX3 can be a surrogate marker of the long-term subclinical inflammation, which is thought to play a major role in the development of retinopathy.

Epicardial fat is normally present between the heart and pericardium and is thought to act as an energy source to the myocardium. Epicardial fat is a visceral fat deposit and a metabolically active organ which has the capacity to secrete several proatherogenic cytokines that may have a role in the pathogenesis of inflammation and atherosclerosis [29]. Increased EFT, as a marker of inflammation and subclinic atherosclerosis, is reported to be associated with major cardiovascular outcomes, independent of other well-known risk factors [30].

It is demonstrated that EFT is higher in diabetic patient than non-diabetic control [31, 32]. In our study, compared to the non-diabetic patients, EFT measurements were higher in diabetic patients without DR. This may be explained by the chronic inflammatory state in diabetes. Furthermore, considering only diabetic patients, patients with DR had higher EFT levels than the patients without DR. As a novel finding, EFT was also independently associated with DR in diabetic patients. Additionally, as a well-known indicator of inflammation, HsCRP was also significantly higher in DR group. We also found that EFT was positively correlated with PTX3 and HsCRP levels and the duration of diabetes.

To the best of our knowledge, this is the first study to describe the association between EFT and DR. Studies evaluating the proatherogenic mechanism of epicardial fat have reported infiltration of chronic inflammatory cells and increased inflammatory gene expression in epicardial adipose tissue [33, 34]. These inflammatory precursors are known to induce persistent inflammation. When all these data are evaluated together, it can be interpreted that EFT may be associated with chronic persistent inflammation and may cause a predisposition to retinopathy. Early detection of diabetic complications may play a key role in reducing diabetes-related morbidity and mortality. Echocardiographic EFT may be useful as an easily accessible and cost-effective tool for assessing subclinical vascular damage in diabetic patients.

Diabetes duration and serum creatinine level were independently associated with DR in diabetic patients. Diabetes duration is reported to be a strong predictor of DR [35]. Elevated serum creatinine level is shown to be associated with DR [36]. Our study was consistent with these results.

Atherosclerosis is a disease in which chronic inflammation is involved and every risk factor contributes to pathogenesis by worsening the primary inflammatory action [37]. DM is a chronic inflammatory state and it is a cardiovascular disease risk equivalent. Our study result showed that diabetes duration, PTX3, EFT, and serum creatinine level have an independent association with diabetic retinopathy. Our results supported the previous data [24, 25] and showed the asymptomatic inflammatory state in DM. Another important result of our study is the relation of PTX3, as a marker of low-grade inflammation, and EFT, as a marker of inflammation and vascular damage, with DR in diabetic patients. These two parameters may be used as a surrogate marker of DR in diabetic patients.

### Limitations

Our study had some limitations: our study had limited number of patients and controls. Our study was a cross-sectional study. The evaluation of EFT was made by two-dimensional echocardiography instead of computerized tomography (CT) or cardiac magnetic resonance imaging (MRI). Longitudinal studies must be planned to show the long-term effect of these parameters on DR.

## Conclusion

Besides, diabetes duration and serum creatinine as well-known predictors, PTX3 and EFT have independent association with diabetic retinopathy. We suggest that echocardiographic EFT measurement and serum PTX3 may be used in the evaluation of DR risk in patients with DM. It is known that EFT is one of the early markers of atherosclerosis, and it is related with inflammation and cardiovascular disease. To our knowledge, this is the first study suggesting that EFT might be associated with DR.

**Funding** This study was funded by the Scientific Research Projects of the University of Bozok.

### Compliance with ethical standards

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

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the Bozok University Clinical Research Ethics Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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

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

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):506–512
https://doi.org/10.1007/s13410-018-0705-3

ORIGINAL ARTICLE

# Clinical, radiological, and histological characteristics of Chinese type 2 diabetic patients with diabetic scleredema: an observational study

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Received: 17 June 2018 / Accepted: 3 December 2018 / Published online: 18 December 2018


## Abstract

**Objectives** Diabetic scleredema (DS) is reported rarely in China and its features are poorly documented. We aim to describe the characteristics of Chinese patients with DS clinically, radiologically, and histologically.

**Methods** Of 9825 hospitalized participants with diabetes older than 18 years, 15 type 2 diabetes patients with DS as well as 15 non-DS patients with type 2 diabetes were included in this observational study.

**Results** All the individuals with DS presented diffuse erythema, non-pitting, and painless indurations on the skin of the nape and upper back. Patients with DS were mainly middle-aged or elderly (male to female ratio, 4:1), with long-standing poorly controlled type 2 diabetes, overweight, or obese. Patients with DS vs non-DS had a higher prevalence of diabetic microvascular complications, neuropathy, hypertension, cerebral infarction, nicotine abuse, and alcohol abuse. LDL-C level was significantly higher in the DS group ($p = 0.0143$), and the skin and subcutaneous fat in T2-weighted MRI were significantly thickened in the DS group than in the non-DS group ($p < 0.01$). Skin biopsies showed thickened collagen bundles throughout the dermis and active fibroblast proliferation.

**Conclusion** Our reports suggest that DS is relatively rare in Chinese patients with diabetes. DS occurs both in middle-aged and elderly patients (male predominantly), overweight or obese, having long-term poorly controlled type 2 diabetes, and presenting a high incidence of microvascular complications, hypertension, neuropathy, and ischemic cerebral stroke. High LDL-C level, nicotine abuse, and alcohol abuse may be risk factors. MRI could be preferable to assess the severity of DS non-invasively.

**Keywords** Diabetic scleredema · Type 2 diabetes · Thickened collagen bundles · Low-density lipoprotein cholesterol · Magnetic resonance imaging

## Introduction

Diabetic scleredema is a rare chronic dermatologic complication of diabetes. It must be distinguished from scleredema adultorum, which is complicated by a preceding streptococcal infection and predominantly affects young adults and children [1, 2]. According to the current reports, DS is characterized by markedly thickened reticular dermis affecting the posterior neck, upper back, shoulders, with occasional extension to the face, arms, chest, or abdomen. However, acral skin is always spared. The involved skin is stiff, thick, and non-pitting, sometimes erythematous and may have a peau d'orange appearance [3–5]. DS is usually asymptomatic, as its onset is generally subtle and progression slow, but in some severe cases, pain and limitation of mobility may present [6, 7].

Worldwide, the prevalence of DS is reported to be 2.5 to 14% among patients with type 2 diabetes. However, it may be under-recognized, particularly in China, as only a few cases of DS have been reported. The prevalence of DS might be higher in China, since, according to a most recent national survey, the prevalence of diabetes in Chinese adults was 9.7%, representing an estimated 92.4 million adults in China with diabetes [8].

We reported here 15 type 2 diabetic patients with DS, wherein clinical features, radiological images, and histological findings were assessed and compared to the non-DS patients. This study is one of the largest cohorts reported to date in a Chinese population.

Yingying Zhou and Chaoming Wu contributed equally to this work.

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## Materials and methods

### Patients

Of 9825 hospitalized participants with diabetes older than 18 years, 15 type 2 diabetes patients with DS (12 male, three female) were identified, and 15 gender-, age- and BMI-matched non-DS patients with type 2 diabetes were also included in this observational study conducted in our hospital between January 2006 and June 2017. Clinical diagnosis of DS was established on the basis of the medical history, typical diffuse erythema, non-pitting and painless induration on the skin and its characteristic distribution. Acute infections were excluded in all cases. Informed written consent for this study was obtained from each patient. The study was also approved by the Hospital Ethics Committee for Human Research of The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University.

### Laboratory measurements

Effective laboratory tests were done according to each instruction. Glucose level was evaluated by enzymatic analysis. HbA1c was detected through iron-exchange high-performance liquid chromatography (IE-HPLC). Lipid profile was conducted using an enzymatic/GPO-PAP assay. The 24-h proteinuria was measured by a pyrogallol red-molybdate (PRM) method.

### MRI and histological analysis

Neck and back MRI was carried out using a 1.5 or 3.0 T scanner (General Electric Medical Systems, Milwaukee, WI). An incisional skin biopsy including the subcutaneous tissue was performed and stained with the standard hematoxylin and eosin.

### Statistical analysis

Data was subjected to independent-samples *t* test analysis using SPSS 17.0. Differences were considered statistically significant at $p$ values < 0.05.

## Results

The main features of patients in the DS and non-DS group were summarized in Table 1.

### Symptoms and signs

As indicated in our cohort, the age distribution of patients with DS was mainly about middle-aged or elderly people ($n = 7$, aged 45–59 years; $n = 6$, aged 60–74 years; $n = 2$, aged 40–44 years). The population was predominantly male, with a male to female ratio of 4:1. Twelve of the 15 individuals with DS have a history of type 2 diabetes for more than 10 years. More than half of the patients (8/15) were treated with insulin. However, there was no significant difference with regard to the diabetic duration or insulin usage between DS and non-DS groups. Seven of the 15 patients with DS were overweight and six subjects with DS were obese. Since the vast majority of the individuals with DS were asymptomatic, only four of them could date the onset of their skin infiltration. Most lesions began on the nape, spreading gradually to the shoulders, and then to the upper back, especially in the interscapular region of the upper back, without lower limbs involvement. The lesion had a hard, thick, and inflexible appearance. There was no clear demarcation between involved and healthy skin. The affected skin of the most patients was not mobile and could not be pinched up, and part of the lesions appeared to have a decreased superficial sensation to pain and a light touch. The lesion also had a diffuse erythema. As shown in Fig. 1, a 43-year-old Chinese male with hypertension and poorly controlled type 2 diabetes (HbA1c, 8.7% or 71.6 mmol/mol; FBG, 8.16 mmol/L) presented for the evaluation of a progressive thickening and erythematous induration of his upper back, nape, and shoulders. In some severe cases (3/15), patients suffered from a limited neck activity which was the main complaint when they came to the hospital. After strict glucose control and intravenous prostaglandin E1 treatment, the clinical features mentioned above were slightly relieved in some DS patients.

### Biochemical analysis

With regard to tests, nearly all (13/15) of the patients with DS exhibited poor glycemic control, reflected by an elevated HbA1c level, although there was no significant difference as compared with the non-DS group (Table 1). LDL-C level was significantly higher in the DS group than in the non-DS group (DS vs. non-DS: $3.00 \pm 0.86$ vs. $2.14 \pm 0.94$ mmol/L, $p = 0.0143$). Lymphocyte ratio analysis was carried out in part of (5/15) the patients with DS. The result showed that the proportion of the total T cells was in the upper limit of normal, and the percentage of CD4 positive T-helper cells was increased in three DS patients. No other biochemical variables were significantly different.

### Diabetes-related complications and comorbidities

Patients with DS vs non-DS had a higher prevalence of diabetic retinopathy (6/15), diabetic kidney disease (6/15), neuropathy (5/15), hypertension (11/15), and cerebral infarction (5/15). No distinct differences were found in the occurrence of

**Table 1** Characteristics of type 2 diabetic patients with DS and without DS

| Parameters | DS | Non-DS | *p* value |
|---|---|---|---|
| Numbers of patients (males) | 15 (12) | 15 (12) | |
| Age (years)[a] | 57.80 ± 10.75 | 59.73 ± 11.39 | 0.52 |
| BMI (kg/m$^2$)[a] | 26.07 ± 7.59 | 27.88 ± 2.03 | 0.42 |
| WHtR[a] | 0.56 ± 0.06 | 0.57 ± 0.04 | 0.57 |
| History of diabetes (years)[a] | 11.13 ± 5.07 | 12.33 ± 5.83 | 0.55 |
| Insulin employment (years)[b] | 1.0 (0–4.0) | 1.0 (0–3.0) | 0.20 |
| Duration of scleredema (months) | 3–48 | | |
| Site involved with scleredema | Posterior neck, back, shoulders | | |
| Biomarkers | | | |
| FBG (mmol/L)[a] | 8.37 ± 2.39 | 8.99 ± 3.51 | 0.57 |
| HbA1c (% or mmol/mol)[a] | 9.37 ± 2.05 (78.91 ± 1.09) | 9.17 ± 2.29 (76.72 ± 1.53) | 0.80 |
| TG (mmol/L)[a] | 1.72 ± 1.36 | 1.85 ± 1.13 | 0.78 |
| TC (mmol/L)[a] | 4.99 ± 1.17 | 4.25 ± 1.22 | 0.10 |
| HDL-C (mmol/L)[a] | 1.13 ± 0.25 | 0.99 ± 0.34 | 0.21 |
| LDL-C (mmol/L)[a] | 3.00 ± 0.86 | 2.14 ± 0.94 | 0.0143* |
| Non-HDL-C (mmol/L)[a] | 3.87 ± 1.27 | 3.26 ± 1.07 | 0.17 |
| Complications/comorbidities (%) | | | |
| Diabetic kidney disease | 6/15 | 3/15 | |
| Retinopathy | 6/15 | 3/15 | |
| Neuropathy | 5/15 | 2/15 | |
| Acute coronary syndrome | 1/15 | 0/15 | |
| Cerebral infarction | 5/15 | 2/15 | |
| Peripheral atherosclerosis | 10/15 | 10/15 | |
| Hypertension | 11/15 | 6/15 | |
| Hyperlipidemia | 9/15 | 8/15 | |
| Non-alcoholic fatty liver disease | 6/15 | 8/15 | |
| Nicotine abuse | 9/15 | 3/15 | |
| Alcohol abuse | 6/15 | 2/15 | |
| Skin and subcutaneous fat thickness | | | |
| NST (mm)[a] | 7.84 ± 2.56 | 3.55 ± 0.94 | < 0.0001** |
| NFT (mm)[a] | 30.58 ± 12.37 | 12.22 ± 5.24 | < 0.0001** |
| BST (mm)[a] | 8.63 ± 3.14 | 4.77 ± 1.55 | 0.0002** |
| BFT (mm)[a] | 32.81 ± 7.94 | 25.11 ± 5.94 | 0.0062** |

*BMI*, body mass index; *WHtR*, waist/height ratio; *FBG*, fasting blood glucose; *HbA1c*, glycated hemoglobin A1c; *TG*, triglycerides; *TC*, total cholesterol; *HDL-C*, high-density lipoprotein cholesterol; *LDL-C*, low-density lipoprotein cholesterol; *Non-HDL-C*, non -high-density lipoprotein cholesterol; *NST*, neck skin thickness; *NFT*, neck fat thickness; *BST*, back skin thickness; *BFT*, back fat thickness

[a] Data represents the means ± SD

[b] Data represents the median (p25-p75)

*$p$ values < 0.05 are considered statistically significant

peripheral artery atherosclerosis and non-alcoholic fatty liver disease (NAFLD).

## Lifestyle

Patients with DS vs non-DS had a higher prevalence of nicotine abuse (9/15) and alcohol abuse (6/15).

## Radiological analysis

We also demonstrated the MRI findings in the present study. T2-weighted MRI of DS patients revealed marked thickening of the involved tissues, which presented as obvious hyperintense signals in the cervical region and upper back (Fig. 2a, b). Surprisingly, we also observed a high-intensity and band-like lesion in the lower dermis of patients with DS in

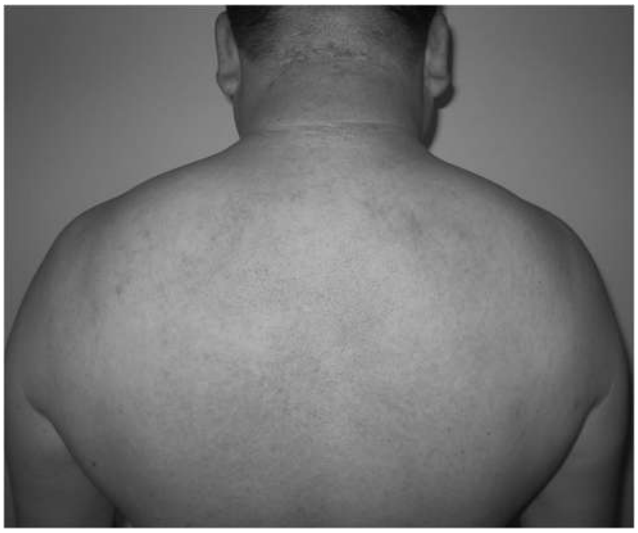

**Fig. 1** A 43-year-old man with type 2 diabetes presenting a symmetrical, non-pitting, asymptomatic, and erythematous lesion on the skin of posterior neck and upper back

T2-weighted MRI (Fig. 2c). Further, we measured the thickness of the neck skin (NST), neck subcutaneous fat (NFT), back skin (BST), and back subcutaneous fat (BFT) in the DS/non-DS groups respectively. Results showed that ones with DS presented a much more expanded skin and subcutaneous fat thickness in the neck and upper back when compared to the non-DS (NST: DS vs. non-DS, $7.84 \pm 2.56$ vs. $3.55 \pm 0.94$ mm, $p < 0.0001$; BST: DS vs. non-DS, $8.63 \pm 3.14$ vs. $4.77 \pm 1.55$ mm, $p = 0.0002$; NFT: DS vs. non-DS, $30.58 \pm 12.37$ vs. $12.22 \pm 5.24$ mm, $p < 0.0001$; BFT: DS vs. non-DS, $32.81 \pm 7.94$ vs. $25.11 \pm 5.94$ mm, $p = 0.0062$).

## Histological analysis

Cutaneous biopsy including subcutaneous tissue was performed and stained with the standard hematoxylin and eosin in three DS patients. Microscopic result demonstrated thickened and swollen collagen bundles separated by spaces by a variable amount of mucin in the deep reticular dermis. Hyaline

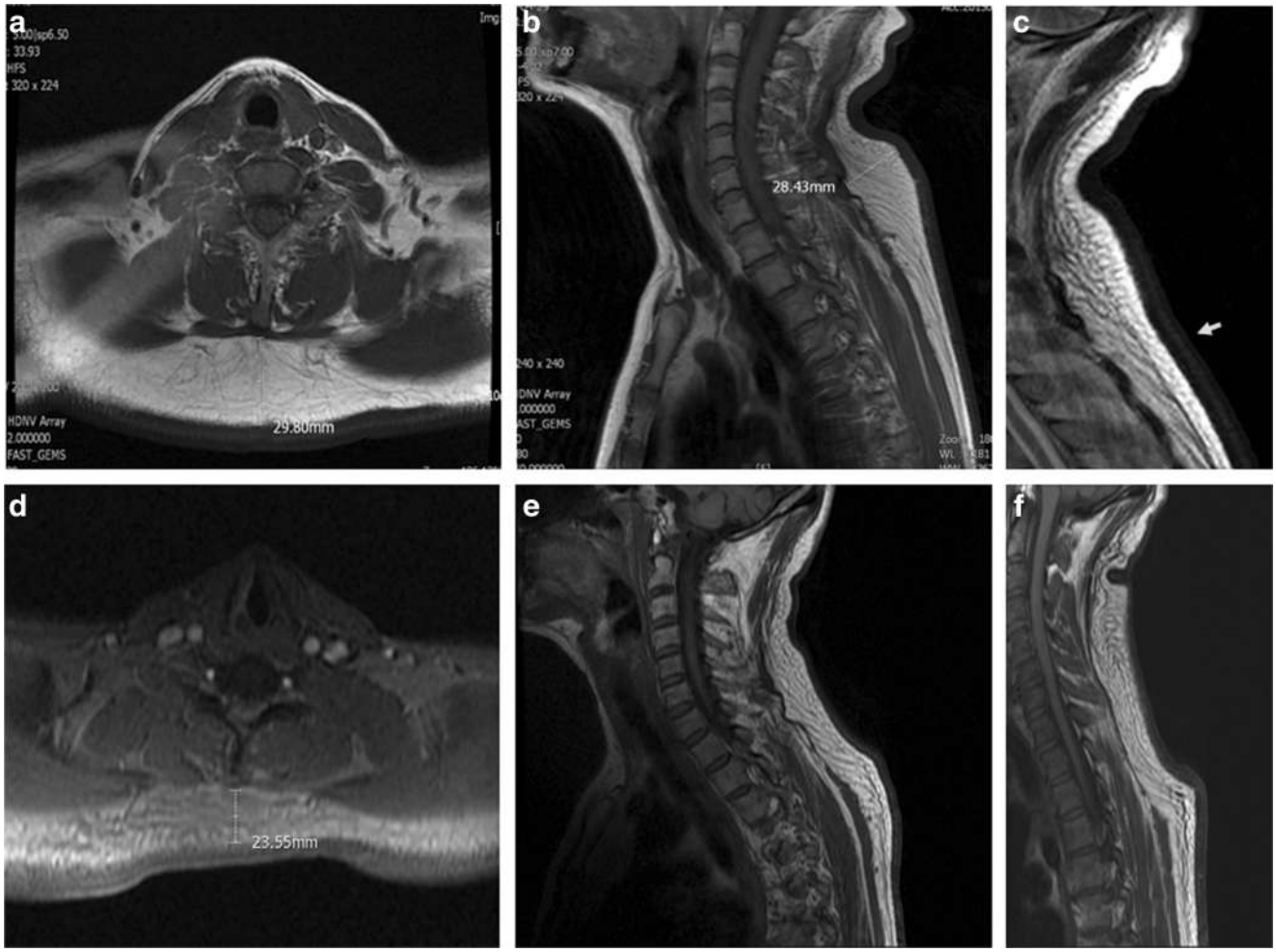


**Fig. 2** **a**, **b** Skin and subcutaneous tissue thickness in neck and upper back of the DS and non -DS patients. **a**-**b** Hyper-intense signals were apparent on T2-weighted magnetic resonance imaging in DS patients. **c** Note the presence of a white lining in the lower portion of the dermis in the DS patients. **d**–**f** The indication of MRI in diabetic patients without DS (d-f)

degeneration was also observed in the involved collagen fibers. No infiltration of inflammatory cells was observed (Fig. 3). Electronic microscopy showed enlarged collagen bundles and active fibroblast proliferation.

## Discussion

DS is a distinct cutaneous manifestation of diabetes. The first account of this condition was described by Krakowski et al. in 1973 in a patient with refractory diabetes and without preceding infection [9]. Previously, DS was described to mainly affect the dermis of the patients, whereas both our research and other recent reports presented that DS was also involved in the subcutaneous fat tissue [10, 11]. The onset of DS is usually subtle and its progression slow, which was confirmed in our study. According to previous reports, DS occurs primarily in obese adults of middle age, with long-standing and poorly controlled type 2 diabetes and those on long-term insulin treatment. Our findings in Chinese patients with DS confirmed most of these characteristics listed above. Nevertheless, our study also revealed that DS can also present in elderly subjects. Furthermore, some of the patients were overweight, not obese. Poor glycemic control was found in DS patients, though no distinctions were found in the DS and non-DS group. Insulin treatment may not be independently associated factor for Chinese subjects with DS, since only half of the patients with DS in our study have been treated with insulin, and the course is less than 5 years. Hyperlipidemia was a common finding in patients with DS. In our research, only LDL-C level was significantly elevated in the DS group compared with the non-DS group. Koga has concluded that weekly LDL apheresis therapy to lower serum lipids was effective to improve the cervical skin thickness of DS patient histologically [12, 13]. Therefore, high LDL-C level, might be another potential risk factor and indicator of DS. The reason may be that excess LDL-C concentration could become cross-linked to collagen that has an advanced glycation end products [14]. Most patients with DS were observed to have various vascular complications [3, 15]. Our study also confirmed this phenomenon. It has been speculated that microvascular damage in the dermis was associated with the progression of DS [4]. Most of the DS patients also had a long history of cigarette smoking and drinking, which may aggravate the vascular lesion to some extent.

The pathogenesis of DS remains unclear and seems to be heterogeneous. An irreversible nonenzymatic glycosylation of collagen fibers and increased dermal deposition of glycosaminoglycans have been previously proposed. Other hypotheses suggest that hyperglycemia may stimulate fibroblast proliferation and the synthesis of extracellular matrix components [16]. In our study, active fibroblast proliferation in the dermis was confirmed through electron microscopy. However, there was no difference in the glucose level between the DS patients and the non-DS patients. Hypoxia caused by microangiopathy may also be an important factor in the development of DS [17, 18]. Lower local oxygen pressure was reported to cause an increased synthesis of collagen and glycosaminoglycan by fibroblasts and improving the blood circulation of dermal capillaries with agents like PGE1 was effective for our DS patients [18]. Immunological response has also been postulated, as some patients have ameliorated following a treatment with cyclosporine; however, the lack of lymphocytic infiltrates in the dermal lesions seems to rule out a T cell–mediated etiologic mechanism [19–21]. Furthermore, in our study, we demonstrated that no inflammatory cells surround the collagen fibers.

Currently, the diagnosis of DS is generally suspected on a clinical base initially and confirmed by an aggressive skin biopsy. Some noninvasive examinations have been recommended before, like computed tomography (CT) and ultrasonography (US) [21–23]. On the basis of our study, we recommend MRI for a quantitative assessment of the changes in DS. MRI involves no exposure to radiation and hence appears to be safer than CT. The soft tissue contrast is better as compared to US or CT, and MRI can effectively distinguish subcutaneous tissue from the muscle [24]. As we delineated, T2-weighted MRI revealed that not only the dermis but also the subcutaneous fat of DS patients was obviously thickened, therefore, suggesting that the collagen might not only accumulate in the skin but also in the subcutaneous tissue. The

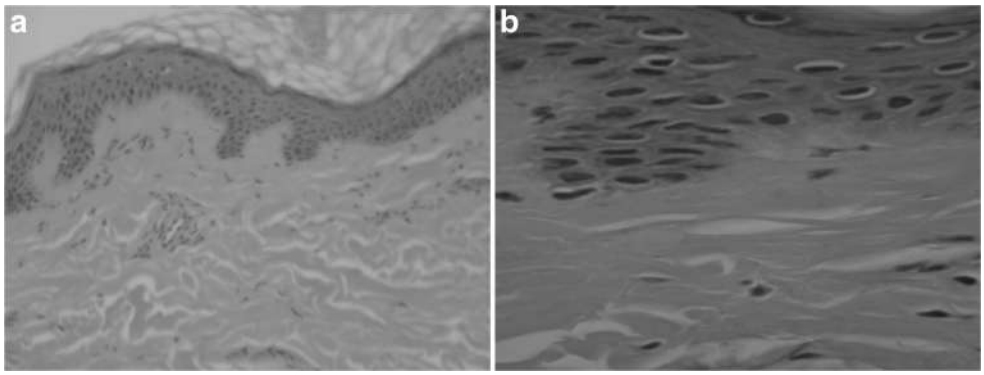


**Fig. 3** Histological appearance of skin biopsy specimen of two DS patients. Microscopy showed thickened dermis with increased spaces between large collagen bundles due to increased deposition of mucopolysaccharide. **a** Hematoxylin–eosin [HE], original magnification × 40. **b** HE, original magnification × 400

increased thickness of the skin and subcutaneous tissue of the DS is statistically significant as compared to the control group, which demonstrated that MRI could be recommended as an informative indicator for diagnosing or staging DS. We further confirmed the deposition of glycosaminoglycans as a white lining in the lower portion of the dermis in T2-weighted MRI, which was in accordance with the histological description. Since the deposited glycosaminoglycans were mainly hyaluronic acids, reported to have water-retention properties, which may explain why T2-weighted MRI of our patient's lesion revealed a high-intensity, band-like lesion in the dermis.

To date, there is no highly effective treatment for DS, though strict glycemic control, PUVA-phototherapy, immunosuppressants, such as cyclosporine and low-dose methotrexate, radiation therapy, potent topical, and intralesional steroids, penicillamine, intralesional insulin, prostaglandin E1, and pentoxifylline seem to be functional to some extent [1, 21, 25–27]. Our recommendation is to make glucose intensification together with lipids control and intravenous PGE1 application as a therapy, a treatment plan which would need further research and confirmation in the future.

In conclusion, DS is a relatively rare cutaneous complication specific to type 2 diabetes, which affects the dermis and the subcutaneous fat tissue. Chinese subjects with DS are mainly those of middle-aged and elderly patients (male predominantly). with long-standing but poorly controlled type 2 diabetes, who are overweight or obese, with increased microvascular complications, neuropathy, hypertension, and ischemic cerebral stroke. High LDL-C level, nicotine abuse, and alcohol abuse may be risk factors. MRI is efficient in determining the extent of disease progression non-invasively. Prolonged intravenous PGE1 application may be effective.

According to the estimated prevalence of diabetes, the morbidity of DS should be higher in China. However, in China, only a few cases have been reported. Whether the racial difference or the missed diagnosis that leads to the decreased reporting of DS needs to be validated in a larger clinical observation in the future.

**Acknowledgements** The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication. We thank all the patients for their cooperation.

**Author contributions** Y.Y.Z. analyzed the data and drafted the manuscript. Z.J.D., J.J., and C.M.W. conceived and designed the study and revised the article. C.M.W., Y.X.Z., and Y.Y.Z. conducted the literature review and interpretation of the study results. Y.Y.Q. edited the final manuscript.

**Funding information** This research was supported by Zhejiang Provincial Natural Science Foundation of China (no. LY15H070003) and Wenzhou Municipal Science and Technology Bureau (no. Y20170253).

## Compliance with ethical standards

**Conflict of interest** None of the authors have any conflicts of interest to declare.

**Human and animal rights** All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):513–522
https://doi.org/10.1007/s13410-018-0700-8

ORIGINAL ARTICLE

# Varied presentations and outcomes of Charcot neuroarthropathy in patients with diabetes mellitus

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Received: 10 August 2018 / Accepted: 20 October 2018 / Published online: 14 November 2018


**Abstract**
Charcot neuroarthropathy (CN) of the foot is uncommon and CN of atypical sites like wrist is sparingly described. CN is grossly under-diagnosed or misdiagnosed by primary care physicians. CN may present in acute phase with classical signs of inflammation or with deformities in chronic phase. The present study describes varied presentations, diagnostic dilemmas, and outcomes of CN in patients with diabetes. Data from the electronic repository of patients with diabetes and foot complications was obtained. The repository has a pre-designed structured format for information pertaining to the patients' anthropometry, presentation, neurological, vascular and foot examination details, treatment offered, and outcomes on follow-up. In addition, literature search for prior reported cases of atypical site (wrist) of CN was conducted. Data of 206 subjects with foot CN and two cases of atypical joint amongst 1297 patients with diabetes mellitus was screened. The particulars of patients with varied presentation viz. acute phase (case 1), stable foot deformities in chronic phase (case 2), and unstable foot with destruction of ankle joint in hindfoot CN (case 3) are presented along with the differential diagnosis and their outcomes on follow-up at 36 months of presentation. A literature search for atypical site of CN revealed only nine cases of wrist CN. The diagnosis of wrist CN was not initially considered and misdiagnosed. Patient with CN may present in active phase or more commonly with deformities and destroyed joints. The acute CN is misdiagnosed as cellulitis and inadvertently treated with antibiotics. Wrist and MCP joint CN is extremely rare and the diagnosis of CN requires a high index of suspicion supported by radiography findings.

**Keywords** Charcot neuroarthropathy · Diabetic foot · Amputation · Diabetic neuropathy

## Introduction

Charcot neuroarthropathy (CN) is a progressive destructive disease of bones and joints of an insensate foot, usually seen in patients with diabetes. CN has an incidence of 0.1–0.5% in patient with diabetes [1, 2] and not commonly seen in day-to-day practice by primary care physicians. As a result, CN is usually missed at first presentation and not considered amongst differentials for unilateral erythematous foot swelling by non-specialists. Hence, the incidence of CN may be much more than thought earlier, though there are no studies to support the same. The prevalence of CN amongst patients with diabetic neuropathy is estimated to be 13% [2]. CN is often misdiagnosed as soft tissue inflammation (cellulitis), inflammatory arthropathy, gouty arthritis, or deep vein thrombosis. A significant delay in diagnosis may culminate in foot deformity, non-healing ulcer, and amputation of limb.

The most common site of CN is the foot joints, especially mid-foot and involvement of the knee and hip is less common in diabetes mellitus. Similarly, CN of the shoulder and knee, though rare, is more frequently described in patients with syringomyelia or cervical spondylopathy. CN of small joints of the hand has not been reported earlier and wrist CN is also extremely rare in patients with diabetes mellitus.

The manifestations of CN may vary from a patient presenting with classical triad of swollen, erythematous, and warmth at local sites, a normal radiograph, and requiring MRI and bone scan to corroborate the diagnosis of active CN to a deformed and unstable foot with destroyed joints. Patient may also present with mid-foot deformity subsequent to a missed diagnosis of active CN serendipitously treated with antibiotics considering cellulitis for a long period. Finally, a patient may come with deformed foot having destroyed foot joints and

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fragmentation of tarsal bones highlighting the devastating consequences of CN [3]. Further, the treatments for different stages of CN are different including total contact cast in active phase of CN to customized footwear and/or surgery for chronic CN.

The present cases highlight the varied presentations, difficulties in diagnosis, and the importance of early diagnosis of foot and atypical site of CN like hand joints in patients with diabetes mellitus to prevent debilitating complications.

## Patients and methods

The clinical data of all patients with diabetes and foot complications presented to diabetic foot care unit at academic tertiary care hospital in north India is prospectively captured and entered in an electronic repository with a standardized data collection format after obtaining a written informed consent. The information related to the anthropometry, diabetes status, duration of diabetes, presence of microvascular complications of diabetes viz. retinopathy on fundus examination, nephropathy (creatinine clearance or presence of micro- or macro albuminuria), other co-morbidities, epidemiology of Charcot neuroarthropathy of foot, approximate date of onset of the acute episode of CN and preceding events (such as foot surgery, ulceration, infection, or trauma), previous treatment received, prior amputation, or corrective surgery like arthrodesis were recorded.

In addition, we retrospectively reviewed the records of patients with diabetic CN, evaluated at our unit, over the past 10 years from 2007 to 2016, for any atypical site of CN joint involvement including wrist and small joints of hand. A thorough search for English-language articles including the headings "diabetic Charcot neuroarthropathy of wrist," "diabetic Charcot arthropathy of wrist," "diabetic Charcot neuroarthropathy of hand," and "diabetic Charcot neuroosteoarthropathy of hand" was conducted in MEDLINE/PubMed, Embase, and Scopus (words used interchangeably) was also conducted.

Neurological examination included vibration (VPT), hot, cold, and monofilament (Semmes-Weinstein) perception at 10 standardized plantar sites on either foot. The presence of peripheral vascular disease (PVD) was assessed by palpation of pedal pulses and ankle-brachial index by Omaron vascular Doppler instrument. The details of presence/absence of foot deformities, co-existing ulcer, prior treatment, and treatment offered, including types of off-loading, use of bisphosphonates for acute CN, and outcome details, were also noted. The outcomes were pre-specified as clinical resolution of active CN (if active CN was the presenting manifestation) or amputation-free survival (inactive CN) or amputation or death.

## Results

A total of 1297 patients with diabetes mellitus and foot complications were recorded in the repository during June 2013 to Dec 2017. A total of 206 subjects had CN of foot including 47 patients with presentation as acute CN and 52 as acute on chronic CN and rest with chronic CN deformities. We subsequently highlight three cases of CN of foot with different presentations and outcomes. There were two cases of atypical CN joint involvement (overall prevalence of < 1%), including an elbow CN [4] and index case with wrist and MCP joint involvement.

**Case 1** A 70-year-old gentleman presented recent onset of swelling, redness, and warmth over the left foot for the past 10 days. He was diagnosed with T2DM 20 years earlier and was on oral anti-hyperglycemic drugs, metformin, vildagliptin, and premix insulin twice a day. He had a history of right distal phalanx amputation after a traumatic ulcer 5 years earlier. He had distal symmetric peripheral neuropathy, severe non-proliferative diabetic retinopathy (received laser photocoagulation), and nephropathy (Table 1). All peripheral pulses were palpable and bounding, and ankle-brachial index (ABI) was 1.1 in either limb. Foot examination revealed a swollen, erythematous, and warm left mid-foot (Fig. 1a). The temperature (infrared thermometer) difference between the two feet was 4 °C at mid-foot region (left mid-foot more than the corresponding area over the right foot). All peripheral pulses were palpable and ABI was normal (Table 2). A diagnosis of active CN was considered and radiograph of the left foot was sought (Fig. 1b) that revealed non-specific degenerative changes. Contrast-enhanced magnetic resonance imaging (CEMRI) (Fig. 1c) and triple-phase bone scan (Fig. 1d) of the left foot were sought that suggested active CN of the left mid-foot (anatomical pattern type III [Sanders and Frykberg]).

**Case 2** A 45-year-old lady presented painless, progressive deformity of the left foot for the past 6 months. She was diagnosed with T2DM 7 years earlier and was on oral anti-hyperglycemic drugs including metformin and sitagliptin along with lifestyle modifications. She had history of prior right great toe amputation 2 years earlier after a traumatic non-healing ulcer over great toe. She had distal symmetric peripheral neuropathy, mild non-proliferative diabetic retinopathy, and nephropathy (Table 1). She had a history of redness and swelling of the left foot 1 year earlier without any antecedent trauma. Considering a possible diagnosis of cellulitis, she was treated with antibiotics like quinolones and amoxicillin, clavulonate for 4 weeks at other centers prior to visiting us but with no relief in swelling. Within the next 2–3 months, the foot swelling reduced; however, gradually progressive deformity of the foot developed. She sought consultation with another physician and was again prescribed antibiotics that did not bring any relief to her symptomatology.

**Table 1** Baseline investigations amongst patients with CN of foot

| | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| Hemoglobin (g/dl) | 12.8 | 10.8 | 10.4 | 12.6 |
| TLC (cells/mm$^3$) | 8200 | 6000 | 9600 | 7800 |
| HbA1c (%) | 12 | 7.8 | 7.8 | 7.1 |
| Creatinine (mg/dl) | 1.16 | 1.06 | 1.0 | 0.8 |
| eGFR (ml/min/1.73 m$^2$) | 66.16 | 34.0 | 63.73 | 118.72 |
| Urine protein (mg/24 h) | 434 | 1240 | 264 | 400 |

At presentation to us, foot examination revealed deformity and swelling of the left foot with collapse of medial longitudinal arch and flattening of the mid-foot (Fig. 2). The temperature (infrared thermometer) difference between the two feet was 0.9 °C at the mid-foot region. A diagnosis of inactive Charcot neuroarthropathy was considered and an X-ray foot was sought (Fig. 3a–c). In retrospect, active CN was misdiagnosed as cellulitis by the treating clinician. Hence, a diagnosis of inactive CN with pes planus deformity was made and she was offered modified sandals (Fig. 4a, b).

**Case 3** A 42-year-old woman presented swelling and progressive deformity of the left ankle over the past 13 months. She had no prior history of trauma, recurrent ulceration, fracture, or amputation. She was diagnosed with T2DM 6 years earlier and was on oral anti-hyperglycemic drugs including metformin and glimepiride. She had distal symmetric peripheral neuropathy but no features of diabetic retinopathy on fundus examination or nephropathy (Tables 1 and 2).

Examination of foot revealed swelling and deformity of left hindfoot and ankle (Fig. 5a) with increased range of movements (plantar and dorsiflexion). There was no crepitus and vascular examination of the foot including palpation of peripheral pulses and ABI was normal (Table 2).

Radiograph of the foot was suggestive of destructive hindfoot inactive CN (anatomical pattern types IV and V [Sanders and Frykberg]) (Fig. 5b).

A differential diagnosis of cellulitis, inflammatory arthropathy, osteoarthritis, gouty arthritis, deep vein thrombosis, underlying fracture, lymphatic obstruction, or even osteomyelitis may be considered in a patient presenting with unilateral warm, erythematous, and swollen foot; otherwise, suggestive of acute CN of foot (as in case 2).

**Case 4** A 62-year-old male subject with type 2 diabetes mellitus for 6 years presented acute onset swelling and stiffness of the left wrist for 2 weeks. He was receiving metformin and glemipiride with sitagliptin. He had difficulty in flexion movement at the wrist and MCP joints with paresthesias and dull aching pain over the left wrist and hand. History of swelling or pain of other joints (arthritis), fever, Raynaud's phenomenon, or early morning stiffness was not present. There was no history of trauma, leprosy, poliomyelitis, cervical cord lesions, syringomyelia, or gout. He was right handed, a shopkeeper by profession, and had no risk factors for precipitation of active CN.

Neurological and vascular examination details are shown in Table 2. Orthostatic hypotension was not observed and no other features of autonomic neuropathy were present. Examination of the left wrist revealed swelling, erythema,

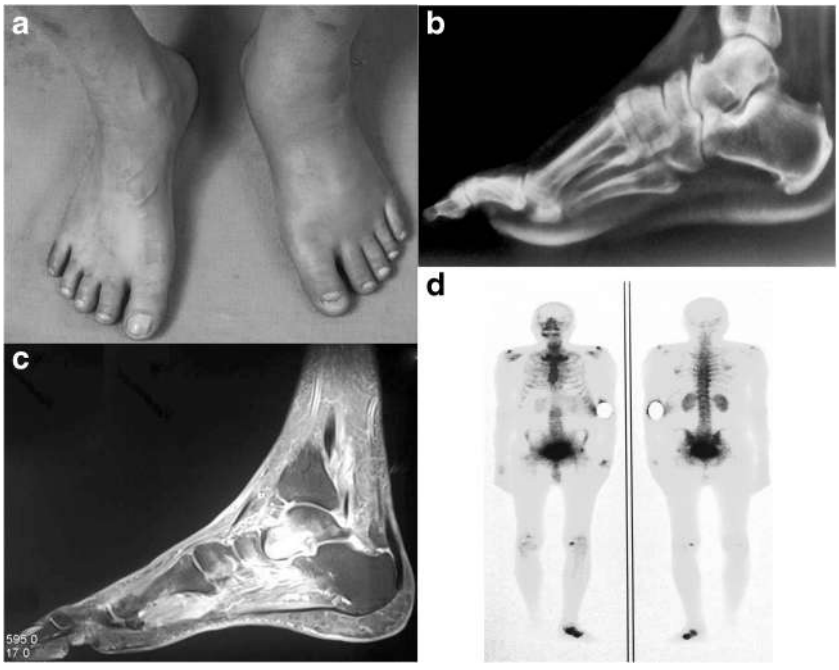


**Fig. 1** **a** Foot examination showing a swollen and erythematous the left mid-foot suggestive of acute CN. **b** X-ray of the foot (lateral view) shows non-specific degenerative changes in the talonavicular joint. **c** CEMRI T2-weighted fat saturated (sagittal image) of the left foot showing patchy marrow edema in the talus, calcaneum, and other mid-foot tarsal bones associated with osteoarthritris changes in talo-navicular joint and surrounding muscle edema. **d** Three-phase $^{99m}$Tc bone scan showing increased uptake in mid- and hindfoot region

**Table 2** Neurological and vascular examination of the foot amongst patients with CN of foot

| Parameters | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| VPT (mV) | 30 | 30 | > 50 | 50 |
| Temperature perception | Absent | Absent | Absent | Absent |
| Monofilament perception | Absent | Absent | Absent | Absent |
| Dorsalis pedis | Palpable and bounding | Palpable | Palpable | Palpable |
| Posterior tibial | Palpable and bounding | Palpable | Palpable | Palpable |
| ABI | 1.01 | 1.18 | 1.0 | 1.0 |

*VPT* vibration perception threshold, *ABI* ankle brachial index

and warmth limited to the wrist and proximal hand (Fig. 6). His grip and pinch strength were reduced compared to the opposite hand. The thenar and hypothenar muscle atrophy was not observed. Finkelstein's test, Phalen's maneuver, and Tinel's sign were negative. In the present context, diagnosis of Charcot neuroarthropathy of the wrist and MCP joints was considered and other differentials like acute inflammatory arthritis, complex regional pain syndromes (CRPS), Dupuytren's contracture, De Quervain's tenosynovitis, limited scleroderma (CREST syndrome), and carpal tunnel syndrome (CTS) were to be evaluated.

Hematological investigations are shown in Table 1. Additional workup included erythrocyte sedimentation rate (ESR) was 35 mm/h, C-reactive protein 0.9 mg/dl, and procalcitonin 0.6 ng/ml. Rheumatoid factor, anti-CCP (cyclic citrullinated peptide), anti-nuclear, anti SCL-70, and anti- centromere antibodies were not detected. Thyroid function tests were normal.

A radiograph of the wrists (Fig. 7), MDP three-phase skeletal scinitigraphy (Fig. 8a, b), and CEMRI (Fig. 9a, b) suggested the diagnosis of CN. Nerve conduction studies (NCS) of the median and ulnar nerves were suggestive of bilateral axonal sensorimotor neuropathy. A diagnosis of active CN of the left wrist was made in view of the clinical presentation, absence of markers of inflammation, and radioimaging.

## Treatment, follow-up, and outcomes

Case 1 was provided with non-walking fiber-glass total contact cast (TCC) that was serially changed weekly to two weekly (depending on resolution of swelling). Patient continued with TCC until the temperature difference between the two feet was < 2 °C, when he was offered customized shoes at 26 weeks (clinical resolution) of initial presentation. The lady in case 2 continued to remain ambulatory over the past 1 year, thereby, leading to progression of mid-foot deformity. She was provided with modified shoes (Fig. 4a, b) having medial longitudinal arch support to prevent collapse of the mid-foot. She was also counseled regarding gait (slow but steady) with short steps. Case 3 with destroyed ankle joint underwent calcaneotibial nailing and arthrodesis. Case 1 and case 2 are ambulatory and able to perform their routine pursuits with no progression of deformity or ulceration of the involved foot. Case 3 is ambulatory with limited walking in air-cast walker. All the three cases have an amputation-free survival of more than 36 months till the last follow-up. For case 4, the wrist was immobilized with a splint in the neutral position. The erythema, swelling, and temperature difference (< 2 °C) decreased after12 weeks suggesting clinical remission. None of the patient received antibiotics at our unit as they were aptly diagnosed as CN.

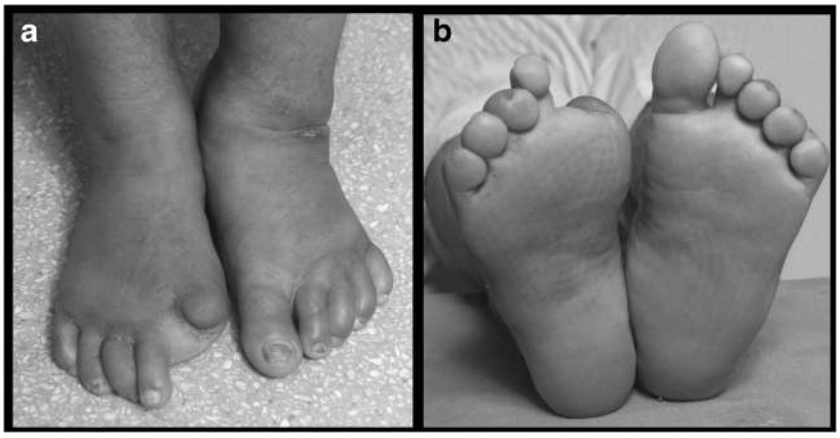


**Fig. 2** **a** (PGIMER): clinical photograph showing collapse of medial longitudinal arch and flattening of the left mid-foot. **b** Right foot showing amputated great toe

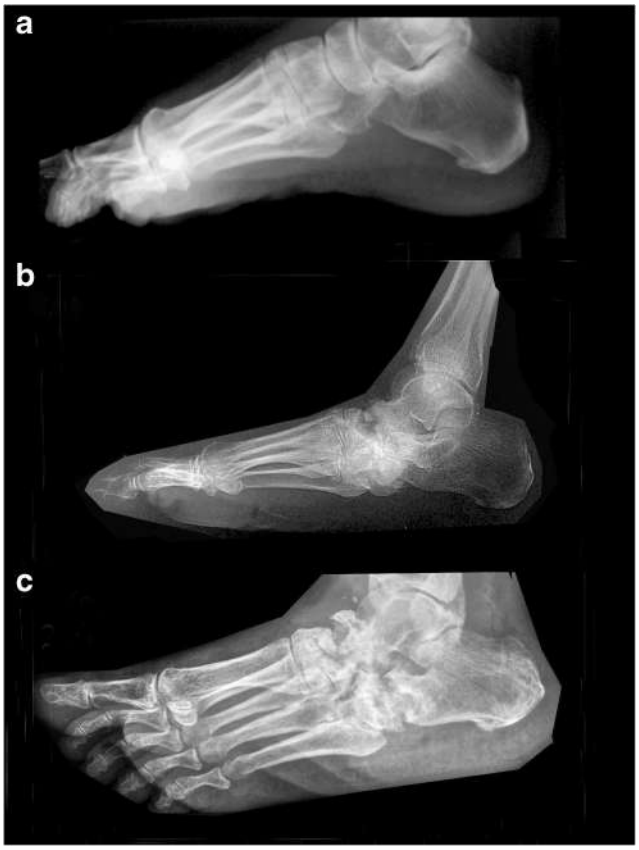


**Fig. 3** **a**–**c** X-ray of the left foot (1 year prior) showing normal radiograph with preserved alignment of foot bones (**a**). Subsequent radiograph showing tarsal bones fragmentation (5 months earlier) (**b**) and present radiograph (**c**) showing collapse and fragmentation of tarsal bones suggestive of anatomical pattern type III (Sanders and Frykberg)

## Literature review of prior reported cases of wrist CN

Literature review revealed nine cases of wrist CN [5–10] and none for MCP joint involvement. The clinical details of these nine cases are summarized in Table 3. Including the present case, wrist CN was equally prevalent in both genders (five each). Seven cases had type 2 diabetes mellitus and the remaining three had type 1 diabetes mellitus. The mean age at presentation was 60.7 years for subjects with type 2 diabetes. All the subjects had long duration of diabetes (> 10 year) and had prevalent microvascular complications of diabetes, including peripheral sensory neuropathy. Seven out of the ten cases had a history of prior involvement of the Charcot foot that supported the diagnosis of wrist CN. The diagnosis of wrist CN was not initially considered in most of the reported cases and were initially treated as gout, osteoarthritis, cellulitis, osteomyelitis, and complex regional pain syndrome (CRPS). The lag time to diagnosis ranged from 7 days to 2 years. Treatment with splint led to improvement in four of the seven cases, three cases had subsequent deformities, and the outcome was not reported in the remaining two cases.

## Discussion

Charcot neuroarthropathy of the foot is relatively uncommon and CN of the wrist is an extremely rare manifestation of diabetes mellitus. However, the actual incidence may be much higher than is usually thought, as foot CN is more often misdiagnosed than considered. The classical presentation of CN is painless, unilateral swelling with erythema and warmth over a localized region of the peripheral joints. We report the first case of MCP joint involvement in CN.

In a patient of diabetes mellitus presenting with unilateral, erythematous, and swollen foot, soft tissue infection (cellulitis) is considered more often and treated with antibiotics without much improvement, as was seen in the present cases. The other differentials considered are deep vein thrombosis, acute gouty arthritis, osteomyelitis, or fractures. In a retrospective series of 36 cases of active CN, only 61% were diagnosed correctly by specialist before referral [11]. The average delay from the first symptoms to the correct diagnosis was 29 weeks. The other diagnosis considered were erysipelas ($n = 10$), deep venous thrombosis ($n = 5$), gout ($n = 4$), osteoarthrosis/arthritis ($n = 5$), fracture ($n = 2$), and non-

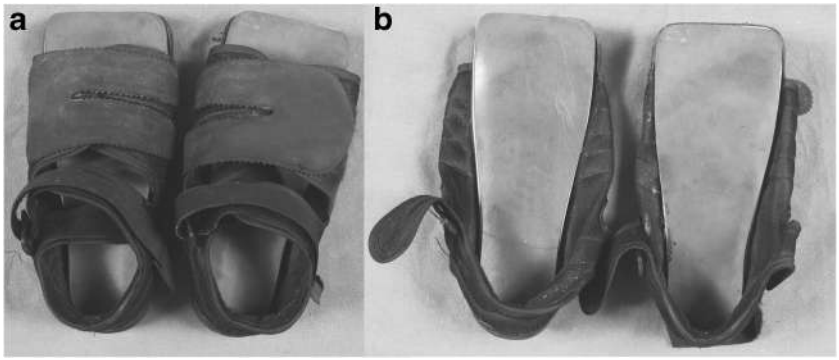


**Fig. 4** **a**, **b** Customized sandals showing wide toe box, cushioned insole, and medial longitudinal arch support with velcro

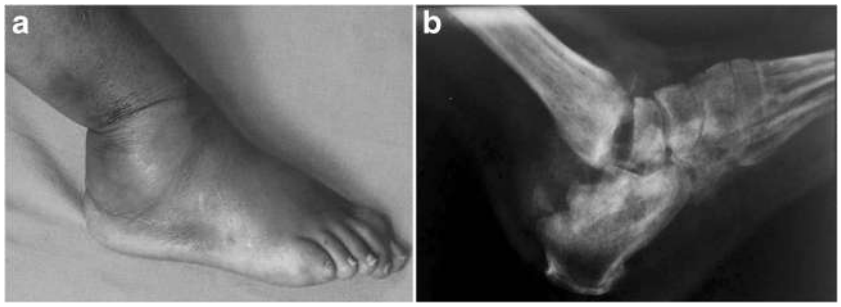


**Fig. 5** **a** Clinical photograph showing swelling and deformity of left ankle and hindfoot. **b** X-ray of the right foot (lateral view) showing features of Charcot neuroarthropathy of the ankle in the form of partial resorption of talus, altered alignment, joint destruction, debris, and soft tissue around the joint

specific inflammation/osteomyelitis/tumor ($n = 4$) [11]. In another study of 101 cases, CN was commonly misdiagnosed as osteomyelitis, cellulitis, or deep vein thrombosis in patients with diabetes mellitus [12].

Similarly, for wrist CN, diagnosis of more prevalent disorders viz. inflammatory arthritis are suspected when a patient presents with swelling, sign of inflammation, and limitation of movements at the wrist because of rarity of CN at this site. CN of the wrist and small joints of the hand is extremely rare because of non-weight bearing joints and lesser susceptibility to trauma which is considered a pre-requisite for neuropathic joint disease. The diagnosis of arthritis was less likely in the index patient due to the presence of sensory neuropathy, sudomotor dysfunction, and absence of markers of inflammation viz. normal ESR, CRP, total blood count, and undetectable antibodies for auto-immune arthritis. Clinically, Dupuytren's contracture, De Quervain's tenosynovitis, or scleroderma (CREST syndrome) may also present with limitation of flexion at the MCP joint, but a unilateral swelling along with signs of inflammation are unusual. A unilateral involvement of the wrist and MCP joints without early morning stiffness, and negative serological markers of inflammation suggests an alternate etiology. The presence of peripheral sensory neuropathy in patients with diabetes and history of prior foot CN, along with the bone and joints changes on radiograph of the wrist and hand are useful to differentiate the etiology. The radio imaging details of the wrist in the present case were similar to those noticed in stage I of Eichenholtz's classification of natural history of Charcot foot [13], suggesting a diagnosis of wrist CN.

Another etiology for paresthesias, dull aching pain, and stiffness of wrist movements could be CTS. CTS and diabetes mellitus are commonly associated and the prevalence of symptomatic CTS is understood to be as high as 30% in patients with diabetic neuropathy [14] and 11% in patients of type 1 diabetes mellitus without neuropathy [15]. CTS usually presents with bilateral involvement of wrists and is more common in females in the fifth or sixth decade of life. The presence of wrist swelling and erythema along with axonal sensorimotor neuropathy pattern on NCS and radiographic changes in the index case did not favor the diagnosis of CTS.

Wrist swelling and sensory neuropathy in a patient with diabetes may be associated with CRPS. CRPS is a rare entity

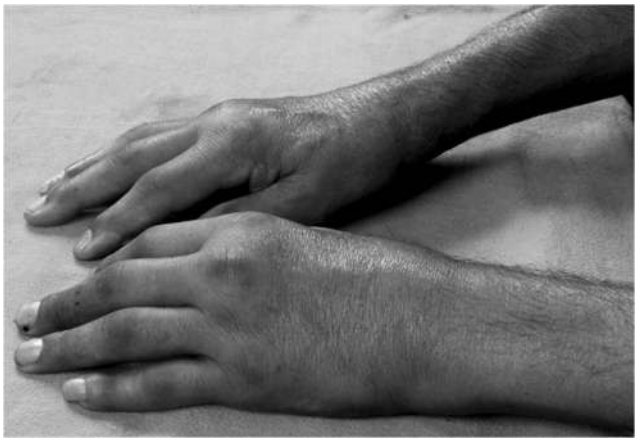

**Fig. 6** Clinical photograph of hands showing swelling limited to the left wrist and meta carpo-phalangeal joint

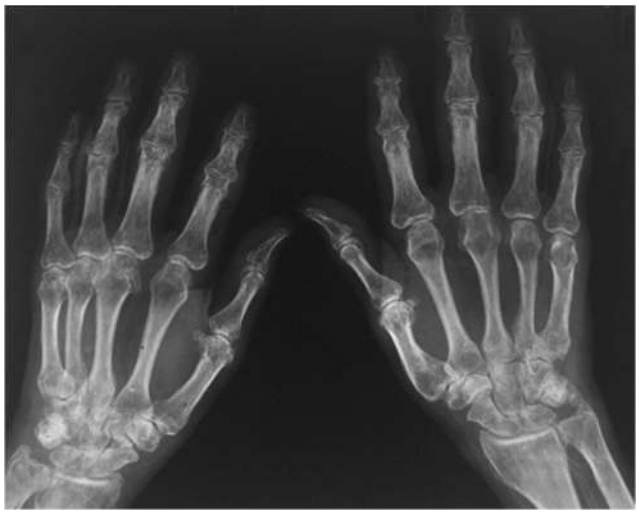

**Fig. 7** Radiograph of the wrist showing periarticular osteopenia, osteophytes, and degenerative changes predominantly at the left radiocarpal and interphalangeal joints

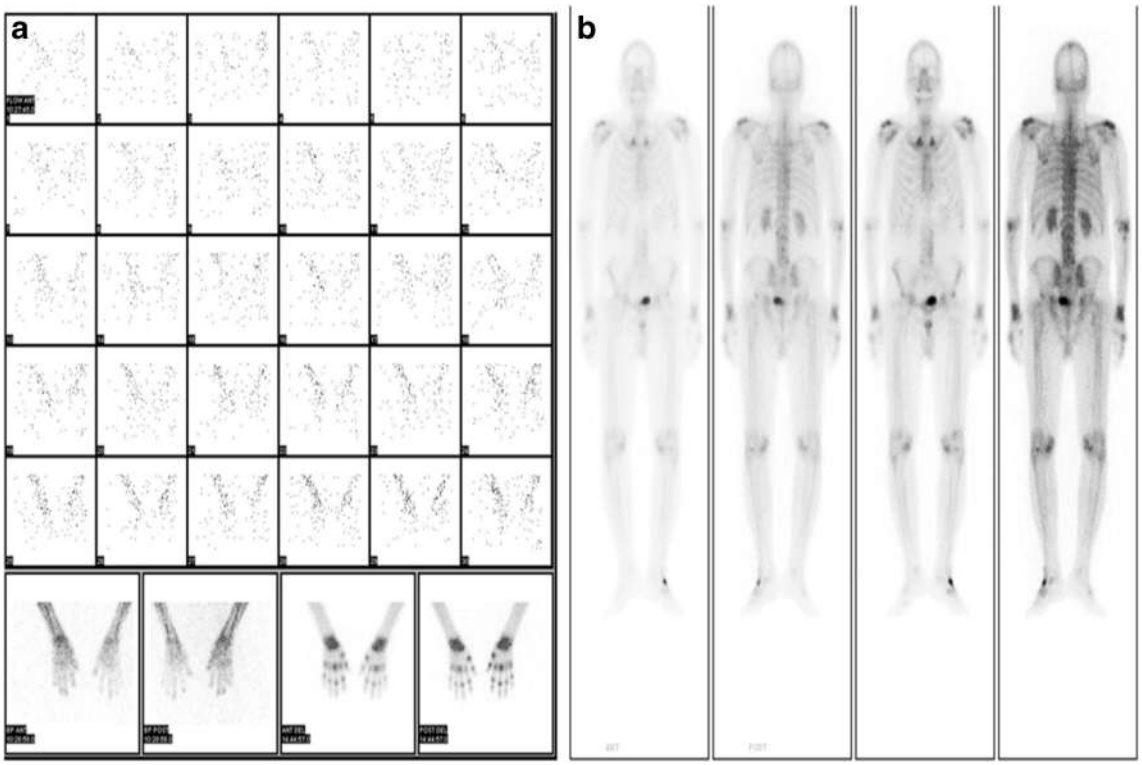


**Fig. 8** **a**, **b** $^{99m}$Tc MDP three-phase skeletal scinitigraphy showing increased flow and soft tissue pooling of tracer around the left wrist; the delayed images showed increased tracer uptake in the carpal bones of the left wrist suggestive of active inflammation

occurring in < 2% of subjects and more common in middle-aged women with a predilection for upper limb involvement [16]. CRPS is a diagnosis of exclusion and is considered when signs and/or symptoms could not be explained by another etiology (Budapest criteria) [17], unlike the index case. Moreover, the radiographic changes of the affected wrist and hand supported the diagnosis of CN.

Hence, unless the physician is aware of CN, the diagnosis is likely to be missed, as there are very few hematological or radiological parameters that may guide to diagnosis of active CN. The total leukocyte count, C-reactive protein, and erythrocyte sedimentation rate are non-specific and may be normal in active CN [2]. X-ray is an useful modality for anatomical extent of CN, but may not show any changes or only subtle

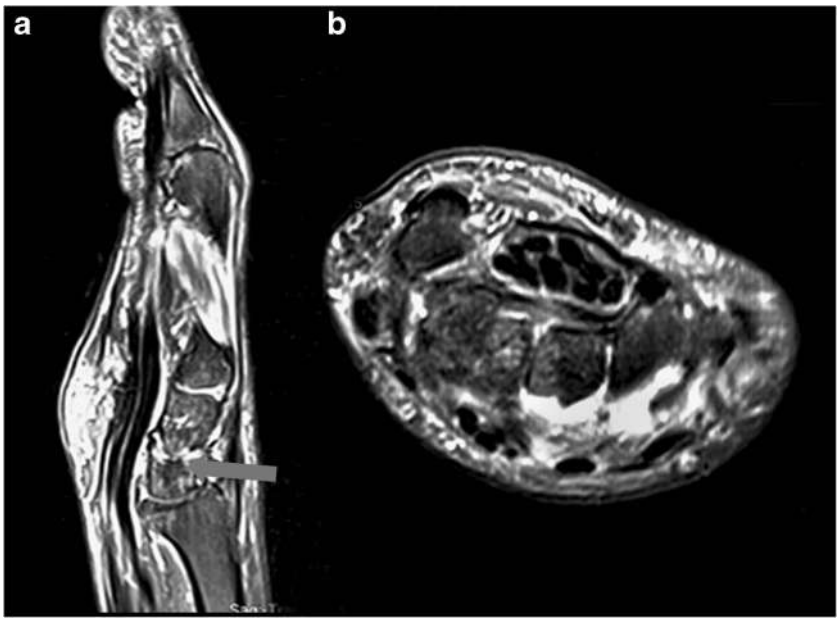


**Fig. 9** **a**, **b** Fat-saturated sagittal and axial T2-weighted MRI images of left wrist and hand showing patchy areas of bone marrow edema in the carpal (red arrow) and metacarpal bones; joint fluid is predominant in the radiocarpal and intercarpal joints (joint effusions) and osteophytes

**Table 3** Clinical data of reported patients with diabetic Charcot neuroarthropathy of wrist

| Case | Age/ gender | Type of diabetes | Duration of DM | Lag time to diagnosis | Complications | Other joint CN | Treatment | Outcome (time to remission) | Ref |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 65/M | T2DM | 16 | 2 years | Neuro, retino | Both feet, left ankle, right knee | Acetaminophen, indomethacin | Deformed wrist (left) | [5] |
| 2 | 55 | T2DM | 24 | 2 years | Neuro | Foot | NA | NA | [6] |
| 3 | 53/F | T1DM | 23 | 2 years | Neuro | Foot | NSAID splint | Deformed wrist (right) | [7] |
| 4 | 57/M | T2DM | 12 | 1 month | Neuro, retino, nephro | No | Immobilizing splints | Improvement in swelling (4 weeks) | [8] |
| 5 | 66/M | T2DM | 9 | 7 days | Neuro, retino, nephro | Both feet | Immobilization and antibiotics | Restriction of wrist movement (7 weeks) | [9] |
| 6 | 58/F | T2DM | 10 | NA | NA | NA | Corticosteroid (initial), pregabalin, NSAID splint | NA | [9] |
| 7 | 25/F | T1DM | > 10 | NA | Neuro, retino, nephro | Feet | Immobilization (casts), orthoses | Remission (? duration) | [10] |
| 8 | 31/F | T1DM | > 10 | NA | Neuro, retino, nephro | Feet | Immobilization (casts), orthoses | Remission (? duration) | [10] |
| 9 | 62/F | T2DM | > 10 | NA | Neuro, retino, nephro | No | Immobilization (casts), orthoses | Remission (? duration) | [10] |
| 10 | 62/M | T2DM | 6 | 2 weeks | Neuro, retino, nephro | No | Immobilization in wrist splint | Clinical remission in 12 weeks | Present study |

*NA* not available

changes at an early stage of CN. Bone and joint destruction, fragmentation of bones, and collapse of mid-foot may only be evident on X-ray after few weeks, once CN is established [3], as highlighted in the present case series. A magnetic resonance imaging is a sensitive modality in early phase of CN, but is costly and not readily available at all centers. In primary and secondary care settings, the main barrier to the diagnosis of CN at first presentation is that CN is usually not considered by the primary care physicians, possibly because of ignorance, as it is not much emphasized during medical training to the internists. Secondly, CN is less often encountered by the primary care physicians and other differentials of CN are much more prevalent in general population. Hence, the diagnosis of CN is essentially clinical and require high index of suspicion and knowledge of this entity.

CN was originally described as denervation-induced joint destruction by the French neurologist Jean-Martin Charcot in 1868 in people with tertiary syphilis [18]. The first description of CN in people with neuropathy complicating diabetes was in 1936 [19]. Patients of diabetes mellitus with peripheral neuropathy continue to be ambulatory on an insensate foot over prolonged duration leading to weight bearing and repeated trauma. Sympathetic vascular denervation due to autonomic neuropathy in patients with diabetes mellitus causes an increase in local arterio-venous shunts, increased venous pressure, capillary leakage, and swelling that is observed in foot with active CN. In addition, vasoregulatory failure due to autonomic neuropathy causes an increased blood flow to the bone, leading to more osteoclast recruitment, local bone resorption, weakening of bones, fractures, and joint dislocation [20]. As a result, there occurs mal-alignment of the joints of the foot, fragmentation with collapse of tarsal bones (the most common region involved), foot deformity (like pes planus, rocker-bottom foot), neuropathic ulcers, and subsequent amputations [2].

A patient with unilateral erythematous, swollen, and warm foot with diabetic sensory neuropathy and without any systemic signs of inflammation should be offered immobilization in non-removable contact cast. One clinical sign that is useful to assess response of the inflamed foot to immobilization in initial few days/week is a decrease in swelling and local temperature. The inflammation associated with active Charcot foot usually settles early, while that of cellulitis or osteomyelitis will not [21]. Hence, local swelling and difference in local temperature between the involved and the other foot measured with non-contact thermometer are useful during follow-up.

The treatment of choice for active CN involves the provision of off-loading in a serially applied non-removable, non-walking TCC, as in case 1. The TCC needs to be changed and reapplied initially weekly and later less frequently until the active CN has abated that is defined as temperature difference between the two feet < 2 °C. Unfortunately, the diagnosis is delayed and most patients are not provided with TCC, as also highlighted in CDUK study in which only 32% of subjects received non-removable TCC [22]. Another treatment modality that is considered for active CN are bisphosphonates. Due to repeated trauma to insensate foot, CN is associated with increased local proinflammatory cytokines, especially TNF-alpha. These local cytokines trigger RANKL (receptor activator of nuclear factor-kB ligand) that induces recruitment of osteoclasts and resorption of bones [23]. Activity of RANKL is antagonized by OPG (osteoprotegerin), which is a decoy receptor for RANK ligand and neutralizes its effects [24]. Patients with CN have elevated RANKL/OPG ratios leading to enhanced osteoclastic resorption of foot bones. Thus, bone resorption-inhibiting agents like bisphosphonates were considered for active CN, as they act on osteoclasts inducing osteoclast apoptosis, decrease bone turnover, and inhibit bone resorption. However, the use of bisphosphonates in CN is controversial; as earlier studies suggested some benefit, [25] but recent randomized controlled studies [26] and systematic reviews suggest that they may not reduce the duration of acute phase of CN [27]. Inactive CN require limited weight bearing in an appropriate foot wear to off-load and accommodate the deformities of the foot and also provide protection to the insensate foot. Similarly, the treatment of wrist CN by immobilization with hand splints in neutral position till the resolution of swelling and warmth could be useful, as also observed in the index case. Wrist immobilization with splint led to resolution of active CN in four of the seven cases where outcomes were reported.

To conclude, CN of foot is not an uncommon manifestation in subjects with diabetic neuropathy. However, CN at sites other than foot joints is rare and requires keen observation supported by radiography findings to make a diagnosis. CN should be considered amongst differentials for unilateral, erythematous, warm, and swollen joints. Patient with CN may present an active phase, inactive phase, or destroyed joints and deformities and missing the diagnosis of CN may lead to non-healing ulcers, bony deformities, and eventual amputation.

**Authors' contribution** AR was involved in the clinical care of the patient, literature search, and writing and editing of the manuscript. MP provided inputs for radiological imaging. AB was involved in writing and editing of the manuscript.

## Compliance with ethical standards

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

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

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):523–527
https://doi.org/10.1007/s13410-018-0669-3

ORIGINAL ARTICLE

# Hand function in people with type 1 and type 2 diabetes

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Received: 3 April 2018 / Accepted: 25 June 2018 / Published online: 7 July 2018


**Abstract**
Impaired hand function has a negative impact on occupational performance and activities of daily living among people with diabetes. Inadequate literature focused on hand function of patients with diabetes in Indian population limits early identification and prevention of hand dysfunction. Therefore, the purpose of our study was to report hand function among people with type 1 and type 2 diabetes. Hand function of 211 people with diabetes (111 males and 100 females) was evaluated on the dominant side using standardized clinical tests—Minnesota Manual Dexterity Test (MMDT), nine hole peg test, pinch and grip strength using pinch meter, and Jamar hand-held dynamometer. Hand function scores were compared between males and females using unpaired *t* test. The mean score of MMDT and nine hole pegboard test was 401.9 and 35.3 s respectively. The mean hand grip strength was 16.70 kg and key pinch strength was 3.7 kg; center pinch was 2.8 kg and palmar pinch was 2.5 kg in patients with diabetes. All test scores of hand function were lower compared to normative values reported in literature recorded with the same standard measurements. Hand function test scores did not vary between males and females ($p > 0.05$). Low hand function score of patients with diabetes warrants an urgent need for implementing an exercise program to improve hand function among people with diabetes. Additionally, reported scores of hand grip strength, pinch strength, and hand dexterity will guide hand therapy for people with diabetes following injury.

**Keywords** Hand function · Diabetes · Type 1 · Type 2

## Introduction

Diabetes mellitus is a systemic disorder with hyperglycemia (increased levels of blood glucose) because of deficiency in insulin secretion or its abnormal action in some target tissues. Hyperglycemia/poor glycemic control in type 1 and type 2 diabetes causes micro- and macrovascular complications leading to pathophysiological and structural changes in musculoskeletal structures causing "diabetic hand" [1, 2]. Due to adverse effects of hyperglycemia on nervous and connective tissues, hand functions in terms of dexterity, grip and pinch strength, hand manipulation skills, etc. may be impaired in patients with both types of diabetes.

Hand function plays an important role in most of the functional abilities of an individual. Persistent metabolic perturbations in diabetes led to impaired hand function and reported limitations in self-care tasks compared to non-diabetic subjects. In approximately 50% of individuals with diabetes, musculoskeletal impairments in hand like limited range of motion, tenosynovitis, Dupuytren's contracture, and altered nerve function are observed due to structural changes in connective tissues [3–5]. Such structural impairments affect hand function in terms of strength, dexterity, fine motor skills, and hand performance [3]. In addition, presence of sensory deficits in people with diabetes due to peripheral neuropathy which advances from distal to proximal direction can adversely affect hand function in later stages [6]. However, limited information is available on functional impact of diabetes on hand function

**Highlights**
- This is the first study to report hand function among people with diabetes in India providing test score values of Minnesota Manual Dexterity Test (MMDT), nine hole peg test, and pinch and grip strength for reference in rehabilitation.
- Hand function did not vary among males and females with diabetes.

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in contrast to the extensive literature on sensorimotor function of the lower extremity and foot among individuals with diabetes [4–6].

Few studies have reported impaired hand function in terms of grip strength in American, Saudi Arabian, Egyptian, etc. people with diabetes [5, 7–9]. However, overall hand function in terms of grip and pinch strength, dexterity, hand-eye coordination, and fine motor skills was not reported among Indian people with diabetes. Reported values of various hand function tests cannot be applicable to Indian people with diabetes due to genetic, ethnic, and anthropometric variations. Therefore, it was deemed essential to evaluate hand function and provide score values for people with diabetes to plan hand therapy and ultimately prevent further deterioration of function.

Therefore, the primary aim of this study was to report hand function score values among people with diabetes in India. The secondary aim was to explore the effect of gender on hand function.

## Methods

This study was approved by an institutional review board and was performed in compliance with the ethical standards laid down in the Declaration of Helsinki (as revised in Brazil 2013), local legal and ethical regulations. A sample of 211 participants (100 females, 111 males) with type 1 and type 2 diabetes aged ≥ 18 years was recruited from two diabetes clinics in the city. Exclusion criteria included any neurological disorder, musculoskeletal conditions such as rheumatoid arthritis involving hand, congenital hand deformities, or visual impairment known to impair hand function. Patients receiving renal dialysis with upper limb fistula secondary to renal dysfunction were excluded.

The procedure for all four tests was explained to participants. After seeking written informed consent, dominant hand function was evaluated in terms of dexterity, hand-eye coordination, grip and pinch strength using standardized tests such as MMDT, nine hole pegboard test, Jamar hand dynamometer, and pinch meter respectively during a single visit. The upper extremity used to eat and write was considered as the dominant side [10]. Three trials were recorded for each test and average scores were considered for further analysis.

### Test procedures and instructions

The Minnesota Manual Dexterity Test (MMDT) is a standardized test for evaluation of an individual's ability to move small objects through various distances. In general, the MMDT measures gross motor skills [11]. The battery of MMDT consists of five subtests, i.e., placing, turning, displacing, one-hand turning and placing, and two-hand turning and placing.

The participant was instructed to place his dominant hand on the first peg and begin the trial immediately after the word "go" and time was recorded using a stopwatch. After the last peg was inserted into the hole, time on stopwatch was recorded. This procedure was followed for all five batteries of MMDT. Each participant was allowed one practice trial, following which three trials were recorded. The total time for performance was obtained by computing the mean of three trials for all subtests.

The nine hole pegboard test is a standard reliable and valid tool used for assessing finger dexterity in healthy adults [12]. This test measures time in seconds required for a subject to place and remove nine pegs on a pegboard with the dominant hand. An average score for the three consecutive trials was calculated. Lower value of time generally indicates better hand function.

Center, key, and palmar pinch strength of the dominant hand was measured with a pinch meter. Scores were read on the needle side of the red readout marker. For each strength test, the average scores of three successive trials were recorded for each side.

Grip strength of the dominant side was measured using Jamar hand dynamometer. The calibrated Jamar hand dynamometer has acceptable validity and reliability for hand grip strength measurement [13]. The test was performed while the participant was seated with shoulder adducted and elbow flexed to 90° with the forearm and wrist in neutral to measure grip strength. The participant was asked to squeeze the Jamar dynamometer with maximum isometric effort, which was maintained for about 5 s. No other body movement was permitted through stabilization. The participant should be encouraged to give a maximum effort without causing pain. The mean value of three trials is recorded.

The mean and standard deviation values of grip and pinch strength recorded from healthy age-matched adults from our previously published study were used for descriptive comparison with the present findings [14].

## Data analysis and results

Statistical analysis was performed using SPSS version 20.0. Out of 211 participants, 53% were males and 47% females with an average of 5.87-year duration of diabetes. Participant's demographic data were presented in Table 1. Mean and standard deviations of all the score of hand function tests are presented in Table 2. Hand function test scores did not differ between males and females ($p$ value > 0.05). A significance level of $p < 0.05$ was set for all analyses.

**Table 1** Characteristics of participants: age, gender, duration, and type of diabetes

| | Type 1 diabetes | | Type 2 diabetes | |
|---|---|---|---|---|
| | Males ($n = 59$) (mean ± SD) | Females ($n = 49$) (mean ± SD) | Males ($n = 52$) (mean ± SD) | Females ($n = 51$) (mean ± SD) |
| Age (year) | 53.1 ± 9.5 | 51 ± 13.3 | 53.9 ± 12.3 | 53.3 ± 13.7 |
| Duration of diabetes (year) | 5.5 ± 5.1 | 4.7 ± 2.8 | 6.7 ± 4.5 | 6.6 ± 0.9 |

## Discussion

Hand function including gross motor skills, finger dexterity, and grip and pinch strength was evaluated using standardized tests in people with diabetes. The mean time required to complete the Minnesota test and the nine hole pegboard test was 401.9 and 35.3 s respectively whereas the mean grip strength was 16.70 kg and mean key, center, and palmar pinch strength was 3.70, 2.80, and 2.5 kg respectively on the dominant side. Hand function did not differ between males and females. People with diabetes reported lower score of hand function tests compared to age-matched healthy adults, which is published earlier.

Patients with diabetes required more time to complete the Minnesota Dexterity test (females 396.4 ± 22.9 s and males 407.5 ± 29.1 s) compared to normative data obtained (females 308.33 s and males 313 s) published by the same authors [14]. Gross motor skill of hand was impaired by approximately 22.2% in females and 23.1% in males with diabetes. Similar findings reported earlier by Julia Pfützner et al. (2011) suggest impaired hand dexterity in insulin-treated people with type 1 and type 2 diabetes compared to healthy controls [7]. The recent systematic review of Gundmi et al. (2018) suggested that dexterity on the dominant side did not differ between people with diabetes and controls, whereas on the non-dominant side, it differs [15].

Both males and females required almost equal time of 35 s to complete the nine hole pegboard test. In comparison with normative reference values (males 18.99 s and females 17.9 s), people with diabetes required almost twice the time to complete the nine hole pegboard test [12]. It is speculated that hand-eye coordination may be impaired among people with diabetes. Hand function impairment due to poor hand dexterity and altered hand-eye coordination may be due to structural changes in connective tissues of hand caused by diabetes leading to increased stiffness in small joints of hand (diabetic cheiroarthropathy) and poor vision caused by diabetic retinopathy [4].

Hand grip strength of males and females with diabetes was 21.4 and 12.2 kg respectively. In comparison with the normative values of grip strength in Indian people (males 33 kg and females 20 kg) reported by Mullerpatan R et al. (2013) [14], grip strength was less by 35.2% in males and 39.3% in females with diabetes. Similar findings were reported earlier in type 2 diabetes patients in Turkey, describing low hand grip strength and key pinch strength compared to age-matched control subjects. However, the grip strength of diabetes people reported in our study was around 40% lower than the grip strength (27.61 kg) reported in people with type 2 diabetes in Turkey [16]. Higher values of grip strength in Turkish people with diabetes may be an influence of apparently longer and broader body segments of typical Turkish adult compared to Indian adult. Whereas another study conducted on Nigerian patients with diabetes reported lower hand grip strength on the dominant side (male 19.56 kg and female 12.17 kg) compared to non-diabetes people (male 21.94 kg and female 14.06 kg) [17]. However, the grip strength values from this study were almost similar to our values among Indian people with type 2 diabetes, probably because of lack of huge variations in anthropometric characteristics between two populations.

Precision hand function was evaluated using three types of pinch strength. Key, center, and palmar pinch strength was

**Table 2** Hand function score among male and female patients with diabetes

| Test | Male ($n = 111$) Mean ± SD | Female ($n = 100$) Mean ± SD | Total ($n = 211$) Mean ± SD | Unpaired $t$ test $p$ value |
|---|---|---|---|---|
| Minnesota test (s) | 407.5 ± 29.1 | 396.4 ± 22.9 | 401.9 ± 26 | 0.66 |
| Hand dynamometer test (lb) | 47.1 ± 10.4 | 26.7 ± 10.8 | 36.9 ± 10.6 | 0.034* |
| Key pinch (lb) | 8.3 ± 2.3 | 8 ± 2.3 | 8.30 ± 2.54 | 0.13 |
| Center pinch (lb) | 6.7 ± 1.9 | 6.4 ± 2 | 6.68 ± 2.18 | 0.06 |
| Palmar pinch (lb) | 5.7 ± 2.9 | 5.9 ± 3 | 5.75 ± 3.03 | 0.24 |
| Nine hole peg test (s) | 35.4 ± 7.3 | 35.3 ± 8.4 | 35.3 ± 7.8 | 0.75 |

*$p < 0.05$ shows a statistically significant difference

reduced by approximately 67% in males and around 52% in females with diabetes compared to available normative data from previous literature [18]. Palmar pinch strength was most affected in both females and males. There is a large engagement of intrinsic muscles in palmer pinch, which are affected due to peripheral sensorimotor neuropathy affecting hand muscles in people with diabetes [19].

Hand function score obtained from all tests did not vary among male and female patients with diabetes may be due to almost similar duration of diagnosed diabetes in both genders. However, contradictorily, the score of grip and pinch strength was more in male than that in female non-diabetes healthy people [17, 18]. A recently published meta-analysis of seven full text-related articles has suggested reduced grip and pinch strength on dominant and non-dominant sides for people with diabetes compared to healthy controls [15]. Decrease in grip and pinch strength was justified by pathophysiological changes occurring during diabetes due to the formation of sorbitol from sugar which causes demyelination of large fibers leading to decrease in fine motor skills affecting hand dexterity function [20]. Reduction in grip and pinch strength may be associated with poor glycemic control and increased systemic inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) which have detrimental effects on overall muscle function [21].

Hand function is known to reduce with increasing duration of diagnosed diabetes [3]. However, data acquired from recall of diagnosis of diabetes in the present study are not reliable. Therefore, the influence of duration of diabetes on hand function was not explored in this study, because it is assumed that people often remain undiagnosed for long periods in India. Secondly, influence of occupation on hand function was not explored in this study.

Reported values of the Minnesota test, nine hole pegboard test, and grip and pinch strength for Indian people with diabetes will provide reference values which will be beneficial in routine clinical practice. Secondly, present findings will urge clinicians to evaluate hand function in people with diabetes along with routine evaluation of diabetic foot evaluation and plan rehabilitation program to maximize hand function. The present study recommends clinicians to implement prophylactic hand mobility and grip strengthening exercise program immediately after the diagnosis of diabetes to delay decline in hand functions.

## Conclusion

Low hand function score of patients with diabetes warrants an urgent need for implementing hand exercise program to improve hand function. Additionally, reported scores of hand grip strength, pinch strength, and hand dexterity will guide hand therapy among people with diabetes following injury.

**Acknowledgements** We thank all participants and medical superintendents of the MGM Hospital and Colony Nursing Home in Aurangabad for their support in data acquisition.

**Authors' contribution** Author 1: has made substantial contributions to the literature search, acquisition of data, analysis and interpretation of data, drafting, and editing the manuscript.

Author 2: has been involved in the concept, design, and definition of intellectual content, statistical analysis and revising it critically for important intellectual content, literature review, and analysis of the manuscript.

**Funding** This research was sponsored by MGM Institute of Health Sciences and did not receive any specific grant from external funding agencies in the public, commercial, or not-for-profit stores.

## Compliance with ethical standards

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

**Research involving human participants and/or animals** This is an observational study. Informed consent from all individual participants was taken prior to enrollment.

**Ethical approval** All procedures performed in the present study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):528–534
https://doi.org/10.1007/s13410-018-0685-3

ORIGINAL ARTICLE

# Therapeutic effect of catalpol on type 2 diabetic mice induced by STZ and high-fat diet and its possible mechanism

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Received: 23 November 2017 / Accepted: 6 August 2018 / Published online: 7 September 2018


**Abstract**
To explore the therapeutic effect and possible mechanism of catalpol on type 2 diabetic mice. Twenty-four C57/BL6 male mice were randomly divided into four groups—normal control group (CON, $n = 6$), diabetic model group (DM, $n = 6$), the lower dose catalpol treatment (80 mg/kg body weight) group (DM+L, $n = 6$), and the higher dose catalpol treatment (160 mg/kg body weight) group (DM+H, $n = 6$). Intraperitoneal glucose tolerance test was performed after 30 days of treatment. Fasting blood glucose (FBG), triglyceride (TG), and total cholesterol (TC) in serum were detected by full automatic biochemical instrument. Enzyme-linked immunosorbent assay was used to detect insulin levels in serum. Insulin resistance level was calculated by trapezoid rule. The morphological changes of pancreatic tissue were observed through HE staining. The protein levels of insulin receptor substrate 1 (IRS1) and glucose transporter type 4 (GLUT4) in the liver and muscle were measured by Western blot (WB). Compared with the DM group mice, the glucose tolerance and insulin resistance in the DM+H group and the DM+L group were improved, the levels of TG, TC, and FBG decreased ($p < 0.01$). The blood insulin levels were elevated in the DM+H group and the DM+L group ($p < 0.01$) and the insulin resistance level decreased ($p < 0.01$, $p < 0.05$). IRS1 and GLUT4 protein levels in the liver and muscle increased in the DM+H group and the DM+L group ($p < 0.01$, $p < 0.05$). Catalpol could be potential medicine to treat type 2 diabetes. Its therapeutic mechanism might be improving insulin-stimulated glucose uptake in the liver and muscle.

**Keywords** Type 2 diabetes · Catalpol · IRS1 · GLUT4

## Introduction

Nowadays, diabetes, including type 1 diabetes and type 2 diabetes, troubles an increasing number of people [1]. Diabetes mellitus type 2 is a kind of chronic metabolic disease due to the relatively insufficient insulin secretion with hyperglycemia and insulin resistance as its main characteristics [2]. These characteristics further influence some important organs. Insulin resistance of liver cells causes fatty deposition in the liver, and impairment of liver cells' function, which further aggravates glucolipid metabolic disorder, promotes the development of insulin resistance [3, 4]. Insulin plays a physiological effect by binding with insulin receptor, which mediated a series of enzymatic actions [5]. This effect of insulin is mediated mainly through insulin receptor substrate (IRS) [6–8]. GLUT4, a kind of glucose carrier protein, after regulated by its upstream, transfers extracellular glucose to the cells in the liver and muscle, and finally reduces the blood glucose [9, 10].

Previous research has reported that catalpol may ameliorate type 2 diabetes of mice. Catalpol, extracted from the root of *Rehmanniae glutinosa* L., can significantly decrease blood glucose and improve glucose tolerance, as well as regulate glucose metabolism of diabetic mice [11–13]. Moreover, studies suggest that a certain dose of catalpol can improve mitochondrial function of skeletal muscle which further reduces blood glucose [14]. Meanwhile, other studies presented that hypoglycemic effect of catalpol on streptozotocin (STZ)-induced diabetic mice may be related to the morphology of pancreas and insulin secretion, which promotes utilization of glucose [15–17]. However, there are few reports about how catalpol affects insulin activation in type 2 diabetic mice. Therefore, to figure out more specific biochemical mechanisms about hypoglycemic effect of catalpol on type 2 diabetic

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mice, we studied the changes of insulin resistance as well as IRS-1 in type 2 diabetic mice with treatment of catalpol.

## Material and methods

### Chemicals and reagent

STZ was purchased from Shanghai Biological Technology Co., Ltd. Catalpol (purity 98%) was provided by Nanjing Zelang Biological Technology Co., Ltd. (Jiangsu, China). Blood glucose meter (GA-3) was obtained from Sannuo Biosensor Inc. (Hunan, China). Insulin (INS) (expressed as mIU/L) enzyme-linked immunosorbent assay kits were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). BCA protein assay kit was obtained from ThermoFisher Scientific (Shanghai, China). IRS1, GLUT4, and β-actin primary antibodies were purchased from Lianke Biotech Co., Ltd. (Hangzhou, China).

### Animals and drugs

Eighteen to 25 g C57BL/6 mice were supplied by Shanghai Laboratory Animal Center, China Academy of Sciences (Shanghai, China). All the mice were maintained in the Laboratory Animal Center of Whenzhou Medical University (Zhejiang, China). The mice had free access to water and a standard diet and were kept in a 12-h dark/light room with temperature at 23–25 °C and humidity at 53–57%. Catalpol (purity 98%) was provided by Nanjing Zelang Biological Technology Co., Ltd. (Jiangsu, China).

### Experimental protocol

After acclimated to the condition of the Laboratory Animal Center for 1 week, the 24 male C57BL/6 mice were randomly divided into four groups: normal control group (CON, $n = 6$), diabetic model group (DM, $n = 6$), the lower dose catalpol treatment (80 mg/kg body weight) group (DM+L, $n = 6$), the higher dose catalpol treatment (160 mg/kg body weight) group (DM+H, $n = 6$). The drug dose was chosen according to the previous work [11]. Mice in the NC group were fed regular diet, while DM, DM+L, and DM+H were fed high-fat and high-carbohydrate diet (regular fodder 66.5% + 20% brown sugar + 10% lard + 2.5% cholesterol + 1% cholic acid salt). Six weeks later, mice in DM, DM+L, and DM+H were induced into type 2 diabetes by intraperitoneal injection of 1% streptozotocin at 40 mg/kg body weight after 12-h fasting for solids, following that the DM+L group and the DM+H group were respectively given a gavage of catalpol suspended in distilled water at 80 mg/kg body weight and 160 mg/kg body weight once a day for 4 weeks. The DM group was given a gavage of distilled water of the same volume and time with the drug. After 26 days of treatment, the mice were fasted 12 h and then intraperitoneally injected with 2.0 g/kg glucose dissolved in saline. Then, blood samples were taken from the tip of the tail at 0, 30, 60, and 120 min, which was used to estimate the level of glucose with blood glucometer (WetRust, Taiwan, China). To value the intraperitoneal glucose tolerance of mice, we calculated the blood glucose incremental area under the glucose-time curve (iAUC) according to trapezoid rule. At the 30th day, all the mice were fasted for 8 h and then sacrificed. The blood sample was collected and centrifuged (4 °C, 1000*g*, 10 min) to get the serum. The pancreatic tissues were taken and fixed in 4% phosphate-buffered paraformaldehyde overnight, and then embedded in paraffin for hematoxylin-eosin (HE) staining. The liver and muscle tissues were quickly frozen by liquid nitrogen and then stored at − 80 °C. All experimental procedures were conducted according to the institutional guidelines for the care and use of laboratory animals in China.

### Serum biochemistry

Glucose, triglyceride (TG), and total cholesterol (TC) in serum were detected with automatic biochemistry analyzer (Hitachi, Japan).

### Enzyme-linked immunosorbent assay

The concentration of insulin (INS) (expressed as mIU/L) in serum was measured with mice enzyme-linked immunosorbent assay kits (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China).

### Histopathological study

The pancreatic tissues were taken and washed in PBS. After being fixed in 4% polyformaldehyde for 24 h, tissues were dehydrated in a graded series of alcohol. Next, xylene was used to clear these tissues which were then infiltrated with molten paraffin (melting point of 52–56 °C) and finally embedded in paraffin (melting point of 58–62 °C). The embedded tissues were sliced up into 4 μm. The paraffin wax slices with tissues were cleared by xylene and then rehydrated in a graded series of alcohol. The slices of tissues were adequately hydrated in distilled water. After that, the slices of tissues were dyed in hematoxylin followed by being washed in distilled water. Again, they were dehydrated by a graded series of alcohol and re-dyed by eosin. At last, the slices of tissues were sealed by resinene and examined under a light microscope, and photomicrographs were taken.

### Western blot determination

Three samples of liver tissues from each group (CON, DM, DM+L, DM+H) were randomly selected. Each sample (approximately 50 mg) was grinded and lysed in 500 μL lysis buffer which contained 1% phosphatase inhibitors and 1% PMSF for 30 min. The grinder was cooled on the ice in advance and the whole process of grinding was operated on the ice. After the lysates were centrifuged at 12000*g* at 4 °C for 10 min, the total proteins were obtained from the supernatants. Concentrations of total protein were determined by BCA protein assay kit (ThermoFisher Scientific). The protein products were used to detect the expressions of IRS1, GLUT4, and β-actin in the liver and muscle using Western blot. Equal amounts of protein product (30 μg) from each sample were electrophoresed through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Afterwards, the separated protein was transferred to nitrocellulose membrane. And then it was blocked with 5% skimmed milk in TBST solution (2.42 g Tris-base, 14.6 g NaCl, and 1 mL Tween-20 in 1 L water) for 1.5 h. Following that, the membrane, with protein on it, was incubated with primary antibodies against IRS1 (1:500), GLUT4 (1:3000), or β-actin (1:6000) at 4 °C for 12 h. Next, the membrane was washed with TBST solution for three times (10 min each time) and then incubated with secondary antibodies for 1 h at room temperature. At last, after washing with TBST solution for three times (8 min each time), the brand was covered with ECL chemiluminescence followed by quantitative gray-scale scanning.

### Statistical analysis

The results were presented as means ± SEM. The data were analyzed by SPSS 19.0 software and performed by one-way analysis of variance (ANOVA). Individual differences among groups were analyzed using Dunnett's test. When $p < 0.05$, the difference was considered statistically significant.

## Results

### The effect of catalpol on serum biochemistry

As shown in Table 1, the TG, TC, and FBG levels of the DM group were significantly higher than those of CON, DM+L, and DM+H. Moreover, TG, TC, and glucose (GLU) levels of the DM+H group were significantly lower than those of the DM+L group.

**Table 1** Effects of catalpol on fasting blood glucose (FBG) total triglyceride (TG) and total cholesterol (TC) in serum of mice (means ± SEM, $n = 6$)

| | FBG (mM) | TG (mM) | TC (mM) |
|---|---|---|---|
| CON | 5.847 ± 0.18 | 0.62 ± 0.03 | 2.32 ± 0.065 |
| DM | 12.40 ± 0.33** | 1.04 ± 0.04** | 3.85 ± 0.36** |
| DM+L | 9.127 ± 0.99## | 0.80 ± 0.04## | 2.83 ± 0.10# |
| DM+H | 8.620 ± 0.68## | 0.73 ± 0.05## | 2.36 ± 0.15## |

Compared with normal the control group, $^{**}p < 0.01$. Compared with the model control group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$

### The effect of catalpol on intraperitoneal glucose tolerance

Glucose tolerance is an important indicator of type 2 diabetes. In this experiment, we chose the method of intraperitoneal glucose tolerance test. As shown in Fig. 1, compared with the CON group, the DM group of AUC levels increased significantly ($p < 0.01$). While compared with the DM group, AUC level of the DM+L group and the DM+H group dropped significantly ($p < 0.01$). There was no significant difference between the DM+H group and the DM+L group. This result indicated that catalpol (80 and 160 mg/kg) obviously improved glucose tolerance in diabetic mice induced by STZ and high-fat diet.

### Effect of catalpol on INS in blood

As presented in Fig. 2, the blood insulin level of the DM group was significantly higher than that of the CON group ($p < 0.01$). Compared with the DM group, blood insulin levels of the DM+L group and the DM+H group were significantly higher than that of the DM group ($p < 0.01$). This result shows that catalpol can obviously reduce the insulin level of diabetic mice induced by STZ and high-fat diet.

### Effect of catalpol on insulin resistance

The insulin resistance index was calculated by method of homeostasis model assessment (HOMA-IR) (HOMA-IR=FBG × insulin/405). As shown in Fig. 3, insulin resistance level significantly increased in the DM group ($p < 0.01$). Compared with the DM group, insulin resistance levels in the DM+L group and the DM+H group were significantly reduced ($p < 0.01$). The results showed that the catalpol might improve the insulin resistance of diabetic mice induced by STZ and high-fat diet.

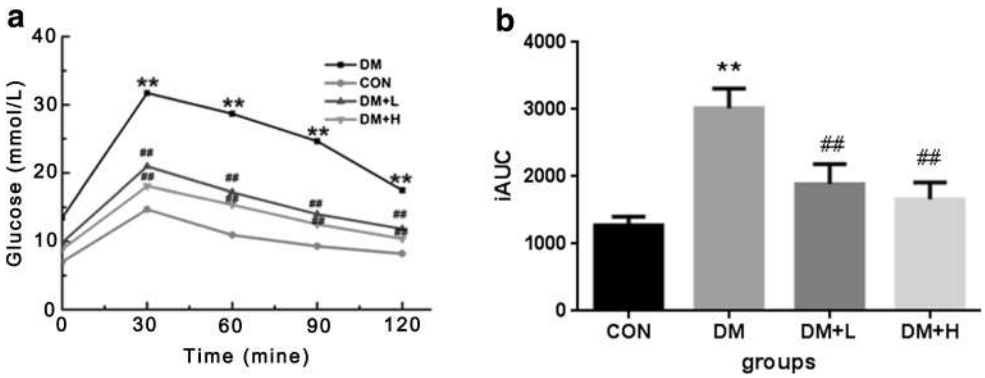


**Fig. 1** Effects of catalpol on intraperitoneal glucose tolerance. **a** Blood glucose levels during intraperitoneal glucose tolerance test. **b** The incremental area under the curve (iAUC) for intraperitoneal glucose tolerance test. Data were presented as means ± SEM ($n = 6$). Compared with the CON group, $^{**}p < 0.01$. Compared with the DM group, $^{\#\#}p < 0.01$

## Effects of catalpol on pancreas

As presented in Fig. 4, the results of pancreatic HE showed that the 30-day catalpol treatment had brought the pancreas of diabetic mice near normal, which indicated that catalpol might have effects to repair the pancreas.

## Effects of catalpol on IRS1 and GLUT4 protein expression in the liver and muscle

As presented in Fig. 5, lower dose catalpol (80 mg/kg) and higher dose catalpol (160 mg/kg) significantly reduce the protein level of IRS1 in liver tissue ($p < 0.01$, $p < 0.01$). Protein level of IRS1 of higher dose catalpol (80 mg/kg)-treated group was significantly lower than that of lower dose catalpol-treated group ($p < 0.05$). There was a significant difference of IRS1 protein level between the DM+L group and the DM+H group ($p < 0.05$). These results indicated that catalpol can improve insulin signaling in the liver. In addition, GLUT4 protein level in the liver was detected. This result shows that both lower dose catalpol and higher dose catalpol significantly increased GLUT4 level of liver tissue in diabetic mice ($p < 0.05$). There was significant difference between the DM+L group and the DM+H group ($p < 0.05$). These results provided evidences for increased insulin-stimulated glucose uptake in liver tissues, which may be related to the catalpol dosage.

At the same time, we evaluated IRS1 and GLUT4 protein levels in muscle tissues. IRS1 protein levels in muscle tissues show the same tendency of those in liver tissue. But there were no significant differences between IRS1 protein levels in the DM+L group and the DM+H group. In addition, GLUT4 protein level of catalpol-treated groups was observed higher than that of the DM group, which is similar to GLUT4 protein level in liver tissue. But there were no significant differences between GLUT4 protein levels in the DM+L group and the DM+H group. These results provided evidences for increased insulin-stimulated glucose uptake in muscle tissues, though the effect of catalpol dosage was unknown.

# Discussion

The main characteristic of type 2 diabetes is that the pancreatic β cells do not produce abundant insulin to maintain the activity of the enzyme associated with glycemic activity [18]. Diabetic animals can be induced by a single high-

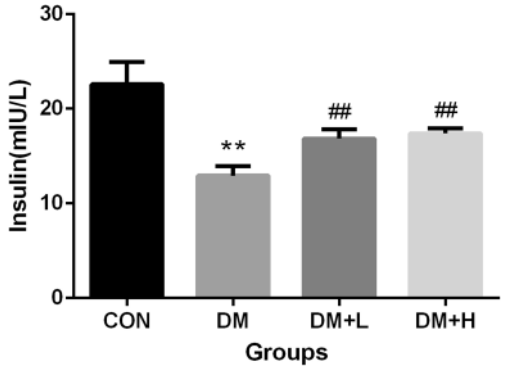


**Fig. 2** Effects of catalpol on serum insulin concentrations in STZ+high-fat diet-induced diabetic mice. Data were presented as means ± SEM ($n = 6$). Compared with the CON group, $^{**}p < 0.01$. Compared with the DM group, $^{\#\#}p < 0.01$

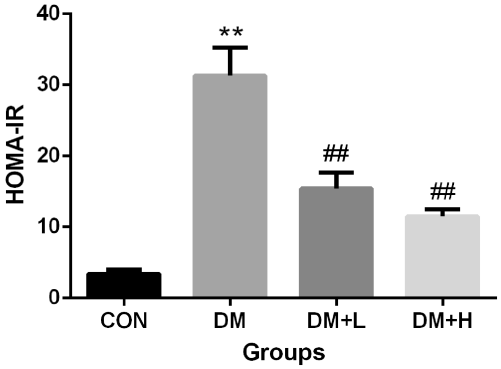


**Fig. 3** Effects of catalpol on HOMA-IR index of STZ+high-fat diet-induced diabetic. Data were presented as means ± SEM ($n = 6$). Compared with the CON group, $^{**}p < 0.01$. Compared with the DM group, $^{\#\#}p < 0.01$

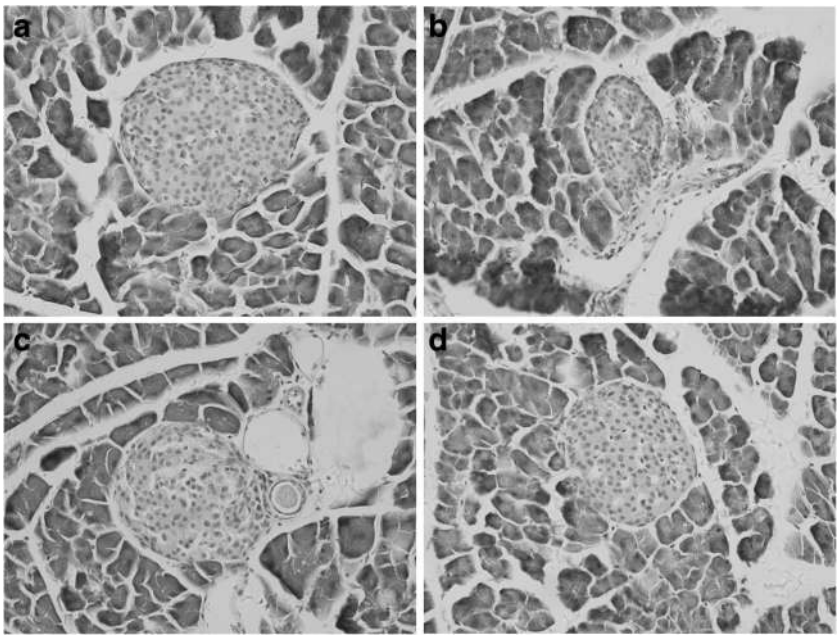


**Fig. 4** Effects of catalpol on pancreas in STZ+high-fat diet-induced diabetic mice. **a** Hematoxylin and Eosin (HE, × 400) staining of normal control mice pancreas. **b** Hematoxylin and Eosin (HE, × 400) staining of diabetic control mice pancreas showing destroyed islets. **c** Diabetic+80 mg/kg catalpol-treated mice pancreas. Showing regenerating islet cells (HE, × 400). **d** Diabetic+160 mg/kg catalpol-treated mice pancreas, showing regenerating islet cells (HE, × 400)

dose STZ injection or low dose of STZ. It was reported that catalpol had therapeutic effects on the diabetic mice and db/db mice [12].

In our current study, the catalpol significantly reduced the blood glucose of STZ and high-fat diet mice and improve intraperitoneal glucose tolerance in mice. This result

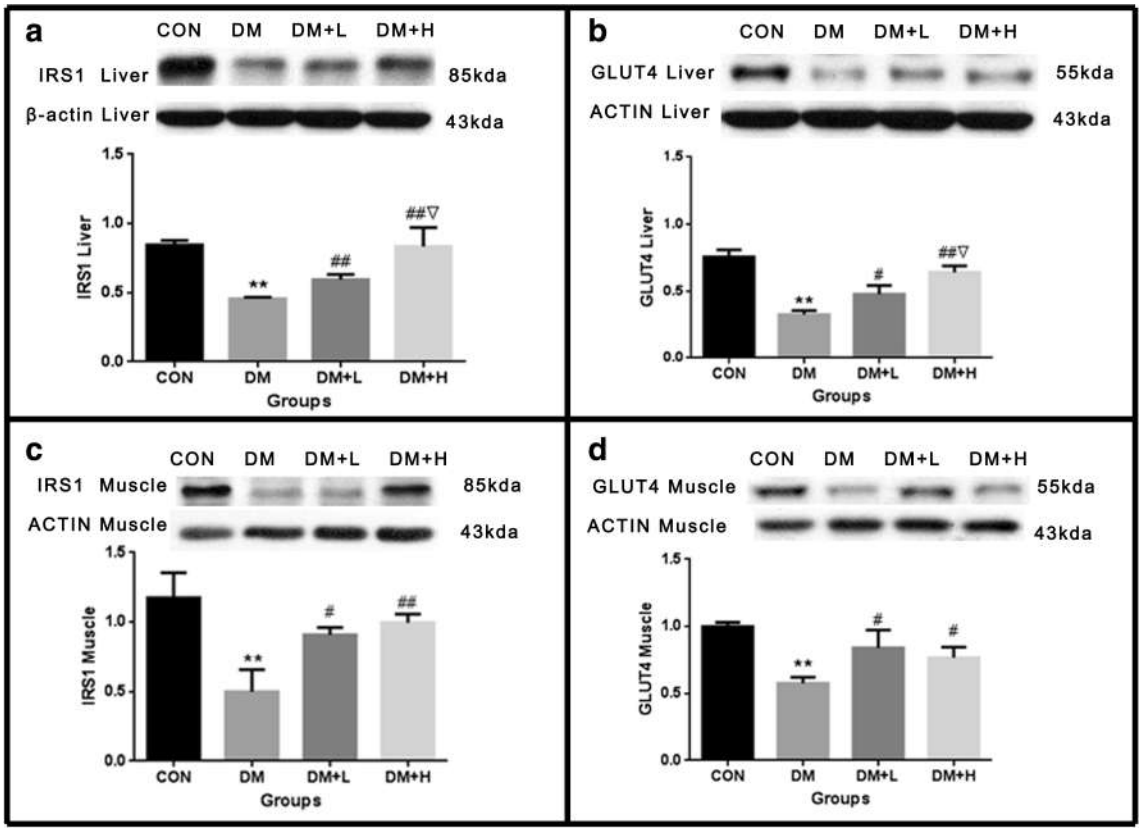


**Fig. 5** Western blot analysis for liver and muscle IRS1 and GLUT4 protein expression levels in type 2 diabetic rats. Each value is mean ± SEM for three replicates. $^{**}p \leq 0.01$, diabetic mice tissues compared with normal. $^{\#}p \leq 0.05$ and $^{\#\#}p \leq 0.01$, catalpol-treated mice tissues compared with diabetic control. $^{\triangledown}p \leq 0.05$, Diabetic+160 mg/kg catalpol-treated mice tissues compared with diabetic+80 mg/kg catalpol-treated mice tissues

is similar to Yan et al. which indicated that study catalpol significantly reduced the blood glucose and improve oral glucose tolerance in type 2 diabetes [19]. Diabetes is associated with insulin secretion and insulin resistance [4]. Our results showed that the serum insulin levels in serum of mice increased and the insulin resistance index decreased in the treatment of catalpol. The results of elevated serum insulin level were the same as Yan et al. but different from those of Bao et al. whose study showed decreased serum insulin level [11, 19]. This may be method different to inducing diabetes, db/db mice were used in their research, but in this case STZ-induced mice. Moreover, in our study, the results of pancreatic HE showed that the 30-day catalpol treatment had brought the pancreas of diabetic mice near normal, which indicated that catalpol might have effects to repair the pancreas. In one word, catalpol might increase the absorption of glucose by improving insulin secretion and insulin sensitivity.

In addition, we evaluated the effect of catalpol on lipid metabolism in diabetic mice. The lipid metabolism disorder is an important factor in diabetes with many diabetics accompanied by high serum lipid. Studies have shown that hyperlipidemia has adverse effects on blood glucose homeostasis [20]. As a result, a drop in blood lipid may be beneficial in controlling blood glucose. In this study, catalpol significantly reduced the levels of TC and TG in diabetic mice induced by STZ, indicating that catalpol may improve lipid metabolism in diabetic mice.

The IRS protein mediates insulin-metabolic activity, and many studies have shown that insulin signaling and the loss of the IRS gene are associated with the development of diabetes [8]. In our study, compared with the normal group, the protein levels of IRS and GLUT4 in the liver and muscle lowered in diabetic mice induced by STZ and high-fat diet. And, under catalpol treatment of different doses, the protein levels of IRS and GLUT4 in the liver and muscle rose. Therefore, we believe that catalpol may reduce blood glucose of diabetic mice by regulating the insulin signaling pathway which involved IRS1.

In conclusion, the hypoglycemic effect of catalpol may be related to insulin signaling pathway. Current researches show that long-term treatment of catalpol may lower blood glucose, promote insulin secretion, and strengthen the glucose tolerance in diabetic mice induced by STZ and high-fat diet. In addition, we found that the hypoglycemic effect of catalpol in this study may be achieved by raising the expression level of IRS1 and GLUT4 in the liver and the muscles.

**Funding** The authors would like to extend their sincere appreciation to the Deanship of Wenzhou Medical University for its funding of this research through the 2015 Zhejiang Public Interest Technology Application Research Project No. 2015C37099.

## Compliance with ethical standards

**Animal ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Eighteen to 25 g C57BL/6 mice were supplied by Shanghai Laboratory Animal Center, China Academy of Sciences (Shanghai, China). All the mice were maintained in Laboratory Animal Center of Whenzhou Medical University (Zhejiang, China). The mice had free access to water and a standard diet and were kept in a 12-h dark/light room with temperature at 23–25 °C and humidity at 53–57%.

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

**Ethical approval** The experiment was approved by the laboratory animal ethics committee of Wenzhou Medical University.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):535–546
https://doi.org/10.1007/s13410-018-0689-z

ORIGINAL ARTICLE

# Effect of whole-grain plant-based diet on the diabetes mellitus type 2 features in newly diagnosed patients: a pilot study

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Received: 7 May 2018 / Accepted: 21 August 2018 / Published online: 13 September 2018


**Abstract**
Fifteen diabetic patients, with a duration of 4.9 months with diabetes and an average age of 49.6 ± 8.6 years, participated in a pilot study for 12 weeks to assess the effect of plant-based diet on their diabetes features. Participants were 2 females and 13 males and their baseline measurements were 30.2 ±5.4 kg/m$^2$, 189.2 ± 76.3 mg/dL and 9.4 ± 2.5% for body mass index [BMI], fasting blood glucose [FBG] and HbA1c, respectively. Participant's adherence to the dietary programme on a scale of 0–10 was 8 ± 1.5. Their endpoint BMI, FBG and HbA1c were 28.7 ± 4.3 kg/m$^2$, 102.6 ± 19.8 mg/dL and 6.2 ± 0.8%, respectively. Weight loss was significantly correlated with baseline body weight, baseline BMI and duration in diabetes, while baseline fasting blood glucose was correlated with baseline and endpoint HbA1c [$p < 0.01$]. However, it was not significantly correlated with endpoint body weight [$p < 0.08$] and BMI [$p < 0.018$]. This study has shown that management of body parameters [weight and BMI] through diet such as plant-based diet has resulted in significant reduction in diabetes parameters such as FBG and HbA1c. A larger number of participants are recommended in similar type of studies to have solid conclusions.

**Keywords** Plant-based diet · Diabetes · Food frequency · Weight management · Risk factor

## Introduction

Modern lifestyle is characterised by a plethora of pros and cons started with the easiness and convenience to get food and high reliance on technology in almost everything. As a result of that, physical inactivity and obesity have become predominant. Chronic diseases such as cardiovascular diseases, cancer and diabetes, which are another face of modern life, are affecting every house in the developed society.

In Palestine, chronic diseases became the major cause of death largely due to a shift in lifestyle from traditional to Western type of life. Palestinians became more reliable on convenient food from restaurants and supermarkets. Palestinians are spending more than a one third of their income on food items such as meat and cereal products [1]. It was reported that the prevalence of diabetes mellitus in Palestine was 9.7% in 2000, increasing to 15.3% by 2010 with a forecast to reach 20.8% by 2020 and 23.4% by 2030 [2]. The primary medical treatment of diabetes is based on medically agreed protocols started with glucose regulators then in advanced cases, insulin injection might be required and in most cases, medications of blood pressure are prescribed. It is very often that physicians flout the potential values of good nutrition and promptly prescribe medications instead of giving patients a chance to cope with their disease through healthy eating and active living.

Changes in lifestyle of diabetics including type of diet are more effective than medications alone not only to establish healthy glucose homeostasis but also to decrease mortality due to diabetes that paradoxically increased with medication while normal glycosylated hemoglobin levels have been achieved [3].

Specific etiology for DM is not known [4]. There is a wide range of risk factors including non-modifiable factors such as ethnicity, race, familial aggregation, genetic susceptibility, age and gender, whereas obesity, percentage of body fat, and the region of distribution, lack of physical activity, diet, smoking, alcohol abuse, changing lifestyle [urbanisation], gestational diabetes and low birth weight are considered modifiable factors [5, 6].

Patients with obesity, high percentage of body fat and especially abdominal fat are of high risks for diabetes [7, 8]. Also, abdominal

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obesity [visceral fat] with lower BMI has a risk. High calorie intake is related to obesity and diabetes [9]. Comorbidities associated with obesity are well documented and prevention of obesity has eliminated a great risk for type 2 diabetes incidence [10]. In a cohort study, an increases of 1 kg in weight increase risk of having diabetes by 4.5% [11], on the other hand, a prospective study for 23 years involving a million individuals, has shown that weight loss has eliminated risk of type 2 diabetes [12]. BMI is associated with risk for type 2 diabetes. However, it differs between black and white Americans due to fat distribution. In this context, high visceral fat was the main player affecting incidence of diabetes regardless of the BMI of an individual [13, 14].

On the other hand, diet was reported to be a major risk factor in developing diabetes qualitatively and quantitatively. Sugar-sweetened beverages increase obesity and further may be associated with diabetes [15]. Consuming fructose-sweetened beverages will increase visceral adiposity leading to decrease in insulin sensitivity and high glucose levels [16]. Increased animal fat intake, edible oil, added sweetener as fructose corn syrup and animal source of food are factors related to obesity and increase risk for type 2 diabetes [17]. Consumption of red meat and processed red meat was considered as a risk factor for the development of type 2 diabetes [18]. Many evidences showed that processed meat increased the risk of diabetes [19–21]. For example, a meta-analysis of cohort studies found that red and processed meat accompanied by high intake was associated with diabetes [18]. It was reported that iron from meat is a high-risk factor [22]. In a cohort study, high heme iron intake increased the risk of type 2 diabetes [23]. Heme iron, which is provided by animal sources, is associated with such a high risk while non-heme dietary iron [from plant sources] is not a risk factor.

Whole-food, plant-based, low-fat diet is the diet that is characterised by high content of plant foods in their whole form, such as vegetables, fruits and legumes and lower amounts of seeds and nuts. For maximal health benefits, this diet limits animal products in all forms with more restriction on processed red meat. Total fat such as pressed and purified vegetable oil is also restricted [24]. Low-fat plant-based diets as planned by a clinical trial usually contain 10% of energy from fat, 15% protein and 75% carbohydrate which include vegetables, fruit, grains and legumes and avoidance of meat, dairy products and eggs, added oils, fried products, avocadoes, nuts and seeds [25].

The aim of this study was to assess the effect of plant-based diet (PBD) on FBG, HbA1c and body anthropometry of diabetic patients.

## Materials and methods

This study was conducted in the city of Nablus, Palestine, during the period April 2017 to February 2018. All patients were randomly recruited and enrolled a 12-week dietary programme to assess the effect of plant-based diet on their diabetes parameters. A flow chart representing the trial procedures is shown in Fig. 1.

### Subjects

Participants who were eligible to enter the study were from both genders. All patients were confirmed to be diabetic by a physician diagnosis accompanied by results of their blood analysis. Exclusion criteria were as follows: pregnancy, having medication for diabetes or its complications or following a diet plan for any reason [i.e. weight loss plan]. None of the patients has received any kind of incentive for their participation. Fifteen patients were eligible and have been recruited in this open randomised clinical trial.

### Measurements

Anthropometric measurements of all patients were taken and body mass index [BMI = person's weight in kilograms [kg] divided by his or her height in meters squared] was calculated at the beginning of the study and at the end.

Baseline fasting blood glucose and HbA1c were filed for each patient and after 12 weeks.

### Food frequency questionnaire

A food frequency questionnaire containing ten questions was filled by all patients prior to the start of the trial. Such a questionnaire comprised of three parts; part 1 included personal information, part 2 health history and part 3 food items that have been frequently consumed in the last 3 months. Food categories mentioned in the questionnaire were based on modified Mediterranean diet [MMD] as a reference and contained the following food items: fruits, vegetables, legumes, red meat, white meat, dairy products, fish, cereals and refined cereal products, processed meat, canned vegetables, nuts, soft drinks, juices and sweetened beverages, tea, coffee, traditional and non-traditional sweet.

Frequency of food intake ranged from more than once a day to none within 3 months. Quantities of each food item were not recorded and food frequency questionnaire (FFQ) was based on qualitative frequency, i.e. the number of intakes of any food item. Scoring of patient's food intake was based on closeness to MMD [Table 1].

### Dietary programme

All participants were interviewed using a face-to-face counselling method. Dietary programme was explained by the researcher. Then, a list of allowed food items and not-allowed

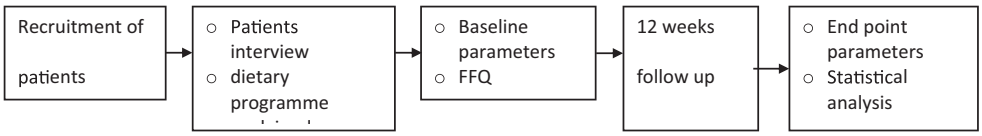


**Fig. 1** Flow chart of the study procedures and stages

food items was given to each patient [Table 2]. All participants were given no restriction to eat from the allowed list [ad libtum]; hence, daily energy intake and other nutrients requirements were not calculated. The participants were advised to maintain their lifestyle apart from diet as before the trial with no extra physical activity or unusual change of their habits.

## Programme compliance and follow-up

During the period of the trial, all patients were followed up and a weekly phone call was made for further counselling and to assess their adherence to the dietary programme. A scoring system was placed to assess such an adherence with a scale from 0 to 10. If a participant fully adhered to the programme for a week, a 10-score adherence level was given. For any intake of the not-allowed list, 1 point was subtracted of 10. For example, if the patient ate red meat once a week and had cheese twice a week, this will add up to 3 points then the patient's score will be 10–3 = 7, for the corresponding week. The average of 12 scores for each patient was calculated. During the follow-up period, patients were also advised not to take any additional supplements or unusual herbal infusion that may interfere with their glucose level.

After the assigned period, all participants were asked a question about their willingness to carry on the programme by their own.

## Statistical analysis

The main endpoints that were determined by this study were fasting blood glucose and HbA1c of the tested group and their correlation with diet. Therefore, different correlations were conducted to (1) determine if there were correlations between baseline and endpoint levels with dietary programme and (2) determine if adherence to the dietary programme was associated with the changes in the endpoints. Another outcome to be evaluated was the change in BMI as a secondary endpoint and if change in BMI was related to dietary programme. Analysis of variance, correlations between variables and correlations between main outcomes (the Wilcoxon sign) were applied using SSPS™ [2013] (IBM SPSS Statistics for Windows, Version 21.0) used for statistical analysis [26].

**Table 1** Scoring methodology of frequently food intake by the participants. Scoring was based on closeness to MMD

| Food item | > once a day | 1–3 a week | Once every 2 weeks | Once a month | Once every 3 months | Not at all |
|---|---|---|---|---|---|---|
| Vegetables | 1 | 0 | − 1 | − 1 | − 1 | − 1 |
| Fruits | 1 | 0 | − 1 | − 1 | − 1 | − 1 |
| Legumes | 1 | 0 | − 1 | − 1 | − 1 | − 1 |
| Red meat | − 1 | | 1 | 1 | 1 | 1 |
| White meat | − 1 | | 1 | 1 | 1 | 1 |
| Fish | 1 | | 0 | − 1 | − 1 | − 1 |
| Dairy products | − 1 | 0 | 1 | − 1 | − 1 | − 1 |
| Refined wheat products, rice, maise etc. | − 1 | 0 | 1 | − 1 | − 1 | − 1 |
| Processed meat−1 | − 1 | | | 0 | 1 | 1 |
| Canned veg. | − 1 | | 0 | 1 | 1 | 1 |
| Nuts | 1 | | 0 | − 1 | − 1 | − 1 |
| Soft drinks | − 1 | | 0 | 1 | 1 | 1 |
| Juices | − 1 | | 0 | 1 | 1 | 1 |
| Tea with sugar | − 1 | | 0 | 1 | 1 | 1 |
| Coffee with sugar | − 1 | | 0 | 1 | 1 | 1 |
| Sweets (variable) | − 1 | | 0 | 1 | 1 | 1 |

**Table 2** List of food categories that are allowed and that are prohibited in the plant-based diet

| Allowed food list | Prohibited food list |
|---|---|
| Dried legumes, beans, peas, chickpeas etc. | Meat (beef, lamb, poultry etc.) |
| Whole grains, oat, barley, brown rice etc. | Dairy products (milk, butter, cheese etc.) |
| Whole-wheat products or fragments | Extracted vegetable oil (olive, soy, corn oils) and margarine from plant, ghee from animal source |
| Green leaves, lettuce, spinach, rockets, radish, onions, garlic etc. | Processed foods such as canned, highly refined products |
| Potatoes and sweet potatoes | Sugary and sweetened drinks (soft and juices) |
| Fresh fruits or their fresh unsweetened juices | White flour pastries, white bread |
| Fresh vegetables, carrots, cucumber, cauliflower etc. | |
| Nuts | |
| Any mixture of the abovementioned items | Any mixture of the abovementioned items |

## Results

### Demographic, physical and clinical criteria of participants

Participants were from both sexes, with male representing 86.7% of the total patients [Table 3]. The average age of the participants was 49.6 ± 8.6 years with a range from 31 to 66 years. The average of BMI of all participants was 30.2 ± 5.2 kg/m$^2$ with a range from 23.4 to 38.2; however, only 3 [20%] out of 15 had normal BMI while 5 [33%] out of 15 were overweight and the rest 47% were obese. All patients were with diabetes for 1 year or less with an average of 4.9 ± 3.8 months and a range of 1 to 12 months. Their initial fasting blood glucose was 189.2 ± 77.1 mg/dL with a range from 95 to 340 mg/dL. They were distributed as follows: 6% < 110, 26.7% from 110 to 120 and 66.7% > 120. Similarly, measurements of HbA1c showed that the average of this group was 9.4 ± 2.5% with a range from 6.4 to 14%.

Other clinical parameters showed that 3 [20%] out of 15 of the patients have had other illness in addition to diabetes with only 2 [13.3%] of them on medication for these diseases.

Moreover, 9 [60%] out 15 of the patients have declared that a close member of the family [parents and parents' brothers and sisters or the patients' brothers or sisters] has diabetes.

### Qualitative food frequency

Data collected from food frequency questionnaire showed that all participants have negative scores. The average score on MMD for this group was − 6.4 ± 2.17 with a range between − 1 to − 10. Only 2 and 4 out of 15 participants have scored

**Table 3** Demographic, physical and clinical status of participants

| | Gender | Age | Weight (kg) | Height (cm) | BMI | Time since diagnosed (m) | Diabetic Relative | Other disease | On medication | FBG mg/dL | HbAc1% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 50 | 104 | 165 | 38.2 | 3 | Yes | No | No | 140 | 10.6 |
| 2 | F | 31 | 75 | 170 | 25.1 | 9 | No | No | No | 270 | 14 |
| 3 | M | 40 | 112 | 173 | 37.4 | 6 | Yes | Yes | Yes | 115 | 6.7 |
| 4 | M | 41 | 85 | 185 | 24.8 | 3 | Yes | No | No | 270 | 12 |
| 5 | F | 50 | 93 | 161 | 35.9 | 1 | No | No | No | 95 | 7 |
| 6 | M | 56 | 78 | 178 | 24.6 | 3 | No | No | No | 250 | 11 |
| 7 | M | 49 | 80 | 176 | 25.8 | 1 | Yes | No | No | 118 | 7 |
| 8 | M | 66 | 76 | 174 | 25.1 | 1 | No | No | No | 157 | 7.2 |
| 9 | M | 51 | 95 | 175 | 31.0 | 3 | Yes | Yes | Yes | 235 | 12.4 |
| 10 | M | 56 | 88 | 165 | 32.3 | 6 | No | No | No | 208 | 9.3 |
| 11 | M | 45 | 75 | 165 | 27.6 | 1 | Yes | Yes | No | 155 | 7 |
| 12 | M | 53 | 95 | 173 | 31.7 | 7 | Yes | No | No | 110 | 6.4 |
| 13 | M | 58 | 70 | 173 | 23.4 | 6 | Yes | No | No | 250 | 10.8 |
| 14 | M | 49 | 110 | 170 | 38.1 | 12 | Yes | No | No | 115 | 7.2 |
| 15 | M | 49 | 91 | 176 | 29.4 | 12 | No | No | No | 340 | 12.3 |

positive intake on a daily basis for vegetables and fruits, respectively, while none of participants has consumed any type of legumes on a daily basis. On the other hand, the main negative scoring contributors in their food intake, which showed common trends in all participants, were meat [red and white] 13 out of 15; dairy products 14 out of 15; white bread 11 out of 15; and soft drinks 12 out of 15, while juices, tea and coffee [with sugar] and sweets were 13, 15 and 9 out of 15, respectively. These food items were taken on regular basis and sometimes more than once a day. It is worth to mention that participants showed positive scores in two food items; processed meat and canned food with 5 out of 15 and 1 out of 15, respectively, had consumed it on regular basis [Fig. 2].

## Compliance with dietary programme

The results of participant's adherence to the dietary programme on a scale of 0–10 are shown in Fig. 3. The average score of participants was 8 ± 1.5 with a range between 5 and 10.

## Effect of the dietary programme on the diabetes parameters

### Fasting blood glucose

Measurements of baseline and endpoint fasting blood glucose are shown in Fig. 4. The average FBG at baseline was 189.2 ± 77.1 mg/dL, while endpoint average FBG was 102.6 ± 19.8 mg/dL. The total average reduction was 84.6 mg/dL which corresponds to 45% reduction in blood glucose from the baseline.

### HbA1c %

Measurements of baseline HbA1c and endpoint HbA1c are shown in Fig. 5. The average value for the group at baseline was 9.4 ± 2.5% while at the endpoint was 6.2 ± 0.8%. This means a total reduction in averaged HbA1c equals to 3.3% which corresponds to 35%.

## Effect of dietary programme on body measurements

### Body weight

The baseline values of individual body weight and at the endpoint are shown in Fig. 6. All participants have shown a reduction in body weight with an average of 4.6 ± 3.9 kg; however, the range was from 1 to 14 kg.

### Body mass index

The baseline and endpoint body mass index of all participants are shown in Fig. 7. There was a reduction in BMI for all participants and the group averaged 28.7 ± 4.3 kg/m$^2$ with a range from 23.1 to 34.9. The total reduction in BMI was 1.5. Moreover, the percentage of normal body weight category has increased from 20 to 33% and all overweight, obese and severe obese BMI have been shifted down to some extent.

## Correlations between baseline and endpoint variables

Sixteen variables [in both baseline and endpoint] were measured and analysed statistically by finding the Pearson square and two-tailed significant differences [Table 4].

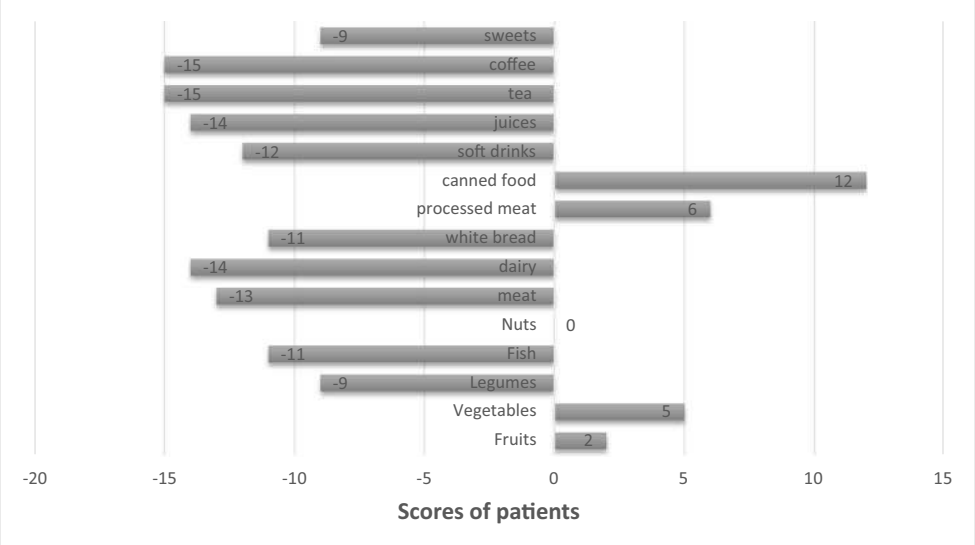


**Fig. 2** Accumulative scores of frequent food consumed by participants over a 12-week period before the beginning of the trial. Based on Table 3, positive scores represent healthier lifestyle either by consuming good food items or avoiding bad food items. While negative scores represent non-healthy lifestyle by eating bad food items or avoiding good food items

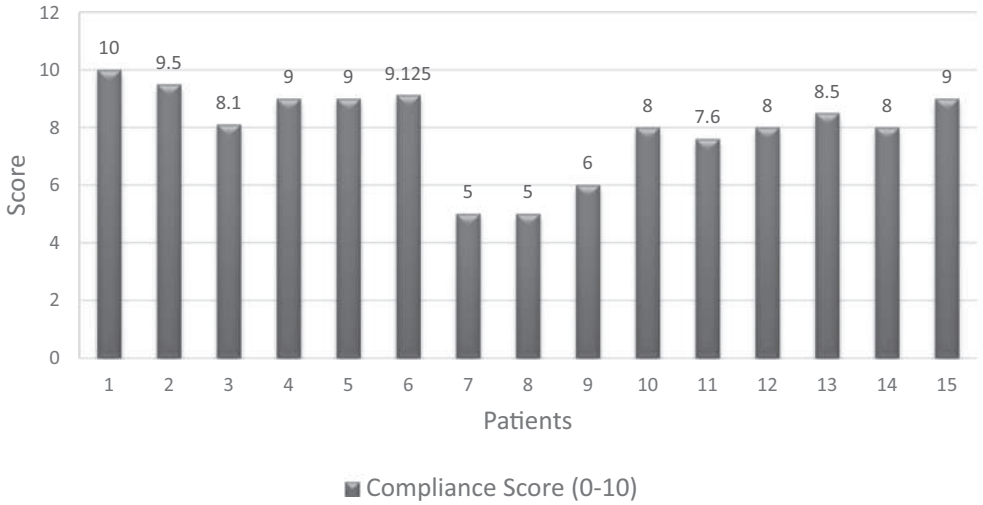


**Fig. 3** Compliance score to the dietary programme by participants over a 12-week period. Each point represents the average of 12 weeks of a weekly recorded score on a scale 0–10 achieved by one patient

Significant differences on two levels of $p$ value [$< 0.01$ and 0.05] were denoted with double asterisks and single asterisk, respectively.

Baseline body weight in a negative manner was significantly correlated with endpoint weight, endpoint BMI and weight loss. Baseline BMI was significantly correlated with baseline fasting blood glucose [$p < 0.05$] and significantly correlated with endpoints body weight, weight loss and BMI. Duration that patient has been with diabetes was correlated only with weight loss [$p < 0.05$]. Baseline fasting blood glucose was significantly correlated with baseline and endpoint HbA1c [$p < 0.01$]; however, it was not significantly correlated with endpoint body weight [$p < 0.08$] and BMI. Baseline HbA1c was significantly correlated with endpoint HbA1c [$p < 0.01$]. Also, using the Wilcoxon sign, it was found that endpoint of weight, BMI, HbA1c and FBG were significantly less and correlated ($Z = -3.4$ and $p = 0.001$) with baseline weight, BMI, HbA1c and FBG, respectively [Table 5].

Total score of qualitative food frequency and compliance were not significantly correlated with any of the variables. Endpoint fasting blood glucose was significantly correlated with baseline fasting blood glucose [$p < 0.01$] and endpoint HbA1c [$p < 0.001$].

It was found that gender and age were not correlated with any of the variables.

## Discussion

To our knowledge, this is the first study of its kind in Palestine addressing both dietary intervention and diabetes. Participants have been involved in an open uncontrolled trial. Although the study was conducted in a small geographic area, i.e. Nablus city, the study can be further expanded to include more participants and geographical regions to draw solid conclusions.

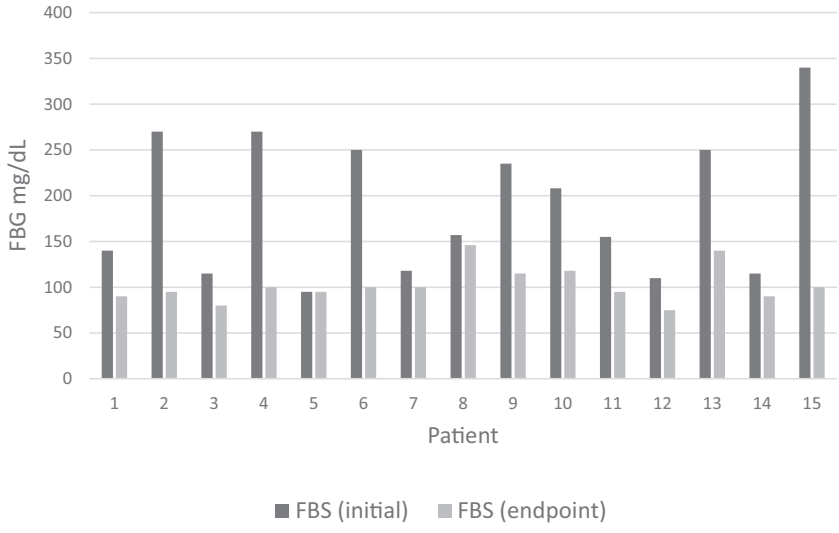


**Fig. 4** Baseline fasting blood glucose (mg/dL) and fasting blood glucose at the end of the trial. Each bar represents a participant, who enrolled a 3-month dietary programme

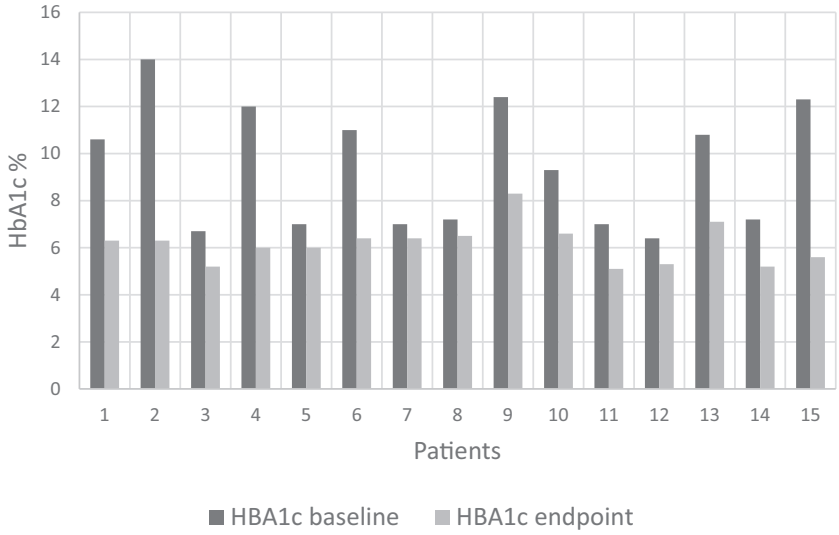


**Fig. 5** Baseline HbA1C (%) and HbA1C at the end of the trial. Each bar represents a participant who enrolled a 3-month plant-based dietary programme

The dietary programme investigated in this study was also practised in many institutions and was recommended as a safe approach. Plant-based diet is a lifestyle for millions of people around the world; moreover, in our area, the Mediterranean diet which was prevalent few decades ago is another version of plant-based, whole-plant diet with a limited consumption of red meat and dairy products. The participants clearly shifted their lifestyle to the Western type of lifestyle with increasing consumption of meat and meat products, refined carbohydrates and sugars with very limited consumption of fruits and vegetables. Based on the United Nations Food and Agriculture Organization data, this change has been especially drastic in Asian countries. More meat and meat products with refined grain products have increased remarkably. This also has increased availability of fast foods which backed the unhealthful diets with high calorie content of sugary beverages and unhealthy fats [15].

In this study, changing the lifestyle towards less risk factors and healthier factors has its impact on diabetes features such as reduction in fasting blood glucose by 45% and HbA1c by 35%. Such reductions can shift diabetic person to be non-diabetic by definition. Another important risk factor that significantly has been affected by the diet was endpoint body weight. This also has affected the endpoint BMI. Participants have lost 4.5 kg on an average but very obese and obese participants have lost more, in a way dropped their BMI sometimes by 7.7%. High BMI [33.5 kg/m$^2$] amongst Palestinian diabetics was also reported by Abu-Halaweh et al. [27]. This result is in agreement with many researches which showed that obesity and weight gain are risk factors of diabetes; moreover, an increase of body weight above normal by 1 kg was associated with 4.5% increase in diabetes incidence. In this trial, an average 1 kg loss of weight has resulted in decrease in average FBG equals 42 mg/dL and 0.7% of

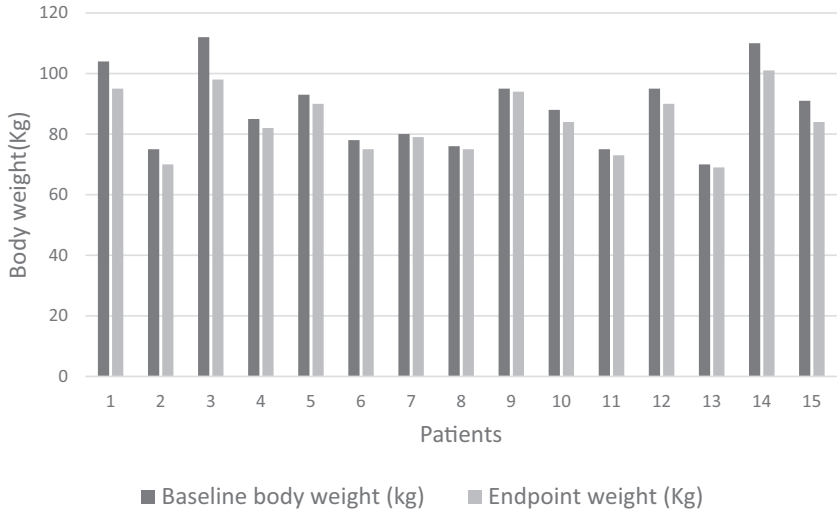


**Fig. 6** Distribution of baseline and endpoint body weight (kg) of participants who involved in 12-week plant-based diet programme. Each bar represents one patient

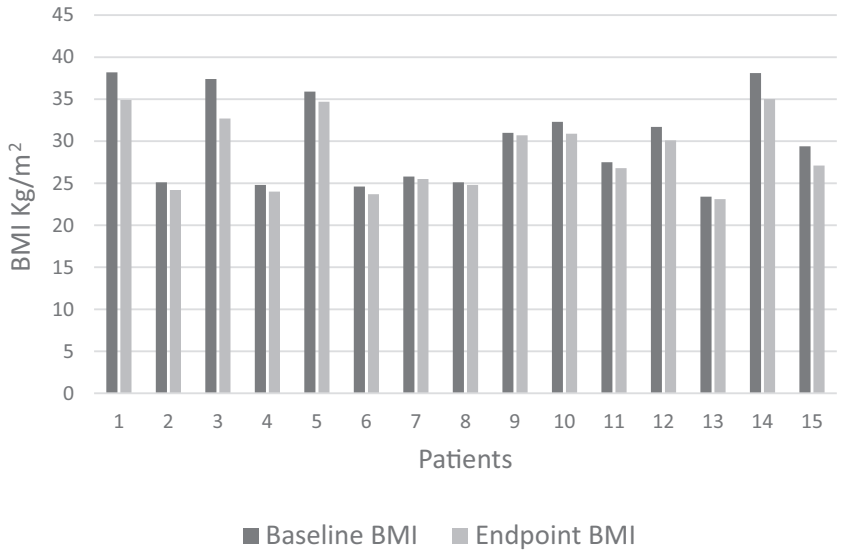


**Fig. 7** Distribution of baseline and endpoint body mass index (BMI, kg/m$^2$) of the participants before and after a 12-week plant-based diet programme. Each bar represents one patient

HbA1c. Similar trend was found by McDougall et al., who reported a 17-mg/dL drop in FBG for 1.7 kg loss body weight in 7-day trial [3]. A meta-analysis systematic review [28] which included six studies found that participants of vegetarian diet (very similar to PBD) had decreases in energy (− 139.8 kcal), protein (− 6.4% energy), total fat (− 11.6% energy) and cholesterol intakes (− 172.5 mg), along with increases in carbohydrate (13.8% energy) and fibre intakes (7.0 g). Such changes can contribute positively to weight management.

Some participants who had normal baseline body weight and have not got changes in their BMI may have their fat distribution changed; however, this was not measured by this study. Other studies have reported that fat distribution especially abdominal fat poses a risk factor for diabetes. All participants showed good level of adherence to dietary programme; however, the level of adherence was not correlated with either level of weight loss or blood parameters, and this can be explained by the fact that other factors, not studied here [either genetic or environmental], may play a complementary effect as diabetes is a multifactorial disease. As the prevalence of obesity in the Palestinian population is worrying and will reach 35% in 2020, this study has its significance in treatment and prevention of diabetes. If obesity declined by 1% every year for 10 years, a 5.3% reduction in diabetes prevalence can be achieved [2]. If obesity prevalence was reduced by 35% in 10 years, as suggested by the WHO, diabetes prevalence might be decreased by 20%. Lifestyle intervention that restricts calories intake and enhances weight loss was reported to significantly reduce transformation of high-risk patients to diabetes by 58% [15].

HbA1c represents average blood glucose over a period of 2–3 months in a single measure which can be performed without having special preparation such as fasting. HbA1c, although widely used as a tool to assess hyperglycaemia, its threshold was variable amongst studies. The broadly used threshold was 6.3% which represents the average endpoint HbA1c in this study. Such a result is considered a huge benefit for diabetic patients if one has to consider that most Palestinian patients with HbA1c 9.4% will suffer from macrovascular complications, including myocardial infarction and/or stroke and who have 9.9% will be suffering from microvascular complications [27]. Roughly, in this study, for every 1% drop in HbA1c, there was a 26-mg/dL drop in FBG. And as a consequence, insulin resistance will be improved.

Similar trends have been reported by other researchers [29]. They conducted a 74-week intervention trial, with a low-fat vegan diet, without energy restriction. Their results showed that weight loss and improved FBG, TG and LDL cholesterol in the low-fat vegan diet were more than a conventional diet.

Insulin resistance is found in patients with type 2 diabetes and also in normal obese and non-obese persons [30]; insulin resistance means that muscle, fat and liver cells do not respond properly to insulin and thus cannot easily uptake glucose from the bloodstream. By administration of intravenous free fatty acids [FFAs] to healthy persons, increased FFAs in plasma cause insulin resistance which proposed the relation between FFA and type 2 diabetes [31]. High intake of lipids and FFA diets cause insulin resistance, which will inhibit glucose transport and phosphorylation in skeletal muscle [32]. A group of normal healthy men without a family history of diabetes were given FFA and challenged with glucose. It was found that FFA cause insulin resistance. In addition, persons with normal glucose level were affected and the glucose absorption delayed after 160 min [33]. Fatty acids reduce glucose transport activity by affecting GLUT4 transporter directly or affecting insulin signalling result in decrease number of GLUT4 transporters and by altering their location towards plasma membrane. Fatty acid metabolites, fatty acetyl CoAs,

**Table 4** Correlations between baseline and endpoints of blood and body parameters with lifestyle (total score before trial and compliance to the dietary programme) of intervention group who enrolled a 12-week plant-based diet

| | | Baseline wt. | Baseline BMI | Interval on diabetes (M) | baseline fast blood sugar | Baseline HbA1c | Total Score | Compliance | Endpoint FBS | Endpoint HbA1c | Endpoint wt. | Endpoint BMI | Wt. loss | Sex | Age |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Baseline wt. | The Pearson correlation | 1 | 0.921** | 0.337 | −0.430 | −0.270 | 0.346 | 0.180 | −0.155 | −0.204 | 0.976** | 0.869** | 0.799** | −0.138 | −0.185 |
| | Sig. (2-tailed) | | 0.000 | 0.219 | 0.110 | 0.330 | 0.207 | 0.520 | 0.581 | 0.466 | 0.000 | 0.000 | 0.000 | 0.624 | 0.510 |
| Baseline BMI | The Pearson correlation | 0.921** | 1 | 0.226 | −0.568* | −0.377 | 0.280 | 0.231 | −0.218 | −0.275 | 0.905** | 0.983** | 0.718** | 0.033 | −0.109 |
| | Sig. (2-tailed) | 0.000 | | 0.419 | 0.027 | 0.166 | 0.313 | 0.407 | 0.435 | 0.321 | 0.000 | 0.000 | 0.003 | 0.906 | 0.699 |
| Interval on diabetes (M) | The Pearson correlation | 0.337 | 0.226 | 1 | 0.335 | 0.266 | −0.152 | 0.380 | 0.404 | 0.160 | 0.236 | 0.141 | 0.527* | 0.007 | −0.264 |
| | Sig. (2-tailed) | 0.219 | 0.419 | | 0.222 | 0.338 | 0.590 | 0.162 | 0.136 | 0.569 | 0.397 | 0.615 | 0.044 | 0.980 | 0.342 |
| Baseline fast blood sugar | The Pearson correlation | −0.430 | −0.568* | 0.335 | 1 | 0.875** | −0.056 | 0.295 | 0.637* | 0.703** | −0.465 | −0.601* | −0.220 | −0.032 | −0.124 |
| | Sig. (2-tailed) | 0.110 | 0.027 | 0.222 | | 0.000 | 0.842 | 0.285 | 0.011 | 0.003 | 0.081 | 0.018 | 0.430 | 0.910 | 0.660 |
| Baseline HbAc1 | Pearson Correlation | −0.270 | −0.377 | 0.266 | 0.875** | 1 | 0.062 | 0.419 | 0.361 | 0.643** | −0.294 | −0.391 | −0.131 | 0.173 | −0.301 |
| | Sig. (2-tailed) | 0.330 | 0.166 | 0.338 | 0.000 | | 0.827 | 0.120 | 0.186 | 0.010 | 0.287 | 0.149 | 0.641 | 0.538 | 0.276 |
| Total score | Pearson Correlation | 0.346 | 0.280 | −0.152 | −0.056 | 0.062 | 1 | −0.376 | 0.129 | 0.404 | 0.421 | 0.316 | 0.045 | −0.206 | −0.168 |
| | Sig. (2-tailed) | 0.207 | 0.313 | 0.590 | 0.842 | 0.827 | | 0.167 | 0.648 | 0.135 | 0.118 | 0.251 | 0.872 | 0.461 | 0.549 |
| Compliance | The Pearson correlation | 0.180 | 0.231 | 0.380 | 0.295 | 0.419 | −0.376 | 1 | 0.023 | −0.092 | 0.068 | 0.163 | 0.441 | 0.334 | −0.413 |
| | Sig. (2-tailed) | 0.520 | 0.407 | 0.162 | 0.285 | 0.120 | 0.167 | | 0.936 | 0.745 | 0.810 | 0.562 | 0.100 | 0.224 | 0.126 |
| Endpoint FBS | The Pearson correlation | −0.155 | −0.218 | 0.404 | 0.637* | 0.361 | 0.129 | 0.023 | 1 | 0.764** | −0.180 | −0.250 | −0.045 | −0.155 | 0.204 |
| | Sig. (2-tailed) | 0.581 | 0.435 | 0.136 | 0.011 | 0.186 | 0.648 | 0.936 | | 0.001 | 0.521 | 0.369 | 0.873 | 0.582 | 0.466 |
| Endpoint HbA1c | The Pearson correlation | −0.204 | −0.275 | 0.160 | 0.703** | 0.643** | 0.404 | −0.092 | 0.764** | 1 | −0.149 | −0.244 | −0.299 | −0.082 | 0.238 |
| | Sig. (2-tailed) | 0.466 | 0.321 | 0.569 | 0.003 | 0.010 | 0.135 | 0.745 | 0.001 | | 0.595 | 0.381 | 0.278 | 0.771 | 0.394 |
| Endpoint wt. | The Pearson correlation | 0.976** | 0.905** | 0.236 | −0.465 | −0.294 | 0.421 | 0.068 | −0.180 | −0.149 | 1 | 0.889** | 0.649** | −0.154 | −0.085 |
| | Sig. (2-tailed) | 0.000 | 0.000 | 0.397 | 0.081 | 0.287 | 0.118 | 0.810 | 0.521 | 0.595 | | 0.000 | 0.009 | 0.584 | 0.762 |
| Endpoint BMI | The Pearson correlation | 0.869** | 0.983** | 0.141 | −0.601* | −0.391 | 0.316 | 0.163 | −0.250 | −0.244 | 0.889** | 1 | 0.584* | 0.086 | −0.048 |
| | Sig. (2-tailed) | 0.000 | 0.000 | 0.615 | 0.018 | 0.149 | 0.251 | 0.562 | 0.369 | 0.381 | 0.000 | | 0.022 | 0.760 | 0.864 |
| Wt. loss | The Pearson correlation | 0.799** | 0.718** | 0.527* | −0.220 | −0.131 | 0.045 | 0.441 | −0.045 | −0.299 | 0.649** | 0.584* | 1 | −0.057 | −0.409 |
| | Sig. (2-tailed) | 0.000 | 0.003 | 0.044 | 0.430 | 0.641 | 0.872 | 0.100 | 0.873 | 0.278 | 0.009 | 0.022 | | 0.839 | 0.131 |
| Sex | The Pearson correlation | −0.138 | 0.033 | 0.007 | −0.032 | 0.173 | −0.206 | 0.334 | −0.155 | −0.082 | −0.154 | 0.086 | −0.057 | 1 | −0.444 |
| | Sig. (2-tailed) | 0.624 | 0.906 | 0.980 | 0.910 | 0.538 | 0.461 | 0.224 | 0.582 | 0.771 | 0.584 | 0.760 | 0.839 | | 0.097 |

**Table 4** (continued)

| | | Baseline wt. | Baseline BMI | Interval on diabetes (M) | baseline fast blood sugar | Baseline HbA1c | Total Score | Compliance | Endpoint FBS | Endpoint HbA1c | Endpoint wt. | Endpoint BMI | Wt. loss | Sex | Age |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | The Pearson Correlation | − 0.185 | − 0.109 | − 0.264 | − 0.124 | − 0.301 | − 0.168 | − 0.413 | 0.204 | 0.238 | − 0.085 | − 0.048 | − 0.409 | − 0.444 | 1 |
| | Sig. (2-tailed) | 0.510 | 0.699 | 0.342 | 0.660 | 0.276 | 0.549 | 0.126 | 0.466 | 0.394 | 0.762 | 0.864 | 0.131 | 0.097 | |

**Table 5** Correlation between endpoints and baselines of paired variables using the Wilcoxon Ranking test

| | Baseline | | Endpoint | | |
|---|---|---|---|---|---|
| | Mean ± S | Median | Mean ± S | Median | *p** |
| Body weight | 88.5 ± 13.1 | 88 | 83.9 ± 10.4 | 84 | 0.001 |
| BMI | 30.2 ± 5.2 | 29.4 | 28.7 ± 4.3 | 27.1 | 0.001 |
| HbA1c | 9.4 ± 2.5 | 9.3 | 6.2 ± 0.8 | 6.3 | 0.001 |
| FBG | 189.2 ± 77.1 | 157 | 102.6 ± 19.8 | 100 | 0.001 |

Wilcoxon asymp. sig. (two-tailed)

diacylglycerol and ceramides affect insulin sensitivity by suppression of its signalling [34]. One can increase glucose uptake by increasing insulin sensitivity via lowering FFA intake [35]. High-fat [HF] diet was found to influence mitochondrial function in skeletal muscle [36]. HF diets increase intramyocellular lipid [IMCL] contents in a short time [37]. On the other hand, concerns about reduction of muscle mass of patients on PBD due to low protein quality was discussed as some essential amino acids can be provided by plant sources and complementary amino acids can be obtained by mixing a range of sources. Therefore, a well-balanced, plant-based diet will provide adequate amounts of essential amino acids and prevent protein deficiency [24].

Diabetes can be viewed as an inflammatory disease. There is increasing evidence that maintained inflammatory status induced by cytokines is closely linked with the generation of insulin resistance and type 2 diabetes mellitus. Researchers have connected T2DM with the presence of inflammatory and immune system biomarkers, including TNF-α, IL-1, IL-6, C-reactive protein [CRP], leptin, adiponectin and resistin [38]. These biomarkers have an adverse impact on the function of beta-cells directly by affecting pancreatic cells or indirectly by prohibiting cells to uptake glucose.

Plant-based diet is one of the healthy regimens that contain a lot of antioxidants, anti-inflammatory, dietary fibre and sources of beneficial microbiota. All of these components, individually or combined, will improve the inflammation status in the body leading to remarkable decrease in insulin resistance.

Another explanation for the improvement seen with low-fat diet with regard to diabetes is that of its effect on gut microbiota. It is well known that diet is a modulating factor of gut microbiota with diet rich in fibre being a good source for energy for a wider microflora community with beneficial metabolic end products such as short-chain fatty acids [SCFA]. SCFAs were reported to increase gene expression to produce peptides related to reduced hunger and appetite.

On the opposite, diet rich in processed meat/high fat will reduce gut microbiota richness and may shift such communities towards gut dysbiosis [39]. Gut dysbiosis eventually will

change bacterial translocation, obesity and weight gain. It was found that gut microbiota may help in fat gain by promoting adipocyte lipogenesis and suppressing fasting-induced adipocyte factor [FIAF] expression. On the other hand, it was reported that *Bifidobacterium adolescentis* negatively associate with HbA1c [40], which may explain glucose lowering effects of diet increasing its level, such as plant-based diet.

Limitations of this study have arisen from the small number of participants partly due to lack of awareness about the effect of diet on diabetes as well as due to the referral process. Increasing the awareness amongst health care providers about benefits of diet as an option for diabetics may help in conducting a wider clinical trial.

## Conclusion

Lifestyle changes including dietary intervention should be considered as first-line prevention for diabetes mellitus; moreover, dietary therapy for diabetes treatment is well documented to be effective alone or in conjunction with medication. Increasing awareness about lifestyle role in the disease progress amongst healthy or diabetic people should also be considered as it showed its efficiency.

Controlling body weight can be easily managed by plant-based diet; this is also an advantage over medical approach alone.

In Palestine where health care system is highly physician-centred and patients may be, mostly, oriented to medications, it is highly recommended to involve dieticians as part of the health care team who will perform counselling and monitor the changes in patient's lifestyle.

**Acknowledgements** The authors would like to thank Hassan Tamimi for his valuable data analysis.

### Compliance with ethical standards

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

**Informed consent** Dietary programme and the study procedures were approved by the Institutional Review Board [IRB], An-Najah National University, number 10 Feb 2017. All patients were interviewed for at least 1 h and given detailed explanation about the dietary programme, and then they signed a written consent before the commencement of the study.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):547–550
https://doi.org/10.1007/s13410-018-0706-2

ORIGINAL ARTICLE

# Impact of calorie restriction on glycemic control in overweight patients with type 2 diabetes mellitus

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Received: 12 July 2018 / Accepted: 3 December 2018 / Published online: 14 December 2018


## Abstract

**Introduction** Calorie restriction is emerging as a tool to achieve remission in type 2 diabetes mellitus. We conducted a clinical trial to assess its effectiveness and durability in our setting.

**Methods** This was a single-arm clinical trial (CTRI/2017/05/008711) conducted at JIPMER hospital, Pondicherry. Nine adult patients with type 2 diabetes mellitus and with a BMI of more than 23 kg/m$^2$ were included into the study from December 2016. They were admitted to the hospital and given a 700 kcal per day balanced diet for 1 week following which either weight maintaining or weight reducing diet was advised. Patients were followed up monthly for the initial 3 months and were reassessed later after 12 months.

**Results** One patient was lost to follow-up and hence results have been provided for eight patients. The mean age was 50.5 years. All patients were males. The duration of diabetes was a median of 2.5 years. All patients tolerated the low calorie diet. The mean weight loss was 4.2 kg after a median of 12 months. The average HbA1c declined from a baseline value of 7.98% to 7.13% after a median of 12 months ($p = 0.27$). Four patients were off any antidiabetic medication at 12 months of follow-up.

**Conclusion** The intervention of a short duration of calorie restriction may be effective in reducing HbA1C in overweight patients with diabetes and the effect can last for a year.

**Keywords** Very low calorie diet · Type 2 diabetes mellitus · Durability

## Introduction

Obesity leads to an insulin resistant state and contributes to the pathogenesis of type 2 diabetes mellitus (DM). Measures to reduce weight mainly include lifestyle modifications like exercise and diet restriction, but the current main treatment modalities (sulfonylurea, insulin) lead to weight gain. Significant improvement in glycemic status and remission has been documented after bariatric surgery [1]. The same has been replicated using calorie restriction. A simultaneous reduction in body weight (15%) and normalisation of blood sugar (with demonstrable improvement in the islet beta cell function) was achieved by reducing the daily calorie intake to 600 kcal for a period of 8 weeks [2]. The suggested mechanism is that the negative calorie balance leads to simultaneous decline in the fat content of the liver and pancreas and hence improving the hepatic insulin sensitivity and beta cell function [3]. This has generated a renewed interest in very low calorie diet as a tool to achieve remission in type 2 DM. The intervention has now been applied at the level of primary care. In a cluster randomised trial involving 306 participants, a weight loss of 15 kg and diabetes remission in 46% of patients has been demonstrated in the intervention group [4]. Such studies are required from India too and only one is available [5]. We have done a clinical trial addressing the efficacy and durability of 1 week of very low calorie diet (VLCD) in overweight individuals with type 2 DM.

## Methods

This was a single-arm clinical trial conducted at JIPMER hospital, Pondicherry. Adult patients with type 2 diabetes mellitus and with a BMI of more than 23 kg/m$^2$ were considered for inclusion into the study. Patients were excluded if they had coronary artery disease, cerebrovascular accident, an eGFR of less than 60 ml/min, an elevation of liver transaminases of more than two times the upper limit of normal, gallstone disease, or required more than 6 units of insulin per day. Nine patients were recruited

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**Table 1** Baseline characteristics ($N = 8$ patients)

| Characteristic | – |
|---|---|
| Mean age (in years) | 50.5 |
| BMI (kg/m$^2$) | 25.26 (IQR 23.88–27.61) |
| Duration of diabetes (in years) | 2.5 (IQR 1.5–6.0) |
| HbA1c (%) | 7.98 ± 2.1 |
| Diabetes treatment | |
| Metformin and sulphonylurea | 75% |
| Metformin alone | 25% |

from December 2016. They were admitted to the hospital and given a 700 kcal per day balanced diet for 1 week. The daily diet consisted of the following: the breakfast consisted of one slice of bread or 50 g of pongal; the lunch had 50 g of rice, 100 g of poriyal (vegetable dish) and salad; the dinner had 50 g of rice and salad. Milk (100 ml) was provided at 6 AM and 10 PM and tea or vegetable soup was given at 11 AM and 4 PM. Blood sugar levels were monitored three times a day. Medications were tailored as required. Sulphonylureas were stopped at the start of low calorie diet. Metformin dose was reduced if fasting plasma glucose levels were normal and stopped if further lower trend was noted. After ensuring that the blood sugar levels were stable, patients were discharged at the end of 1 week. Patients were given a diet plan with the calorie content appropriate for their ideal body weight. Physical activity of 200–300 min/week was also advised. Patients were followed up monthly for the initial 3 months and on each visit, body weight was measured and fasting plasma glucose and post prandial plasma glucose were estimated. Later they continued usual treatment from the clinic and were reassessed after a median period of 12 months. Antidiabetic agents could be restarted if the plasma glucose levels rose on follow-up. Fasting and post prandial glucose values, HbA1C levels and body weight recorded at baseline and after median period of 12 months were compared using paired $t$ test.

## Results

Thirty-two patients were screened and nine patients were recruited to the study. All the participants included received an in-hospital balanced diet containing 700 cal/day for a week. No participant had symptomatic or any documented episode of hypoglycemia. All of them tolerated the low calorie diet

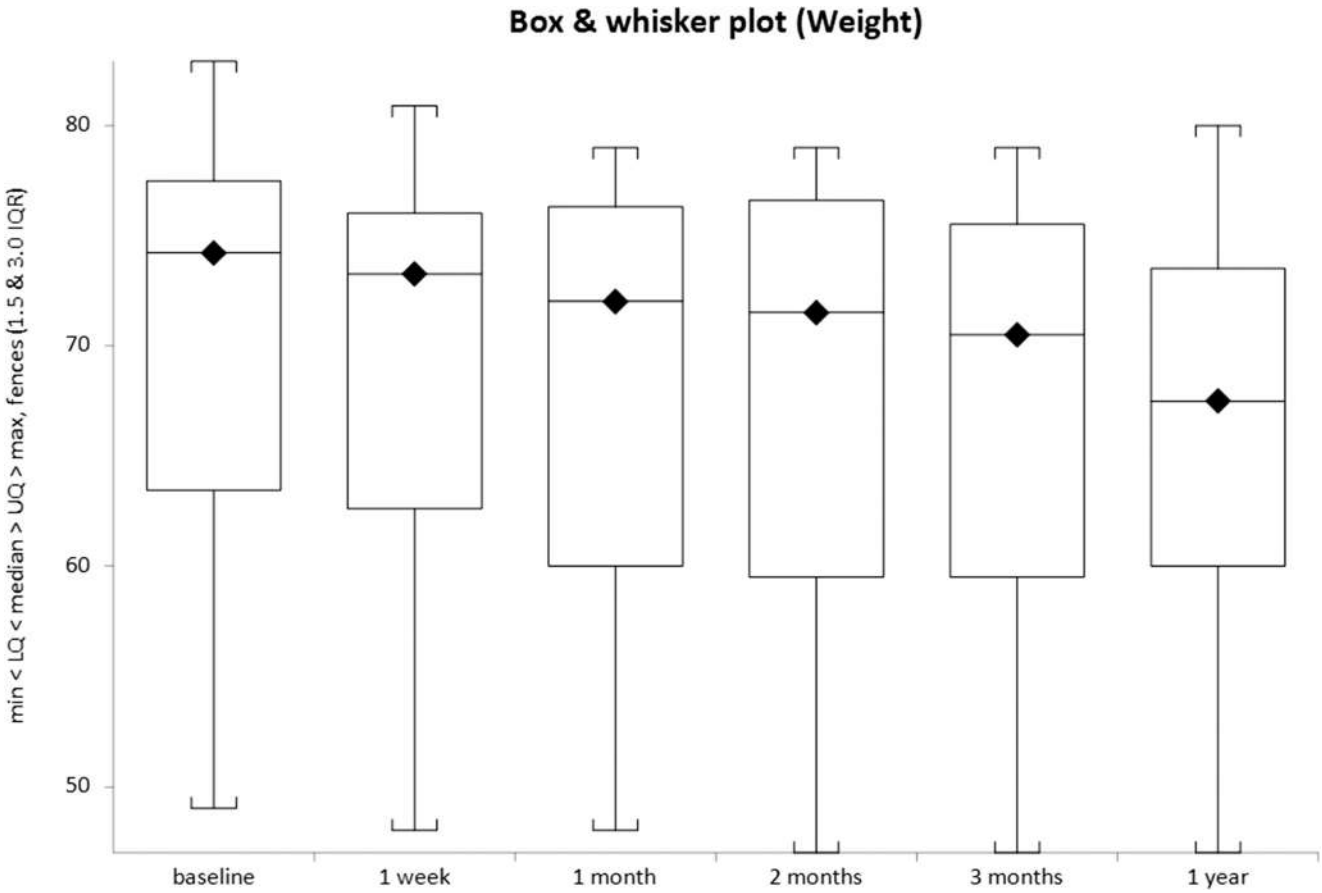


**Fig. 1** Box and Whisker plot depicting the weight change over a period of 1 year. The mean weight loss was 4.2 kg

**Table 2** Change in HbA1c and body weight after 12 months ($N = 8$)

| Characteristic | Baseline | At 12 months | Change (CI) | $p$ value |
|---|---|---|---|---|
| HbA1C (%) | 7.98 | 7.13 | − 0.85 (− 0.65 to 1.99) | 0.27 |
| FPG (mg/dl) | 143.5 | 114.7 | − 28.7 (− 64.85 to 7.35) | 0.10 |
| PPPG (mg/dl) | 230.6 | 157.3 | − 73.78 (− 132.78 to − 13.97) | 0.02 |
| Weight (kg) | 70.3 | 66.1 | − 4.2 (− 2.07 to − 6.21) | 0.002 |

without any side effects. Patients had continued routine treatment in the Diabetes Clinic and were reassessed after a median period of 12 months. One patient had completed the calorie restriction phase but later had started following up elsewhere; hence, the results have been presented for the remaining eight patients.

## Baseline characteristics

The mean age was 50.5 years. All patients were males. The duration of diabetes was a median of 2.5 years. The average BMI at baseline was 25.3 kg/m$^2$. Fifty percent of the participants were obese (BMI > 25). Pre-intervention average calorie intake was 2170± 260 cal/day. The treatment at the time of intervention consisted of metformin and sulphonylurea in six patients (75%), metformin alone in two patients (25%). The glycemic status as measured by HbA1c was an average of 7.98. Two of them (25%) were also on treatment for systemic hypertension. All the participants had dyslipidemia and were on statin treatment for the same (Table 1).

## Efficacy

All but one patient had experienced weight loss; the mean weight loss was 4.2 kg, achieved over a period of 1 year. The average baseline weight was 70.3 kg and it had reduced to 66.1 kg at 12 months ($p = 0.002$). The decline was gradual and it had continued after the initial monitoring period of 3 months (Fig. 1). The fasting plasma glucose declined from a baseline mean of 144 to 115 mg/dl after a median period of 12 months ($p = 0.1$). Similarly, post prandial plasma glucose declined from 231 to 157 mg/dl ($p = 0.02$). (Table 2). The decline in post prandial glucose was more evident than fasting plasma glucose (Fig. 2). The average HbA1c declined from a baseline value of 7.98% to a post-intervention value 7.13% at 12 months ($p = 0.27$). (Table 2) The HbA1c values decreased by more than 0.5 in three participants; while it increased by more than 0.5 in two participants.

After the in-hospital calorie restriction, four patients were off any antidiabetic medication and the same patients continued to be off any antidiabetic drugs after a median of 12 months of follow-up. The recent average HbA1c of these four patients was 6.95%. Two of them maintained a HbA1C below 6.5% and they could be classified as having complete remission of diabetes.

There were no significant changes in blood pressure throughout the in-hospital stay as well as on the follow-up. The anti-hypertensives were being continued as that before the intervention. The cholesterol lowering drugs were continued as before.

# Discussion

We have reported 1-year outcome of a small group of selected overweight patients with type 2 diabetes who

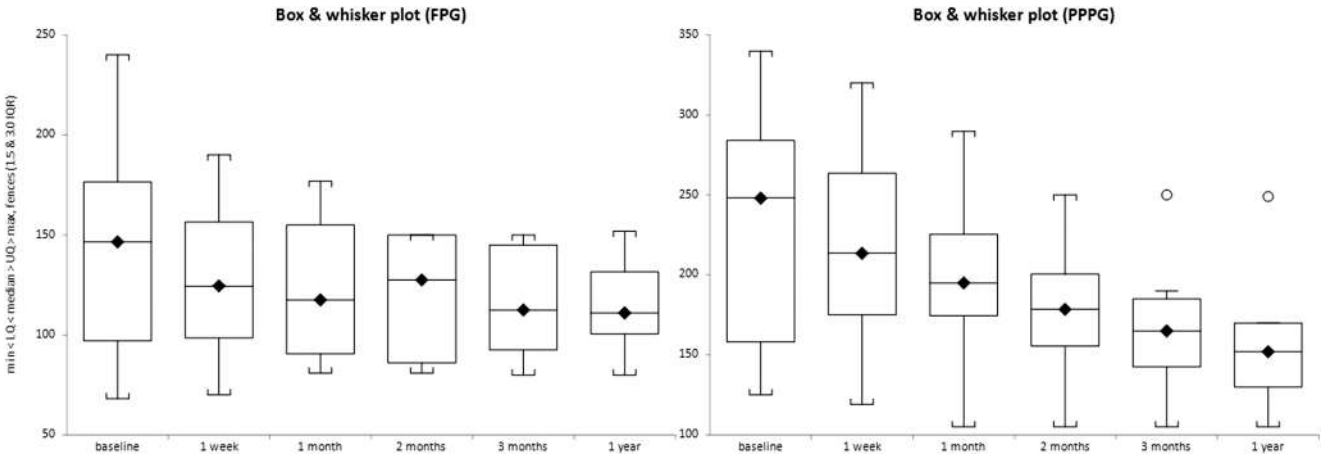


**Fig. 2** Box and Whisker plot depicting the changes in fasting and post prandial plasma glucose

underwent monitored 1 week very low calorie dietary intervention. An overall 0.8% reduction in HbA1C was achieved and the effect persisted for a year. Four patients (50%) were completely off medications and their HbA1C remained below 7 after 1 year. The limitation of this study is its small sample size.

VLCD is one of the few measures available for treatment of diabetes which leads to weight loss. A systematic review of VLCD interventions done until 2013 showed that the low calorie diets were tolerable and the intervention was associated with significant weight loss and reduction in blood glucose profile [6]. The mean weight reduction was 13.2 kg and the mean reduction in HbA1C was 1.4% [6]. A weight reduction of 6% (4.2 kg) and a HbA1C reduction of 0.8% at 1 year of follow-up was noted in our study with two patients (25%) achieving remission. Lean ME et al. have reported the results at 1 year after providing 825–853 kcal diet per day for 3–5 months in a trial involving 306 individuals. Forty-six percent of the participants had achieved remission to a non-diabetic state [4]. As yet there is only one published study relating to VLCD in diabetes from India. Bhatt AA et al. have reported the effects of calorie restriction of 1000 kcal per day for 12 weeks involving 12 participants [5]. The results were impressive with a median decline in HbA1C of 2.8%. This was measured at 12 weeks after the initiation of low calorie diet. The duration of intervention with VLCD has been used variably, with most trials applying it for 12 weeks. The duration in our study was for 1 week. We have found it to be effective and if replicated, it can be a more practical option.

The effects of glycemic control and weight reduction have lasted a year in our study. Studies by Steven S et al. and Lean ME et al. have demonstrated that the beneficial effects in terms of weight loss and reduction in plasma glucose levels can last up to 6 months and 1 year respectively [4, 7]. Steven S et al. have further shown that there is no fat redistribution to the liver after restarting isocaloric diet (ensuring weight stability) [7]. This may be the reason for the continued benefits after the initial calorie restriction. Still, the durability of response to a short-term VLCD intervention remains to be established [8] as regaining of the lost weight is common after any weight loss intervention. Hence, any VLCD intervention has to be considered in conjunction with a sustained weight loss program [9].

## Conclusion

It appears that the intervention of short duration of VLCD is effective in reducing HbA1C in overweight patients with type 2 DM and the effect can last for a year.

**Authors' contributions** SBS: concepts, design, literature search, statistical analysis, manuscript preparation, manuscript editing, and manuscript review; NDT: literature search, clinical studies, data acquisition, data analysis, statistical analysis, manuscript preparation. Both the authors have read and approved the final manuscript.

## Compliance with ethical standards

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

**Ethics approval and consent to participate** The study was approved by the Institute Ethics Committee (JIP/IEC/2016/1150) and written informed consent was obtained from all the participants.

**Ethics statement** All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

**Consent for publication** Not applicable as this contains only de-identified information.



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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):551–556
https://doi.org/10.1007/s13410-018-0676-4

ORIGINAL ARTICLE

# Treatment with combination of pioglitazone and glimepiride decreases levels of chemerin and asymmetric dimethylarginine (ADMA) in obese type 2 diabetic patients

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Received: 13 May 2018 / Accepted: 1 July 2018 / Published online: 12 July 2018


**Abstract**
Chemerin is an adipokine that plays a crucial role in adipocyte differentiation and development, as well as in glucose and lipid metabolism. High levels of asymmetric dimethylarginine (ADMA), a naturally occurring product of metabolism, inhibit nitric oxide (NO) synthesis and are related to endothelial dysfunction. The aim of this study was to investigate the effect of vildagliptin therapy and the combination of pioglitazone and glimepiride on the levels of NO, ADMA, and chemerin in diabetic patients. The study was conducted on 140 subjects, including 40 apparently healthy subjects, and 100 type 2 diabetic obese patients; 50 of them were treated with vildagliptin, and the other 50 patients revived combination of pioglitazone and glimepiride, both groups were treated for 12 months. For all participants, the levels of fasting blood glucose (FBG), fructosamine, HbA1c, lipid profile, ADMA, NO, and chemerin were determined. The levels of those parameters were compared before and after treatment. In both treated groups, levels of FBG, fructosamine, HbA1c, TC, and LDL-C decreased after treatment. Levels of chemerin and ADMA decreased significantly after treatment, whereas the levels of NO increased compared to the baseline values. Additionally, levels of chemerin and ADMA in the group treated with combination of pioglitazone and glimepiride were significantly lower compared to the group treated with vildagliptin. In conclusion, treatment with combination of pioglitazone and glimepiride had a favorable effect on chemerin and ADMA levels in obese type 2 diabetic patients.

**Keywords** Asymmetric dimethylarginine · Chemerin · Nitric oxide · Obesity · Type 2 diabetes mellitus

**Abbreviations**

| | |
|---|---|
| ADMA | Asymmetric dimethylarginine |
| BMI | Body mass index |
| FBG | Fasting blood glucose |
| HbA1c | Glycated hemoglobin |
| HDL-C | High-density lipoprotein cholesterol |
| LDL-C | Low-density lipoprotein cholesterol |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| PPARγ | Proliferator-activated receptor gamma |
| T2DM | Type 2 diabetes mellitus |
| TAG | Triacylglycerols |
| TC | Total cholesterol |
| TZD | Thiazolidinedione |

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## Introduction

Type 2 diabetes mellitus (T2DM) is characterized by increased levels of blood glucose, insulin resistance, and relative insulin deficiency [1]. Obesity, particularly abdominal adiposity, is linked to hyperglycemia, insulin resistance, dyslipidemia, and hypertension, a cluster of disorders collectively known as metabolic syndrome. Obesity is considered a major risk factor for development of insulin resistance and diabetes via mobilizing free fatty acids and increased secretion of inflammatory cytokines [2]. Adipokines secreted by adipose tissue are thought to play a role in regulation of insulin sensitivity, inflammation, and lipid and carbohydrate metabolism [3]. They are also suggested to be part of the pathophysiologic link between obesity and T2DM [4].

Chemerin, a recently identified adipose tissue-specific adipokine, has a crucial role in adipocyte differentiation and development, as well as in glucose and lipid metabolism [5]. Chemerin may play a role in pathogenesis of obesity and T2DM [6]. Raised chemerin levels were found in obese subjects and prediabetic states. Chemerin levels are also usually high in lean, overweight, and obese T2DM patients [7]. Chemerin has considerable roles in energy metabolism, adipogenesis, and inflammation. It has been hypothesized as a possible link between obesity and insulin resistance in T2DM [8].

Asymmetric dimethylarginine (ADMA) is a natural analogue of the conditionally essential amino acid arginine and a metabolic by-product of cellular protein turnover processes. Arginine is the precursor of nitric oxide (NO), an important regulator of immune and endothelial functions [9].

NO is known to have a role in regulation of insulin transport and glucose uptake in several tissues including the endothelium, liver, pancreas, and skeletal muscle. ADMA acts as an endogenous inhibitor of the nitric oxide synthase (NOS) enzyme and can therefore impair NO bioavailability; ADMA is eliminated from body via both urinary excretion and degradation by the enzyme dimethylarginine dimethylaminohydrolase [10].

Vildagliptin, a selective inhibitor of dipeptidyl peptidase-4, increases the availability of glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, and endogenous incretin hormones, and consequently enhances glycemic control [11]. Vildagliptin is approved as a T2DM treatment, either as a monotherapy or in combination with other drugs [12]. It has been reported in many clinical trials to reduce glycated hemoglobin (HbA1c), fasting blood glucose (FBG), and prandial plasma glucose significantly [13].

Pioglitazone is used as an antidiabetic drug for T2DM. It could enhance insulin sensitivity by stimulating a certain type of nuclear receptors, called peroxisome proliferator-activated receptor gamma (PPAR$\gamma$) [14].

Glimepiride, as all sulfonylureas, is an insulin secretagogue. It stimulates insulin release by pancreatic beta cells and induces increased activity of intracellular insulin receptors. Glimepiride has a potent effect on glycemic control and body weight reduction [15].

The aim of this study was to investigate the effect of vildagliptin therapy and the combination of pioglitazone and glimepiride on the levels of NO, ADMA, and chemerin in diabetic patients.

## Subjects and methods

The current study involved 140 subjects divided into three age-matched groups. The first group included apparently healthy 40 subjects with BMI less than 25 kg/m$^2$ and FBG < 100 mg/dL. The other two groups included 100 type 2 diabetic obese patients selected from the outpatients' clinic of diabetes, Suez Hospitals, Egypt. All patients had uncontrolled FBG levels with body mass index (BMI) greater than 30 kg/m$^2$. Fifty patients were treated with vildagliptin (50 mg twice daily) for 12 months (group 2), and the other 50 patients received combination of pioglitazone (30 mg/day), and glimepiride (2 mg/day) for 12 months (group 3). The present study was conducted according to the principles of the Declaration of Helsinki, and all the participants provided written informed consent, following a protocol approved by the Suez Canal University Research Ethics Committee (code # 201611MH2). Subjects suffering from overt macrovascular complications such as myocardial infarction, angina pectoris, stroke, and peripheral vascular diseases were excluded.

Five milliliters of blood samples were collected by vein puncture after 10-h fasting, divided into two portions, and 2 ml collected in EDTA anticoagulant tube for measurement of HbA1c that was quantitatively determined in whole blood by colorimetric method (Stanbio, USA). The other 3 ml were collected in plain tube and centrifuged for separation of serum.

Levels of FBG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triacylglycerols (TAG) were all determined in serum calorimetrically (Spectrum Diagnostics, Egypt). Fructosamine was determined using nitro blue tetrazolium (NBT) method [16], and nitric oxide was determined by colorimetric determination of total nitrate/nitrite in serum by the Greiss reaction [17]. Serum chemerin levels were measured using human chemerin ELISA kit (Glory Science Co. Ltd., China), and serum ADMA levels were quantified using human ADMA ELISA kit (Sigma, Germany).

All parameters were determined for patients before the treatment was started (baseline values), then determined again after 1 year of treatment, whereas they were determined only once for healthy subjects.

## Statistical analysis

The values of measured parameters were compared before and after treatment using paired $t$ test, and comparison between different groups (normal, patients treated with vildagliptin, and patients treated with combination of pioglitazone and glimepiride) was conducted using analysis of variance (ANOVA) followed by Tuckey's post hoc test. Values of $p < 0.05$ were considered significant, and data were represented as mean ± SD.

## Results

In the current study, there was a significant increase in BMI, FBG, fructosamine, HbA1c, TAG, TC, and LDL-C in the patients with T2DM and obesity compared to the normal

group, with a significant increase in the levels of HDL-C in patients (Table 1). Treatment with vildagliptin for 12 months significantly decreased levels of FBG, fructosamine, and HbA1c compared to the baseline values (before treatment). Similar results were obtained in the case of treatment with combination of pioglitazone and glimepiride for 12 months. It was noticed that in both treated groups, no significant decrease in the BMI or TAG levels in comparison to the baseline values was shown; however, treatment with either vildagliptin or the combination of pioglitazone and glimepiride significantly decreased TC and LDL-C levels. Only the combination of pioglitazone and glimepiride led to a significant increase in HDL-C compared to baseline values. When both treated groups were compared, the levels of HDL-C after treatment with the combination of pioglitazone and glimepiride were found to be significantly higher compared to the vildagliptin-treated group at $p < 0.05$ (Table 1).

Table 2 shows that levels of chemerin and ADMA were significantly higher in diabetic obese patients compared to the apparently healthy group at $p < 0.05$. Levels of NO were significantly lower in the patients compared to the normal group. In both treated groups, levels of chemerin and ADMA decreased significantly after treatment, whereas the levels of NO increased when compared to the baseline values. Notably, comparison of the two treated groups showed that the levels of chemerin and ADMA in the group treated with combination of pioglitazone and glimepiride were significantly lower compared to the group treated with vildagliptin. Additionally, the levels of NO after treatment with the combination of pioglitazone and glimepiride were significantly higher than the levels shown after treatment with vildagliptin at $p < 0.05$ (Table 2).

## Discussion

Adipocytes are active endocrine secretory cells that release free fatty acids and produce several cytokines including chemerin, resistin, leptin, adiponectin, visfatin, and omentin [18]. Chemerin is a relatively newly identified adipokine whose levels are elevated in obesity and are positively correlated with markers of metabolic syndrome such as BMI, TAG, and cholesterol [19].

In the current study, the levels of chemerin were found to be significantly higher in diabetic obese patients compared to the apparently healthy group. Serum chemerin levels were previously found to be elevated in obese diabetic patients [20]. Additionally, studies have shown link between elevated concentrations of circulating chemerin and insulin resistance in both type 1 and type 2 diabetic patients [21]. Chemerin and its receptor were expressed in β cells where chemerin regulates β cell function and plays an important role in glucose homeostasis in a tissue-dependent manner [22].

Our results showed that levels of ADMA, the endothelial NOS inhibitor, were significantly higher in diabetic obese patients, whereas NO levels were significantly lower in patients compared to the apparently healthy group. Increased levels of ADMA were reported in pathological conditions related with endothelial dysfunction, including diabetes, dyslipidemia, and obesity [23]. On the other hand, impaired glucose tolerance

**Table 1** Clinical parameters, glucose homeostasis traits and lipid profile of the study groups

| Groups/parameters | Normal ($n = 40$) | Patients with type 2 diabetes and obesity | | | |
|---|---|---|---|---|---|
| | | Vildagliptin-treated group ($n = 50$) | | Pioglitazone and glimepiride -treated group ($n = 50$) | |
| | | Baseline values | After 1 year treatment | Baseline values | After 1 year treatment |
| Age (year) | 47 ± 5 | 49 ± 3 | | 50 ± 4 | |
| BMI (kg/m$^2$) | 21.72 ± 1.24 | 35.62 ± 1.44* | 33.32 ± 2.56* | 33.96 ± 2.00* | 31.20 ± 2.66* |
| FBG (mg/dL) | 90.40 ± 7.02 | 269.60 ± 24.77* | 183.40 ± 19.88*# | 281.50 ± 28.11* | 169.80 ± 25.12*# |
| Fructosamine (μmol/L) | 214.80 ± 22.57 | 416.80 ± 25.66* | 324.40 ± 24.97*# | 425.67 ± 23.67* | 308.50 ± 11.05*# |
| HbA1c % | 5.12 ± 0.64 | 11.78 ± 1.48* | 8.21 ± 0.90*# | 12.37 ± 1.82* | 7.56 ± 1.71*# |
| TAG (mg/dL) | 98.95 ± 28.57 | 322.30 ± 69.54* | 288.16 ± 38.76* | 290.10 ± 40.99 | 263.11 ± 45.12* |
| TC (mg/dL) | 153.20 ± 19.30 | 294.90 ± 26.54* | 234.65 ± 17.81*# | 284.00 ± 42.50* | 230.76 ± 19.63*# |
| HDL-C (mg/dL) | 54.95 ± 5.90 | 31.03 ± 4.35* | 33.61 ± 5.90*# | 33.75 ± 5.62* | 40.03 ± 4.25*#$ |
| LDL-C (mg/dL) | 86.35 ± 21.40 | 198.60 ± 40.84* | 143.12 ± 24.79*# | 193.70 ± 20.11* | 138.20 ± 25.02*# |

Data are represented as mean ± SD

*BMI* body mass index, *FBG* fasting blood glucose, *HbA1c* glycosylated hemoglobin, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TAG* triacylglycerols, *TC* total cholesterol

* Significantly different from normal group at $p < 0.05$

# Significantly different from baseline values (before treatment) at $p < 0.05$

$ Significantly different from vildagliptin-treated group at $p < 0.05$

**Table 2** Levels of chemerin, nitric oxide (NO), and asymmetric dimethylarginine (ADMA) in the study groups

| Groups/parameters | Normal ($n = 40$) | Patients with type 2 diabetes and obesity | | | |
|---|---|---|---|---|---|
| | | Vildagliptin-treated group ($n = 50$) | | Pioglitazone and glimepiride -treated group ($n = 50$) | |
| | | Baseline values | After 1 year treatment | Baseline values | After 1 year treatment |
| Chemerin (pg/mL) | 24.02 ± 11.72 | 73.18 ± 13.98* | 51.73 ± 9.35*# | 71.20 ± 12.87* | 36.03 ± 15.08*#$ |
| NO (μmol/L) | 32.77 ± 8.79 | 21.84 ± 6.95* | 44.74 ± 5.71*# | 20.43 ± 7.20* | 63.55 ± 5.80*#$ |
| ADMA (ng/mL) | 21.23 ± 8.99 | 72.22 ± 15.29* | 48.24 ± 12.84*# | 74.18 ± 23.50* | 32.76 ± 13.10*#$ |

Data are represented as mean ± SD

* Significantly different from normal group at $p < 0.05$

# Significantly different from baseline values (before treatment) at $p < 0.05$

$ Significantly different from vildagliptin-treated group at $p < 0.05$

may result in a decrease of NO production and inactivation of NOS by reactive oxygen and nitrogen species [24]. Sarwar et al. [25] reported that elevated blood glucose levels drive production of ROS via multiple pathways, resulting in uncoupling endothelial NOS activity and reducing NO availability.

ADMA pathway has been proposed as a link between inflammation and endothelial dysfunction [26]. It was demonstrated that ADMA levels, independent of the other risk factors, is related to insulin resistance [27]. Baum et al. [28] observed that ADMA concentrations were higher in obese and overweight individuals compared with control subjects, and that there was a relationship between BMI and ADMA concentrations. Elevated ADMA levels have been suggested to have a role in cardiovascular system disorders, DM, and in the pathogenesis of hypertension [29].

The results of this study show that treatment with vildagliptin for 12 months decreased FBG, fructosamine, and HbA1c significantly with decreased TC and LDL-C levels. Vildagliptin also decreased levels of chemerin and ADMA and increased NO levels compared to the baseline values.

Dipeptidyl peptidase-4 inhibitors as vildagliptin were shown to improve lipid profile and inflammation parameters in patients with T2DM [30]. Vildagliptin is known to improve the sensitivity of both α and β cells to glucose, leading to improved glucose tolerance and reduced FBG [31]. Additionally, vildagliptin seems to have a positive action on the levels of some adipocytokines that are related to inflammation as chemerin [13]. Cakirca et al. [32] observed that treatment with vildagliptin decreased serum ADMA levels significantly in T2DM patients.

Treatment with combination of pioglitazone and glimepiride for 12 months in the current study decreased FBG, fructosamine, HbA1c, TC, and LDL-C levels and increased HDL-C. Combination of pioglitazone and glimepiride decreased levels of chemerin and ADMA, and increased NO levels relative to their values before treatment. Interestingly, both treatments had similar effects on chemerin, ADMA, and NO levels; however, the chemerin and ADMA-lowering effects observed in the group treated by the combination of pioglitazone and glimepiride were significantly stronger compared to that shown in vildagliptin-treated group.

Pioglitazone, a member of the thiazolidinedione (TZD), improves blood glucose control through alleviation of insulin resistance. This aim is achieved through binding PPARγ [21]. Pioglitazone also improves lipid profiles in patients with T2DM. Levels of TC were significantly decreased and HDL levels were significantly increased upon treatment with pioglitazone [33].

Glimepiride was reported to provide a potent effect on glycemic control and body weight reduction [15]. Significant reductions in TC and LDL-C levels and significant increases in HDL-C levels were previously observed in patients treated with glimepiride [34].

Pioglitazone was shown to decrease levels of ADMA in rats in a glucose-lowering independent manner via inducing dimethylarginine dimethylaminohydrolase enzyme [35, 36]. Pioglitazone was also observed to decrease levels of chemerin [21]. PPAR-γ agonists, including pioglitazone, were shown to suppress the expression of certain white adipose tissue genes, including chemerin and resistin [37].

In conclusion, treatment with either vildagliptin or combination of pioglitazone and glimepiride significantly lowered chemerin and ADMA levels and increased NO. The lowering effect caused by the combination of pioglitazone and glimepiride was more potent compared to that caused by glimepiride treatment.

**Acknowledgements** The authors are thankful to the staff members of the outpatients' clinic of diabetes in Suez Hospitals for their help in collection of samples.

**Authors' contributions** All authors have contributed significantly to the study. Dina M. Abo-Elmatty and Hussein Al-Sawaf designed the study. Ahmed A. Youssef and Omnia I Ezzat were responsible for collection of samples and laboratory work. Eman T. Mehanna was responsible for data analysis. Ahmed A. Youssef and Eman T. Mehanna wrote the manuscript. This work was funded by the authors.

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** The present study was conducted according to the principles of the Declaration of Helsinki. The study protocol was approved by the Suez Canal University Research Ethics Committee (code # 201611MH2).

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

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):557–567
https://doi.org/10.1007/s13410-018-0701-7

ORIGINAL ARTICLE

# A 2018 clinical practice pattern in the management of diabetes in India and Nepal: a three-city study

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Received: 21 July 2018 / Accepted: 20 October 2018 / Published online: 10 November 2018


## Abstract

Knowledge, awareness, and practices with regard to diabetes management in south Asia are not known. This study aimed to determine current clinical practices with regard to managing diabetes in Nepal and India. Doctors attending conferences in Delhi, Guwahati, and Kathmandu were evaluated regarding their diabetes treatment preferences using a standardized questionnaire having 34 multiple-choice questions. The questionnaire evaluated doctor's preferences on therapeutic lifestyle modification, pharmacotherapy, and insulin injection practices. From a total of 409 filled questionnaires which were collected, 261, 75, and 73 questionnaires from New Delhi, Guwahati, and Kathmandu, respectively, which fulfilled all criteria, were analyzed. The mean age of doctors was 42.26 ± 11.99 years. Low carbohydrate, high protein was the most frequently recommended diet plan. The use of carbohydrate counting in practice was non-existent. Self-monitoring of blood glucose (SMBG) is commonly used in practice with 1–2 times per day checking. The preferred second, third, and fourth oral agents after metformin were sulfonylureas (53.05%), glucosidase inhibitors (37.16%), and dipeptidyl-peptidase-4 inhibitors (DPP4i) (42.79%) respectively. Glimepiride (73.83%) and gliclazide (28.60%) were the most preferred sulfonylureas. Teneligliptin (31.54%), sitagliptin (23.71%), linagliptin (22.24%), and vildagliptin (16.38%) were the preferred DPP4i. Dapagliflozin (30.56%), empagliflozin (20.29%), and canagliflozin (8.85%) were the preferred sodium-glucose co-transporter-2 inhibitor (SGLT2i). Insulin use was delayed in type-2 diabetes, typically initiated only after glycemic control not adequate with five oral anti-diabetes agents (OADs) (50.36%). The most preferred insulin was basal insulin analogue (47.68%) followed by neutral protamine hagedorn (NPH) insulin (22.24%). Most patients received < 20 U of insulin/day (56.96%). The most commonly used short-acting insulin in pregnancy was lispro (204; 49.88%). The preferred long-acting insulin in pregnancy was NPH insulin (180; 44.01%). Lack of use of digital technology, less use of lifestyle modifications, delayed use of insulin, preference for multiple OADs, popularity of sulfonylureas and alpha-glucosidase inhibitors (AGIs), and late use of SGLT2i were some of the key highlights of diabetes practice in south Asia.

**Keywords** Diabetes therapy · India · Nepal · Treatment practices · Pattern · Guidelines

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## Introduction

India is the diabetes capital of the world. With a diabetes prevalence of 9% [1], an additional 14–18% of the population having prediabetes [2], one of the highest global rates of prediabetes progression to diabetes (18% per annum), diabetes onset nearly two decades earlier than rest of the globe and less than half the treated patients achieving glycemic targets [3], there is an urgent need to document and understand how to improve diabetes treatment practices in India [4]. A large number of guidelines are available from different societies across the globe to standardize and improve diabetes care. However, individualization remains the key, and the treatment protocol does vary from patient to patient depending on the metabolic and end-organ damage profile of the patient. We previously did studies assessing the knowledge and awareness with regard to thyroid disorders among doctors and patients, and documented that there remained significant gaps between what the clinical practice guidelines said, and what was actually happening in the day-to-day clinical practice [5, 6]. Such assessments are necessary as they help to gauge our limitations and how we can go about improving them. A similar assessment with regard to diabetes is lacking from this part of the globe.

Hence, this study aimed to determine the current clinical practices and preferences with regard to managing diabetes among doctors of different specialties in Nepal and India. This study also aimed to compare how the clinical practices in Nepal and India are different from those in the USA as per the American Diabetes Association (ADA) and American Association of Clinical Endocrinologist (AACE) guidelines.

## Methods

Doctors/physicians attending medical conferences in New Delhi (Annual Update of Society for the Promotion of Education in Endocrinology and Diabetes (SPEED) Annual Conference 10th, 11 February 2018) and Guwahati (Metabolic Conclave organized by Society for Promotion of Research in Metabolic and Endocrine Disorders on 4 March 2018) in India and Kathmandu, Nepal (1st Global Endocrine Summit, 7 April 2018, organized by the Diabetes & Endocrinology Association Of Nepal (DEAN)), were evaluated regarding their diabetes treatment preferences using a standardized questionnaire. At the beginning of the conference, all the doctors were given a questionnaire including 34 multiple-choice questions with the scope of marking more than one choice as the preferred answer. A time of 15 min was allocated for filling the questionnaire based on a pilot study done in 30 doctors before the events. Questions in the questionnaire were primarily based on evaluating the doctor's preferences on therapeutic lifestyle modification in diabetes management, pharmacotherapy in diabetes (oral antidiabetes agents and insulin), and insulin injection practices.

### Questionnaire

Questions 1–7 evaluated the non-pharmacology aspects of diabetes management. These included questions on how often diet and lifestyle changes were recommended to the patients, person taking care of diet and lifestyle changes advice to the patients, type of diet recommended to the patients, recommendations on carbohydrate counting, and self-monitoring of blood glucose (SMBG) in clinical practice. Questions 8–31 evaluated the pharmacotherapy aspects of diabetes management. These included questions evaluating the preferred second-, third-, and fourth-line oral agents in diabetes management, preferred sulfonylurea, dipeptidyl-peptidase-4 inhibitors (DPP4i), and sodium-glucose co-transporter-2 inhibitor (SGLT2i) in clinical practice and position of insulin among various pharmacotherapeutic agents in clinical practice. Questions evaluated the preferred insulin formulation, regimen, and administration methods in practice. Question 32 was on the clinical specialty of the doctor, question 33 on the gender of the doctor, and question 34 on age of the doctor. The questionnaires were blinded and personal details like name and contact details were not collected, to avoid any bias while filling of the questionnaires. The detailed questionnaire has been elaborated at the end of the manuscript.

### Study participants

Study participants were a diverse group of practicing doctors ranging from family physicians/general practitioners, internal medicine specialists, and endocrinologists practicing in New Delhi, Guwahati, and Kathmandu who voluntarily chose to attend the conferences at the respective cities. The venue, the timing, and the program content of the conferences were advertised in local newspapers, radio, and all major hospitals of the respective cities for the prior 1 month to the actual event.

### Statistical analysis

Summary statistics were prepared for responses to each question. Because not every participant answered all questions, the percentage of respondents providing a given answer was calculated individually for each question, using the number of respondents to that question as the denominator. The analysis was performed using the Statistical Package for the Social Sciences (SPSS) (Chicago, IL, USA) version-20 software.

## Results

Three hundred five, ninety-eight, and eighty-eight doctors returned filled questionnaires at the conferences at New Delhi, Guwahati, and Kathmandu respectively. Forty-four questionnaires from Delhi, 23 from Guwahati, and 15 from Kathmandu with less than 80% answered questions were excluded from analysis. A total of 261, 75, and 73 questionnaires from New Delhi, Guwahati, and Kathmandu, respectively, which fulfilled all, were analyzed. The mean age of the doctors participating in this study was 42.26 ± 11.99 years (44.68 ± 11.85 years, 40.59 ± 14.23 years, and 36.28 ± 6.90 years at New Delhi, Guwahati, and Kathmandu respectively). 74.71% ($n = 195$), 56% ($n = 42$), and 80.82% ($n = 59$) of the study participants at New Delhi, Guwahati, and Kathmandu were males. The majority of the doctors who participated in this study were seeing 10–30 patients of diabetes per day in their practice (Table 1). However, nearly 1 in 5 doctors was also seeing more than 40 patients per day in their practice (Table 1).

### Non-pharmacotherapy practices

A large majority of the doctors were giving diet and lifestyle changes advice to their patients as a part of the clinical visit (Table 2). Nearly 60% of the doctors were doing it themselves (Table 2). The remaining 40% doctors were getting it done through dieticians/trained staff at their clinics. There was low penetrance of use of online tools/software for giving diet and lifestyle advice to patients with only eight and two doctors at New Delhi and Kathmandu respectively using such technology. Among the different diet plans available, low carbohydrate, high protein was the most frequently recommended diet plan to patients with diabetes (Table 2). However, a large number of doctors did not choose to recommend a specific type of diet plan to patients. In spite of recent hype about ketogenic diet in media, social, and scientific circles, none of the doctors who participated in this study recommended ketogenic diet to their patients as a part of diabetes management (Table 2). The use of carbohydrate counting in clinical practice was rare with only 3, 0, and 2 doctors in New Delhi, Guwahati, and Kathmandu respectively frequently teaching carbohydrate counting to their patients as a part of therapeutic lifestyle modification in diabetes management (Table 2). The use of SMBG in diabetes management was much more prevalent with only 15, 3, and 4 doctors (New Delhi, Guwahati, and Kathmandu respectively) never recommending SMBG to their patients for diabetes management (Table 2). One to two times per day blood glucose checking was the most frequently recommended SMBG pattern by doctors to their patients (199; 48.65%) with a significant number of doctors recommending < 1 time per day blood glucose monitoring (108; 26.41%) (Table 2).

### Pharmacotherapy practices

The preferred second-line oral agent after metformin in the management of diabetes in the decreasing order of preference was sulfonylureas (217; 53.05%), alpha-glucosidase inhibitors (78; 19.07%), pioglitazone (62; 15.16%), dipeptidyl-peptidase-4 inhibitors (DPP4i) (16; 3.91%), and sodium-glucose linked transporter-2 inhibitor (SGLT2i) (8; 1.96%) (Table 3). As a third-line oral anti-diabetes add-on medication, the most preferred were alpha-glucosidase inhibitors (AGIs) (152; 37.16%), followed by sulfonylureas (91; 22.24%), SGLT2i (61; 14.91%), DPP4i (33; 8.07%), and pioglitazone (24; 5.86%). The preferred fourth-line add-on medication in patients with diabetes not controlled with three medications was DPP4i (175; 42.79%), SGLT2i (124; 30.31%), AGIs (69; 16.87%), pioglitazone (64; 15.65%), and sulfonylureas (38; 9.29%) (Table 3). Glimepiride (302; 73.83%) and gliclazide (117; 28.60%) were the two most preferred sulfonylureas in clinical practice. Teneligliptin (129; 31.54%), sitagliptin (97; 23.71%), linagliptin (91; 22.24%), and vildagliptin (67; 16.38%) were the preferred DPP4i in clinical practice. Among SGLT2i, the decreasing order of preference was dapagliflozin (125; 30.56%), empagliflozin (83; 20.29%), and canagliflozin (35; 8.85%) (Table 3). The number of oral anti-diabetes agents (OADs) after which insulin was added to the treatment regimen for glycemic control was after 5 OADs (206; 50.36%), followed by 1 OAD (190; 44.01%), 4 OADs (95; 23.23%), 3 OADs (70; 17.11%), and 3 (14; 3.4%). GLP1a use was low in clinical practice with 96 (23.47%) doctors reporting never to have used GLP1a. A majority of the doctors (172; 42.05%) only rarely used GLP1 in clinical practice (< 5% of patients). Eighty-seven (21.27%)

**Table 1** Patients with diabetes seen in clinical practice per day

| Patients with diabetes seen in clinical practice (per day) | New Delhi ($n = 261$) | Guwahati ($n = 75$) | Kathmandu ($n = 73$) |
|---|---|---|---|
| < 10 | 66 (25.4%) | 18 (24%) | 33 (45.2%) |
| 10–20 | 72 (27.3%) | 36 (48%) | 22 (30.1%) |
| 20–30 | 61 (23.5%) | 9 (12%) | 4 (5.5%) |
| 30–40 | 30 (11.5%) | 6 (8%) | 10 (13.7%) |
| > 40 | 32 (12.3%) | 6 (8%) | 4 (5.5%) |

**Table 2** Patterns of therapeutic lifestyle practices recommended by doctors

| Parameter | | New Delhi ($n = 261$) | Guwahati ($n = 75$) | Kathmandu ($n = 73$) |
|---|---|---|---|---|
| Almost always (> 90% times) giving diet & lifestyle advice to patients during consultation | | 202 (77.39%) | 51 (68.00%) | 63 (86.30%) |
| Frequently (50–90% times) giving diet & lifestyle advice to patients during consultation | | 46 (17.62%) | 21 (28.00%) | 6 (8.21%) |
| Diet & lifestyle advice given by self | | 152 (58.23%) | 54 (72.00%) | 35 (47.94%) |
| Diet & lifestyle advice given by dietician | | 101 (38.69%) | 18 (24.00%) | 22 (30.13%) |
| Type of diet recommended | Low carb, high protein | 145 (55.55%) | 39 (52.00%) | 40 (54.79%) |
| | Low carb, high fat | 6 (2.29%) | 3 (4.00%) | 0 (0%) |
| | Normal diet | 5 (1.91%) | 3 (4.00%) | 2 (2.73%) |
| | No specific diet type | 100 (38.31%) | 30 (40.00%) | 25 (34.24%) |
| Rarely/never teach carbohydrate counting (< 10%) | | 148 (56.70%) | 57 (76.00%) | 52 (71.23%) |
| Frequently (> 50% patients) recommending SMBG | | 93 (35.63%) | 18 (24.00%) | 47 (64.38%) |
| Number of times SMBG recommended per day | < 1 per day | 73 (27.96%) | 21 (28.00%) | 14 (19.17%) |
| | 1–2 per day | 135 (51.72%) | 48 (64.00%) | 16 (21.91%) |
| | > 3 per day | 67 (25.67%) | 18 (24.00%) | 29 (39.72%) |

*carb*, carbohydrate; *SMBG*, self-monitoring of blood glucose

infrequently (5–50% patients) used GLP1a in clinical practice. Pharmacotherapy practices were overall similar in Nepal and India, few exceptions being virtual lack of use of SGLT2i and gliclazide for diabetes management in Nepal.

**Table 3** Diabetes pharmacotherapy practices among doctors at different cities

| Parameter | | New Delhi ($n = 261$) | Guwahati ($n = 75$) | Kathmandu ($n = 73$) |
|---|---|---|---|---|
| Preferred second-line oral agent after metformin | Sulfonylureas | 98 (37.54%) | 60 (80.00%) | 59 (80.82%) |
| | AGIs | 45 (17.24%) | 21 (28.00%) | 12 (16.43%) |
| Preferred third-line oral agent | AGIs | 125 (47.89%) | 15 (20.00%) | 12 (16.43%) |
| | Sulfonylureas | 65 (24.90%) | 12 (16.00%) | 14 (19.17%) |
| Preferred fourth-line oral agent | DPP4i | 92 (35.24%) | 36 (48.00%) | 47 (64.38%) |
| | SGLT2i | 73 (27.96%) | 45 (60.00%) | 6 (8.21%) |
| Preferred sulfonylurea in clinical practice | Glimepiride | 181 (69.34%) | 64 (85.33%) | 67 (91.78%) |
| | Gliclazide | 77 (29.50%) | 36 (48.00%) | 4 (5.47%) |
| Preferred DPP4i in clinical practice | Teneligliptin | 75 (28.73%) | 36 (48.00%) | 18 (24.65%) |
| | Sitagliptin | 51 (19.54%) | 9 (12.00%) | 37 (50.68%) |
| Preferred SGLT2i in clinical practice | Dapagliflozin | 87 (33.33%) | 36 (48.00%) | 2 (2.73%) |
| | Empagliflozin | 70 (26.81%) | 9 (12.00%) | 4 (5.47%) |
| Number of OADs after which insulin is initiated | Five | 130 (49.80%) | 33 (44.00%) | 43 (58.90%) |
| | One | 133 (50.95%) | 42 (56.00%) | 15 (20.54%) |
| | Four | 52 (19.92%) | 13 (17.33%) | 10 (13.69%) |
| Preferred statin in clinical practice | Rosuvastatin | 230 (88.12%) | 69 (92.00%) | 71 (97.26%) |
| | Atorvastatin | 213 (81.60%) | 66 (88.00%) | 59 (80.82%) |
| | Pitavastatin | 82 (31.41%) | 12 (16.00%) | 0 |
| | Simvastatin | 23 (08.81%) | 0 | 0 |
| Preferred ARB in clinical practice | Losartan | 118 (45.21%) | 36 (48.00%) | 36 (49.31%) |
| | Telmisartan | 109 (41.76%) | 33 (44.00%) | 35 (47.94%) |
| | Olmesartan | 212 (81.22%) | 62 (82.66%) | 33 (45.20%) |
| | Valsartan | 53 (20.30%) | 6 (8.00%) | 9 (12.32%) |
| | Others | 6 (2.29%) | 0 (0%) | 2 (2.73%) |

*AGI*, alpha-glucosidase inhibitors; *DPP4i*, dipeptidyl-peptidase-4 inhibitors; *SGLT2i*, sodium-glucose co-transporter inhibitors; *ARB*, aldosterone receptor blockers

Most of the doctors preferred to start insulin for glycemic control after trying 5 OADs (172; 42.05%). At the other end of the spectrum, 96 doctors (23.47%) initiated insulin after single OAD use in clinical practice (Table 4). Overall, the most preferred insulin in the management of T2DM was basal insulin analogue (glargine/degludec/detemir) (195; 47.68%) followed by neutral protamine hagedorn (NPH) insulin (91; 22.24%) (Table 4). Most of the patients received less than 20 U of insulin per day (233; 56.96%). The most commonly used short-acting insulin in pregnancy was lispro (204; 49.88%) followed by regular insulin (105; 25.67%) and glulisine (78; 19.07%). The preferred long-acting insulin in pregnancy was NPH insulin (180; 44.01%), followed by almost similar preference for glargine (120; 29.34%) and detemir (119; 29.09%) (Table 4).

Regarding insulin injection practices, both abdominal wall and thighs were equally preferred sites for insulin injection (Table 5). However, a significant number of doctors (303; 74.08%) were also using arms as sites for insulin injection (Table 5). Regarding insulin injection techniques, not all doctors were routinely recommending a 90° angle of the needle with regard to the skin surface for insulin injection (Table 5). Nearly 321 doctors (78.48%) were also suggesting a 60° angle for insulin injection (Table 5).

## Discussion

This first ever study on diabetes clinical practices among physicians in south Asia gave rise to interesting observations. There was low penetration of use of digital diabetology in clinical practice. In spite of a large variety of diet plans available, including the recent increased popularity of "keto diet" and "paleo diet" in social media and certain urban populations [7], the low-carbohydrate diet pattern was the most common diet recommended by doctors to their patients. This is especially important, as traditionally Indian diet has been a high-carbohydrate diet. The STARCH study demonstrated that irrespective of any part of India, Indians were consistently taking 64–66% carbohydrates in their meals [8]. This also contributes to the greater challenges faced in controlling post-prandial glucose [8]. This may explain the increased use of AGIs in south Asia, which are especially effective in controlling post-prandial hyperglycemia. A good diet control would play a major role in controlling diabetes [9]. The doctors themselves were predominantly doing therapeutic diet and life style counseling of patients. This study highlights that use of carbohydrate counting in clinical practice is virtually nonexistent in this part of the world. Hence, increased awareness and training regarding carbohydrate counting among doctors managing patients with diabetes is warranted [9]. For monitoring of glycemic control, SMBG was commonly recommended by doctors to their patients with 1–2 monitoring per day being the most recommended pattern. Studies have shown that regular use of SMBG itself is associated with better glycemic control, and better quality of life, as patient feels more empowered and has more awareness of his disease [10]. This is different from western guidelines where they recommend more frequent SMBG, especially in patients on multiple subcutaneous insulin injections (MSII). Costs associated with SMBG may explain their

**Table 4** Patterns and position of insulin use in managing type-2 diabetes

| Parameter | | New Delhi ($n = 261$) | Guwahati ($n = 75$) | Kathmandu ($n = 73$) |
|---|---|---|---|---|
| Number of OADs use after which insulin is initiated in uncontrolled T2DM* | | 5 (3–5) | 4 (3–5) | 5 (3–5) |
| Preferred insulin in clinical practice | Human insulin regular/short-acting analogues | 77 (29.50%) | 16 (21.33%) | 14 (19.17%) |
| | Premixed insulin (regular + NPH / analogue/ analogue + long-acting insulin) | 15 (5.74%) | 0 (0) | 0 (0) |
| | NPH insulin | 36 (13.79%) | 38 (37.84%) | 17 (23.28%) |
| | Long-acting insulin analogue (detemir/glargine/degludec) | 132 (50.57%) | 21 (28.00%) | 42 (57.53%) |
| Average total daily dose of insulin received per day | < 20 U/day | 160 (61.30%) | 45 (60.00%) | 28 (38.35%) |
| | 20–40 U/day | 45 (17.24%) | 12 (16.00%) | 19 (26.02%) |
| | > 40 U/day | 56 (21.45%) | 18 (24.00%) | 26 (35.61%) |
| Preferred short-acting insulin in pregnancy | Lispro | 128 (49.04%) | 33 (44.00%) | 43 (58.90%) |
| | Regular | 73 (27.96%) | 18 (24.00%) | 14 (19.17%) |
| | Aspart | 15 (5.74%) | 3 (4.00%) | 4 (5.47%) |
| | Glulisine | 45 (17.24%) | 21 (28.00%) | 12 (16.43%) |
| Preferred long-acting insulin in pregnancy | NPH insulin | 103 (39.46%) | 39 (52.00%) | 38 (52.05%) |
| | Detemir | 91 (34.86%) | 12 (16.00%) | 16 (21.91%) |
| | Glargine | 67 (25.67%) | 24 (32.00%) | 19 (26.02%) |

*OAD*, oral anti-diabetes agent; *T2DM*, type-2 diabetes; *median [25th–75th percentile]; *NPH*, neutral protamine hagedorn

**Table 5** Insulin injection practices among doctors in different cities

| Parameter | | New Delhi ($n = 261$) | Guwahati ($n = 75$) | Kathmandu ($n = 73$) |
|---|---|---|---|---|
| Preferred site for insulin injection | Abdomen | 205 (78.54%) | 66 (88.00%) | 69 (94.52%) |
| | Thighs | 223 (85.44%) | 72 (96.00%) | 73 (100%) |
| | Arms | 195 (74.71%) | 63 (84.00%) | 65 (89.04%) |
| | Others | 31 (11.87%) | 0 (0%) | 4 (5.47%) |
| Angle of the needle from skin during injection | 90° | 182 (69.73%) | 57 (76.00%) | 57 (78.08%) |
| | 60° | 208 (79.69%) | 66 (88.00%) | 67 (91.78%) |
| | 30° | 2 (0.76%) | – | – |
| Needle used more than once | | 184 (70.49%) | 66 (88.00%) | 63 (86.30%) |
| Number of times needle is re-used | Up to 2 times | 142 (54.40%) | 42 (56.00%) | 51 (69.86%) |
| | 3–5 times | 45 (17.24%) | 6 (8.00%) | 10 (13.69%) |
| | 6–10 times | 45 (17.24%) | 0 (0%) | 6 (8.21%) |
| Device used for insulin injection | Syringe | 152 (58.23%) | 39 (52.00%) | 53 (72.60%) |
| | Reusable pen | 193 (73.94%) | 63 (84.00%) | 59 (80.82%) |
| | Disposable pen | 164 (62.83%) | 48 (64.00%) | 38 (52.05%) |
| Cleaning injection site with alcohol swab before injection | | 158 (60.53%) | 24 (32.00%) | 26 (35.61%) |

decreased use, especially when health care expenditure is primarily out of pocket. Sulfonylureas followed by AGIs continue to be the preferred second- and third-line oral anti-diabetes medication, after metformin. DPP4i and SGLT2i were most commonly used as fourth-line agents in the management of diabetes. In contrast to the West, there was a low penetration of use of GLP1a in the management of diabetes with nearly a quarter of doctors in this study, never having used GLP1a.

Glimepiride was the most commonly used sulfonylurea in clinical practice. Glimepiride was the preferred sulfonylurea for nearly three-quarters of doctors surveyed. Gliclazide was the second most commonly used sulfonylurea. However, use of gliclazide was negligible in Nepal in contrast to India. Lack of availability may explain the limited use of gliclazide in Nepal. With regard to use of DPP4i, no molecule had clear majority with regard to preference of use. However, teneligliptin was marginally ahead with regard to preference of use, perhaps due to the low associated costs, followed by sitagliptin, linagliptin, and vildagliptin. Lack of availability of teneligliptin in Nepal may explain its minimal use there in clinical practice. Dapagliflozin was the most commonly used SGLT2i, followed by empagliflozin and canagliflozin. From a glycemic control point of view, as per the data from the clinical trials, all SGLT2i are equivalent [11–13]. South Asia being a price-sensitive society, the mildly lower cost of dapagliflozin may explain its increased preference. Lack of availability in Nepal may explain the negligible use of SGLT2i in clinical practice there.

It is interesting to note that although most of the diabetes practice guidelines from the western world like those from the American Association of Clinical Endocrinologists (AACE) and the American Diabetes Association (ADA) recommend early use of SGLT2i in clinical practice and have now recommended this class of drug as a compelling indication in patients with established coronary artery disease [14, 15], as per our study, the use of this class of drug is pretty late among the entire basket of anti-diabetes medications. The causes may be multifactorial, with medication costs perhaps being an important factor, considering the lower per capita income in this part of the world, coupled with predominantly out of pocket expenditure for pharmacotherapy. Also, SGLT2i is comparatively new in clinical practice, and its acceptability among doctors may take some time [12]. Concerns about euglycemic ketosis, genital tract infection, acute kidney injury, osteoporosis, fractures, and trends for increased cerebrovascular accidents and amputations with some of the drugs in this class may limit their use in clinical practice, in spite of the overall cardiovascular superior outcomes in patients with established coronary artery disease (CAD) or high risk for CAD [13].

Position of insulin in the management of T2DM is late with 50% doctors typically initiating insulin when the patient is already on five different OADs, and the patient had yet to attain glycemic control. Needle prick phobia, myths, and misinformation regarding insulin use in the general population may contribute to this delayed insulinization in the management of type-2 diabetes in south Asia. Our observations are in accordance with those of a recent study from 748 patients with diabetes from eastern India where insulin initiation was late with mean diabetes duration being $8.80 \pm 6.42$ years [16].

The most commonly used insulin therapy is basal insulin therapy along with different OADs, with long-acting insulin analogues more commonly used than NPH insulin. A single prick per day with long-acting insulin resulting in better glycemic control along with OADs may explain the increased popularity of basal insulin therapy. NPH insulin was the most preferred long-acting insulin in pregnancy, followed by glargine and detemir insulin. Regarding insulin injection practices, the most preferred sites for insulin injection were the abdomen and thighs. However, a significant number of doctors also recommended insulin injections in the arms. A limitation with insulin injection in the arms is that it cannot be self-administered there, and in a thin lean person, there remains a possibility of the injection becoming intramuscular instead of subcutaneous. Insulin when administered intramuscularly has totally different pharmacokinetics as compared to when administered subcutaneously, which is the ideal way. Studies have shown that wrong insulin injection technique, injection at inappropriate sites, and needle reuse are associated with increased occurrence of lipohypertrophy [16]. There is a need to increase awareness about good clinical practices in insulin injection therapy as a significant number of doctors were not keeping the needle perpendicular to the skin while injecting insulin. Decreasing angle of insulin injection increases the risk of injection being administered intra-dermally, which again alters the insulin kinetics and may be associated with increased risk of allergic reactions [17]. It must be highlighted that although this study gives important insights into the diabetes clinical practices among doctors from three different corners of the Indian subcontinent, it may not be reflective of the general clinical practices of the entire Indian subcontinent. A much larger study systematically covering the different parts of the subcontinent is warranted.

To summarize, this study highlighted the type-2 diabetes treatment practices in Nepal and India, which were largely similar. Lack of use of digital technology, less use of lifestyle modifications, non-existent use of carbohydrate counting, variability in diet plan preferred for diabetes management, delayed use of insulin, preference for multiple OADs, with popularity of sulfonylureas and AGIs, relatively late use of SGLT2i, preference for basal insulin when initiating insulin therapy, and a greater preference for insulin analogues were some of the key highlights of diabetes practice in south Asia.

## Compliance with ethical standards

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

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

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

## Appendix

### SOCIETY FOR THE PROMOTION OF EDUCATION IN ENDOCRINOLOGY & DIABETES

#### DIABETES CLINICAL PRACTICES AMONG DOCTORS SURVEY

The idea of this survey is to determine the clinical practice preferences of doctors in our country with regards to diabetes management in clinical practice. We understand that diabetes management is highly variable, individualized and varies from patient to patient. We intend to understand the general preferences and trends. This will help us in better framing of diabetes based clinical update programs in the future, addressing the specific needs and desires. It takes approximately 6-8 minutes to complete 34 questions. Your answers will remain strictly confidential. As you answer the questions please consider recent patients you have treated, as this research survey is intended to assess actual current practices rather than idealized approaches. Please assume that the patient wants to defer to your judgment as to the preferred approach. We are maintaining total confidentiality and hence not collecting any personal details

### SECTION-1: Non-pharmacology diabetes management:

1) **How many patients with diabetes are you seeing in your clinical practice per day?**

a) 0-10
b) 10-20
c) 20-30
d) 30-40
e) >40

2) **Are you giving diet and life style advice to your diabetes patients while prescribing medications?**

a) Almost Always (>90%)
b) Frequently (50-90%)
c) Infrequently (10-50%)
d) Rarely (<10%)

3) **Who take care of the diet and life style advice management of your patients?**

a) Self
b) Certified dietician in Clinic/Hospital
c) Self trained medical assistant

d) Online tools/software
e) Fixed diet plan printouts

4) **What type of diet do you recommend your diabetes patients to have?**

a) Low carbohydrate high protein diet
b) Low carbohydrate high fat diet
c) Keto-diet
d) Normal balanced diet
e) Others (Kindly specify……)

5) **Do you teach your patients carbohydrate counting in clinical practice?**

a) Frequently (>50%)
b) Infrequently (10-50%)
c) Rarely (0-10%)
d) Never (0%)

6) **How often do you recommend self-monitoring of blood glucose (SMBG) in your patients with T2DM?**

a) Never
b) Rarely (<5%)
c) Infrequently (5-25%)
d) Often (25-50%)
e) Frequently (50-90%)
f) Almost always (>90%)

7) **In a patient doing SMBG, how many times in a day you recommend SMBG?**

a) Less than 1 per day
b) Once daily
c) Twice a day
d) Thrice daily
e) More than 3 times a day

## SECTION-2: Diabetes pharmacotherapy practices:

8) **What is your preferred second line oral anti-diabetes medication after metformin in clinical practice?**

a) Sulfonylureas
b) Pioglitazone
c) Alpha-glucosidase inhibitors
d) DPP-4 inhibitors
e) SGPT2 inhibitors

9) **What is your preferred third line oral anti-diabetes medication in clinical practice?**

a) Sulfonylureas
b) Pioglitazone
c) Alpha-glucosidase inhibitors
d) DPP-4 inhibitors
e) SGPT2 inhibitors

10) **What is your preferred fourth line oral anti-diabetes medication in clinical practice?**

a) Sulfonylureas
b) Pioglitazone
c) Alpha-glucosidase inhibitors
d) DPP-4 inhibitors
e) SGPT2 inhibitors

11) **What is your preferred sulfonylurea in clinical practice?**

a) Glibenclamide
b) Glipizide
c) Glimeperide
d) Gliclazide
e) Others (Kindly specify……..)

12) **What is your preferred DPP4 inhibitor in clinical practice?**

a) Sitagliptin
b) Vildagliptin
c) Saxagliptin
d) Linagliptin
e) Teneligliptin
f) Gemigliptin

13) **What is your preferred SGLT2i in clinical practice?**

a) Dapagliflozin
b) Canagliflozin
c) Empagliflozin
d) None of the above

14) **After how many oral anti-diabetes medication uses in T2DM, do you prefer starting insulin, when diabetes remains uncontrolled?**

a) One
b) Two
c) Three

d) Four
e) Five

15) **Which insulin do you generally start with when initiating insulin in a patient with T2DM?**

a) Human insulin regular/short acting analogues
b) Premixed insulin (regular+NPH/ analogue+NPH/ analogue+long acting insulin)
c) NPH insulin
d) Long acting insulin (detemir/glargine/degludec)

16) **What is the average total daily dose of insulin received by your patients (short acting and/or long acting summed up)?**

a) <10U/day
b) 10-20U/day
c) 20-30U/day
d) 30-40U/day
e) >40U/day

17) **How frequently do you use GLP1 analogue in your clinical practice?**

a) Never
b) Rarely (<5% patients)
c) Infrequently (5-50%)
d) Frequently (>50% patients)

18) **What is your preferred short acting insulin in pregnancy?**

a) Human insulin regular
b) Insulin aspart
c) Insulin lispro
d) Insulin glulisine

19) **What is your preferred long acting insulin in pregnancy?**

a) NPH Insulin
b) Detemir
c) Glargine
d) Degludec
e) U-300 Glargine

20) **What is your preferred site for insulin injection?**

a) Arms
b) Abdominal wall
c) Thighs
d) Calf
e) Any other site (please specify……)

21) **What is the approximate angle of needle entry used by the patient?**

a) 30 $^{o}$
b) 45 $^{o}$
c) 60 $^{o}$
d) 90 $^{o}$

22) **Does the patient use his/her needle more than one time?**

a) Yes
b) No

23) **If Yes, how many times does he/she use a single needle?**

a) 2 times
b) 3 to 5 times
c) 6 to 10 times
d) More than 10 times

24) **What device does this patient use to inject insulin? (tick, all that apply)**

a) Disposable Pen
b) Reusable Pen
c) Insulin syringe

25) **What length of needle do you currently use to inject (tick all answers that apply)?**

a) 12.7 mm
b) 12 mm
c) 10 mm
d) 8 mm
e) 6 mm
f) 5 mm
g) 4.5 mm
h) 4mm
i) Don't know

26) **Before the injection do you clean the skin with disinfectant (e.g. an alcohol swab)?**

a) Yes
b) No

27) **Before inserting the needle into the vial or attaching a pen needle to the pen, do you clean the stopper with disinfectant (e.g. an alcohol swab)?**

a) Yes
b) No

28) **What is your preferred ARB in clinical practice?**

a) Losartan
b) Telmisartan
c) Olmesartan
d) Valsartan
e) Azilsartan
f) Others (Kindly specify……..)

29) **What is your preferred statin in clinical practice?**

a) Atorvastatin
b) Rosuvastatin
c) Pitavastatin
d) Simvastatin
e) Others (Kindly specify……..)

30) **Who teaches insulin injection techniques and compliance to your patients?**

a) I do it myself
b) Have a diabetic nurse
c) Diabetes trainer trained by self
d) The pharmacist
e) Representative of the insulin manufacturer
f) Others (Kindly specify……..)

31) **How frequently do you notice urinary infections with SGLT2 inhibitors?**

a) <5% patients
b) 5-15% patients
c) 15-25% patients
d) 25-35% patients
e) 35-50% patients
f) >50% patients

## SECTION-3: Demography:

32) **What is your specialty?**

a) Family/General Physician
b) Internal Medicine specialist
c) Surgical specialties
d) Obstetrics & Gynecology
e) Pediatrics
f) Endocrinology
g) Others (Kindly specify……..)

33) **What is your gender**

a) Male
b) Female
c) Choose not to state

34) **What is your age? …… years**

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):568–578
https://doi.org/10.1007/s13410-018-0673-7

ORIGINAL ARTICLE

# Factors associated with consumption of sugar-sweetened foods and beverages in Malaysia: an ethnic comparison

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Received: 3 April 2018 / Accepted: 25 June 2018 / Published online: 12 July 2018


**Abstract**
High intake of added sugar is related to various diseases. One of the main objectives of public health administrators is to reduce the consumption of sugar-sweetened foods and beverages. The present study attempts to examine sociodemographic factors associated with consumption of sugar-sweetened foods and beverages in Malaysia. The Malaysian Household Expenditure Survey (HES) 2014 ($n = 14,838$) is used. The dependent variable is monthly household expenditures on sugar-sweetened foods and beverages. The independent variables are gender, age, ethnicity, marital status, education, employment status, household income, household size, region, location of residence and expenditures on tobacco and alcohol. The present study uses a two-part model to estimate the factors associated with consumption decision and amount decision of sugar-sweetened foods and beverages. Analyses stratified by ethnicity are performed. Age, household size, marital status, education level, employment status, region, location of residence, tobacco and alcohol expenditures are associated with consumption of sugar-sweetened foods and beverages. For Bumiputera and non-Bumiputera households, those who have a higher likelihood of consuming sugar-sweetened foods and beverages are less-educated, reside in Peninsular Malaysia and rural areas and spend less on tobacco, while those who spend more on sugar-sweetened foods and beverages are females, married, well-educated, employed and spend more on alcohol. In conclusion, household expenditures on sugar-sweetened foods and beverages are associated with numerous sociodemographic factors. Findings of the present study can assist policy makers in developing a more effective intervention measure directed towards reducing the intake of added sugar among people in Malaysia.

**Keywords** Beverage · Consumption · Ethnicity · Food · Sugar

## Introduction

There is a sharp rise in the number of people who suffer from diabetes across the globe. The number of diabetic patients in the world more than tripled from 108 million in 1980 to 422 million in 2014 [1]. In 2014, the prevalence of diabetes was 8.5%, compared to only 4.7% in 1980 [1]. Diabetes is one of the main contributing factors of cardiovascular diseases, kidney failure, visual impairment and stroke [2]. Worse still, diabetes was responsible for 1.6 million mortalities in 2015. In terms of economic costs, one study estimated that the burden of diabetes in the United States (US) was the United States Dollar (USD) 245 billion in 2012 [3].

Diabetes is more prevalent in developing countries than in developed countries. In Malaysia, the report of National Health and Morbidity Survey showed that the prevalence of diabetes increased more than two times from 6.3% in 1986 to 15.2% in 2011, which amounted to 2.6 million adults with diabetes [4]. A study conducted based on elderly sample showed that approximately three in every ten Malaysian elders suffer from diabetes [5]. Diabetes also resulted in huge

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medical costs as Ringgit Malaysia (RM) 2.4 billion was allocated for diabetes treatments in 2010.

Diabetes is a major public health issue that has various associated factors. One of the factors is consumption of sugar-sweetened foods and beverages. There are evidences suggesting that individuals who often consume sugar-sweetened foods and beverages are more likely to develop diabetes and various diseases than individuals who seldom consume sugar-sweetened foods and beverages [6–8]. In 2009, Malaysia was ranked as the eighth highest sugar consumers worldwide. The total teaspoons of sugar consumed by a Malaysian in a day increased from 17 in 1970 to 26 in 2009 [9]. As the Malaysian Adult Nutrition Survey (MANS) showed, about 80% of Malaysian adults consumed sugary beverages, such as carbonated drinks in 2014 [10]. Furthermore, the demand for sugar in Malaysia was estimated to increase from 1.4 million tonnes in 2011 to 1.9 million tonnes in 2020 [11].

In spite of the alarming facts and figures of diabetes and sugar intake, no study has examined the factors associated with consumption of added sugar in Malaysia. Previous studies often examined the types of sugary foods consumed by the consumers, but did not focus on how sociodemographic factors were associated with the consumption [12]. Considering this research gap, the present study analyses data from a nationwide survey of Malaysia to identify sociodemographic factors associated with consumption of sugar-sweetened foods and beverages. It is important to obtain a better understanding of the associated factors of consumption of sugar-sweetened foods and beverages if a more effective public policy directed towards reducing the intake of sugar is to be implemented.

The present study has several contributions. First, the present study is the first study to investigate sociodemographic factors associated with consumption of sugar-sweetened foods and beverages amongst a large nationwide sample of Malaysian households. Second, the present study offers an in-depth investigation of the sociodemographic differences in consumption of sugar-sweetened foods and beverages across ethnic groups. It is important to understand the factors associated with consumption of sugar-sweetened foods and beverages among people from different ethnic groups, especially given that Malaysia has a multi-ethnic population. Third, data used in the present study is a nationally representative sample consisting of a large sample size, which allows the present study's analysis to be stratified by ethnicity and includes various sociodemographic variables. Fourth, the present study uses a rigorous statistical model to examine factors associated with consumption decision and amount decision of sugar-sweetened foods and beverages. Fifth, findings of the present study can facilitate a comparison of the factors associated with consumption of sugar-sweetened foods and beverages between a developing country and the results for the developed countries documented in the literature.

## Methods

### Data

Data from the Malaysian Household Expenditure Survey (HES) 2014 was used in this study. HES was a nationwide survey conducted by the Department of Statistics Malaysia once every 5 years. The main purpose of the survey was to study the overall consumption pattern of Malaysian households. In order to ensure that the sample represented all the households in Malaysia, the sample was designed based on a two-stage stratified sampling. In the first stage, the selection was based on Enumeration Blocks (EBs) designed for the Population and Housing Census. The EBs were classified into urban and rural areas. A gazetted area with ≥ 10,000 population was considered to be an urban area, while a gazetted area with < 10,000 population was considered to be a rural area. In the second stage, households in the selected living quarters (LQs) were canvassed using face-to-face interview. In particular, each EB consisted of 80 to 120 LQs. Exclusion criteria were those staying at residential institutions, such as hotels, hospitals, welfare homes and prisons.

During the survey, only the head of each household was interviewed. The head was asked to report the total expenditures made by every household member. Then, the amounts were summed up. All the interviewers were given training prior to the survey. In an effort to reduce errors, the data collected by the interviewers were screened by the professionals and experienced staff before it was analysed. The data consisted of information about the characteristics of households and the total monthly household expenditure. The items recorded include food and beverages, clothing and footwear, housing, health and transport. The sample size was calculated based on three criteria: (i) findings of previous HES, (ii) level of sampling design and (iii) desired error. Overall, a total of 14,838 households were surveyed. More details about HES were described elsewhere [13].

### Variables

The dependent variable of the present study is the total monthly household expenditure on sugar-sweetened foods and beverages (in RM). These foods and beverages include sugar, jam, honey and confectioneries. Additionally, the amount spent by households on sugar-sweetened foods and beverages from household budget (in percentage) is calculated and used as another dependent variable. The formula for this calculation is household expenditure on sugar sweetened foods and beverages divided by household income. Then, the amount is converted to percentage. The sociodemographic characteristics of household head (gender, age, ethnicity, marital status, education, employment status), household income, household size, region, location of residence and monthly household

expenditure on tobacco and alcohol are used as the independent variables.

Age of household head and monthly household income (in RM) are formatted as continuous variables to allow for linear relationships. Household income refers to the total monthly income earned by all the household members. Household head's ethnicity is categorised into two categories: Bumiputera and non-Bumiputera. Bumiputera are Malaysians of indigenous Malay origin who have ethnic privilege in Malaysia, while non-Bumiputera are Malaysians from the ethnic minorities. Marital status of household head is grouped into three categories: single, married and widow/divorce. Following previous studies, the highest education level of household head is collapsed to form four categories: no formal education, primary (< 7 years of schooling), secondary (7–11 years) and tertiary (≥ 12 years) [14, 15].

To facilitate comparisons, employment status variable is categorised into two groups: employed and unemployed. Household heads who are employers, government employees, private sector employees or self-employed are considered to be employed, otherwise they are considered to be unemployed. Household size is taken into account and is formatted as a continuous variable. Region is categorised into Peninsular Malaysia and East Malaysia, while location of residence is grouped into urban and rural areas. To measure smoking and drinking behaviours, total monthly household expenditures on tobacco and alcohol are taken into account. These include spirits, liquors, wine, beer, cigarettes, cigar and tobacco.

### Statistical analyses

Descriptive statistics of all the independent variables was calculated. For continuous variables, mean and standard deviation were estimated. For categorical variables, percentage and frequencies were calculated. The present study used a two-part model to estimate demographic and lifestyle factors associated with consumption decision and amount decision of sugar-sweetened foods and beverages. In the first part, probit was used to examine whether or not the households consumed sugar-sweetened foods and beverages (i.e. consumption equation). The marginal effects were calculated to assess the change in probability of consuming sugar-sweetened foods and beverages resulted from the change in the independent variable. In the second part, ordinary least square (OLS) was utilised to evaluate how much the households spent on sugar-sweetened foods and beverages (i.e. amount equation). Because the second part was a linear regression, the estimated coefficients could be interpreted directly.

Simply put, the two-part model was a method to deal with the data that had a lot of zeros. In the model, a dichotomous model was developed in order to obtain the probability of observing the positive values of expenditure on sugar-sweetened foods and beverages. Then, a linear regression for the positive values was estimated. Consumption decision referred to decision of household to consume sugar-sweetened foods and beverages. Amount decision referred to the amount of money that household spent on sugar-sweetened foods and beverages. According to the assumptions of two-part model, there were three reasons explaining zero observations. First, the survey period was too short. For instance, some people purchase sugar-sweetened foods and beverages every 2 months instead of every month. Second, not all the respondents preferred sugar-sweetened foods and beverages because they had good health awareness. Third, not all the individuals could afford sugar-sweetened foods and beverages.

Since not every household consumed sugar-sweetened foods and beverages, the observations used in the amount eq. (14,028 households) were less than those used in the consumption eq. (14,838 household). In the consumption equation, all the households were included for analyses, so that the probability of consumption could be estimated. In the amount equation, only households that consumed sugar-sweetened foods and beverages were used for estimations because those that did not consume sugar-sweetened foods and beverages reported zero expenditure. In addition, analyses stratified by ethnicity were conducted to compare the factors affecting consumption decision and amount decision of sugar-sweetened foods and beverages in the two ethnic groups. Hence, a separate two-part model for Bumiputera and non-Bumiputera was estimated. Moreover, OLS was also used to estimate demographic and lifestyle factors associated with the amount spent by households on sugar-sweetened foods and beverages from household budget across ethnic groups. Statistical analyses were performed using Stata statistical software [16]. See Wooldridge for further discussion on two-part model [17].

## Results

Descriptive statistics for the HES sample is presented in Table 1. The average age of household heads, monthly household income and household size are 46 years, RM 5978.02 and four members, respectively. The majority of the household heads are males (84.78%). Slightly more than two third of the households are from Bumiputera ethnic groups (68.18%), while only 31.82% are from non-Bumiputera ethnic groups. This is similar to the ethnic composition of the Malaysian population. A large proportion of the household heads are married (79.09%), followed by single (12.08%) and widowed or divorced (8.83%). More than half of the household heads have secondary-level education (56.69%), while only 21.30, 17.58 and 4.42% have tertiary-level, primary-level and no formal education, respectively. The sample consists of 92.51% of employed household heads and

**Table 1** Summary statistics of the independent variables

| Variables | Mean/frequency | Std. dev./percent |
|---|---|---|
| Age | 46.46 | 13.01 |
| Income | 5978.02 | 6193.27 |
| Household | 4.32 | 2.04 |
| Gender | | |
| Male | 12,580 | 84.78 |
| Female | 2258 | 15.22 |
| Ethnicity | | |
| Bumiputera | 10,116 | 68.18 |
| Non-Bumiputera | 4722 | 31.82 |
| Marital status | | |
| Married | 11,735 | 79.09 |
| Widowed/divorced | 1310 | 8.83 |
| Single | 1793 | 12.08 |
| Education | | |
| No formal | 656 | 4.42 |
| Primary | 2609 | 17.58 |
| Secondary | 8412 | 56.69 |
| Tertiary | 3161 | 21.30 |
| Employment status | | |
| Employed | 13,727 | 92.51 |
| Unemployed | 1111 | 7.49 |
| Region | | |
| Peninsular | 10,665 | 71.88 |
| East | 4173 | 28.12 |
| Location | | |
| Urban | 10,246 | 69.05 |
| Rural | 4592 | 30.95 |
| Tobacco | 61.09 | 113.66 |
| Alcohol | 16.93 | 73.95 |
| Observations | 14,838 | |

For continuous variables, the values refer to mean and standard deviation. For categorical variables, the values refer to frequency and percent

Source: Malaysian Household Expenditure Survey 2014

7.49% of unemployed household heads. The majority of the households reside in Peninsular Malaysia (71.88%) and urban areas (69.05%). The average monthly household expenditures on tobacco and alcohol are RM 61.09 and RM 16.93, respectively.

Three separate multivariable regressions are estimated: pooled sample, Bumiputera sample and non-Bumiputera sample. The likelihood ratio (LR) statistics and F-statistics of all the regressions are highly significant, thus concluding that all the independent variables are jointly significant in explaining the consumption decision and amount decision of sugar-sweetened foods and beverages. The majority of the independent variables are significant. It is not surprising that the constants are significant in all the regressions because sugar-sweetened foods and beverages are somewhat like necessity goods, which people consume regardless of their income and sociodemographic status.

Table 2 shows the factors associated with consumption decision and amount decision of sugar-sweetened foods and beverages for the pooled sample. Age of household head is positively associated with monthly household expenditure on sugar-sweetened foods and beverages. An additional member of household increases the likelihood of consuming sugar-sweetened foods and beverages, and monthly expenditure by 1.2% and RM 2.08, respectively. Households headed by males are 1% less likely to consume sugar-sweetened foods and beverages and also spend RM 1.52 less than households headed by females. In terms of ethnicity, Bumiputera households are 2% more likely to consume sugar-sweetened foods and beverages than non-Bumiputera households. Households headed by married individuals are 1.4% more likely to consume sugar-sweetened foods and beverages than households headed by single individuals.

Compared to households with heads having tertiary-level education, household with heads having no formal education, primary-level education and tertiary-level education spend RM 11.98, RM 10.45 and RM 8.09 less on sugar-sweetened foods and beverages, respectively. Households headed by employed individuals spend RM 3.53 more on sugar-sweetened foods and beverages than households headed by unemployed individuals. Households residing in Peninsular Malaysia are 1.3% more likely to consume sugar-sweetened foods and beverages and also spend RM 4.26 more than households residing in East Malaysia. Although urban households are 1.2% less likely to consume sugar-sweetened foods and beverages than rural households, they spend RM 1.73 more. An increase of RM 1 in monthly household expenditures on tobacco and alcohol, the likelihood of consuming sugar-sweetened foods and beverages reduces by 0.1%, but the amount spent increases by RM 0.03.

Tables 3 and 4 show the factors associated with consumption decision and amount decision of sugar-sweetened foods and beverages for Bumiputera sample and non-Bumiputera sample. Among Bumiputera households, an additional year of age of household heads increases the amount spent on sugar-sweetened foods and beverages by RM 0.05. For both Bumiputera and non-Bumiputera households, an increase of one unit of household size raises the probability of consuming sugar-sweetened foods and beverages, and the amount spent by about 1% and RM 2.10, respectively. Non-Bumiputera households headed by males spend RM 3.91 less on sugar-sweetened foods and beverages than their counterparts headed by females. Bumiputera households with married heads spend RM 2.15 more on sugar-sweetened foods and beverages compared to Bumiputera households with single heads.

Among Bumiputera households, having low education level seems to increase the likelihood of consuming sugar-

**Table 2** The factors associated with consumption decision and amount decision of sugar-sweetened foods and beverages: pooled sample

| Variables | Consumption | | Amount |
|---|---|---|---|
| | Coefficients | Marginal effects | Coefficients |
| Constant | 0.889 (0.134)* | – | 10.365 (1.746)* |
| Age | 0.001 (0.002) | 0.001 (0.001) | 0.067 (0.022)* |
| Income/100[#] | − 0.001 (0.001) | − 0.001 (0.001) | 0.002 (0.004) |
| Household | 0.116 (0.011)* | 0.012 (0.001)* | 2.079 (0.116)* |
| Gender | | | |
| Male | − 0.103 (0.056)* | − 0.010 (0.005)* | − 1.522 (0.739)* |
| Female | – | – | – |
| Ethnicity | | | |
| Bumiputera | 0.194 (0.038)* | 0.020 (0.004)* | 0.585 (0.502) |
| Non-Bumiputera | – | – | – |
| Marital status | | | |
| Married | 0.130 (0.055)* | 0.014 (0.006)* | 1.340 (0.771) |
| Widowed/divorced | − 0.103 (0.077) | − 0.011 (0.009) | − 1.174 (1.099) |
| Single | – | – | – |
| Education | | | |
| No formal | 0.080 (0.098) | 0.007 (0.008) | − 11.977 (1.244)* |
| Primary | 0.033 (0.061) | 0.003 (0.006) | − 10.453 (0.788)* |
| Secondary | 0.080 (0.044) | 0.008 (0.004) | − 8.093 (0.569)* |
| Tertiary | – | – | – |
| Employment status | | | |
| Employed | 0.082 (0.069) | 0.009 (0.008) | 3.531 (0.954)* |
| Unemployed | – | – | – |
| Region | | | |
| Peninsular | 0.122 (0.040)* | 0.013 (0.004)* | 4.259 (0.510)* |
| East | – | – | – |
| Location | | | |
| Urban | − 0.122 (0.044)* | − 0.012 (0.004)* | 1.725 (0.514)* |
| Rural | – | – | – |
| Tobacco | − 0.001 (0.001)* | − 0.001 (0.001)* | − 0.001 (0.002) |
| Alcohol | 0.001 (0.001) | 0.001 (0.001) | 0.025 (0.003)* |
| Likelihood ratio | 287.990* | | – |
| F-statistic | – | | 60.42* |
| Observations | 14,838 | | 14,028 |

*Significance at the 5% level ($p < 0.05$). [#] income divided by 100

Source: Malaysian Household Expenditure Survey 2014

sweetened foods and beverages, but reduces the money spent. The negative relationship between monthly household expenditure on sugar-sweetened foods and beverages and low education level is also evidenced in non-Bumiputera sample. Bumiputera households with employed heads spend RM 2.97 more on sugar-sweetened foods and beverages than Bumiputera households with unemployed heads. Similarly, non-Bumiputera households headed by employed individuals spend RM 4.81 more on sugar-sweetened foods and beverages than non-Bumiputera households headed by unemployed individuals.

Bumiputera households who reside in Peninsular Malaysia are 2.3% more likely to consume sugar-sweetened foods and beverages and also spend RM 5.04 more than Bumiputera households who reside in East Malaysia. However, region is not associated with consumption of sugar-sweetened foods and beverages among non-Bumiputera households. Urban Bumiputera households spend RM 1.68 more on sugar-sweetened foods and beverages than rural Bumiputera households. For non-Bumiputera sample, urban households are 2.1% less likely to consume sugar-sweetened foods and beverages than rural households. Monthly household expenditure on tobacco is negatively related to the likelihood of consuming sugar-sweetened foods and beverages among Bumiputera households, whereas monthly household expenditure on alcohol is positively associated with the money spent on sugar-sweetened foods and beverages among non-Bumiputera households.

The factors associated with the amount spent by households on sugar-sweetened foods and beverages from household budget across ethnic groups are presented in Table 5. An additional year of age of household heads, the proportion of household income allocated for sugar-sweetened foods and beverages increases by 0.002%. An increase of RM 100 in household income, the proportion of household income spent on sugar-sweetened foods and beverages reduces by 0.004–0.005%. Household size is positively associated with the amount spent from household budget. The proportion increases by 0.063–0.069% when household size increases by one member.

**Table 3** The factors associated with consumption decision and amount decision of sugar-sweetened foods and beverages: Bumiputera sample

| Variables | Consumption | | Amount |
|---|---|---|---|
| | Coefficients | Marginal effects | Coefficients |
| Constant | 0.947 (0.168)* | – | 9.104 (1.797)* |
| Age | 0.001 (0.002) | 0.001 (0.002) | 0.052 (0.023)* |
| Income/100[#] | 0.001 (0.001) | 0.001 (0.001) | 0.003 (0.004) |
| Household | 0.123 (0.013)* | 0.010 (0.001)* | 2.087 (0.118)* |
| Gender | | | |
| Male | − 0.133 (0.074) | − 0.010 (0.005) | − 0.505 (0.797) |
| Female | – | – | – |
| Marital status | | | |
| Married | 0.113 (0.075) | 0.010 (0.007) | 2.154 (0.862)* |
| Widowed/divorced | − 0.165 (0.103) | − 0.015 (0.011) | − 0.484 (1.200) |
| Single | – | – | – |
| Education | | | |
| No formal | 0.279 (0.127)* | 0.018 (0.006)* | − 9.486 (1.321)* |
| Primary | 0.207 (0.083)* | 0.015 (0.005)* | − 8.894 (0.868)* |
| Secondary | 0.202 (0.057)* | 0.017 (0.005)* | − 6.916 (0.611)* |
| Tertiary | – | – | – |
| Employment status | | | |
| Employed | 0.045 (0.093) | 0.004 (0.008) | 2.969 (1.034)* |
| Unemployed | – | – | – |
| Region | | | |
| Peninsular | 0.259 (0.051)* | 0.023 (0.005)* | 5.038 (0.536)* |
| East | – | – | – |
| Location | | | |
| Urban | − 0.094 (0.051) | − 0.008 (0.004) | 1.682 (0.499)* |
| Rural | – | – | – |
| Tobacco | − 0.001 (0.001)* | − 0.001 (0.001)* | − 0.001 (0.002) |
| Alcohol | 0.001 (0.001) | 0.001 (0.001) | 0.006 (0.004) |
| Likelihood ratio | 177.730* | | – |
| F-statistic | – | | 54.710* |
| Observations | 10,116 | | 9669 |

*Significance at the 5% level ($p < 0.05$). [#] income divided by 100

Source: Malaysian Household Expenditure Survey 2014

If households are headed by males, the proportion of household income spent on sugar-sweetened foods and beverages is 0.059% lower compared to households headed by females. Compared to non-Bumiputera households, Bumiputera households spend 0.038% higher on sugar-sweetened foods and beverages from household budget. The amount of budget allocated for sugar-sweetened foods and beverages among households headed by married individuals is approximately 0.075–0.094% higher than households headed by single individuals. Setting tertiary-level education as the reference group, having no formal education, primary-level education and secondary-level education reduce the amount of household budget spent on sugar-sweetened foods and beverages by 0.156–0.325%. Households with employed heads spend around 0.120–0.213% more on sugar-sweetened foods and beverages from household budget compared to households with unemployed heads. Residing in Peninsular Malaysia is positively associated with the proportion of household budget allocated for sugar-sweetened foods and beverages (0.136–0.177%). Alcohol drinking is also positively associated with the amount spent by household on sugar-sweetened foods and beverages from household budget (0.001%).

## Discussion

HES provides information for analysing the consumption behaviour of Malaysian households, which is important for policy development. Although HES is not a panel data, it has a high quality for research use. It has a very large sample size and various sociodemographic variables and allows for analyses stratified by ethnic groups. The objective of the present study is to examine the sociodemographic factors associated with consumption of sugar-sweetened foods and beverages. The results show that age, household size, gender, marital status, education level, employment status, region and location of residence are associated with consumption of sugar-sweetened foods and beverages among Bumiputera and non-Bumiputera households in Malaysia. In addition, tobacco and alcohol expenditures are found to be associated with consumption of sugar-sweetened foods and beverages.

Previous studies' findings pertaining to age are noteworthy. Mullie et al. surveyed military men in Belgium and found that older individuals are less likely to consume sugar-sweetened beverage compared with younger individuals [18]. Similarly, using the National Health Interview Survey, Park et al. found

**Table 4** The factors associated with consumption decision and amount decision of sugar-sweetened foods and beverages: Non-Bumiputera sample

| Variables | Consumption | | Amount |
|---|---|---|---|
| | Coefficients | Marginal effects | Coefficients |
| Constant | 1.189 (0.213)* | – | 14.922 (3.738)* |
| Age | 0.001 (0.003) | 0.001 (0.001) | 0.081 (0.046) |
| Income/100[#] | − 0.001 (0.001) | − 0.001 (0.001) | 0.002 (0.007) |
| Household | 0.108 (0.018)* | 0.015 (0.002)* | 2.097 (0.284)* |
| Gender | | | |
| Male | − 0.076 (0.085) | − 0.010 (0.011) | − 3.913 (1.564)* |
| Female | – | – | – |
| Marital status | | | |
| Married | 0.153 (0.082) | 0.022 (0.013) | 0.311 (1.545) |
| Widow/divorce | − 0.026 (0.118) | − 0.004 (0.017) | − 1.876 (2.285) |
| Single | – | – | – |
| Education | | | |
| No formal | − 0.168 (0.158) | − 0.026 (0.027) | − 16.029 (2.788)* |
| Primary | − 0.191 (0.091)* | − 0.028 (0.015)* | − 12.855 (1.626)* |
| Secondary | − 0.084 (0.070) | − 0.011 (0.010) | − 10.133 (1.214)* |
| Tertiary | – | – | – |
| Employment status | | | |
| Employed | 0.152 (0.104) | 0.022 (0.017) | 4.806 (1.998)* |
| Unemployed | – | – | – |
| Region | | | |
| Peninsular | − 0.129 (0.071) | − 0.017 (0.009) | 0.934 (1.181) |
| East | – | – | – |
| Location | | | |
| Urban | − 0.174 (0.092)* | − 0.021 (0.010)* | 2.001 (1.498) |
| Rural | – | – | – |
| Tobacco | − 0.001 (0.001) | − 0.001 (0.001) | − 0.001 (0.004) |
| Alcohol | 0.001 (0.001) | 0.001 (0.001) | 0.038 (0.005)* |
| Likelihood ratio | 80.360* | | – |
| F-statistic | – | | 18.130* |
| Observations | 4722 | | 4359 |

*Significance at the 5% level ($p < 0.05$). [#] income divided by 100

Source: Malaysian Household Expenditure Survey 2014

that age is negatively associated with consumption of sports drinks, energy drinks and sugar-added beverages [19, 20]. Based on another sample collected in the US, Zytnick et al. and Xu et al. also identified that adults who are in the older age groups have lower odds of consuming sports drinks and sugar-sweetened beverages than their peers who are in the younger age groups [21, 22]. Similar findings were evidenced by Rehm et al. and Bleich and Wang [23, 24]. However, the results of the present study suggest that among households that consume sugar-sweetened foods and beverages, those headed by older adults tend to spend more on sugar-sweetened foods and beverages than those headed by younger adults. These are somewhat in contrast to the findings evidenced by previous studies. Surprisingly, there is no age difference in the likelihood of consuming sugar-sweetened foods and beverages. The plausible reason for these unexpected outcomes needs to be identified by a future qualitative study. An important policy implication of these findings is that an intervention measure directed towards reducing the consumption of sugar-sweetened foods and beverages should focus primarily on households headed by older individuals, especially those from Bumiputera ethnic groups because age variable is highly significant in this ethnic sample.

Household size is associated with increased consumption of sugar-sweetened foods and beverages. The explanation is quite straightforward. Since overall consumption of foods and beverages increases with the number of family members, the likelihood of consumption and the amount spent on sugar-sweetened foods and beverages increase as well. This argument is further supported by the finding on marital status that being married increases the consumption of sugar-sweetened foods and beverages as households headed by married individuals tend to have more household members than households headed by single individuals. However, findings of Park et al. and Xu et al. suggested otherwise [19, 22]. The authors found that being unmarried is positively related to the consumption of sports and energy drinks. These differences may be due to the fact that findings of the present study are based on household data, whereas findings of Park et al. and Xu et al. are based on individual data [19, 22]. In terms of policy implication, government should pay special attention to households with a large family size and headed by married individuals if the goal of lowering the demand for sugar-sweetened foods and beverages is to be achieved. However, because there is no data that

**Table 5** The factors associated with the amount spent by households on sugar-sweetened foods and beverages from household budget

| Variables | Pooled | Bumiputera | Non-Bumiputera |
|---|---|---|---|
| Constant | 0.446 (0.063)* | 0.511 (0.072)* | 0.428 (0.119)* |
| Age | 0.002 (0.001)* | 0.001 (0.001) | 0.004 (0.001)* |
| Income/100# | − 0.005 (0.001)* | − 0.005 (0.001)* | − 0.004 (0.001)* |
| Household | 0.067 (0.004)* | 0.069 (0.005)* | 0.063 (0.009)* |
| Gender | | | |
| Male | − 0.059 (0.027)* | − 0.044 (0.032) | − 0.095 (0.049) |
| Female | – | – | – |
| Ethnicity | | | |
| Bumiputera | 0.038 (0.018)* | – | – |
| Non-Bumiputera | – | – | – |
| Marital status | | | |
| Married | 0.075 (0.028)* | 0.094 (0.034)* | 0.046 (0.048) |
| Widowed/divorced | − 0.014 (0.039) | 0.012 (0.047) | − 0.052 (0.072) |
| Single | – | – | – |
| Education | | | |
| No formal | − 0.294 (0.045)* | − 0.257 (0.053)* | − 0.325 (0.089)* |
| Primary | − 0.276 (0.029)* | − 0.251 (0.035)* | − 0.307 (0.051)* |
| Secondary | − 0.163 (0.021)* | − 0.156 (0.024)* | − 0.169 (0.039)* |
| Tertiary | – | – | – |
| Employment status | | | |
| Employed | 0.120 (0.034)* | 0.076 (0.041) | 0.213 (0.063)* |
| Unemployed | – | – | – |
| Region | | | |
| Peninsular | 0.136 (0.019)* | 0.177 (0.021)* | − 0.002 (0.038) |
| East | – | – | – |
| Location | | | |
| Urban | 0.033 (0.019) | 0.027 (0.020) | 0.047 (0.048) |
| Rural | – | – | – |
| Tobacco | − 0.001 (0.001) | − 0.001 (0.001) | 0.001 (0.001) |
| Alcohol | 0.001 (0.001) | 0.001 (0.001) | 0.001 (0.001)* |
| Likelihood ratio | – | – | – |
| F-statistic | 129.240* | 110.760* | 33.890* |
| Observations | 14,838 | 10,116 | 4722 |

*Significance at the 5% level ($p < 0.05$). # income divided by 100

Source: Malaysian Household Expenditure Survey 2014

compares the prevalence of consumption before and after any intervention, the present study is unable to conclude which policy should be adopted.

Findings of the present study that families headed by males are less likely to consume and also spend less on sugar-sweetened foods and beverages than families headed by females somewhat contradict findings of previous studies [19, 21, 23, 24]. In one of those studies, males were found to have a higher likelihood of drinking soft drinks frequently than females [23]. In another study, being males was positively associated with the grams of sugar intake [24]. Furthermore, Park et al. and Zytnick et al. found that males are more likely to consume sports and energy drinks than females [19, 21]. These contradictory findings evidenced in the present study may be explained by the fact that men take more responsibilities for their households and consequently are more aware of their health. Since consumption of sugar-sweetened foods and beverages is bad for health, men are unlikely to indulge in it. Given these findings, policy makers are suggested to devote their attention to households headed by women, instead of those headed by men. Nationwide policies that can help to discourage women from consuming sugar-sweetened foods and beverages are worthy of consideration.

Ethnic factor, as measured by Bumiputera and non-Bumiputera, is associated with consumption of sugar-sweetened foods and beverages. The results show that Bumiputera households are more likely to consume sugar-sweetened foods and beverages than non-Bumiputera households. Why being Bumiputera is related to increased likelihood of consuming sugar-sweetened foods and beverages is ambiguous, but culture may be a contributing factor. In a previous study conducted in the US, Berger et al. found that non-Black minority is more likely to consume energy drinks than the White [25]. Findings of Bleich and Wang and Park et al. suggested likewise. Taken together, it can be concluded that ethnicity plays an important role in determining added sugar intake [24, 19]. While the present study finds sociodemographic and lifestyle differences in consumption of sugar-sweetened foods and beverages across ethnic groups, additional qualitative studies should be conducted in order to obtain a better understanding of how ethnicity affects sugar-sweetened foods and beverages consumption.

The findings on education are interesting. Households with less-educated heads are more likely to consume sugar-sweetened foods and beverages but spend less than households with well-educated heads. The results are more obvious among Bumiputera than non-Bumiputera. The relationship between education and consumption of sugary beverage was also evidenced by previous studies but the results were mixed. On one hand, using a nationwide data of South Korea, Han et al. found that individuals with higher education levels display a higher likelihood of consuming sugar-sweetened beverages than their peers with lower education levels [26]. On the other hand, Thompson et al., in examining the socioeconomic factors associated with intake of sugar-added beverages, found a negative relationship between academic qualification and mean intake of sugar-added beverages [27]. This outcome was also shared by other studies [20–23, 28]. Surprisingly, there were also evidences suggesting that education is not associated with sugar-sweetened beverage intake [24, 29]. It is noteworthy that the explanations provided by Cheah and Tang and Cheah and Goh that education promotes healthy lifestyle from mediation through the health knowledge and time preference are not strongly supported by the findings pertaining to added sugar consumption [15, 14]. Therefore, one cannot simply conclude that providing the less-educated segments of the population with more education can definitely help to reduce the consumption of sugar-sweetened foods and beverages.

Employment is associated with consumption of sugar-sweetened foods and beverages, as households with employed heads spend more on sugar-sweetened foods and beverages than households with unemployed heads. Findings based on Bumiputera and non-Bumiputera samples show likewise. These findings highlight the financial capability associated with household expenditure behaviour. Since households headed by employed individuals have a better financial capability, they tend to spend more on sugar-sweetened foods and beverages. In a Denmark population, Friis et al. also found that employed individuals have a higher likelihood of consuming energy drinks than unemployed individuals [28]. It is worthy to note that region and location of residence are also associated with consumption of sugar-sweetened foods and beverages independent of other sociodemographic factors. Similarly, these findings are evidenced in both Bumiputera and non-Bumiputera samples. Households who reside in Peninsular Malaysia are more likely to consume sugar-sweetened foods and beverages and also spend more than household reside in East Malaysia. However, households in urban areas are less likely to consume sugar-sweetened foods and beverages but spend more than households in rural areas. Taken together, it can be concluded that there are geographical differences in the intake of added sugar. The reason may be pertaining to the food environment. An in-depth research should be conducted to supplement a better knowledge of the reason explaining the relationship between geographical factor and consumption of sugar-sweetened foods and beverages. Nevertheless, these findings have important implications for policy. First, policy makers should pay special attention to households who reside in Peninsular Malaysia, especially those from Bumiputera ethnic group. Second, policies aimed at reducing the likelihood of consuming sugar-sweetened foods and beverages among urban households can be considered, as households induce to convert from non-consumption to consumption will spend more on sugar-sweetened foods and beverages.

Holding sociodemographic factors constant, household expenditure on tobacco is negatively associated with the odds of consuming sugar-sweetened foods and beverages, especially among Bumiputera households. This means that smoking can reduce consumption of sugar-sweetened foods and beverages, which contradicts findings of previous studies. Based on a sample of US households, Park et al. found that smokers have a higher intake of added sugars than non-smokers [20]. An older study conducted by Park et al., as well as others, such as Mullie et al. and Friis et al. observed similar findings [19, 18, 28]. Using Brazilian data, Rombaldi et al. also found that smokers are more likely to consume soft drinks than non-smokers [30]. The explanation for this unexpected finding may be related to an economic factor. Owing to budget constraint, households that purchase tobacco are less inclined to allocate their budget for sugar-sweetened foods and beverages compared with households that do not purchase tobacco. Furthermore, unlike alcohol, tobacco and sugar-sweetened beverages are not complementary goods. It is worthy of note that although the marginal effect of tobacco expenditure on consumption of sugar-sweetened foods and beverages is highly significant, it is very small. Thereby, it should not be the main concern of policy makers.

Another non-sociodemographic variable that is found to be significantly associated with consumption of sugar-sweetened foods and beverages is alcohol consumption. It seems that household expenditure on alcohol is positively associated with the amount spent on sugar-sweetened foods and beverages. However, alcohol only explains the consumption behaviour of non-Bumiputera. Because of the prohibition of alcohol in Islam, that is, the main religion of Bumiputera, non Bumiputera is likely to report consuming alcohol [14, 15]. The positive relationship between alcohol and sugar-sweetened foods and beverages is in agreement with the findings of previous studies that alcohol drinkers have a higher likelihood of consuming sugar-added beverages than non-alcohol drinkers [19, 20, 28]. This is partly due to the fact that sugar-added beverages, such as energy drinks and soft drinks are often used to mix with alcohol [25]. In other words, alcohol and sugary beverages are complementary goods. Given these findings, nationwide policies directed toward reducing consumption of sugar-sweetened foods and beverages among alcohol drinkers may

be worthwhile. Particular attention should be paid to non-Bumiputera households, instead of Bumiputera households.

Although the present study uses a nationally representative data to offer an in-depth examination of the factors associated with consumption of sugar-added foods and beverages among different ethnic groups of households, there are several limitations. First, all the information obtained in the survey is self-reported. Hence, reporting error may occur. Nevertheless, the data has a large sample size. Second, because the data used in the present study is a cross-sectional data, the causal relationship between sociodemographic factors and consumption of sugar-sweetened foods and beverages is not well-understood. Third, household data is used instead of individual data, thus individuals' consumption behaviour cannot be well-identified. Fourth, other carbohydrate foods that have the same negative effects on health, such as grains, maize and wheat are not taken into account for analyses because of data limitation. HES does not survey household expenditure on these foods. In spite of these limitations, the present study has significant contributions to literature and policy development. With data availability, future study may want to estimate separate models for the expenditure on soft drinks, energy drinks and sport drinks, as well as other carbohydrate foods.

**Acknowledgements** The authors would like to thank the Department of Statistics Malaysia for sharing the data from the Malaysian Household Expenditure Survey and to publish this paper.

**Funding** This study received financial support from the CIMB Bank Berhad (KOD SO 13743).

## Compliance with ethical standards

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

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):579–584
https://doi.org/10.1007/s13410-019-00712-7

ORIGINAL ARTICLE

# Technical accuracy of ten self-monitoring blood glucose devices commonly used in Dhaka City of Bangladesh

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Received: 31 July 2018 / Accepted: 13 January 2019 / Published online: 29 January 2019


**Abstract**
Due to inadequate regulatory mechanisms, the accuracy of the self-monitoring blood glucose (SMBG) devices is not ensured leading to potentially serious clinical consequences in Bangladesh. The present study was undertaken to evaluate the technical accuracy of ten most commonly used SMBG devices marketed in Dhaka City of Bangladesh. Top ten SMBG devices sold in Dhaka City were studied on a group of 100 type 2 diabetes mellitus subjects. Blood glucose values estimated (at fasting and 2 h after breakfast) by SMBG devices, using a blinding technique, were compared with the corresponding laboratory values by enzymatic method using the Dimension RXLMax automated chemistry analyzer. Hematocrit was measured using the Sysmex XT 2000 hematology autoanalyzer. The mean absolute relative error (MARE, %) was used as an indicator of accuracy and precision together. A highly significant correlation was observed between the device and laboratory values. However, none of the devices showed an acceptable accuracy at 5–10% deviation from the corresponding laboratory values either at fasting or postprandial states. On pooling together data from two prandial states, even at 15% deviation limits, 70% of the devices failed to show accurate results. On calculation of MARE, 60% devices were found to be beyond 15% error limit at 95% accuracy level. Corresponding analysis with 90% accuracy level showed 30% beyond the limit. Blood glucose results from around 30% of the top ten SMBG devices sold in Dhaka City do not have even minimum level of technical accuracy, and many others are not optimum in their accuracy levels.

**Keywords** Self-monitoring blood glucose devices · Glucometer · Glycemic control tools · Technical accuracy

## Introduction

Optimum glycemic control is of central importance in diabetes management [1]. Introduction of dry chemistry-based blood glucose monitors [often called self-monitoring blood glucose (SMBG) devices or home blood glucose meters (HMBG) devices] has revolutionized the management of the disease due to self-empowerment of the patients. However, at the same time, it has increased the risk of mismanagement of diabetes due to the often incorrect results obtained from low-quality devices particularly in unregulated and ill-supervised settings. Technical accuracy of blood glucose monitors refers to the agreement of the analytical results with a comparative laboratory method [2]. The same or different clinical decisions may be made despite noticeable analytical differences in results, depending on how the result will be utilized in patient care: screening, diagnosis, or management. Glucose meter variability or precision also contributes to differences in glucose meter analytical and clinical agreement. A glucose meter total variability of 10% leads to different insulin dosage in 16–45% of

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**Table 1** SMBG devices included in the study with models, analytical methods, manufacturers, and country of origin

| Device no. | Device name | Analytical technology | Manufacturer | Country |
|---|---|---|---|---|
| 01 | Free style—Optimum | Glucose oxidase biosensor | Abbott | USA |
| 02 | Quick Check | Glucose oxidase biosensor | Major Biosystem Corp. | Taiwan |
| 03 | OneTouch SelectSimple | Glucose oxidase biosensor | LifeScan | Switzerland |
| 04 | Accu-check Active | Dehydrogenase | Roche Diagnostics | Germany |
| 05 | GlucoLab | Glucose oxidase biosensor | Infopia Co. Ltd. | South Korea |
| 06 | Omnitest Plus | Glucose oxidase biosensor | Braun | Germany |
| 07 | Sensocard Plus | Glucose oxidase biosensor | 77Elektronika kft | Hungary |
| 08 | HGM-111 | Glucose oxidase biosensor | Omron | Japan |
| 09 | GlucoLeader Enhance | Glucose oxidase biosensor | HMD Biomedical Inc. | Taiwan |
| 10 | Rightest GM100 | Glucose oxidase biosensor | Bionime Corporation | Taiwan |

cases, and a glucose meter variability of > 10–15% leads to a twofold or greater discrepancy in insulin dosage [3]. Glucose meter total precision of < 1–2% would be required to ensure similar insulin dosage compared to the laboratory methods more than 95% of the time [2].

Due to high importance of technical accuracy, as stated above, it is very important to evaluate the SMBG devices before allowing those to be marketed. But, unfortunately, regulatory and monitoring system in this respect is virtually nonexistent in many countries, and people are driven mainly by aggressive marketing promoted by pharmacy, salesman, and advertisement as well as cheaper and cheaper options. In line with this situation, Bangladesh market is now flooded with low-grade meters without any standardization. In the long run, this is not only damaging the health of the patients but it is also leading to substantial socioeconomic burden to the individuals, families, and state due to the high cost of managing diabetic complications. Under the above perspective, the present study was planned to evaluate the technical accuracy of blood glucose measurement results of ten most commonly used SMBG devices sold in the Bangladesh market.

## Methods

### Study design

It was an experimental study.

### SMBG devices

From among the most commonly sold blood glucose meters in Dhaka City, as found out through a retailer-based survey [4], 10 m was collected from the major markets in Dhaka. The models, manufacturers (with country), and the basic technology used by the respective devices (as per their user manuals) are given in Table 1. Except the Manual of OneTouch Select Simple, no other user manual provided any information regarding the compliance with International Standard

**Table 2** Comparison of fasting blood glucose values obtained from the SMBG devices and chemistry analyzer

| Device no. | Device value (mmol/l) (mean ± SD) | Lab value (mmol/l) (mean ± SD) | *t*/*p* value |
|---|---|---|---|
| 1 | 10.33 ± 3.3 | 10.71 ± 3.6 | − 1.448/0.001 |
| 2 | 9.70 ± 3.2 | 10.71 ± 3.6 | − 2.717/0.001 |
| 3 | 9.30 ± 2.7 | 9.96 ± 3.0 | − 4.204/0.001 |
| 4 | 9.41 ± 2.6 | 9.96 ± 3.0 | − 3.106/0.001 |
| 5 | 9.69 ± 4.66 | 9.76 ± 4.8 | − 0.257/0.001 |
| 6 | 9.99 ± 4.5 | 9.76 ± 4.8 | 0.816/0.001 |
| 7 | 9.65 ± 4.17 | 10.6 ± 4.5 | − 2.709/0.001 |
| 8 | 10.72 ± 4.7 | 10.61 ± 4.5 | 0.457/0.001 |
| 9 | 11.22 ± 5.9 | 11.53 ± 5.5 | − 1.77/0.001 |
| 10 | 11.5 ± 5.9 | 11.53 ± 5.5 | − 0.116/0.001 |

Data are expressed as M ± SD. Statistical differences between device and analyzer values were analyzed by paired *t* test

**Table 3** Comparison of postprandial (2-h ABF) blood glucose values obtained from the SMBG devices and chemistry analyzer

| Device no. | Device value (mmol/l) (mean ± SD) | Lab value (mmol/l) (mean ± SD) | *t/p* value |
|---|---|---|---|
| 1 | 12.92 ± 3.3 | 13.74 ± 3.9 | −2.871/0.001 |
| 2 | 12.89 ± 4.1 | 13.74 ± 3.9 | 2.651/0.001 |
| 3 | 12.79 ± 3.2 | 13.14 ± 2.9 | −1.315/0.001 |
| 4 | 12.57 ± 3.1 | 13.14 ± 2.9 | −3.063/0.001 |
| 5 | 12.70 ± 4.4 | 12.87 ± 4.3 | −0.465/0.001 |
| 6 | 12.99 ± 4.5 | 12.87 ± 4.3 | 0.404/0.001 |
| 7 | 12.46 ± 3.9 | 13.79 ± 4.0 | −4.676/0.001 |
| 8 | 14.21 ± 4.4 | 13.79 ± 4.0 | 1.340/0.001 |
| 9 | 13.29 ± 4.9 | 14.62 ± 5.0 | −2.582/0.001 |
| 10 | 13.35 ± 4.4 | 14.62 ± 5.0 | −2.678/0.001 |

Data are expressed as M ± SD. Statistical differences between device and analyzer values were analyzed by paired *t* test

Organization (ISO) or any other regulatory authority criteria. The OneTouch Select Simple Manual stated that the model was ISO 15197:2003 compliant. The technical details of the devices and directions or precautions on their use were noted from the written instruction of the manufacturers. The devices were blinded during the study to avoid possible bias.

## Subjects

A total of 100 type 2 diabetic subjects were recruited from the Outpatient Department of the BIHS Hospital, a tertiary hospital in Dhaka City operated by the Diabetic Association of Bangladesh.

## Inclusion criteria

- Age—30 to 60 years
- T2DM subjects

**Table 4** Correlation between SMBG device and corresponding laboratory analyzer values of blood glucose at fasting and postprandial (2-h ABF) states

| Device no. | Fasting (*r/p*) | Postprandial (*r/p*) |
|---|---|---|
| 1 | 0.924/0.001 | 0.967/0.001 |
| 2 | 0.936/0.001 | 0.951/0.001 |
| 3 | 0.989/0.001 | 0.870/0.001 |
| 4 | 0.977/0.001 | 0.930/0.001 |
| 5 | 0.995/0.001 | 0.950/0.001 |
| 6 | 0.987/0.001 | 0.956/0.001 |
| 7 | 0.866/0.001 | 0.913/0.001 |
| 8 | 0.973/0.001 | 0.930/0.001 |
| 9 | 0.996/0.001 | 0.869/0.001 |
| 10 | 0.997/0.001 | 0.886/0.001 |

Correlation analysis was done using Pearson's correlation test

## Exclusion criteria

- Pregnancy or lactation period for female subjects
- Acute or chronic complications of DM
- Any other notable disorder detected clinically

## Experimental procedure

**Patient preparation** The patients were ambulatory and were on a normal-to-high carbohydrate intake for 3 days before the test. They were advised to fast for at least 10 h (and not more than 16 h), and the tests were performed in the morning because of the hormonal diurnal effect on glucose. Patients were advised to refrain from exercise, eating, drinking (except that they may drink water), and smoking before the test was completed.

**Sample collection** Capillary blood samples were collected from fingertips in the fasting state and 2 h after breakfast. Measurement was done in random order as per instructions given by the respective manufacturers. A venous blood sample was drawn immediately by skin puncture both during fasting and postprandial states, and the sample was kept with an anticoagulant.

**Biochemical analysis** All measurements were done in the analytical laboratory of BUHS where ambient temperature was controlled within 23–28 °C. All samples were centrifuged for 3 min at 10,000×*g* within 30 min. Plasma glucose was measured by enzymatic method (hexokinase glucose-6-phosphate dehydrogenase) using the Dimension RXLMax Autoanalyzer (Siemens, Germany). Hematocrit was measured using Sysmex XT-2000 (Sysmex, Japan).

**Statistical analysis** Average results from duplicate measurements were taken. Absolute as well as relative (%) errors were

**Table 5** Accuracy of SMBG devices in measuring the fasting and postprandial (2-h ABF) blood glucose values at various preset levels of deviation from the analyzer values ($n = 42$)

| Device no. | < 5% | | < 10% | | < 15% | | < 20% | |
|---|---|---|---|---|---|---|---|---|
| | Fasting % | ABF % | Fasting % | ABF % | Fasting % | ABF % | Fasting % | ABF % |
| 1 | 26 | 31 | 60 | 62 | 76 | 79 | 93 | 95 |
| 2 | 24 | 26 | 50 | 57 | 60 | 67 | 74 | 83 |
| 3 | 24 | 40 | 64 | 67 | 90 | 90 | 95 | 90 |
| 4 | 48 | 55 | 64 | 86 | 83 | 90 | 93 | 86 |
| 5 | 33 | 43 | 64 | 57 | 83 | 79 | 95 | 90 |
| 6 | 31 | 40 | 50 | 69 | 69 | 86 | 83 | 93 |
| 7 | 24 | 14 | 55 | 40 | 62 | 74 | 86 | 90 |
| 8 | 38 | 36 | 71 | 55 | 90 | 79 | 90 | 90 |
| 9 | 38 | 38 | 71 | 64 | 98 | 90 | 95 | 90 |
| 10 | 50 | 36 | 74 | 67 | 90 | 90 | 100 | 93 |

expressed as M ± SD. Data from the SMBG devices were compared with laboratory results by paired *t* test, and comparison between individual devices was done by unpaired *t* test. Pearson correlation test was done to test the agreement between laboratory and device results at different ranges of blood glucose. Agreements were also analyzed by matching of the results between the two systems at various preset levels of accuracy (90% and above of the SMBG device results to be within the respective accuracy limits to become comparable). The mean absolute relative error (MARE) was calculated following Ginsberg (2009) by taking the average of the two individual absolute errors (one for fasting and one for postprandial) for each device relative to its reference value.

## Ethical approval

Ethical approval was obtained from the Ethics and Research Review Committee of BUHS. Informed written/thumb print consent was obtained from all respondents after a full explanation of the nature, purpose, and procedures used for the study. Participants were informed about their right to withdraw from the study at any stage of the study.

**Table 6** Accuracy of SMBG devices in measuring the fasting and 2-h ABF blood glucose values (combined analysis) at various preset levels of deviation from the corresponding laboratory values ($n = 84$)

| Device no. | < 5% | < 10% | < 15% | < 20% |
|---|---|---|---|---|
| 1 | 29 | 61 | 77 | 94 |
| 2 | 25 | 54 | 63 | 79 |
| 3 | 32 | 65 | 90 | 93 |
| 4 | 51 | 75 | 86 | 89 |
| 5 | 38 | 61 | 81 | 93 |
| 6 | 36 | 60 | 77 | 88 |
| 7 | 19 | 48 | 68 | 87 |
| 8 | 37 | 63 | 83 | 90 |
| 9 | 38 | 68 | 93 | 93 |
| 10 | 43 | 70 | 90 | 96 |

## Results

A total of 100 subjects participated in this study. The mean age (years) of the subjects was 45. The fasting and postprandial blood glucose values (mean ± SD), as measured by the SMBG devices and the chemistry analyzer, are shown in Tables 2 and 3, respectively. Statistical analysis of the differences between the corresponding values, as assessed by paired *t* test, showed significantly ($p < 0.05$ to $p < 0.001$) different results between the two equipments at both prandial states. In all cases, the device values were lower than those of the analyzer values. On Pearson's correlation analysis, the device and analyzer values showed highly significant correlation in all the individual devices (Table 4).

In correspondence with the findings of the group difference analysis, many of the SMBG devices failed to show an acceptable accuracy at the lower levels (5–10%) of deviation from the corresponding analyzer values, at both prandial states (Table 5). At the 15% error level, four (40%) out of ten devices (nos. 3, 8, 9, and 10) and four (40%) out of ten devices (nos. 3, 4, 9, and 10) showed acceptable results from analyzer values at both the fasting and postprandial states, respectively. Similar analysis at 20% error level showed that three (30%) devices (nos. 2, 6, and 7) failed to show accurate results in the fasting state and two (20%) of the devices (device nos. 2 and 4) failed to show accurate results in the postprandial states. When accuracy analysis was repeated with the combined values of fasting and postprandial states (taking 84 data points for 21 subjects), no device showed accuracy within 5–10% of deviation and 30% of the devices

**Table 7** Precision and accuracy of SMBG devices from MARE values (at 95% accuracy level)

| Maximum level of deviation (+ or −) | Number (%) | SMBG device nos. with accurate results |
|---|---|---|
| 5% | 0 | – |
| 10% | | – |
| 15% | 4 (40) | 1, 3, 4, 8 |
| 20% | 6 (60) | 1, 3, 4, 5, 8, 10 |

(nos. 3, 9, and 10) showed acceptable accuracy at the 15% error level (Table 6). The corresponding value improves to 60% at the 20% error level (device nos. 1, 3, 5, 8, 9, and 10). When precision and accuracy analysis was based on MARE, at 95% accuracy level (Table 7), four (40%) devices (nos. 1, 3, 4, and 8) were found to be accurate within the 15% error limit and six (60%) devices (nos. 1, 3, 4, 5, 8, and 10) were within the 20% error limit. Corresponding analysis with 90% accuracy level (Table 8) showed that seven (70%) devices (nos. 1, 3, 4, 5, 8, 9, and 10) were within 15% and nine (90%) devices (nos. 1, 3, 4, 5, 6, 7, 8, 9, and 10) were within 20% error limit. Again, none of the devices were within 10% and 5% error limits.

A wide variation was found in the market price of both the devices (from US$7.5 to US$30) and the strips (from US$0.15 to US$1.02 per strip). On analysis of results and costs, higher price of a device or strip was not found to be necessarily associated with the quality of the result.

## Discussion

The objective of the present study was to explore the technical accuracy of some SMBG devices commonly used in Dhaka City. Comparison of results by paired *t* test shows that results from every device significantly differ from the chemistry analyzer values at both prandial states (Tables 2 and 3). Considering the nature of the sample (capillary blood vs venous plasma), this variation is expected, but the magnitude of the variation ($p < 0.001$ for every devices) indicates that a substantial proportion of the devices may not provide accurate results. In contrast to this finding, 100% of the devices, both in the fasting and postprandial states, generate results which show highly significant ($p < 0.001$) correlation with their analyzer counterpart. The later result may provide a false notion regarding the concordance of analyzer and SMBG device results. Statisticians warn about the inappropriate use of correlation analysis in method comparison studies [5]. The present data reemphasizes this warning. The possibility of relative inaccuracy is reflected in the fact that none of the devices in the present study generates results which are within 90% accuracy of the analyzer results (Tables 5 and 6). It is also noteworthy that only 30% of the devices show an accuracy level of 85%. The MARE is regarded as the best single measure of both accuracy and precision [6], and based on MARE analysis, 60% and 40% of the present devices fail to be within the inaccuracy margins of 15% and 20%, respectively, at the 95% accuracy level (Table 7). When the bare minimum of 80% accuracy of the ISO 15197:2003 criteria [7] is considered, a good proportion (20%) of the devices still fall outside the acceptance limit. As per the presently followed ISO criteria, the ISO 19157:2013 [8], 70% of the devices in the present study fall outside the acceptance limit. Since all phases of analytic errors were carefully avoided in the present study, the inaccuracy of the results seems to originate from the devices themselves. The present data leads to a cautionary note regarding the blind use of devices from Bangladesh market as a great number of people are exposed to substantial risk from inaccurate results.

Although the great importance of SMBG devices in clinical care and public health strategies (particularly in the field of diabetes mellitus) are generally acknowledged, the criteria for technical accuracy are still not universally agreed. In the EU, the consensus standard (ISO 15197) is applied for the premarketing evaluation of SMBG systems. The standard was established by the ISO in conjunction with the international regulatory authorities, health care providers, and device manufacturers in many countries. It was revised in 2013 with more stringent accuracy criteria [8] for which compliance was recommended in 2016. ISO 15197:2013 suggests that 95% of all measured values should fall within 15% of lab glucose values

**Table 8** Precision and accuracy of SMBG devices from MARE values (at 90% accuracy level)

| Maximum level of deviation (+ or −) | Number (%) | SMBG device nos. with accurate results |
|---|---|---|
| 5% | 0 (0) | – |
| 10% | 0 (0) | – |
| 15% | 7 (70) | 1, 3, 4, 5, 8, 9, 10 |
| 20% | 9 (90) | 1, 3, 4, 5, 6, 7, 8, 9, 10 |

above 100 mg/dl (5.5 mmol/l) and 15 mg of glucose values below 100 mg/dl (5.5 mmol/l). The American Diabetes Association (ADA), on the other hand, desires only 5% deviation (expressed in concentration units [mg/dl] for glucose values below 75 mg/dl) [9]. Some other authorities have suggested even greater accuracy. Specially, the USFDA has released its Guidance Documents in 2016 (drafted in 2014) with requirements for SMBG systems intended for professional settings and, separately, for self-monitoring by lay persons [10]. The FDA documents have suggested more stringent accuracy criteria compared to the ISO 15197:2013 ones. As per the present FDA criteria for OTC systems, at least 95% of measurement results have to fall within ± 15% and at least 99% within ± 20% of the reference measurement values across the entire claimed measurement range of the SMBG system. Experts from industry, science, and medicine are concerned about these quite strict minimum accuracy criteria [11]. In practice, the levels of accuracy other than that suggested by ISO 15197 are rarely met. In one review [6], at 5% inaccuracy, the most accurate meter today has only 63% acceptable values. As per that review, the average meter has less than half of the values in the highly accurate range. There is a suggestion [12] that a standard label, similar to a nutrition label, is made mandatory for helping people to have informed choice regarding their SMBG devices. The label should contain standard accuracy and the percentage of measurements falling within 20%, 15%, 10%, and 5% (in terms of MARE) inaccuracy levels. By showing the MARE and the percent of values having inaccuracies, patients could select the meter with the proper accuracy and other features that are best for them. If the inaccuracy level is fixed at 10% and 5%, none of the devices in Dhaka market qualifies to be acceptable.

It should be noted that values at lower levels of the blood glucose (< 5.5 mmol/l) have not been tested in the present study. The accuracy of these devices needs to be also tested at the lower ranges of blood glucose. However, even from the present data, it may be concluded that a substantial proportion of SMBG devices, presently sold in Dhaka market, do not show minimum level of technical accuracy and many others are not optimum in their accuracy levels. It is the responsibility of appropriate authorities and professional societies to generate suggestions and regulations regarding the allowable error margins before marketing a device in a particular market. Patients' awareness and education as well as appropriate intervention by regulatory authorities should urgently be promoted to address this alarming situation.

**Acknowledgements** We gratefully acknowledge the contribution of the study subjects with type 2 diabetes for their cooperation. We also thank the Bangladesh University of Health Sciences (BUHS) for the financial and logistic support.

## Compliance with ethical standards

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

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):585–589
https://doi.org/10.1007/s13410-018-0692-4

ORIGINAL ARTICLE

# Self-management practices of type 1 diabetes mellitus

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Received: 19 May 2018 / Accepted: 25 August 2018 / Published online: 8 September 2018


**Abstract**
The effective management of Type 1 Diabetes (T1D) involves self-management practices and education about self-monitoring of blood glucose (SMBG) and insulin therapy. Data on self-management practices in India is sparse. The aim of the study was to assess the self-management practices in young subjects with T1D. The frequency and timing of SMBG, the types of insulin used and insulin regimens, and the sites of administration and rotation patterns were analyzed in the study. This was an observational cross-sectional study carried out in young subjects with T1D using a pre designed, pretested questionnaire. Eighty (80) subjects were enrolled; age ranges from 5 to 25 years with a mean duration of T1D of 5 years. HbA1c was less than 8.5% in 47.4% of subjects. Fifty-seven percent monitored SMBG daily and majority monitored only once in the morning and once in the evening. The combination of fasting, pre-lunch, and pre-dinner was most recorded. The types of insulin used are regular, premixed, NPH, and glargine. Majority (62%) used premixed and regular insulin with a thrice-daily regimen. Site rotation was seen in 75.5% of subjects. An association between SMBG and HbA1c was observed. Eighty-six percent of the subjects with SMBG thrice or more in a day had HbA1C of less than 8.5%. The study concludes that SMBG in T1D is underused and highlights the need for educating subjects with T1D and their families further about SMBG, insulin regimens, and the importance of taking insulin diligently as advised by the doctor for better glycemic control.

**Keywords** Self-management practices · SMBG · Insulin · Type 1 diabetes

## Introduction

The prevalence of Type 1 diabetes (T1D) is increasing all over the world including India. India has 128,500 children with T1D [1]. The effective management of T1D involves self-management practices and education about self-monitoring of blood glucose (SMBG) and insulin therapy. Self-management is tedious and often ignored. However, it is the key to achieve glycemic goals and prevent complications in T1D. Data on self-management practices in India is sparse; hence, this study was conducted to assess self-management practices in young subjects with T1D.

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## Objective

The objective of the study was to assess the self-management practices in young subjects with T1D. The frequency and timing of SMBG, the types of insulin used and insulin regimens, and the sites of administration of insulin and rotation patterns were analyzed in the present study.

## Methodology

### Study design

Observational cross-sectional study.

### Study subjects

The subjects who were attending the Type 1 Diabetes OP of the Endocrinology Department of King George hospital from May–July 2016 were included in the study.

## Study tool

A predesigned, pretested, and close-ended questionnaire was prepared based on the objective of the study. Pretesting was carried out in a pilot study with ten patients to check for clarity of the questionnaire. Necessary changes were then made.

## Data collection

A detailed demographic history was collected in all the subjects. Socioeconomic status was assessed using the updated Kuppuswamy's socioeconomic scale [2]. Literacy was defined as any subject above the age of 7 years able to read and write any one language. Written consent was taken from all the subjects. Questions were asked based on the predesigned questionnaire in their local language.

The questionnaire used for SMBG

1) Number of times monitored

   a) Weekly
   b) Twice weekly
   c) Thrice weekly
   d) Daily

2) Time in the day chosen to monitor

   a) Fasting blood glucose
   b) Pre-lunch
   c) Pre-dinner
   d) Post breakfast
   e) Post lunch
   f) Post dinner
   g) In hypoglycemic state

The questionnaire for insulin usage:

A. Type of insulin used

1. conventional vs. analogs
2. regular
3. premixed
4. NPH
5. glargine

Regimen

1. Twice daily
2. Thrice daily
3. Four times daily

B. Rotation of insulin sites: yes/no

C. Frequency of rotation

1. daily
2. 2–3 times weekly
3. weekly

D. Sites used

1. arms
2. thighs
3. abdomen
4. buttocks

E. Delivery device: pen/syringe

## Statistical analysis

Quantitative data was presented as mean ± SD. Categorical data was presented as percentages. The test of significance used was chi square test. $p$ value < 0.05 was considered statistically significant.

# Results

The number of subjects was 80 (42 males, 38 females), and baseline characteristics are shown in Table 1. The age ranged from 5 to 25 years. The duration of diabetes ranged from 9 months to 12 years. Majority of the subjects belonged to the lower and lower-middle socioeconomic status who were attending the government hospital.

Figure 1a shows the SMBG frequency in the subjects. More than half of the subjects (57%) monitored their blood glucose daily. Monitoring patterns for daily checking were as follows: once a day in 20 subjects, twice a day in 12 subjects, and thrice a day in 14 subjects. Thirty-three percent of the subjects monitored at least twice or thrice a week. Two percent had irregular monitoring and generally checked only in a hypoglycemic state. Figure 1b shows the timing of SMBG done by the subjects' genderwise. Almost all the subjects monitor fasting blood glucose most regularly (96%), followed by dinner—that is to say they

**Table 1** Baseline characteristics of the subjects

| Parameter | Value |
|---|---|
| Mean age (years) | 16 ± 5.25 |
| Mean HbA1c (%) | 10.17 ± 2.4 |
| Mean duration of diabetes (years) | 5 ± 2.81 |
| Literacy rate (%) | 97 |
| Proportion of subjects in HbA1c | |
| < 7.5% | 13.7% |
| 7.5–8.5% | 33.7% |
| > 8.5% | 52.6% |

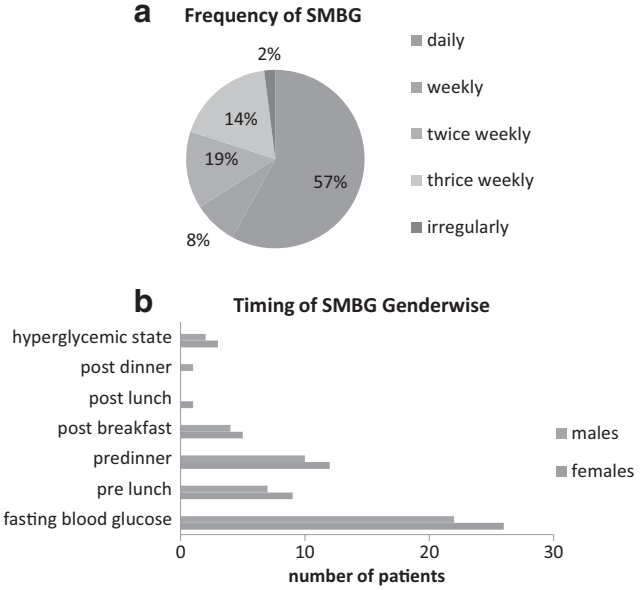


**Fig. 1** Frequency (**a**) and timing (**b**) of SMBG

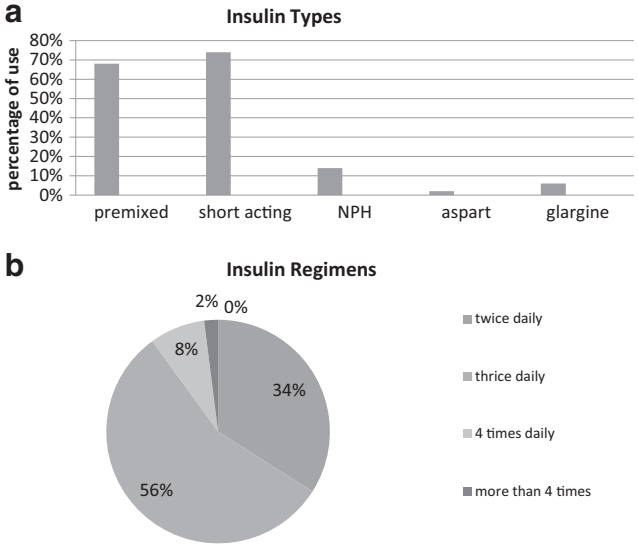


**Fig. 2** Insulin types (**a**) and regimens (**b**)

monitor once in the morning and once in the evening. The combination of fasting blood glucose, pre-lunch, and pre-dinner was most recorded. A few patients claimed that they checked blood glucose in hypoglycemic states only or along with other monitoring times (14%). Postprandial monitoring was done less frequently. Table 2 shows the association between SMBG and HbA1c.

Figure 2a shows the types of insulin used by the subjects. Majority used premixed and regular insulin. Other insulin types recorded were NPH, aspart, and glargine. Figure 2b shows the different types of insulin regimens used. The most widely used regimen was thrice-daily at 56%, followed by a twice-daily regimen with premixed 19% and split mixed 15%. Basal-bolus regimen was seen in 10%.

The subjects were questioned about rotation of insulin sites. Figure 3a shows that 75.5% rotated injection sites while 24.5% did not rotate. The frequency of site rotation is shown in Fig. 3b. Only 41% of the subjects rotated sites daily, 49% of subjects rotated only twice or thrice weekly, and 10% of subjects had irregular rotation. The sites of administration of insulin in the subjects were as follows: abdomen (88%), thighs (64%), arms (14%), and buttocks (12%). All except three subjects were using syringes as the delivery device.

**Table 2** Association between SMBG and HbA1c

| SMBG frequency | < 7.5% | 7.5–8.5% | > 8.5% |
|---|---|---|---|
| More than three times daily | 8 | 5 | 2 |
| SMBG less than three times daily | 3 | 22 | 40 |

## Discussion

Self-management practices are a core component for achieving glycemic control in young subjects with T1D [3]. The present study highlights the self-management practices of children and adolescents with T1D attending the tertiary government hospital in South India. The study shows the poor self-management practices leading to inadequate glycemic control of T1D. The reasons for the poor self-management practices in the present study could be lower socioeconomic status, sub-standard dietary habits, adolescent age, negative attitude, and lack of awareness of the disorder. Toljamo et al. from Finland studied adherence to self-care and glycemic control in adults with insulin-dependent diabetes mellitus and showed that adherence to self-care does not always lead to good metabolic control, but neglect of self-care is likely to lead to poor metabolic control [4]. The American Association of Clinical Endocrinologists also highlights the importance of knowledge of self-management among the patients and the need for them to be involved in their care [5]. According to the ADA review of the standards of self-management education, there is a fourfold increase in complications to those who do not partake in proper self-management [6].

SMBG is one of the most important self-management practices in T1D and an integral part of glycemic treatment. It improves blood glucose levels and outcomes greatly in these patients [3]. The present study showed that SMBG is not done regularly by children and adolescents with T1D despite giving free glucometers and strips. Very few have adhered to SMBG as advised by the health professionals. As explained by Ranjit Unnikrishnan and V Mohan in Suggested Protocols for Self-Monitoring of Blood Glucose in India, patients with T1D should perform SMBG at least three times a day. [7] It is more practical to check the before meal values including fasting,

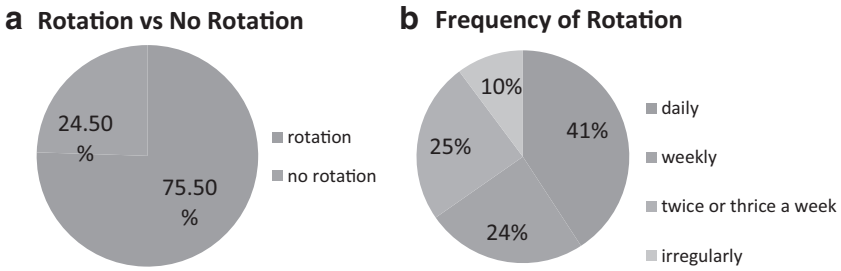


**Fig. 3** Site rotation (**a**) and frequency of rotation (**b**)

pre-lunch, and pre-dinner at the time of initiation of insulin therapy, thereby fixing the dose of long-acting or intermediate insulin. When the pre-prandial values are stable, one can concentrate on recording post meal values, which will help to fix the rapid-acting insulin dosage. In our study, only 57% monitored their blood glucose daily and of these, only one third monitored three or more times a day as recommended. The combination of fasting blood glucose, pre-lunch, and pre-dinner was most frequently done which reveals that initial adherence was adequate; however, it was not optimized by many subjects. A multicenter analysis from Germany and Austria clearly showed that more frequent SMBG is associated with better metabolic control in type 1 diabetes [8]. The poor glycemic control of the subjects in this study is due to poor SMBG which can be improved by more awareness and diabetes self-management and education [6].

Insulin therapy is a crucial aspect to achieve metabolic control in T1D. The different aspects include insulin regimen, site of injection, and rotation of sites. Insulin regimens are not adhered to in the present study because of many reasons which include lack of awareness, low socioeconomic status, improper storage of insulin, and no proper facilities at school or work place to store and inject insulin and social stigma. Farsaei et al. have studied the sociodemographic, disease, and insulin-related barriers to compliance with insulin therapy [9]. Some of the reasons they explained are time consuming, embarrassing, painful, non-affordability, due to forgetfulness, or lack of education causing difficulty in administration. The premixed insulin is chosen mostly because of patient and doctor convenience and the advantage of twice-daily dosing; however, it does not offer the dosing flexibility of basal-bolus regimens. The basal-bolus insulin regimen uses long-acting insulin combined with rapid-acting insulin given before meals and snacks and has been documented to result in stable glycemic control compared with premixed regimens. Basal-bolus, though ideal, is tedious because of a more number of insulin shots and is less acceptable by the patients [10].

In the present study, 75.5% of the subjects rotated sites regularly while 24.5% of the patients did not rotate sites of administration at all despite being educated about the importance of site rotation. Among the 75.5% who follow site rotation, 41% rotate daily but others rotate irregularly. Patton et al. reported similar findings, that despite being educated, 80% do not follow the instructions [11]. Some stated reasons are fear of pain in a new site, being comfortable with sites already being used, forgetting to rotate sites, having problems with new sites, and lack of proper education.

HbA1c levels are satisfactory in 47.4% of the subjects. It was observed that depending on how well the subject followed self-management practices the values of HbA1c varied. Subjects who monitored SMBG thrice daily and followed their insulin regimens properly had a relatively lower HbA1c. Eighty-six percent of the subjects with SMBG thrice or more in a day had HbA1C of less than 8.5% as compared to only 38% who monitored SMBG less than thrice a day. Evans Josie M et al. showed a direct association between HbA1c and SMBG, thus indicating the importance of SMBG for optimal glycemic control [12].

The limitations of the present study are the small sample size, and relying on the questionnaire answered by the patient. The values and timing of SMBG could not be checked as the machines were not having memory.

## Conclusion

The study concludes that SMBG in T1D is underused and a majority monitor only fasting blood glucose regularly. The types of insulin used are regular, premixed, long-acting NPH, and glargine. The most commonly used regimen is thrice daily followed by twice daily. Site rotation is seen in 75.5% of subjects. HbA1c levels are satisfactory in 47.4% of the subjects. The study highlights the need for educating subjects with T1D and their families further about SMBG, insulin regimens and the importance of taking insulin diligently as advised by the doctor, and the necessity of site rotation for better glycemic control.

### Compliance with ethical standards

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

**Ethical approval** The study has approval from the Institutional Ethics Committee of the Hospital.

**Informed consent** A written informed consent was obtained from all the subjects.

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International Journal of Diabetes in Developing Countries (July–September 2019) 39(3):590–595
https://doi.org/10.1007/s13410-018-0691-5

ORIGINAL ARTICLE

# Annual cost incurred for the management of type 2 diabetes mellitus—a community-based study from coastal Karnataka

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Received: 3 May 2018 / Accepted: 23 August 2018 / Published online: 5 September 2018


**Abstract**
Diabetes is projected to become one of the world's main disabler and killer within the next 25 years. Type 2 diabetes mellitus (T2DM) due to its chronicity and incident complications is not only consuming patient's household budget but also the overall healthcare budget. To estimate the annual cost incurred and proportion of family income spent for management of T2DM in the community, a community-based cross-sectional study was conducted among T2DM patients aged ≥ 40 years residing in the field practice area of a Medical College in Karnataka. A total of 809 subjects were included using stratified random sampling with proportionate sample technique. A pretested questionnaire was used to assess direct and indirect costs incurred for disease management in the last 1 year. Data was entered and analyzed using SPSS version 15.0. The annual median (IQR) total cost incurred for the management of diabetes mellitus was estimated to be USD 73.2 which included direct cost of USD 62.2 (27.9–138.2) and indirect cost of USD 6.7 (2.2–22.6). The direct medical cost included USD 5.2 (< 1–9.43), USD 5.2 (2.9–10.4), USD 58.6 (20.8–124.0), and USD 217.7 (116.1–718.6) per person annually for consultation, investigation, medications, and hospitalization, respectively. The direct non-medical cost included USD 3.8 (1.7–10.8) and USD 3.0 (2.0–5.2) annually for the travel and food. On an average, 3% and 21% of the total family income was spent on outpatient and inpatient services, respectively. Annual cost incurred predominantly included direct costs of which medication and hospitalization expenses contributed a major portion.

**Keywords** Type 2 diabetes mellitus · Annual cost · Direct and indirect costs · Karnataka · Community-based cross-sectional study

## Introduction

Type 2 diabetes mellitus (T2DM), the pandemic of twenty-first century, is a chronic and potentially disabling disease that represents an important public health and clinical concern in both developed and developing countries [1, 2]. Globally, it is a major threat to social and economic development as it affects both men and women of working age who are not able to secure a productive employment. Eighth edition of International Diabetes Federation has documented that there are 425 million people with diabetes in the world and is projected to reach 629 million by 2045; in addition, the burden is compounded by one in two adults being undiagnosed [3]. India being the diabetes capital of world is predicted to witness a large burden of diabetics, 105 million by 2035 [4, 5]. The total cost of diabetes and pre-diabetes in the USA for the year 2017 was estimated to be USD 322 billion, and it is speculated to be 2.3 times the non-diabetic healthcare cost. Similarly, the cost of illness studies from different parts of the world has estimated that among diabetic patients, there is a fourfold rise in the costs in comparison to non-diabetic patients [6–8]. The available literature from countries like UK, Brazil, Argentina, and Mexico estimate the annual cost of diabetes to be £9.8 billion, USD 3.9 billion, 0.8 billion, and 2.0 billion, respectively [9]. In India, 5% to 25% of the average family income translating to USD 2.2 billion is attributed to diabetes [10, 11]. The Cost of Diabetes in India (CODI) study reported an expenditure of 65% of cost on ambulatory care services and 35% on hospitalization [9]. The study also documented that 31% of the total cost were medication expenses, a major share of the total cost in the management of diabetes. In India, the lowest income groups bear the greatest

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burden, paying a larger proportion of household income towards diabetes care [9, 12].

As community-based studies with respect to expenditure on management of T2DM from developing countries are scarce, this study was designed to estimate the direct and indirect costs involved in the management of T2DM among residents of coastal Karnataka.

## Materials and methods

A community-based cross-sectional study was carried out in the field practice area of the Department of Community Medicine at a Medical College situated along the coastal area of Karnataka. The field practice area covers a population of 42,190 living in 8635 families spread out in 12 villages. Department of Community Medicine provides healthcare services like consultation, free medications, and investigations at a subsidized cost to this population through a network of five outreach centers.

The study population included patients with T2DM aged ≥ 40 years of either gender who were on allopathy or combination therapy with alternate system of treatment for at least the last 2 years and currently continuing the medications. Individuals suffering from type 1 DM and gestational diabetes were excluded.

A pilot study was conducted to estimate the range of expenditure on diabetes management which provided the anticipated standard deviation (SD) of 500 (Tables 1 and 2). With 20% relative precision at 95% confidence interval and anticipating a non-response rate (NR) of 10% for fasting blood sugar estimation, 783 subjects were required to be studied. A stratified random sampling technique based on the rural centers was carried out which included a final total sample of 809 subjects.

The localities and households of patients with T2DM were identified with the help of the auxiliary nurse midwives (ANMs) attached to the rural outreach health centers. The first household was selected at random, and then, the rest of the households were serially visited in one particular direction until the proportionate sample for the respective center was achieved. Personal interviews were conducted using a pre-tested, structured questionnaire which was pilot tested and validated by experts before data collection. The variables included sociodemographic details and the disease profile with respect to disease duration, place of diagnosis, coexisting comorbidities, and direct and indirect costs of diabetes management. The socioeconomic status of the study subjects was assessed using modified Udai Parikh scale as the study participants were from rural community [13]. The choice of this scale was made, because literature has reported better reliability with Udai Parikh scale in assessing socioeconomic status of the rural population as compared to other scales [14, 15].

**Table 1** Operational definitions for calculation of direct cost incurred for diabetes management

| |
|---|
| Direct medical expenses |
| 1. Annual consultation cost |
| Amount paid per visit × number of visits for which fees paid |
| 2. Annual investigation cost |
| Frequency of investigation × amount paid per investigation |
| 3. Annual medication cost |
| Total amount spent on drugs for diabetes and associated comorbidities. |
| *For the drugs purchased by the patient from pharmacy* |
| Annual cost of each drug = number of tablets being consumed by the patient for a day × cost per tablet × 365 days |
| 4. Cost of hospitalization due to diabetic complications |
| Total amount spent by the patient for each episode of hospitalization in the last 1 year. |
| Direct non-medical expenses |
| 1. Annual expense on travel |
| Frequency of travel for consultation and investigation × amount spent on respective mode of transport (to and fro)/visit |
| - For travel by bus and auto rickshaw the amount stated by patient was taken for calculation. |
| - Travel by personal two wheelers: number of kilometers (km.) traveled for the visit × Rs. 1.5/km. |
| - Travel by personal four wheelers: number of km. traveled for the visit × Rs. 6/km. |
| - Traveled by walk: no amount was taken into consideration. |
| 2. Annual expense on food |
| Amount spent on food × number of visit in the last 1 year |

Fasting blood sugar (FBS) was estimated at participant's residence on a pre-informed date to assess the glycemic status. Fasting was defined according to American Diabetic Association guidelines, as no caloric intake for at least 8 h. FBS (capillary blood) was estimated by finger prick method using standardized ACCU CHECK ACTIVE Glucometer (Batch No. 91000443). Subjects with FBS < 126 mg/dl were considered to be having good glycemic control [16]. The subjects who were not available for FBS estimation even after two visits were considered as non-respondents.

### Calculation of cost expenditure

#### Direct cost (Table 1)

The cost expenditure incurred by the study subjects was calculated for the previous year. The direct cost expenditure was further classified as medical which included the cost of consultation, investigation, medications, and hospitalization

**Table 2** Operational definitions for calculation of indirect cost incurred for diabetes management

For patient

1. Indirect cost for consultation:
   Number of hours spent for consultation per visit × frequency of visits in the last 1 year × value of each working hour
   Similarly, the indirect cost of investigation was also calculated.
2. Indirect cost for hospitalization:
   Number of episodes of hospitalization × number of days of hospitalization per episode × value of each working day
3. Due to temporary/permanent disability:
   Loss in earning as a result of diabetes or its complications.
   Loss in income = lost years of productive work × current monthly income of the subjects

For accompanying person

1. Annual expense on travel:
   Total frequency of travel with the patient for consultation and investigation × amount spent on respective mode of transport (to and fro)
2. Annual expense on food:
   Amount spent on food × number of visits in the last 1 year
3. Loss of wages due to consultation visit:
   Number of hours spent for consultation per visit × frequency of visits in last 1 year × value of each working hour
   Similarly, the lost income on days of investigation was calculated.
4. Loss of wages due to hospitalization:
   Annual loss in income = daily income × total number of working days lost
5. Loss of wages due to premature termination of job.
   Loss in income = lost years of productive work × anticipated monthly income

while the non-medical costs included expenditure on travel and food on the days of consultation and investigation.

The details about cost expenditure incurred for travel and food in the previous year is subject to recall bias; in addition, due to the inability to verify with actual bills; the average annual expenses have been projected rather than cost/person/visit. Disease-related expenditure, namely, consultation, investigation, and hospitalization, which was quantifiable and verifiable with bills were used for calculation of cost/person/visit.

The insurance and health schemes available at the study settings provide cashless benefits; hence, those subjects seeking treatment did not have any out of pocket expenditure. Therefore, cost of treatment for insurance and healthcare schemes patients and those subjects procuring medicines from government health facilities and outreach rural centers of the department were not included in the expenditure calculation.

### Indirect cost (Table 2)

The indirect cost estimated included the man-days lost for the patients due to diabetes and the loss of personal as well as family income. It also included the amount spent for the travel and food on the days of consultation and investigation by the accompanying person in the last 1 year.

The monthly income of the subjects was calculated depending on their employment status. For the subjects who were unemployed, a monetary value of a daily wage for unskilled workers in the local area was taken as proxy considering the payment on all working days (Rs. 300/day for males and Rs. 250/day for females). Similarly, for homemakers, the monthly salary of the house maids in the local area was taken as proxy (Rs. 1500/month). According to Labour wages Karnataka 2014–2015, the expected amount paid for the semi-skilled and skilled workers was considered for the calculation. For employed subjects, their salary was considered while for the daily wage workers, their daily wages were taken into consideration for cost estimation [17]. The monetary amount for businessmen and professionals was calculated depending on the prevailing local area businesses and professions. For retired subjects, pension was considered for calculation of their expenses.

## Statistical methods

The data was entered and analyzed using Statistical Package for Social Sciences (SPSS version 15.0). Categorical data was summarized using percentages. The data related to cost was summarized using median and interquartile range (IQR).

# Results

The study included a total of 809 diabetic subjects. The participation was complete for the questionnaire-based data collection while the non-response was only with respect to fasting blood glucose estimation among 26 subjects. Of the total study subjects, 40% of the participants belonged to age group of 60–69 years with most of them being Hindu and married as depicted in Table 3. There was a near equal representation of males and females with mean (± SD) age of the study participants being 63.08 ± 9.6 years. More than half of the participants (60%) were educated up to secondary school. The monthly family income of 70% of the subjects was more than Rs. 10,000, and the per capita income among more than half of the subjects was Rs. 1000–5000.

The median (IQR) duration of diabetes was 7 (4–12) years and with respect to glycemic status 544 (69.5%) had good glycemic control with FBS < 126 mg/dl. It was observed that 80% of the study subjects opted private healthcare facility for consultation, investigations, and medications and reported visiting their treating physician once in 2 months. Subjects utilizing services at our outreach centers and government facility visited the health facility once weekly and monthly, respectively. Due to the subsidized services and free medications

**Table 3** Characteristics of the study subjects ($n = 809$)

| Variables | Number (%) |
|---|---|
| Age group (years) | |
| 40–49 | 69 (8.5) |
| 50–59 | 194 (24.0) |
| 60–69 | 335 (41.4) |
| ≥70 | 211 (26.1) |
| Gender | |
| Male | 380 (47.0) |
| Female | 429 (53.0) |
| Literacy status | |
| Illiterate | 139 (17.2) |
| Primary (1st–4th std) | 118 (14.6) |
| Secondary (5th–12th std) | 488 (60.3) |
| Graduation and above | 64 (7.9) |
| Occupation | |
| Skilled | 149 (18.4) |
| Unskilled | 57 (7.1) |
| Unemployed | 159 (19.6) |
| Homemaker | 360 (44.5) |
| Retired | 84 (10.4) |
| Monthly family income (Rs.) | |
| < 5000 | 119 (14.7) |
| 5000–10,000 | 146 (18.0) |
| > 10,000 | 544 (67.3) |
| Socioeconomic status (according to modified Udai Pareek Scale) | |
| Low | 198 (24.4) |
| Middle | 591 (73.1) |
| High | 20 (2.5) |

available at our outreach centers and government health facility, the subjects utilizing these facilities spent less in contrast to the subjects visiting private health facilities in the ratio of 1:8, respectively.

It was observed that 652 (80%) subjects were on oral hypoglycaemic agents, while only a small proportion, 21 (2.6%) were on insulin, and 64 (8%) were on combination therapy of both oral hypoglycemic agents and insulin. It was also noted that 36 (4.4%) study subjects were exclusively on Ayurveda medication for diabetes. Of the total, 588(72.7%) reported to be suffering from one or more comorbidity, with hypertension being the most common (84.3%). Less than one fifth of the study subjects reported one or more hospitalization episodes in the preceding year.

It was observed that the direct cost incurred doubled among subjects with associated comorbidities, but there was not much difference observed in the indirect expenditure incurred, Table 4.

Table 5 describes the direct costs incurred for management of T2DM. Medication and hospitalization constituted three fourths of the direct costs while consultation and investigations contributed to a smaller proportion. As compared to subjects on OHAs, those on insulin therapy alone or in combination with OHA spent thrice and seven times more on treatment. A strikingly high expenditure was noted among subjects on Ayurvedic medications, which was 1.5 to 3 times higher as compared to subjects on OHAs.

As expected, inpatient services costed more than outpatient services (Rs. 30,320 vs 5393) (USD 440.2 vs 78.3) although only 160 reported admissions for diabetes-related complications in the preceding year. On an average, 3% of the total family income was used for outpatient services while hospitalization consumed 21% of the total family income.

## Discussion

Diabetes due to its chronicity exerts a substantial economic burden on patients, healthcare systems, and society not only due to day to day costs but also due to long-term consequences [18]. Population-based studies estimating the cost of management of T2DM are few; the present study was planned to estimate the cost of management of diabetes.

In the present study, the average annual total cost of diabetes doubled with the presence of comorbidities and this was in concurrence to the DEDICOM survey results [19]. Similarly, Akari et al. [20] and Tharkar et al. [21] have also documented that there was added cost of managing comorbidities. Among the urban slum residents of Mumbai, Rs. 5120.83 was the mean total cost for 6 months which is much higher than the present study results which could be attributed to the higher cost of living in the metropolitan city [22].

In the present study, the direct cost constituted the major proportion of the total cost (> 85%) which was similar to Akari et al. and Fernandes et al. who noted that 91.4% and 80.5% of the total cost in their respective studies was constituted by the direct expenses [20, 22].

In a study among residents of Haryana [23], the estimated average indirect cost for 1 month was found to be Rs. 271. Annualization of this cost would lead to higher indirect costs which are considerably greater than the present study annual estimate of Rs. 508. Among the residents of Chennai, Loganathan et al. have reported that the indirect medical cost spent annually was Rs. 250 [24].

As noted earlier, the direct cost was the major component of the overall cost and the expenditure was mainly on medication and inpatient services followed by investigation and consultation services. Our estimates appear to be of the same order as those of a community-based study by Loganathan with respect to annual consultation cost and annual travel cost, but the reported medication costs were much higher. This could be attributed to zero cost of medications procured from government facilities and from rural centers in the present

**Table 4** Summary table on cost incurred in the management of diabetes mellitus

| Disease status | No. of subjects | Direct cost, median (IQR) | | Indirect cost, median (IQR) | | Total cost, median (IQR) (Rs.) | |
|---|---|---|---|---|---|---|---|
| | | In Rs. | In USD | In Rs. | In USD | In Rs. | In USD |
| Diabetes alone | 809 | 4282 (1926–9521) | 62.2 (27.9–138.2) | 462 (150–1557) | 6.7 (2.2–22.6) | 5041 (2516–11,224) | 73.2 (36.5–162.9) |
| Diabetes with comorbidities | 588 | 8185 (3414–17,939) | 118.8 (49.6–260.4) | 508 (150–1586) | 7.34 (2.1–23.0) | 9133 (4034–19,053) | 132.6 (58.6–276.6) |

(INR. 68.88 = 1 USD, 2018)

study [24]. Similarly, Grover et al. [25] reported an average cost of Rs. 78.58, Rs. 277.80, Rs. 458.96, Rs. 72.66, and Rs. 3076 on consultation, investigation, travel, food, and medications over 6 months, respectively. Among Pakistani residents, an expenditure of Pakistani rupees 5542 per month was the estimated direct medical expenditure and Rs. 200/visit and 1000/month, respectively, on travel and food as direct non-medical expenses [26].

Diabetes is associated with poor health outcomes and increased use of health services including unplanned hospitalization and the related expenditure [27]. Among residents of coastal Karnataka, Adiga [28] and Kapur [29] estimated the average annual hospitalization cost to be Rs. 14, 213 ± 2224.8 and Rs. 12,781, respectively, which is concurring with the present study findings.

The scenario becomes more complex in cases of catastrophic health expenditure, when healthcare spending increases beyond income and the individual begins to sacrifice items of basic consumption [7]. The present study documented a significant proportion of family income being spent on diabetes, ranging from 3 to 21%. Ramachandran et al. [30] have reported that on an average 7% and 17.5% of the family income was used for outpatient and inpatient services, respectively. Chandra et al. documented an average of 2% to 5% of their family income being spent on disease management among urban residents which is in concordance with the present study findings [31].

The current research was a community-based study estimating the cost of management of T2DM and to the best of our knoweldge is the first population-based estimate on the economic burden of diabetes from the present study settings. Data collection was done by a single trained interviewer thereby ensuring uniformity in data collection and cost calculation. As the cost incurred for the management of T2DM was calculated for the preceeding 1 year, there could be an element of recall bias, which is difficult to avoid unless all disease-related records are carefully preserved by the subjects. Self-reporting of cost expenditure pertaining to hospitalization of the study subjects may have led to a biased estimate, in some cases, when hospital bills were not available.

As the cost incurred on medications was high, it could be economized by improving the availability of generic drugs. A good insurance system covering even the cost of consultation, investigation, and hospitalization especially in private healthcare facilities could reduce the financial burden on the individual and the family.

## Conclusion

Annual cost incurred for management of T2DM predominantly included direct costs. Of these, medication and hospitalization expenses contributed to the major portion of direct expenditure. It was also noted that a significant proportion of family

**Table 5** Annual direct cost incurred by the study subjects for the management of diabetes mellitus ($n$ = 809)

| Components | Number | Median (IQR) total cost/person | | Median (IQR) cost/person/visit | |
|---|---|---|---|---|---|
| | | In Rs. | In USD | In Rs. | In USD |
| Consultation | 693 | 360 (50–650) | 5.2 (< 1–9.4) | 58 (6–114) | < 1 (<1–1.6) |
| Investigation | 777 | 360 (200–720) | 5.2 (2.9–10.4) | 60 (40–100) | < 1 (<1–1.4) |
| Travel expenses | 613 | 264 (120–748) | 3.8 (1.7–10.8) | – | |
| Food expenses | 197 | 210 (140–360) | 3.0 (2.0–5.2) | – | |
| Hospitalization | 160 | 15,000 (8000–49,500) | 217.7 (116.1–718.6) | 14,250 (7062–30,000) | 206.8 (102.5–435.5) |
| Medication cost/person | 809 | 4043 (1432-8543) | 58.6 (20.8–124.0) | – | |

(INR. 68.88 = 1 USD, 2018)

income was being utilized for both outpatient and inpatient services.

**Acknowledgements** We are grateful to the department of Community Medicine for the cooperation and support for carrying out the research. We would like to thank all the research participants who took part in the study. We also acknowledge the invaluable help of the Auxiliary Nurse Midwives (ANMs) during the process of data collection.

## Compliance with ethical standards

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

**Research involving human participants** Institutional ethical committee clearance (IEC 419/2012) was obtained, and the study has been performed in accordance with ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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

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## VISION STATEMENT

To be recognized as a global leader for clinical care, education, training, research, advocacy and capacity building in the field of diabetes.

## MISSION STATEMENT

1. Promotion of excellence in diabetes care to make India the Diabetes Care Capital
2. Empowerment of persons living with diabetes
3. Support for diabetes research
4. Dissemination of information and knowledge in diabetes care
5. Advocacy for the cause of diabetology

## RSSDI Research Grants

- For providing research grants, RSSDI invites proposals from Indian scientists, interested in conducting original research in the field of diabetes mellitus. Furthermore, limited grants are also available for the students of medical colleges for smaller projects.
- There is no deadline for submission of the proposals, which can be sent throughout the year. These proposals may fall into one of the following three categories:

1. Projects involving funding up to Rs 40,000 per project (preference will be given to young scientists <40 years).
2. Projects involving funding up to 10 lakhs.
3. We also invite proposals for more than 10 Lakhs as major projects but these have to be preferably multicentric.

- 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 and bibliography. Brief biodata of principal investigator and other co-investigators
  - ◊ Importance of work in the context of national priorities. 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.
  - ◊ Ethical committee clearance of the institution or other bonafide body.

## Travel grants for young diabetes researchers to attend International Conferences

Criteria's for the travel grant are as follows:

- Applicant should apply 2 months in advance.
- Travel Grant is open only to the RSSDI members.
- Applicant should submit Oral paper / Poster acceptance document to RSSDI Secretariat.
- Applicant should submit Declaration that he/she has not receiving grant from any other agency / Organization – In case of receiving grant from any other Organization, RSSDI shall pay only the exceeding amount not covered by that agency.

## ADVANCED CERTIFICATE COURSE IN DIABETOLOGY

(IN ASSOCIATION WITH JAIPUR NATIONAL UNIVERSITY)

Research Society for the Study of Diabetes in India (RSSDI) was founded by Prof. M.M.S. Ahuja in 1972. RSSDI is the largest body of professional doctors and researchers in Asia, working in the area of Diabetes & is the National Body recognized by IDF (International Diabetes Federation). One of the key areas of focus is to train doctors at all levels to better manage Diabetes and its complications. RSSDI recognizes this problem and runs a well-structured, full time, residential "Advanced Certificate Course in Diabetology". This two-year course is like any other post graduate course and has immensely helped doctors to practice better diabetes care. RSSDI has carefully looked into all aspects of this course & has accredited & recognized 18 centres across India at present and more centers are being inspected for accreditation. National Faculties and experts of RSSDI chosen from Academia visit these centers from time to time to ensure high standards. Now this Advanced Certificate Course has Dual Accreditation from RSSDI and Jaipur National University.

**List of RSSDI Accredited Centres**

| S.N. | Institute Name | Institute Location |
|---|---|---|
| 1. | Diacon Hospital | Bengaluru, Karnataka |
| 2. | North Delhi Diabetes Centre | New Delhi, Delhi |
| 3. | Prithvi Hospital | Tumkur, Karnataka |
| 4. | Totall 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. | G D Hospitals and Diabetes Institute | Kolkata, West Bengal |
| 10. | Aditya Diagnostics & Hospital | Dibrugarh, Assam |
| 11. | Sunil's Diabetes Care N' Research Centre Pvt Ltd. | Nagpur, Maharashtra |
| 12. | Marwari Hospital and Research Centre | Guwahati, Assam |
| 13. | Down Town Hospital | Guwahati, Assam |
| 14. | St.Theresa's Hospital | Hyderabad, Telangana |
| 15. | Aegle Clinic | Pune, Maharashtra |
| 16. | Tulip Hospital | Sonipat, Haryana |
| 17. | Lilavati Hospital & Research Centre | Bandra West, Mumbai |
| 18. | Srajan Hospital | Udaipur, Rajasthan |

## 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 preference.

**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)

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BB Tripathy Nutritional Symposium : "Diet and Inflammation"

Registered with Registrar of News Papers, India vide no. RNI-43019/85

# International Journal of Diabetes in Developing Countries

Volume 39 | Issue 3 | July–September 2019



Springer