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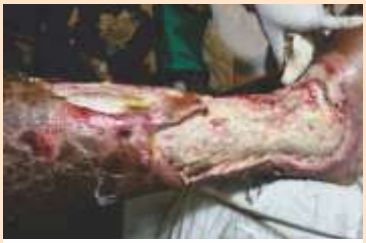
In Chronic Wound Management

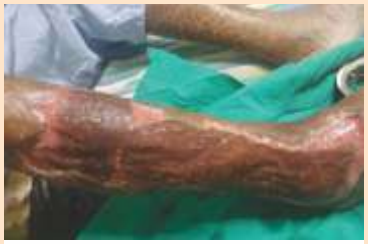
ZiFi TURBO

LINEZOLID 600MG + CEFIXIME 200MG **Tablets**

A Case of Chronic Wound



Before Treatment



After Treatment

Case : 45 year old male patient H/O Diabetic foot ulcer.

Treatment : ZiFi TURBO b.i.d. for 14 days.

In Acute Wound Management

ZiFi TURBO 600

LINEZOLID 600MG **Tablets**

A Case of Acute Wound

Before Treatment

After Treatment

Case : 34 year old Male Patient H/O **Traumatic wound**.

Treatment : ZiFi TURBO 600 b.i.d. for 21 days

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International Journal of Diabetes in Developing Countries
Incorporating Diabetes Bulletin

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International Journal of Diabetes in Developing Countries targets a readership consisting of clinicians, research workers, paramedical personnel, nutritionists and health care personnel working in the field of diabetes. Original research work and reviews of interest to the above group of readers is considered for publication in the journal.

The journal has a goal of serving as an important resource material in diabetes for its readers, mainly in the developing world.

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EDITORIAL

Diabetes mellitus and tuberculosis—a double whammy

S. V. Madhu [1]

Published online: 27 May 2017

India has the second highest prevalence of diabetes mellitus in the world with over 69.2 million diabetes subjects [1]. So also, India accounts for a significant proportion of cases of tuberculosis in the world. It is also estimated that the highest number of cases of tuberculosis associated with diabetes are reported from India and a large proportion of diabetic patients in our country suffer from tuberculosis [2–4]. This suggests a huge double burden of diabetes and tuberculosis in our country.

The study by Hussain et al. from Odisha in this issue is from Eastern India and reports that 14% of active tuberculosis patients in the region have diabetes mellitus and a further 24% have prediabetes. The authors also report that nearly half of those detected with diabetes and over half of those detected with prediabetes were unaware of their glucose intolerance. The study thus underlines the need for and demonstrates the feasibility of screening for early diagnosis and treatment of diabetes mellitus if we have to improve tuberculosis-related outcomes.

Prevalence of diabetes in patients with tuberculosis from different states of India has varied from 11.6% in Punjab to 44% in Kerala [5, 6]. Southern Indian states (Kerala 44%, Tamil Nadu 25%, and Pudicherry 29%) have reported higher prevalence of diabetes mellitus in such cases as compared to the Western (Ahmadabad 15%) or North Indian (Punjab 11.6%) states [5–8].

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The regional variation in the prevalence of coexistent diabetes and tuberculosis within India is both striking and interesting. Whether this merely reflects the regional differences in prevalence of diabetes within the country is unclear. Data from INDIAB [9] indicates that the prevalence of diabetes mellitus is lower in Maharashtra in the West and in rural Jharkhand in the East compared to that in South and North India. However, this does not fully explain the regional variation of cases of diabetes and tuberculosis in the country, and it will be interesting to investigate if other regional factors favoring the coexistence of diabetes mellitus and tuberculosis have a role to play.

The coexistence of diabetes mellitus and tuberculosis has adverse consequences for both conditions. Diabetes predisposes to tuberculosis due to the profound effects of hyperglycemia on body defense mechanisms including suppression of cell-mediated immune mechanisms [10]. It is also well known that presence of diabetes mellitus also alters the way pulmonary tuberculosis manifests and adversely affects clinical and treatment outcomes [11]. It also results in slow conversion to a sputum-negative state [12]. Similarly, the presence of active tuberculosis leads to unmasking of higher levels of glucose intolerance and poses serious challenges to glucose control among diabetic patients. Tuberculosis is an important cause of insulinization and hospitalization among diabetic patients that also increases the cost of diabetes care besides affecting quality of life in these patients.

The original article by Dwivedi et al. in this issue evaluates the CT-based radiological manifestations of pulmonary tuberculosis among diabetic patients. The study confirms higher prevalence of lower lobe consolidation and cavitary lesions in them when compared to those without diabetes. The patients of tuberculosis with diabetes mellitus also showed higher rates of endobronchial spread (57%) compare to non-diabetic subjects (30%). The authors conclude that the

presence of lower lobe consolidation or cavitation in a case of pulmonary tuberculosis should alert the clinician to coexistent diabetes mellitus.

While the association of diabetes mellitus and tuberculosis poses a serious challenge to our efforts to control both diseases, the coexistence of these two conditions also provides a huge public health opportunity. Strategies for early detection of diabetes and tuberculosis when they coexist with each other will be critical in controlling the rising prevalence and impact of both. National guidelines for bi-directional screening of diabetes mellitus and tuberculosis when patients present with either of these two conditions is a welcome step in this direction.

There is also a need to undertake further studies particularly cohort studies to unravel some of the missing links in the coexistence of diabetes mellitus and tuberculosis. More importantly, there is an urgent need to develop clinical practice recommendations for diagnosis, prevention, and control of diabetes mellitus–tuberculosis to facilitate a uniform and effective approach that will help contain this twin problem.

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ORIGINAL ARTICLE

Targeting inflammation using celecoxib with glimepiride in the treatment of obese type 2 diabetic Egyptian patients

Hoda El-Bahrawy · Sahar Hegazy · Wael Farrag · Rehab Werida

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Abstract Obesity, insulin resistance (IR), inflammation, and progressive decline in pancreatic β cell function are major features of type 2 diabetes mellitus (T2DM). We aimed to investigate the effect of co-administration of celecoxib (CE) with glimepiride (GL) in the treatment of obese T2DM patients. Body Mass Index (BMI), serum glucose, C-peptide, lipid profile, adiponectin, tumor necrosis factor-α (TNF-α), visfatin, and leptin levels were determined in 40 obese T2DM patients before and after treatment with GL alone or in combination with a selective cyclooxygenase-2 (COX-2) inhibitor CE for 3 months. Homeostasis model assessment of insulin resistance (HOMA2-IR) and atherogenic index (AI) was calculated. Increased levels of serum glucose, C-peptide, total cholesterol (TCH), low-density lipoprotein (LDL-C), triglycerides (TGs), visfatin, TNF-α, leptin, AI, and HOMA2-IR shown in obese diabetic patients were significantly decreased after co-treatment with GL plus CE compared to patients who received GL alone. On the other hand, adiponectin levels showed a significant increase after treatment. The obtained results demonstrate that targeting inflammation using celecoxib with glimepiride improves insulin resistance, glycemia, and inflammatory process in obese type 2 diabetics.

Keywords Visfatin · Celecoxib · Adiponectin · Leptin · T2DM · Obesity

Abbreviations

BG	Blood glucose
COPD	Chronic obstructive pulmonary disease
COX-2	Cyclooxygenase-2
CVD	Cardiovascular disease
ELISA	Enzyme-linked immunosorbent assay
GIT	Gastrointestinal tract
HDL-C	High-density lipoprotein
HOMA2-IR	Homeostasis model assessment of insulin resistance
HOMA2-β	Homeostasis model assessment of beta cell functionality
LDL-C	Low-density lipoprotein
T2DM	Type 2 diabetes mellitus
TCH	Total cholesterol
TGs	Triglycerides
TNF-α	Tumor necrosis factor alpha

Introduction

Diabetes is a complex, heterogeneous condition associated with beta cell dysfunction which is caused by many factors (e.g., hyperglycemia/glucotoxicity, lipotoxicity, autoimmunity, inflammation, adipocytokines, islet amyloid, incretins, and insulin resistance) [1]. Obesity is a chronic and low-grade inflammation that is involved in the pathogenesis of several chronic diseases, such as type 2 diabetes [2]. Inflammation is a physiological process characterized by elevated number of white

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blood cells and increased levels of pro-inflammatory cytokines in the circulation or tissue [3]. These proinflammatory cytokines, produced by adipose tissue, known as "adipocytokines" or "adipokines," include tumor necrosis factor-α (TNF-α), adiponectin, leptin, resistin, and visfatin [4]. Adipocytokines have been implicated as active participants in the development of insulin resistance and the increased risk of cardiovascular disease associated with obesity [5]. Chronic cyclooxygenase-2 (COX-2)-mediated inflammation seems to be involved in the development of insulin resistance in type 2 diabetes mellitus [6]. A previous study has demonstrated that COX-mediated inflammation and oxidative stress has been related to type 2 diabetes mellitus (T2DM) in elderly men [7]. Moreover, another report suggests that chronic COX-2-mediated inflammation in fat is crucial for obesity-linked insulin resistance [8, 9]. Alpert et al. has shown that the selective inhibitors of COX-2 augment the rate of glucose uptake to the plasma membrane in an insulin-independent manner [10]. The beneficial effect of COX-2 inhibitor on insulin resistance is partial via indirectly attenuating COX-2-mediated inflammation in adipose tissue [9]. On the other hand, Coll et al. demonstrated that COX-2 inhibition could enhance palmitate-induced inflammation in mouse [11]. However, the involvement of COX-2-mediated low-grade inflammation in the development of insulin resistance in obesity and T2DM remains controversial. Therefore, the present study was undertaken to examine the effect of co-administration of selective COX-2 inhibitors (celecoxib, CE) with oral hypoglycemic agent (glimepiride, GL) on the development of insulin resistance and increased inflammatory adipocytokines in obese diabetic Egyptian patients.

Subjects and methods

Patients

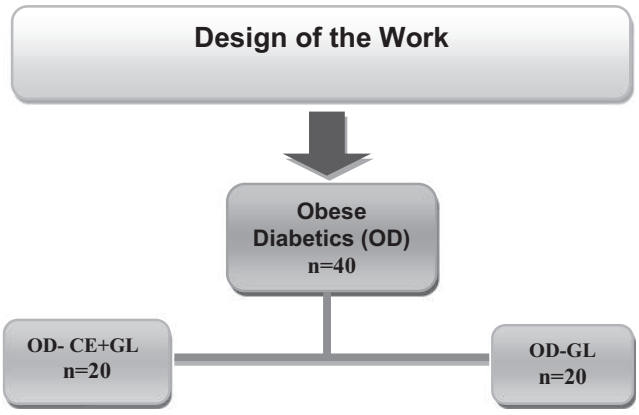
Forty obese diabetic patients were enrolled in a controlled random study from the Outpatient Clinics of Internal Medicine Department, Tanta University Hospitals, Tanta, Egypt. The protocol and potential risks and benefits of the study were fully explained to each subject before he or she provided their written informed consent. All experimental procedures followed the ethical standards and were approved by the Human Ethics Committees of Tanta University. Subjects were asked to maintain their usual dietary and physical activity habits throughout the study. All subjects' health status was evaluated by a complete medical examination. Obese diabetic patients (OD) (body mass index (BMI)≥30 kg/m^2) were randomly assigned into two groups (n=20 each) using a computer-generated random number: One group (OD-GL) received glimepiride (2 mg/day). The second group (OD-GL+CE) received glimepiride 2 mg/day plus celecoxib (100 mg twice daily) for 3 months.

Study design

Patients accepted in the present study were fulfilling the following criteria:

Inclusion criteria:

The selected diabetic patients control the serum glucose level with glimepiride for at least 3 months before the study. Age of the enrolled subjects, range from 30 to 65 years old. Glycated hemoglobin A1c % (HbA1c %) level ≤10 and fasting serum glucose levels between 130 and 300 mg/dl.



Exclusion criteria:

Subjects that were excluded from the study are those with type 1 diabetes mellitus, pregnant women, unstable cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD), impaired liver failure, impaired renal failure, smokers, findings of infection or autoimmune diseases or other chronic disease as determined by history, physical examination, and screening tests. Diabetic patients with diabetic complication were also excluded.

Assessment

Subjects' height and weight were recorded and BMI was calculated using the equation (BMI = weight (kg)/height (m)^2) [12]. Serum samples were obtained by centrifugation (10 min at 3500 r.p.m.) and immediately frozen at −20 °C until analysis. Fasting glucose determined using glucose oxidase method [13]. HbA1c % was determined by ion exchange method [14]. Serum visfatin [15], TNF-α [16], and adiponectin levels [17] were determined using enzyme-linked immunosorbent assay (ELISA) kits. Serum C-peptide quantified using immunoenzymometric assay kit [18]. Leptin serum level was determined using ELISA Kit [19]. Homeostasis model assessment (HOMA) was calculated using HOMA Calculator

version 2.2, where, C-peptide values are used [20]. Triglycerides (TGs) [21], total cholesterol (TCH), and high-density lipoprotein (HDL-C) [22] were measured calorimetrically. Low-density lipoprotein (LDL-C) was calculated [23]. Atherogenic index (AI) was calculated using the following ratio AI = log (TG)/HDL-C [24].

Statistical analysis

Data were analyzed using SPSS statistic 17.0 or Microsoft Excel 2010. Values are expressed as means±SD. Statistical analysis was performed according to the repeated measurements of one-way analysis of variance (ANOVA) followed by LSD as post hoc test. A probability $P<0.05$ was considered statistically significant.

Results

All the patients completed the study with no major side effects. The only minor side effects were tinnitus and mild gastrointestinal tract (GIT) disturbances, which tended to be decreased with continuation of the treatment and disappeared completely after stopping of celecoxib. In the present study, BMI was not changed after 3 months of treatment in both groups. Our results showed that fasting serum blood glucose (BG), C-peptide, and HbA1c % significantly decreased after treatment with GL+CE compared to treatment with GL alone. HOMA2-IR significantly reduced after treatment with GL+CE compared to treatment with GL alone. On the other hand, HOMA2-β % values significantly increased after GL+CE treatment compared to GL treatment (Table 1).

Serum adiponectin level increased significantly after 3 months of treatment with a significant difference when comparing group the GL group with the GL+CE group. On the other hand, serum visfatin and leptin concentrations significantly decreased after 3 months of treatment in both groups, with no difference when comparing the GL group to the GL+CE group. Serum TNF-α level decreased significantly after treatment in both groups with a significant difference comparing group received GL alone to group received GL+CE. TCH and LDL-C levels were not changed in patients who received GL, whereas significantly decreased in patients who received GL+CE treatment, with a significant difference when comparing OD-GL and OD-GL+CE groups. Serum HDL level exhibited no change in both groups. Triglyceride TGs level showed significant decrease upon treatment and when comparing the OD-GL+CE and OD-GL groups, it was found a significant difference. On the other hand, AI decreased significantly in OD-GL+CE group compared to before treatment with a significant difference compared to OD-GL group (Table 2).

Discussion

The present study evaluate whether the co-administration of anti-inflammatory agent celecoxib with glimepiride could have an impact on glycemia, insulin resistance, and adipocytokines levels in obese diabetic subjects. The hallmark of obesity and type 2 diabetes is insulin resistance, a result of decreased insulin metabolic signaling due to enhanced serine phosphorylation and/or degradation of the insulin receptor substrate [25]. The major factors for progressive loss of beta cell function and mass are glucotoxicity, lipotoxicity, proinflammatory cytokines, leptin, and islet cell amyloid. Impaired beta cell function possibly appears to be reversible, particularly at early stages of the disease [26]. Our results implicate that COX-2 inhibition could be a therapeutic drug for treatment of obese subjects with T2DM and may prevent associated complications by suppressing the increased level of adipocytokines and insulin resistance in OD patients. The obtained results showed that fasting serum BG, C-peptide, HbA1c %, and HOMA of insulin resistance (HOMA2-IR) significantly decreased after treatment with GL+CE compared to treatment with GL alone. HOMA of beta cell functionality (HOMA2-β %) values significantly increased after GL+CE treatment compared to GL treatment. In consistence with a recent study that examined the effect of COX-2 inhibition on the development of muscular insulin resistance in high-fat-induced obese rats, emphasized that chronic COX-2 inhibition could significantly suppress insulin resistance via other mechanism than its direct action [27]. Previous study demonstrated that COX-2 inhibition significantly suppressed the whole body and muscular insulin resistance, implying the importance of COX-2-mediated low-grade inflammation in the pathogenesis of insulin resistance in metabolic syndrome and T2DM [28]. These observations indicate that COX-2-mediated inflammatory response might be an important cause of the development of insulin resistance in T2DM and obesity. Nevertheless, some case reports showed that COX-2 inhibitors could induce hypoglycemic episode when over consumed or taken in combination with oral hypoglycemic drugs [29, 30]. Recent study suggested that the changes in COX activity are involved in the regulation of glucose homeostasis under the states of normal and insulin resistance [31]. The present study showed that serum adiponectin was significantly increased whereas, serum visfatin, leptin, and TNF-α levels were significantly decreased after co-administration of CE with GL. Adiponectin and TNF-α are important inflammatory products with potential involvement in the pathogenesis of tissue insulin resistance [32]. Consistent with the present finding, Hsieh et al. have shown that the COX-2 inhibitors could significantly reverse the enhanced TNF-α and the decreased adiponectin as well as in obese rats [9]. Furthermore, the present results showed that the elevated serum values of TC, LDL-C, TGs, and AI in obese diabetics were corrected with

Table 1 Effect of treatment on BMI, blood glucose, glycated hemoglobin, C-peptide, and HOMA levels

Group	OD-GL		OD-GL+CE	
Parameter	B	A	B	A
Age	48±5.52	–	51.3±6.28	–
Gender (F/M)	11/9	–	10/10	–
BMI	32.93±1.97	32.82±1.96	33.12±1.61	32.90±1.56
FBG mg/dl	186.25^{b}±13.80	177.05^{ab}±16.05	181.95^{b}±16.97	136.8^{a}±19.83
HbA1c %	8.11^{b}±0.45	7.71^{ab}±0.76	8.01^{b}±0.52	7.05±0.44
C-peptide ng/ml	1.91^{b}±0.30	1.80^{ab}±0.25	1.96^{b}±0.33	1.58^{a}±0.31
HOMA2-IR	1.77^{b}±0.28	1.64^{ab}±0.23	1.81^{b}±0.31	1.33^{a}±0.28
HOMA2-β %	32.30^{b}±5.78	33.84^{b}±6.89	34.4^{b}±7.32	50.75^{a}±18.13

Data are presented as mean±SD; $P<0.05$

A after treatment, *B* before treatment, *SD* standard deviation, *BMI* body mass index, *FBG* fasting blood glucose, *HbA1c %* glycated hemoglobin percent, *HOMA2-IR* homeostasis model assessment of insulin resistance, *HOMA2-β* homeostasis model assessment of beta cell functionality, *OD-GL* obese diabetic group treated with glimepiride drug, *OD-GL+CE* obese diabetic group treated with glimepiride plus celecoxib drugs, *F* female, *M* male

[a] Significant before versus after treatment

[b] Significant versus after treatment with GL+CE

combination treatment of celecoxib and glimepiride. Accumulation of muscle TGs has been proposed to inversely relate with the defective glucose uptake in insulin-resistant subjects [33]. Jeuestte et al.[34] proposed that insulin resistance in obese diabetic subjects may be responsible for enhanced overproduction of TGs and cholesterol-rich lipoprotein by liver. Pro-inflammatory molecules produced by adipose tissue have been implicated as active participants in the development of insulin resistance [5]. This is explained by the increased oxidative stress, which could originate from adipose tissue in obesity, possibly leads to impaired insulin production and insulin action [31]. Taken together, these results implicate that COX-2 mediated generation of oxidative stress might play an important role in the development of obesity and T2DM in humans. COX-2 inhibition could be a therapeutic drug target to treat obese subjects with T2DM and prevent complications.

Table 2 Effect of treatment on visfatin, adiponectin, leptin, TNF-α levels, and lipid profile

Group	OD-GL		OD-GL+CE	
Parameter	B	A	B	A
Adiponectin (pg/ml)	0.55^{b}±0.09	1.17^{ab}±0.38	0.57^{b}±0.11	1.80^{a}±0.51
Visfatin (ng/ml)	91.69^{b}±16.64	84.33^{a}±15.41	91.09^{b}±12.00	77.18^{a}±11.90
Leptin (ng/ml)	11.96^{b}±2.03	11.02^{a}±2.12	12.17^{b}±3.43	9.34^{a}±4.18
TNF-α (pg/ml)	1.91^{b}±0.43	1.70^{ab}±0.42	1.93^{b}±0.30	1.33^{a}±0.29
Total cholesterol mg\dl	196.31^{b}±19.38	191.66^{b}±19.24	194.18^{b}±14.66	177.75^{a}±12.82
LDL-C mg/dl	129.02^{b}+19.35	125.63^{b}+19.95	126.68^{b}+13.56	117.47^{a}+15.31
HDL-C mg/dl	32.82±1.25	32.98±1.23	32.75±1.25	33.16±1.37
Triglycerides mg/dl	172.36^{b}+14.13	165.25^{ab}±11.75	173.73^{b}+16.39	135.65^{a}±22.41
AI	0.72^{b}±0.04	0.70^{b}±0.04	0.72^{b}±0.05	0.61^{a}±0.07

Data are presented as mean±SD. $P<0.05$

A after treatment, *B* before treatment, *TNF-α* tumor necrosis alpha, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *AI* atherogenic index, *OD-GL* obese diabetic group treated with glimepiride drug, *OD-GL+CE* obese diabetic group treated with glimepiride plus celecoxib drug

[a] Significant before versus after treatment

[b] Significant versus after treatment with GL+CE

The present study demonstrates that COX-2 inhibition could alleviate obesity-induced insulin resistance indirectly via suppressing adipocytokines released in obese diabetics.

Study limitations

Limitations of the study are the small number of participated patients and as with all NSAIDs, the potential GIT, CVD, and renal risks of CE must be weighed against the potential benefits in each individual.

Conclusions

Increased insulin resistance in obese diabetics due to increased inflammatory adipocytokines visfatin, leptin, and TNF-α and decreased adiponectin levels can be reversed by co-treatment with COX-2 inhibitor (celecoxib). So, COX-2 inhibition could be a therapeutic drug target for treatment of obese subjects with T2DM and prevent or delay complications. More extensive studies are needed to evaluate the use of anti-inflammatory strategies, especially COX-2 inhibitors, as preventative and therapeutic interventions in obesity and type 2 diabetes.

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Conflict of interest The authors declared that there are no conflicts of interest.

Author's contribution Hoda El-Bahrawy did the conception and design of the study and final approval of the version to be published. Sahar Hegazy followed up the patients and drafted the article or revised it critically for important intellectual content. Wael Farrag took the detailed history from patients, examined the patients clinically, and analyzed and interpreted the data. Rehab Werida followed up the patients and collected samples from them; performed anthropometric evaluations and biochemical assay for selected parameters; analyzed obtained data; and drafted the article or revised it critically for important intellectual content, and gave final approval of the version to be published.

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ORIGINAL ARTICLE

Lipid peroxidation, antioxidants, lipid profile, and HbA1c in diabetic patients

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Abstract Diabetes mellitus is characterized by hyperglycemia together with biochemical changes in glucose, lipid profile, lipid peroxidation, and antioxidants status. This study aims to assess lipid profile, lipid peroxidation, antioxidants, and glycated hemoglobin (HbA1c) in type 1 and type 2 diabetic subjects. Type 1 and type 2 diabetic patients were selected from the subjects attending OPD in Nepalgunj Medical College, Nepal, for medical checkup. Fasting blood sugar (FBS), lipid profile, lipid peroxidation (malondialdehyde), and antioxidants status (reduced glutathione and vitamin E) were estimated in both groups and were compared with healthy controls. Low-density lipoprotein (LDL)/high-density lipoprotein (HDL) ratio was calculated to assess the cardiovascular risk factors. When type 1 diabetic patients were compared with type 2 diabetic patients, it showed statistically significant increase in the levels of HbA1c, triglycerides (TGs), and high-density lipoprotein cholesterol (HDL-C), whereas statistically significant decreased level was found in malondialdehyde (MDA). FBS, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), reduced glutathione (GSH), vitamin E, and HDL/LDL ratio were not significant. Early diagnosis of dyslipidemia and oxidative stress can be used as a preventive measure for the development of microvascular and macrovascular complications in type 1 and type 2 diabetes mellitus.

Keywords Glycated hemoglobin · Diabetes mellitus · Lipid peroxidation · Antioxidants · Lipid profile

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Introduction

Diabetes mellitus (DM) is one of the most common metabolic disorders, and its prevalence is estimated to be approximately 177 million, and it is expected to reach 366 million by 2030 [1]. Among the glycated hemoglobins, the most abundant form is glycated hemoglobin (HbA1c), where glucose is attached to the N-terminal valine at β chain of hemoglobin [2]. The HbA1c level reveals the mean glucose level over a period of 10–12 weeks, unaffected by recent food intake or recent changes in blood sugar levels. The risk of retinopathy and renal complications are proportionately increased with elevated glycated hemoglobin value [3]. In diabetes mellitus, there are evidences that the changes in lipid metabolism have a specific role in pathogenesis and complication of the disease [4].

Malondialdehyde is a stable end product of lipid peroxidation and is commonly used as an indicator of lipid peroxidation [5]. The major natural antioxidant is vitamin E [6]. Non-enzymatic antioxidant, reduced glutathione, acts as a primary line of defense to cope with the deleterious effects of reactive oxygen species. High levels of peroxidation and the simultaneous decrease of antioxidant defense mechanisms can lead to damage of cellular organelles and oxidative stress [7]. Low levels of vitamin E and antioxidants are associated with increased incidence of diabetes [8, 9]. The study therefore aims

to evaluate HbA1c, lipid profile, lipid peroxidation, and antioxidant status in both type 1 and type 2 diabetic patients.

Methods

The study was designed as a case-control study. The study was conducted from March 2011 to March 2012. Eighty-eight type 1 diabetic patients and 92 type 2 diabetic patients who were treated in outpatient facility in Nepalgunj Medical College, Nepal, were included in the study. Eighty normal healthy individuals with no symptoms of diabetes were taken as control. Diabetes mellitus subjects aged from 20 to 80 years. Type 1 and type 2 diabetic patients' age were between 20 and 35 years and between 40 and 80 years, respectively. The criteria for diagnosis of diabetes mellitus were on the basis of American Diabetes Association guidelines [10]. Ethical approval for the study was taken from the institutional research ethical committee.

Inclusion criteria

Freshly diagnosed type 1 and type 2 diabetic patients were included in the study.

Exclusion criteria

Patients with history of smoking and alcoholism, those without complications and secondary causes of hyperglycemia and any chronic diseases, and patients on hypolipidemic drugs or other antioxidant therapies were excluded in the study.

Data collection

Subjects were mainly volunteer and recruited with mutual consent. All the patients filled up a detailed form which included age, sex, duration of diabetes, history of hypertension, and past history of any cardiovascular or kidney diseases. Blood was collected from the patient after overnight fasting (8–10 h). Six milliliters of blood was collected from each subject by venipuncture for the study of various parameters and was distributed in the following vials: heparinized sample for the estimation of serum vitamin E, citrated sample for the estimation of malondialdehyde (MDA) in plasma, and reduced glutathione (GSH) in erythrocyte, and ethylenediaminetetraacetic acid (EDTA) sample for the estimation of HbA1c and in a plain vial (without anticoagulant) for estimation of fasting blood sugar (FBS) and lipid profile. Lipid profile, FBS, and reduced GSH were done on the same day, and for other tests, the samples were stored at −70 °C until the analyses were carried out.

HbA1c measurement was done using ion-exchange chromatography (LifeChem). Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using the kits supplied by BioSystems, S.A. Costa Brava, 30 Barcelona, Spain. Serum low-density lipoprotein cholesterol (LDL-C) was calculated according to computational procedures of Friedewald et al. [11]. MDA in plasma was assayed using the method of Yagi [12]. Serum vitamin E estimation was based on the Emmerie-Engel procedure [13]. The red blood cells left were washed in cold saline, and diluted hemolysates were prepared for estimation of GSH. Erythrocyte GSH was estimated using the method of Beutler et al. [14].

Statistical analysis

Data entry and analysis were done using SPSS software, version 16.0. Comparison of mean and tests of association was done using independent *t* test. p value <0.05 was regarded as statistically significant.

Results

Out of 88 type 1 diabetic patients, 48 (54.54 %) were males and 40 (45.45 %) were females. Out of 92 type 2 diabetic patients, 48 (52.17 %) were males and 44 (47.82 %) were females. Among the controls, 48 were males and 32 were females, respectively. The mean ± SD age of control, type 1 DM patients, and type 2 DM patients were 39.96 ± 14.84, 27.72 ± 4.82, and 58.19 ± 9.93, respectively (Table 1).

Table 2 shows the comparison of type 1 and type 2 diabetic patients with control in different biochemical parameters. When all the parameters were compared between type 1 and type 2 diabetic patients, HbA1c, TGs, and HDL-C were found to be increased significantly, whereas MDA was found to be decreased significantly. The increase in HbA1c, FBS, lipid profile (TC, TG, and LDL-C), and MDA and the decrease in GSH and vitamin E for type 1 diabetic patients when compared to control subjects showed statistical significance. Increase in serum HDL-C level was insignificant. HDL/LDL ratio was found to be significant. For type 2 diabetic patients, an increase in HbA1c, FBS, lipid profile (TC, TG, LDL-C, and HDL-C), and lipid peroxidation (MDA) and a decrease in

Table 1 Age and sex distribution of study subjects

	Control	Type 1 DM	Type 2 DM
Age in years (range)	20–65	20–35	40–80
Mean ± SD	39.96 ± 14.84	27.72 ± 4.82	58.19 ± 9.93
Sex distribution			
Males, *n* (%)	48 (60)	48 (54.54)	48 (52.17)
Females, *n* (%)	32 (40)	40 (45.45)	44 (47.82)

Table 2 Biochemical parameters of control and diabetic patients

Parameters	Control	Type 1 DM	Type 2 DM
HbA1c (%)	4.83 ± 0.57	7.41 ± 0.66*$	7.75 ± 1.12#$
(95 % CI)	(4.71, 4.94)	(7.28, 7.56)	(7.54, 8.01)
FBS (mg/dl)	87.11 ± 11.67	159.82 ± 29.39*	150.98 ± 47.21#
(95 % CI)	(84.79, 89.43)	(153.59, 166.05)	(141.20, 160.76)
TC (mg/dl)	172.25 ± 14.97	217.48 ± 52.59*	229.35 ± 48.14#
(95 % CI)	(169.28, 175.22)	(206.33, 228.62)	(219.38, 239.32)
TG (mg/dl)	106.04 ± 22.32	147.18 ± 47.35*$	165.59 ± 51.60#$
(95 % CI)	(101.61, 110.47)	(137.15, 157.12)	(154.90, 176.27)
HDL-C (mg/dl)	50.21 ± 6.54	51.20 ± 8.35$	55.41 ± 9.66#$
(95 % CI)	(48.91, 51.51)	(49.43, 52.98)	(53.41, 57.42)
LDL-C (mg/dl)	115.10 ± 9.68	142.89 ± 33.53*	152.04 ± 36.87#
(95 % CI)	(113.18, 117.02)	(135.78, 149.99)	(144.41, 159.68)
MDA (mmol/ml)	2.91 ± 0.74	4.71 ± 1.76*$	4.21 ± 1.19#$
(95 % CI)	(2.76, 3.06)	(4.34, 5.09)	(3.97, 4.46)
GSH (mg/dl)	15.52 ± 1.25	12.7 ± 2.17*	13.11 ± 1.74#
(95 % CI)	(15.27, 15.77)	(12.25, 13.17)	(12.75, 13.48)
Vitamin E (mg/dl)	0.99 ± 0.41	0.57 ± 0.46*	0.52 ± 0.46#
(95 % CI)	(0.42, 0.44)	(0.43, 0.62)	(0.43, 0.62)
LDL/HDL	2.32 ± 0.32	2.81 ± 0.60*	2.80 ± 0.78#
(95 % CI)	(2.25, 2.39)	(2.68, 2.94)	(2.64, 2.96)

*# p value < 0.001, when type 1 and type 2 DM patients were compared with control subjects; $ p value < 0.05, when type 1 DM patients were compare with type 2 DM patients

antioxidants (GSH and vitamin E) and HDL/LDL were statistically significant when compared with normal healthy control subjects.

Discussion

In the present study with the exception of HDL-C in type 1 diabetic patients, all the biochemical parameters are found to be significantly increased in HbA1c, FBS, lipid profile (TC, TG, and LDL-C), and MDA and decreased in GSH and vitamin E for both type 1 and type 2 diabetic patients. Poor glycemic control deteriorates lipid and lipoprotein metabolism [15]. Our results failed to demonstrate a significant difference in the HDL-C concentration in type 1 diabetic patients, whereas in type 2 diabetic patients, the HDL-C level was raised significantly. This finding is in agreement with that of Nikkila and Hormila [16]. Maharjan et al. and Aleyassine et al. reported that there was no significant difference between HDL-C and diabetes [17, 18]. HDL play a role in reverse cholesterol transport, and it also carries cholesterol from the atherosclerotic plaques to the liver for removal. So, it is considered to be inversely related to the development of atherosclerosis [19]. The raised HDL levels should protect the diabetic patients from atherosclerotic complications. In the present study, it was observed that the levels of HbA1c and TG in type 2 DM were higher than their levels in type 1 DM. Our finding is in accordance with the findings of Masram et al. [20]. It has been demonstrated that high levels of serum TC, TG, LDL-C, and HbA1c and low concentration of HDL-C were significantly associated with cardiovascular disease [21]. High LDL/HDL ratio combined with hypertriglyceridemia is associated with high risk of coronary heart disease [22]. Lipid peroxidation (MDA) level was significantly increased in both types of diabetes mellitus when compared with control subjects. Similar results were reported by Singh and Shin [23] and Nacitarhan et al. [24]. Increase peroxide may be due to the increased glycation of proteins in diabetes mellitus [25]. MDA was found to be higher in type 1 DM as compared to type 2 DM. It may be due to poor metabolic control in type 1 DM. Reduced GSH is significantly decreased in type 1 and type 2 diabetic patients when compared with control subjects, and it is correlated with the study done by Giugliano et al. [26] and Ceriello et al. [27]. Hydrogen peroxide is detoxified with the help of reduced GSH, and it is regenerated with the help of NADPH supplied from hexose monophosphate shunt pathway, which is stimulated by insulin. In diabetes mellitus, NADPH production may be sluggish, which results in lowered reduced glutathione reductase enzyme and reduced regeneration of GSH. In addition to this, in diabetes mellitus, increased sorbitol synthesis occurred which depletes NADPH and limits the GSH regeneration [28, 29]. Significantly reduced vitamin E level in diabetic patients observed in this study is similar with previous studies [30, 31]. Abnormally increased activity of free radical induced lipid peroxidation, and simultaneous decline of antioxidant defense mechanisms

can lead to damage of cellular organelles and enzymes and development of complications of diabetes mellitus [32].

Conclusion

This study has shown that dyslipidemia, poor glycemic control, and increased oxidative stress are highly prevalent among diabetic subjects in the population of far western region of Nepal. Efforts should be made in the area of glycemic control, lipid lowering, and lifestyle modifications to reduce the risk of microvascular and macrovascular complications in diabetic subjects.

Conflicts of interest None

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ORIGINAL ARTICLE

Effects of pre-pregnancy weight on incidence of large for gestational age newborn in pregnant women with gestational diabetes mellitus

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Abstract The aim of this study is to compare the incidence of large for gestational age (LGA) infants between gestational diabetes mellitus (GDM) women whose pre-pregnancy weight was in normal and overweight/obese category. Possible associated factors for LGA were evaluated and pregnancy outcomes were compared. A total of 272 singleton pregnant women with GDM were enrolled, 136 overweight/obese women (BMI ≥25 kg/m^2) were in study group and another 136 normal weight women (BMI <25 kg/m^2) were served as comparison group. Data were retrieved from medical records, including demographic data, GDM diagnosis and risks, labor and delivery data, and pregnancy outcomes. Baseline characteristics and incidence of LGA were compared between groups, and logistic regression analysis was performed in order to determine independent risk factors for LGA, adjusted for potential confounders. Baseline demographic data were comparable between groups, except that study group was significantly more likely to have excessive weight gain and more likely to need insulin treatment than control group. The rate of LGA and macrosomic infants was significantly higher in study group than in control group (p=0.038 and 0.024, respectively). Logistic regression analysis showed that only gestational weight gain was significantly associated with LGA. Gestational weight gain less than recommendation significantly decreased the risk of LGA by 60 % (adjusted odds ratio (OR) 0.39, 95 % confidence interval (CI) 0.17–0.96, p=0.04). On the other hand, gestational weight gain greater than recommendation significantly doubled the risk of LGA (adjusted OR 2.03, 95 % CI 1.11–3.71, p=0.022). Gestational weight gain, but not pre-pregnancy BMI was independently associated with LGA in GDM pregnant women.

Keywords Gestational diabetes · Gestational weight gain · Large for gestational age · Pre-pregnancy BMI

Introduction

The term large for gestational age (LGA) has been used to define the newborns whose birth weight is above the 90th percentile for their gestational age [1]. It is generally accepted that LGA was associated with many maternal and neonatal morbidities, including prolonged labor, operative delivery, shoulder dystocia, brachial plexus injury, stillbirth, postpartum hemorrhage, and laceration of the anal sphincter [2–4].

Incidence of LGA newborn has been associated with gestational diabetes mellitus (GDM). In pregnant women with GDM, maternal hyperglycemia can lead to fetal hyperglycemia and hyperinsulinemia which are responsible for subsequent increases in fetal growth [5]. Previous studies reported that the incidence of LGA was significantly higher in GDM than non-GDM pregnant women [6, 7]. However, maternal diabetes might not be the only factor responsible for fetal overgrowth. Other associated factors included genetics, race, and ethnic factors, maternal obesity, excessive weight gain during pregnancy, multiparity, duration of gestation, and previous delivery of macrosomic infant [8–10]. Therefore, controlling glucose level might not be sufficient to reduce the incidence of LGA.

Previous studies have reported the association between pre-pregnancy weight, gestational weight gain, and birth weight in nondiabetic pregnant women [11, 12]. However, there are still

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limited information on association between pre-pregnancy weight and birth weight among GDM women [13–15].

The objective of this study was to compare the incidence of LGA infants between GDM women whose pre-pregnancy weight were in normal and overweight/obese category. In addition, other possible associated factors were also evaluated, such as gestational weight gain and GDM classification. Pregnancy outcomes were also compared between the two groups.

Materials and methods

After ethical approval from Siriraj Institutional Review Board (SIRB), a retrospective cohort study was conducted at the Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital.

According to institutional guideline, selective screening and diagnostic scheme for GDM was offered to all pregnant women using a two-step approach. A 50-g glucose challenge test (GCT) was used to screen pregnant women at risk, and the diagnosis of GDM was based on a 100-g oral glucose tolerance test (OGTT), using Carpenter and Coustan criteria. The process was offered during first visit and repeated during 24–28-week gestation [16, 17]. After diagnosis of GDM, dietary counseling were offered and follow-up visits were scheduled to determine adequacy of glycemic control. Insulin treatment was initiated for those with inadequate glycemic control (fasting plasma glucose >95 mg/dL and/or 2-h postprandial glucose >120 mg/dL) [16]. Well glycemic control was considered when women achieved glycemic control goals over 80 % of times.

A total of 272 pregnant women with GDM were enrolled. Study group comprised of 136 GDM women who were classified as overweight or obese, and comparison group comprised of 136 GDM women whose pre-pregnancy BMI was in normal range, according to the Institute of Medicine (IOM) guideline [18]. Pregnant women with underlying diseases, such as hyperthyroidism, heart disease, or overt DM, and those with documented fetal anomalies were excluded.

Data were retrieved from medical records, including demographic data, GDM characteristics, labor and delivery data, and pregnancy and neonatal outcomes. Adequacy of gestational weight gain was evaluated according to the IOM recommendation and were classified as less than, within, and greater than recommendation [18]. Large for gestational age (LGA) was diagnosed when birth weight was ≥90th percentile of normal newborns according to institutional standard.

Descriptive statistics, including number, percentage, mean, and standard deviation were used to describe various characteristics as appropriate. Student *t* test and chi-squared test or Fisher's exact test were used to compare various characteristics between groups. Logistic regression analysis was performed in order to determine independent risk factors for LGA infants, adjusted for potential confounders. Adjusted odds ratio (OR) and 95 % confidence intervals (CI) were estimated. A p value <0.05 was considered statistically significant.

Table 1 Demographic data of GDM pregnant women between the two groups (N=272)

Characteristics	Comparison group (N=136)	Study group (N=136)	p value
Maternal age ± SD (years)	33.1±5.2	33.1±4.2	0.888
Parity			
0	68 (50.0 %)	56 (41.2 %)	0.311
1	47 (34.6 %)	58 (42.6 %)	
≥2	21 (15.4 %)	22 (16.2 %)	
Pre-pregnancy BMI (kg/m^2)	21.9±1.9	29.3±3.2	<0.001
Gestational weight gain ± SD (kg)	12.7±5.3	11.0±4.1	0.004
Weight gain category			<0.001
Less than recommendation	65 (47.8 %)	9 (6.6 %)	
Within recommendation	39 (28.7 %)	58 (42.6 %)	
Greater than recommendation	32 (23.5 %)	69 (50.7 %)	
GA at first ANC ± SD (weeks)	12.4±5.5	13.3±5.5	0.202
GA at GDM diagnosis ± SD (weeks)	22.4±8.6	21.8±8.4	0.549
GDM classification			0.001
Dietary control	120 (88.2 %)	98 (72.1 %)	
Insulin treatment	16 (11.8 %)	38 (27.9 %)	
Well glycemic control	123 (90.4 %)	118 (86.8 %)	0.125

Table 2 Comparison of risk factor of GDM between the two groups

Risk factors	Comparison group (N=136)	Study group (N=136)	p value
Age ≥30 years	107 (79.3 %)	107 (78.7 %)	0.906
Family history of diabetes	48 (35.3 %)	49 (36.0 %)	0.899
Previous unexplained fetal death	0 (0 %)	5 (3.7 %)	0.060
Previous history of GDM	3 (2.2 %)	6 (4.4 %)	0.500
Previous history of fetal macrosomia	0 (0 %)	4 (2.9 %)	0.122
Previous history of unexplained congenital anomalies	0 (0 %)	1 (0.7 %)	1.000
Pre-pregnancy BMI ≥25 kg/m^2	0 (0 %)	48 (35.3 %)	<0.001
History of HT or gestational HT	1 (0.7 %)	3 (2.2 %)	0.622

Results

A total of 272 pregnant women diagnosed with GDM were included in this study. Study group were 136 overweight or obese women and another 136 normal weight women were served as comparison group. Table 1 demonstrated demographic data of both groups. Both groups were comparable in terms of age, parity, gestational age at first ANC, and at GDM diagnosis. Mean gestational weight gain was significantly greater among control group than study group (12.7± 5.3 and 11.0±4.1 kg, p=0.004). However, women in study group were significantly more likely to have excessive weight gain than comparison group (50.7 and 23.5 %, p<0.001). In addition, women in study group were significantly more likely to require insulin treatment than those in comparison group (27.9 and 11.8 %, p=0.001). Well glycemic control was observed in majority of women in both groups without significant differences (p=0.125).

Risks for GDM according to the institutional guideline were compared between the two groups as shown in Table 2. No significant difference was observed, except pre-pregnancy BMI that was greater among study group.

Table 3 showed comparison of pregnancy outcomes between the two groups. There was no significant difference between groups regarding GA at delivery and route of delivery. Overall and primary cesarean delivery was only slightly higher in study group than comparison group.

Comparisons of neonatal outcomes between the two groups are shown in Table 4. Infants of women in study group had significantly greater birth weight than those in comparison group with approximated difference of 200 g (3344.2±468.6 and 3149.8±469.8 g, p=0.001). In addition, the rate of LGA infants was significantly higher in study group than in comparison group (33.8 and 23.5 %, p=0.038). Significant increase in macrosomic infants was also observed (9.6 and 2.9 %, p=0.024). Regarding neonatal complications, infants of women in study groups had significant greater rate of hypoglycemia than control group (14 and 5.1 %, p=0.013). No differences were observed between groups regarding birth asphyxia, NICU admission, and neonatal jaundice.

Logistic regression analysis was performed in order to determine independent risk factors associated with LGA, taken into account of potential confounders, including gestational weight gain and GDM classification. The results are shown in Table 5 and demonstrated that after adjusting for potential confounders, pre-pregnancy BMI and GDM classification were not significantly associated with LGA. However, gestational weight gain was significantly associated with LGA. Gestational weight less than recommendation significantly decreased the risk of LGA by 60 % (adjusted OR 0.39, 95 % CI 0.17–0.96, p=0.04). On the other hand, gestational weight gain greater than recommendation significantly doubled the risk of LGA (adjusted OR 2.03, 95 % CI 1.11–3.71, p=0.022).

Discussion

Recently, there is an increasing trend of obese and advanced-age pregnant women. As a consequence, the incidence of GDM

Table 3 Comparison of pregnancy outcomes between the two groups

Pregnancy outcomes	Comparison group (N=136)	Study group (N=136)	p value
GA at delivery ± SD (weeks)	38.2±1.3	38.1±1.4	0.508
Route of delivery			0.238
Normal delivery	77 (56.6 %)	72 (52.9 %)	
Vacuum extraction	6 (4.4 %)	2 (1.4 %)	
Cesarean section	53 (39.0 %)	62 (45.6 %)	
Primary cesarean section	45 (33.1 %)	55 (40.4 %)	0.209

Table 4 Comparison of neonatal outcomes between the two groups

Neonatal outcomes	Comparison group (*N*=136)	Study group (*N*=136)	*p* value
Birth weight ± SD (g)	3149.8±469.8	3344.2±468.6	0.001
Birth weight for GA			0.038
SGA	6 (4.4 %)	1 (0.7 %)	
AGA	98 (72.1 %)	89 (65.4 %)	
LGA	32 (23.5 %)	46 (33.8 %)	
Macrosomia	4 (2.9 %)	13 (9.6 %)	0.024
Birth asphyxia	7 (5.1 %)	7 (5.1 %)	1.000
NICU admission	2 (1.5 %)	3 (2.2 %)	0.652
Hypoglycemia	7 (5.1 %)	19 (14 %)	0.013
Neonatal jaundice	43 (38.7 %)	27 (26.5 %)	0.057

increased, as well as related maternal and neonatal morbidity and mortality. Maternal diabetes was among the strong determinants for fetal overgrowth and the incidence of LGA newborn.

From initial univariate analysis, the rate of LGA infants was significantly higher among overweight/obese GDM women. However, after controlling for potential confounders, gestational weight gain was the only independent factor associated with the incidence of LGA. Gestational weight gain less than recommendation significantly decreased the risk of LGA by 60 % (adjusted OR 0.39), and gestational weight gain greater than recommendation significantly doubled the risk of LGA (adjusted OR 2.03).

The results were similar to some previous studies that gestational weight gain was the independent predictor of LGA among GDM pregnant women and the risk was minimized when weight gain was less than recommendation [19–22]. However, conflicting results have also been reported. Other studies have reported that pre-pregnancy BMI or obesity was an important risk for LGA [16, 17, 21, 23, 24]. Other factors that have been associated with LGA in GDM women included HbA1c level [16], previous macrosomia [23], and GDM severity [17, 19, 21]. Different results between studies could be from differences in population in terms of race and ethnicity, GDM screening, diagnostic, and management scheme, dietary and lifestyle during pregnancy, and pre-pregnancy BMI and gestational weight gain classification.

Table 5 Logistic regression analysis for independent risks for LGA

Risks	Adjusted OR	95 % CI	*p* value
Pre-pregnancy weight			
Normal	1.0		
Overweight/obese	0.96	0.52–1.77	0.901
Gestational weight gain			
Within recommendation	1.0		
Less than recommendation	0.39	0.17–0.96	0.040
Greater than recommendation	2.03	1.11–3.71	0.022
GDM classification			
Dietary control	1.0		
Insulin treatment	1.09	0.55–2.13	0.813

In general, it appeared that gestational weight gain might be a more important determinant of birth weight and fetal growth than pre-pregnancy BMI. In a systematic review, the association between gestational weight gain and birth weight was observed despite the use of different standard references and various methods of characterizing gestational weight gain (total, rate, or by trimester) [14]. This was consistent with the results among GDM pregnant women in this study.

Some limitations in this study should be noted. This study has smaller samples than other previous studies which might result in less accurate estimates. The study has not aimed primarily to evaluate effects of gestational weight gain that the study might have limited statistical power in estimating such associations. In addition, although every woman received similar dietary counseling, effects of dietary control could not be determined and quantified. It might be possible that dietary control had greater effects on overweight/obese than normal weight women, resulting in comparable outcomes between groups.

Currently, all women with GDM receive intensive dietary counseling during their antenatal care at our institution which can help pregnant women control their blood glucose level and weight gain during pregnancy. However, 23.5 % of GDM women with normal pre-pregnancy BMI and as many as 50.7 % of overweight/obese women have gained weight more than recommended level despite well-glycemic control was observed in majority of the study population. This reflected the effectiveness of such counseling procedure and highlights the need for improvement.

The results of this study emphasized the importance of gestational weight gain control among this subgroup of pregnant women, regardless of pre-pregnancy BMI. Since gestational weight gain is modifiable, clinicians, and health care providers should always pay more attention to gestational weight gain and put more efforts in counseling and close

follow up among this group of pregnant women. This could help reduce the risk of LGA infants and related complications. Future research with larger samples that aims primarily to investigate the effects of gestational weight gain among this group of women should be considered.

In conclusion, gestational weight gain, but not pre-pregnancy BMI, was independently associated with LGA in GDM pregnant women. Gestational weight gain less than recommendation significantly decreased the risk of LGA by 60 %. On the other hand, gestational weight gain greater than recommendation significantly doubled the risk of LGA. However, the observed associations might partly be secondary from effects of dietary control provided to both groups of GDM women.

Conflict of interest The study received no funding from any sources and there are no conflicts of interest of any nature at all.

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. For this type of study, formal consent is not required.

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ORIGINAL ARTICLE

Can either oral glucose challenge test or oral glucose tolerance test parameters predict gestational diabetes mellitus?

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Abstract This study aims to evaluate the relationship between the glucose challenge test (GCT) levels and any of the oral glucose tolerance test (OGTT) parameters (fasting plasma glucose (FPG), 1-, 2-, or 3-h plasma glucose levels) and their effect on predicting gestational diabetes mellitus (GDM). This analysis was carried out as a retrospective study at Obstetrics and Gynecology Clinic of Turgut Özal University Hospital. Oral GCT were conducted on patients who are at 24–29 weeks' gestation. The study participants with positive GCT results underwent a 3-h, 100-g OGTT, and the resulting values were evaluated using Carpenter and Coustan diagnostic criteria to determine the gestational glucose tolerance status of patients. The data obtained from both tests (GCT, FPG, 1-, 2-, 3-h OGTT values) were analyzed to observe the effect of each group on predicting GDM. Although all of the GCT and OGTT values were found to be statistically significant ($p<0.001$) in determining GDM, the 2-h values of OGTT detected almost all GDM cases with a very high sensitivity level (94.5 %). The 1-h values on the other hand identified 87.6 % of GDM ($p<0.001$). The GCT value with the highest sensitivity and specificity for predicting GDM was calculated as 154.50 mg/dl (sensitivity and specificity rates were 79.2 and 72.8 %, respectively). A 2-h OGTT glucose level can detect GDM with 94.5 % sensitivity. This result can guide clinicians to evaluate the patients with GDM.

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Keywords Gestational diabetes mellitus · Oral glucose challenge test · Oral glucose tolerance test · Prediction

Introduction

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance with onset or first recognition during pregnancy [1]. It affects approximately 1–16 % of pregnant women worldwide depending on the type of population, the geographic location, ethnicity, and the diagnostic criteria used [2–6]. GDM is associated with adverse pregnancy outcomes, such as large-for-gestational age infants, neonatal hypoglycemia, neonatal hyperinsulinism, and pre-eclampsia [7, 8]. Imbalance in glucose metabolism returns to normal in the majority of women during postpartum period but one third to two thirds of women will have glucose intolerance in subsequent pregnancies [6, 9].

While failure to detect GDM may result in severe complications such as harming the fetus and the mother, too aggressive screening on the other hand may result in too many expectant mothers getting unnecessary blood tests, many false-positive GDM diagnoses that are followed by redundant interventions. Although there is no widespread consensus on how to screen and diagnose GDM [10], an acceptable standard identifying approach remains as performing 3-h, 100-g oral glucose tolerance test (OGTT) at 24–28 weeks of gestation, preceded by positive or abnormal 50-g, 1-h glucose challenge test (GCT) in many countries. This two steps approach is also recommended by the American Diabetes Association (ADA) for women with risk factors [11]. The World Health Organization (WHO) recommends all pregnant women be screened at the beginning of third trimester of pregnancy using 2-h, 75-g oral glucose tolerance test (75-g OGTT) which can be simultaneously used both for screening and diagnosis [12].

Although there is an extensive literature dealing with screening and diagnosing GDM, we are not aware of any study which examines the effect of individual OGTT parameter on identifying GDM. In the current study, we aim to demonstrate whether any of 3-h, 100-g OGTT parameters (fasting plasma glucose (FPG), 1-, 2-, and 3-h glucose values) or GCT can solely predict GDM by itself with high sensitivity and specificity. If any of the above parameters is found to be a successful predictor of GDM, then unnecessary interferences on either diagnosing or treating GDM can be prevented.

Material and methods

This analysis was carried out as a retrospective study at Obstetrics and Gynecology Clinic of Turgut Özal University Hospital. An approval of the study protocol was obtained from the ethics committee at Turgut Özal University and written informed consent was signed by the attending women. The current study involved 810 healthy women with singleton pregnancies. Demographic characteristics of participants including age, gravida, parity, body mass index (BMI), and gestational age were recorded. The exclusion criteria for the participants included the existence of any type of abnormal pregnancies (polyhydramnios, oligohydramnios, fetal abnormality, etc.), systemic or infectious diseases, pregestational type 1 and type 2 diabetes, and smoking habit.

In order to screen the GDM, oral glucose challenge tests were conducted on patients who are at 24–29 weeks' gestation, regardless of fasting status. A venous blood sample was obtained from all women an hour after the ingestion of 50 g oral glucose load. The study participants with positive GCT results subsequently underwent a 3-h, 100-g OGTT. In order to determine the gestational glucose tolerance status of patients, OGTT results were evaluated using Carpenter and Coustan diagnostic criteria. Accepting the cutoff value of 130 mg/dl to entitle GCT as a positive test has been demonstrated to improve the sensitivity of test results up to 100 % [13]. Therefore, this study classified 1-h venous plasma glucose concentration of ≥130 mg/dl as a positive screening result.

Women with a positive test result subsequently received a 3-h, 100-g OGTT, which was considered as the diagnostic test for GDM. A positive OGTT was defined as venous plasma glucose concentration of ≥95 mg/dl for fasting, ≥180 for 1 h, ≥155 for 2 h, and ≥140 for 3 h. A patient was diagnosed as having GDM when two or three of the OGTT values at or above these thresholds. Serum glucose levels were measured with using the hexokinase method (COBAS Integra 800, Roche, Germany). Blood glucose values for all women were recorded and then classified as follows: GCT, fasting plasma glucose (FPG), 1-, 2-, and 3-h glucose values. All parameters were statically analyzed to determine whether any of them predict or point GDM by itself.

Statistical analyses

The statistical analyses were conducted using the SPSS 15.0 statistical software package (SPSS Inc., Chicago, IL, USA). ROC analysis was performed to evaluate the sensitivity and specificity of GCT and OGTT values for detecting GDM. In order to evaluate the effects of these tests on the prediction of the development of GDM, logistic regression analysis was used. A value of $p<0.05$ was considered as statistically significant.

Results

Our analysis was comprised of 810 subjects with median age of 28 years (range 16–46 years), gravida of 2 (range 1–9), parity of 1 (range 0–8), gestational age of 26 weeks (range 24–29 weeks), and BMI of 28 kg/m^2 (range 20–38.8 kg/m^2). Out of 810, 228 women (28.1 %) were found to have GCT values at or above 130 mg/dl. This group received 100-g OGTT in order to evince GDM. The gestational diabetes was diagnosed in 70 (8.6 %) of the 810 participants. Only 46 (5.7 %) pregnant women had impaired glucose tolerance (IGT). One hundred fifty-eight (19.5 %) cases had normal OGTT after observing a positive 50 g OGL test. The remaining subjects exhibited normal results following 50 g screening test.

Table 1 presents the empirical results of our analyses. Although all of the GCT and OGTT values were found to be statistically significant ($p<0.001$) in determining GDM, the 2-h values of OGTT detected almost all GDM cases with a very high sensitivity level (94.5 %). The 1-h values on the other hand identified 87.6 % of GDM ($p<0.001$) The GCT value

Table 1 ROC results for GCT and OGTT values in predicting GDM

	AUC	SE	*p*	95 % confidence interval	
				Lower bound	Upper bound
GCT	0.774	0.037	*<0.001*	0.701	0.846
FPG	0.772	0.044	*<0.001*	0.684	0.859
1 h	0.876	0.029	*<0.001*	0.819	0.932
2 h	0.945	0.015	*<0.001*	0.917	0.974
3 h	0.852	0.035	*<0.001*	0.784	0.921

$p<0.05$ was considered as statistically significant

AUC area under the curve, *SE* standard error, *GCT* glucose challenge test, *FPG* fasting plasma glucose

with the highest sensitivity (79.2 %) and specificity (72.8 %) in predicting GDM was calculated as 154.50 mg/dl.

Sensitivity and specificity of cutoff values in different levels for detecting GDM are shown in Table 2. With the GCT cutoff value of 132.5 mg/dl, we found the specificity level very low (23 %), in determining GDM with 100 % sensitivity. The highest values of both sensitivity and specificity for predicting GMD were found at 83.5 mg/dl for FPG (75.0 and 77.5 %, respectively), 175.50 mg/dl for 1 h (79.2 and 86.4 %, respectively), 144.00 mg/dl for 2 h (91.7 and 82.2 %, respectively), and 118 mg/dl for 3 h (75.0 and 80.5 %, respectively).

The result of the logistic regression analysis is presented in Table 3. Specifically, we found that 1-, 2-, and 3-h values are effective parameters in predicting GDM ($p=0.001$, $p=0.002$, and $p<0.001$, respectively). GCT and FPG values, on the other hand, did not produce statistically significant results for the same analysis ($p=0.544$ vs. $p=0.817$).

Table 2 Sensitivity and specificity values for GCT and OGTT at various cutoff values

	Cutoff	Sensitivity	Specificity
GCT	132.50	1.000	0.230
	138.50	0958	0.302
	142.50	0.917	0.467
	145.50	0.833	0.538
	154.50	0.792	0.728
FPG	69.50	1.000	0.118
	73.50	0.875	0.249
	76.50	0.833	0.450
	83.50	0.750	0.775
	90.50	0.542	0.917
1 h	133.00	1.000	0.349
	146.50	0.958	0.509
	153.50	0.875	0.615
	161.50	0.833	0.734
	175.50	0.792	0.864
	181.50	0.750	0.911
2 h	136.50	1.000	0.704
	144.00	0.917	0.822
	150.50	0.833	0.905
	154.50	0.792	0.941
	163.50	0.583	0.953
3 h	55.50	1.000	0.059
	90.50	0.958	0.396
	105.50	0.917	0.598
	110.50	0.833	0.680
	118.00	0.750	0.805
	133.50	0.667	0.923

GCT glucose challenge test, *FPG* fasting plasma glucose

Table 3 Logistic regression results for GCT and OGTT in predicting GDM

	B	S.E.	*p*	Exp (*B*)
@GCT	0.016	0.027	0.544	1.016
@FPG	0.005	0.022	0.817	1.005
@1 h	0.075	0.023	*0.001*	1.078
@2 h	0.087	0.028	*0.002*	1.091
@3 h	0.059	0.017	*<0.001*	1.061
Constant	−34.389	7.904	*<0.001*	0.000

$p<0.05$ was considered as statistically significant

B regression coefficient, *SE* standard error, *Exp (B)* odds ratio, *GCT* glucose challenge test, *FPG* fasting plasma glucose

Discussion

GDM is a common disease occurring in 1–16 % of pregnancies [2–6]. This condition is highly associated with increased risk of maternal and fetal morbidity and mortality [14–16]. Therefore, screening and diagnosing this condition with serious consequences should be given utmost importance. American Diabetes Association (ADA) recommends all pregnant women be screened with 50 g GCT for GDM after reaching 24–28 weeks of gestation [15]. A plasma glucose value between 130 and 140 mg/dl is commonly used as a threshold for performing a diagnostic oral glucose tolerance test (OGTT). A positive GCT requires subsequent OGTT to establish a diagnosis of GDM. According to ADA, if GCT threshold is accepted as ≥140 mg/dl, 80 % of GDM cases can be recognized, while cutoff value is set at 130 mg/dl, GDM cases reach 90 % [17]. There are a number of studies focusing on the most sensitive test for diagnosing GDM accurately. One of them indicates that high-sensitivity cutoff values of GCT for predicting GDM were 142 mg/dl [18]. Juntarat et al. included 838 pregnant women in their study and they identified a GCT value of ≥140 mg/dl as the cutoff value for detecting GDM, which showed the sensitivity and specificity of 95.3 and 48.6 %, respectively [19]. In our study, 50 g GCT by itself detected GDM cases with a sensitivity of 77.4 % (Table 1). When we accepted GCT cutoff value as 132.5 mg/dl, all of GDM cases were identified with GCT. Apart from our study, Maritta et al. cited that when GCT cutoff value was set as 7.3 mmol/l (130 mg/dl), 79 % of GDM cases were determined with 1-h, 50-g GCT without regarding risk factors [20]. They concluded that 50 g GCT appeared to identify a higher number of GDM than risk factor-based screening. On the other hand, Coustan et al. [21] found that 35 % of pregnant women with GDM could not be diagnosed by performing 50 g GCT in all pregnant women over 30 years of age and younger ones with risk factors. In another study, Corrado et al. evaluated the correspondence between the first-trimester FPG and 2-h, 75-g OGTT which were performed at 24–28 weeks gestation in

order to diagnose gestational diabetes [22]. They included 738 pregnant women in their study and they declared that even though there was no complete association in diagnosing GDM between the first-trimester FPG value and the parameters of a 2-h, 75-g OGTT, FPG ≥92 mg/dl could be accepted as highly predictive for GDM. In current study, the highest value of both sensitivity and specificity for predicting GMD was found as 83.5 mg/dl for FPG (75.0 and 77.5 %, respectively).

In this study, 2-h values of OGTT detected almost all GDM cases with a high sensitivity (94.5 %). On the other hand, 1-h values turned out to be identifying 87.6 % of GDM. This is the first study to reveal the importance of 2-h OGTT values. To the best of our knowledge no other study has demonstrated this significant relationship. The success of one of the OGTT parameters in pointing out the GDM may come in handy during clinical practice.

The primary limitation of this study is that it relied solely on retrospective data. A further potential limitation of our study is that the small number of patients may have been diagnosed with GDM. In conclusion, 2-h OGTT values appeared to identify the highest number of GDM than the other parameters. So clinicians must take into account this significant result and scrutinize what they have in their hands while assessing their patient's test results. The effects of each OGGT parameters and GCT values on predicting GDM need to be further studied.

Conflicts of interest None.

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ORIGINAL ARTICLE

Insulin lispro low mixture twice daily versus basal insulin glargine once daily and prandial insulin lispro once daily in patients with type 2 diabetes mellitus requiring insulin intensification—a randomized phase IV trial: Indian subpopulation analyses

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Abstract The aim of this study was to describe the efficacy and safety of two insulin intensification strategies in patients recruited in India with type 2 diabetes mellitus inadequately controlled on basal insulin glargine with metformin and/or pioglitazone. This multinational, open-label, randomized, parallel-arm, noninferiority, phase IV clinical trial evaluated insulin lispro low mixture (LM25) and basal insulin glargine administered with prandial insulin lispro (IGL) for 24 weeks. Patients were male and female, aged ≥18 to ≤75 years, with screening glycosylated hemoglobin (HbA_{1c}) concentration ≥7.5 to ≤10.5 % and fasting plasma glucose ≤121 mg/dL. The primary efficacy end point was change in HbA_{1c} from baseline to 24 weeks of treatment. Secondary efficacy end points included change in HbA_{1c} from baseline to 12 weeks and change in fasting blood glucose (FBG) from baseline to 12 and 24 weeks. Safety and tolerability were measured by treatment-emergent adverse events and the incidence, rate, and severity of hypoglycemic episodes. Of 81 patients randomized to LM25 (n=40) or IGL (n=41), 80 patients completed the trial and one patient discontinued due to subject decision. Mean (SD) change in HbA_{1c} from baseline to week 24 was −1.2 % (1.11) for the LM25 group and −1.0 % (1.18) for the IGL group. Safety profile, mean (FBG), glycemic variability, hypoglycemic episodes per patient-year, and health outcome measures were numerically similar between the two groups. The results of this post hoc analysis in an Indian subpopulation were consistent with results reported for the trial-level population and provide information to the consideration of LM25 as treatment option for intensification.

Keywords Analog · HbA_{1c} · Insulin · Insulin glargine · Insulin lispro · Premixed

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Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by progressive decline of insulin secretion by pancreatic β cells [1]. The International Diabetes Federation estimates that by 2035, the prevalence of diabetes will increase from 382 million to 592 million people, many of whom will reside in low and middle income countries and will be younger than 60 years [2]. The prevalence of diabetes is more than 65 million in India and is likely to reach 79.4 million by 2030 [2, 3]. T2DM is more predominant in India, where one of three patients with T2DM is overweight or obese [4]. The increase in prevalence in India is mainly due to urbanization, sedentary lifestyle, and rising prevalence of obesity [5].

Various guidelines have recommended different approaches for management of diabetes. The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) focused on patient-

centered management that includes providing respectful care, understanding individual patient needs and preferences, and responding accordingly. Current guidelines from ADA/EASD recommend patient education and changes in lifestyle, including diet and exercise, as the foundation of any T2DM treatment regimen. Metformin is usually the first-line drug unless contraindications exist. If glycosylated hemoglobin (HbA_{1c}) targets are not achieved or maintained after 3 months, two-drug combination therapy (i.e., metformin with a sulphonylurea, thiazolidinedione, dipeptidyl peptidase-4 inhibitor, glucagon-like peptide-1 receptor agonist, or basal insulin) is recommended. When two-drug combination therapy fails to achieve the desired HbA_{1c} target, then three-drug combination therapy (with or without basal insulin) is started. Ultimately, complex insulin therapy alone or in combination with other agents is recommended when HbA_{1c} remains uncontrolled [6–8].

Globally, basal insulin is often the starting insulin for patients with T2DM. When combination therapy with oral antihyperglycemic medication (OAM) and basal insulin fails to achieve adequate glycemic control, intensification of insulin therapy is recommended. Several recommended administration methods exist, including the gradual addition of prandial insulin to basal insulin therapy to minimize postprandial hyperglycemia caused by progressive β cell dysfunction [7]. Another strategy is to start premixed insulin (a fixed proportion of intermediate insulin with regular insulin or a rapid analog) administered traditionally twice daily [6].

Racial, cultural, and ethnic disparities are the key factors to consider when choosing antidiabetic therapy. Variation in glucose control in different ethnic populations is probably due to variations in dietary patterns, insulin resistance, glucose metabolism, etc. Guidelines from the Indian National Consensus Group on the use of insulin in patients with diabetes in India recommended premixed insulin as a safe, simple regimen that is easy to start and stay on [9].

Head-to-head data comparing insulin mixtures versus the addition of prandial insulin in patients inadequately controlled using basal-only insulin regimens are lacking. Therefore, this study described the efficacy and safety of two insulin intensification strategies in patients with inadequate glycemic control on once-daily basal insulin glargine plus metformin and/or pioglitazone. This manuscript describes the results of a post hoc analysis of the Indian subpopulation; the trial-level results have been described elsewhere [10].

Materials and methods

Design

This was a multinational, multicenter, open-label, randomized, parallel-arm, noninferiority (margin of 0.4 %), phase IV clinical trial. Patients were enrolled in 55 study centers in 11 countries (Argentina, Brazil, China, Egypt, India, Republic of Korea, Mexico, Romania, Russian Federation, Spain, and Turkey). In India, patients were enrolled in eight study centers.

Eligible patients were randomized in a 1:1 ratio to subcutaneous insulin lispro low mixture (LM25) twice daily or basal insulin glargine once daily, administered with prandial insulin lispro once daily (IGL) for 24 weeks and a stable dose of metformin and/or pioglitazone. LM25 was administered before breakfast and dinner (100 U/mL prefilled pens), basal insulin glargine at bedtime, and prandial insulin lispro before the largest meal of the day (100 U/mL prefilled pens). The largest meal of the day was defined as the meal with the highest 2-h postprandial blood glucose concentration recorded during the 2-week screening period using three separate 7-point self-monitoring of blood glucose (SMBG) profiles. The patient's last dose of insulin glargine during the screening period was considered the initial total dose of LM25 and split into two equal doses per day. Patients in the IGL group were initiated with insulin lispro 4 IU daily and continued on the same dose of insulin glargine they received during the screening period. Randomization was stratified by country and HbA_{1c} concentration at baseline (<8.5 or ≥8.5 %) [10]. All patients provided written informed consent, and the study was conducted in accordance with the ethical principles of the Declaration of Helsinki and is consistent with good clinical practices and applicable local laws and regulations.

Patients

Male and female patients aged ≥18 to ≤75 years with a diagnosis of T2DM based on history and clinical impression consistent with the World Health Organization's Classification of Diabetes [11] were recruited. At screening, patients were to have had HbA_{1c} concentrations ≥7.5 to ≤10.5 %, be taking stable doses of metformin and/or pioglitazone, and have received treatment with basal insulin glargine injected once daily for ≥90 days before the screening visit. Patients were also required to have a fasting plasma glucose (FPG) concentration ≤121 mg/dL, determined by the central laboratory, or >121 mg/dL if the investigator determined further titration of basal insulin glargine was not possible for safety reasons. Patients were excluded from the study if they had a screening body mass index >45 kg/m^2 or more than one episode of severe hypoglycemia within 24 weeks before screening [10].

Outcome measures and assessments

The primary efficacy end point was change in HbA_{1c} from baseline to 24 weeks of treatment. Secondary efficacy end points were change in HbA_{1c} from baseline to 12 weeks of treatment; change in fasting blood glucose (FBG) concentration from baseline to 12 and 24 weeks; 7-point SMBG profiles

at 12 and 24 weeks; glycemic variability (defined as the SD in 7-point SMBG profiles) at 12 and 24 weeks; daily total, basal, and prandial insulin doses at 12 and 24 weeks; and change in weight from baseline at 12 and 24 weeks.

Safety end points were measured by treatment-emergent adverse events (TEAEs); incidence, rate, and severity of hypoglycemic episodes (categorized as documented symptomatic [≤70 mg/dL], nocturnal, and severe); and vital signs. Patients insulin treatment satisfaction was assessed using the 22 grouped items on the Insulin Treatment Satisfaction Questionnaire (ITSQ). In addition, patients perceptions about the acceptability and effectiveness of their diabetes medications and perceived emotional and physical adverse events were assessed using the 21-item Perceptions About Medications-Diabetes 21 (PAM-D21) questionnaire, comprising four subscales (convenience/flexibility, perceived effectiveness, emotional effects, and physical effects) [10].

Statistical analysis

The intention-to-treat (ITT) population comprised all randomized patients who received at least one dose of study medication. The per-protocol (PP) population comprised all randomized patients except those who did not complete the study, received study drug different from their randomized study treatment, had any significant protocol violations, or were significantly noncompliant [10].

All measures were summarized using descriptive statistics including counts and percentages for categorical variables, and mean, SD, median, and interquartile range (IQR) for continuous variables. No treatment comparisons were performed because this was a subgroup analysis with a relatively small sample size. Noninferiority was not assessed in this Indian subpopulation.

Results

Patient disposition

A total of 143 patients were screened for study entry in India (Fig. 1), of which 81 patients were randomized to 24 weeks of treatment either with LM25 (n=40) or IGL (n=41). Eighty patients completed the study, and one patient discontinued from the IGL group due to subject decision.

Baseline characteristics

Patient demographics and baseline disease characteristics are shown in Table 1. At baseline, the mean (SD) age was 53.8 years (10.37) and mean (SD) HbA_{1c} levels were 8.6 % (0.73); 40 patients (49.4 %) had baseline HbA_{1c} <8.5 %. Overall, prior to baseline, 80 patients (98.8 %) were receiving concomitant metformin, 14 patients (17.3 %) were receiving concomitant pioglitazone, and 13 patients (16.0 %) were receiving both concomitant metformin and pioglitazone. A total of 51 (63.0 %) patients had a preexisting condition, most commonly vascular (37 patients [45.7 %]) and metabolic and nutritional (20 patients [24.7 %]) disorders, and 65 patients (80.2 %) were receiving concomitant medications for conditions other than diabetes.

Efficacy

For the primary end point, mean (SD) change in HbA_{1c} from baseline to week 24 was −1.2 % (1.11) for the LM25 group and −1.0 % (1.18) for the IGL group for the PP population. The corresponding mean (SD) change in HbA_{1c} for the ITT population was −1.2 % (1.11) for the LM25 group and −1.0 % (1.16) for the IGL group.

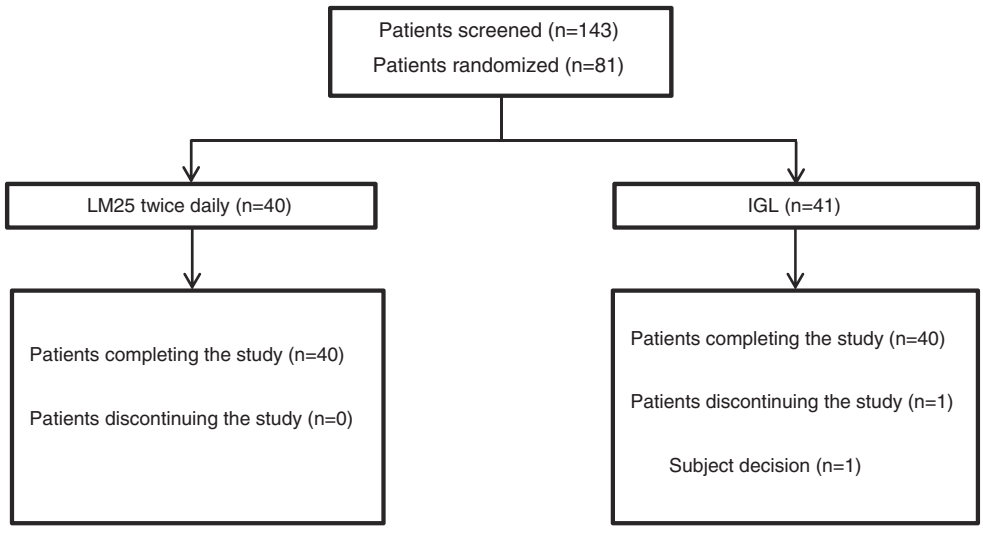

Fig. 1 Summary of patient disposition. *LM25* insulin lispro low mixture (insulin lispro protamine suspension 75 % and insulin lispro solution 25 %), *IGL* insulin glargine once-daily and prandial insulin lispro once-daily

Table 1 Baseline patient characteristics for the intention-to-treat population

Characteristic	LM25[a] (*N*=40)	IGL[b] (*N*=41)	Overall (*N*=81)
Male sex (*n* [%])	24 (60.0)	27 (65.9)	51 (63.0)
Age, years (mean [SD])	53.7 (10.94)	54.0 (9.92)	53.8 (10.37)
Weight, kg (mean [SD])	70.3 (13.00)	72.2 (12.16)	71.3 (12.54)
BMI, kg/m^2 (mean [SD])	26.3 (4.08)	26.9 (4.15)	26.6 (4.10)
Duration of diabetes, years (mean [SD])	10.2 (8.43)	7.7 (4.72)	8.9 (6.88)
HbA_{1c}, % (mean [SD])	8.5 (0.74)	8.6 (0.73)	8.6 (0.73)
HbA_{1c} <8.5 % (*n* [%])	20 (50.0)	20 (48.8)	40 (49.4)
Fasting blood glucose, mg/dL (mean [SD])	98.7 (13.08)	97.3 (13.08)	98.0 (13.0)
Insulin glargine dose at screening visit, IU (mean [SD])	21.2 (11.10)	21.9 (10.98)	21.5 (10.98)
Concomitant oral antihyperglycemic medication			
Metformin (*n* [%])	40 (100.0)	40 (97.6)	80 (98.8)
Daily dose, mg (mean [SD])	1797.6 (267.53)	1799.4 (278.19)	1798.5 (271.18)
Pioglitazone (*n* [%])	4 (10.0)	10 (24.4)	14 (17.3)
Daily dose, mg (mean [SD])	30.0 (0.00)	30.0 (0.00)	30.0 (0.00)
Metformin and pioglitazone (*n* [%])	4 (10.0)	9 (22.0)	13 (16.0)

BMI body mass index, *HbA$_{1c}$* glycosylated hemoglobin A_{1c}, *IGL* insulin glargine plus prandial insulin lispro, *LM25* insulin lispro low mixture

[a] Insulin lispro low mixture (insulin lispro protamine suspension 75 % and insulin lispro solution 25 %)

[b] Basal insulin glargine once-daily and prandial insulin lispro once-daily

The mean (SD) changes in HbA_{1c} from baseline to week 12 were −0.7 % (1.20) and −0.7 % (1.44) in the LM25 and IGL groups, respectively. The observed HbA_{1c} levels throughout the study are presented in Fig. 2a.

Median FBG concentration (IQR) at baseline was 101.80 mg/dL (91.89–107.21 mg/dL) for the LM25 group and 97.30 mg/dL (88.29–106.31 mg/dL) for the IGL group. Median changes in FBG (IQR) from baseline to week 12 were 17.12 mg/dL (3.60–36.94 mg/dL) for the LM25 group and 11.71 mg/dL (−6.31–36.04 mg/dL) for the IGL group. In addition, median changes in FBG (IQR) from baseline to week 24 were 13.51 mg/dL (−12.61–36.04 mg/dL) for the LM25 group and 21.62 mg/dL (−5.41–45.95 mg/dL) for the IGL group.

The mean unadjusted 7-point self-monitoring of blood glucose levels at baseline and 24 weeks are presented in Fig. 2b. The mean (SD) daily average 7-point SMBG profile values were 165.6 mg/dL (26.13) and 162.0 mg/dL (30.81) at baseline and 138.7 mg/dL (21.08) and 135.9 mg/dL (13.33) at week 24 for the LM25 and IGL groups, respectively. At week 24, the total mean (SD) daily doses were 46.9 IU (18.14) for the LM25 group and 41.8 IU (14.07) for the IGL group. Daily basal insulin doses for the LM25 and IGL groups at end point were 35.2 IU (13.60) and 27.4 IU (11.61), respectively. In addition, doses of daily prandial insulin at end point for the LM25 and IGL groups were 11.7 IU (4.53) and 14.5 IU (4.88), respectively.

Safety

At least one TEAE was reported by 20 patients (50.0 %) in the LM25 group and 15 patients (36.6 %) in the IGL group. Two patients (5.0 %) in the LM25 group and one patient (2.4 %) in the IGL group experienced events that were considered possibly related to the study treatments. Two serious TEAEs (myocardial infarction and hypoglycemia) were reported in two patients from the LM25 group. No patient discontinued because of adverse events or died during the study. Eight patients (20.0 %) in the LM25 group and 14 patients (34.1 %) in the IGL group experienced at least one episode of documented symptomatic hypoglycemia. Ten patients (25.0 %) in the LM25 group and 13 patients (31.7 %) in the IGL group experienced at least one episode of asymptomatic hypoglycemia. Six patients (15.0 %) in the LM25 group and five patients (12.2 %) in the IGL group experienced at least one episode of nocturnal hypoglycemia. One patient (2.5 %) in the LM25 group experienced severe hypoglycemia, and no patients reported severe hypoglycemia in the IGL group (Table 2). The mean (SD) change in body weight from baseline to week 24 was 0.0 kg (1.86) in the LM25 group and −0.2 kg (2.08) in the IGL group.

Health outcomes

Mean baseline and end point subscale scores on the ITSQ and PAM-D21 are summarized in Table 3. Total scores on the

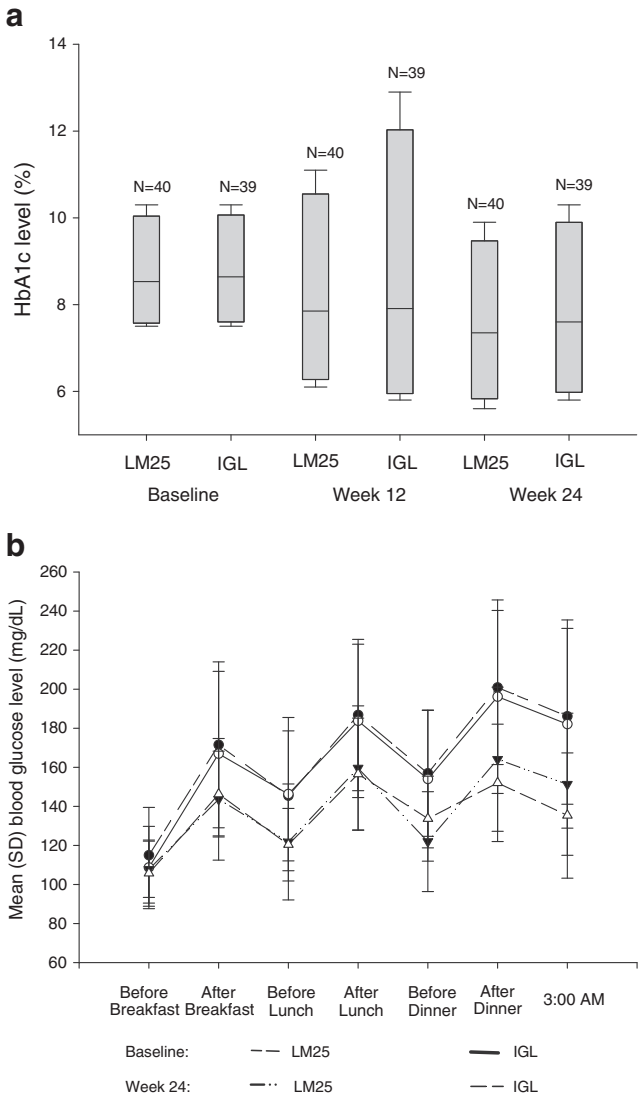

Fig. 2 Glycemic control throughout the study for the intention-to-treat population receiving insulin lispro low mixture (*LM25*; insulin lispro protamine suspension 75 % and insulin lispro solution 25 %) twice daily or basal insulin glargine once–daily and prandial insulin lispro once-daily (*IGL*). *HbA_{1c}* glycosylated hemoglobin A_{1c}. **a** Box plot of observed HbA_{1c} levels (per patient population). **b** Mean unadjusted 7-point self-monitoring of blood glucose levels at baseline and 24 weeks

ITSQ range from 0 to 100, where 100 indicates complete satisfaction with insulin treatment. Mean (SD) total scores on the ITSQ were 79.6 (12.67) in the LM25 group and 80.0 (14.14) in the IGL group at baseline, and 84.2 (10.55) in the LM25 group and 84.6 (11.53) in the IGL group at week 24. The mean (SD) change from baseline to week 24 was 4.5 (9.93) in the LM25 group and 4.3 (11.32) in the IGL group.

Subscale scores on the PAM-D21 range from 0 to 100, where higher scores indicate better perceptions about diabetes medications. There were no obvious differences in mean scores between treatment groups at baseline in the four subscales (convenience/flexibility, perceived effectiveness, emotional effects, and physical effects).

Discussion

When combination therapy with OAM and basal insulin fails to achieve adequate glycemic control, intensification of insulin therapy is recommended. Per guidelines, different approaches exist for intensifying insulin therapy in patients with T2DM, including adding a prandial insulin or switching patients from their basal insulin regimen to a premixed insulin regimen. In India, the preferable prescribing pattern of OAMs is either metformin alone or metformin in combination with glimepiride [12–14]. Both LM25 and IGL regimens are currently used in clinical practice in India. For patients with T2DM failing to reach glycemic targets on basal insulin, National Guidelines on Initiation and Intensification of Insulin Therapy recommend intensification with premixed insulin analogs (grade A, evidence level 2). Premixed insulin preparations are simple and convenient regimens that provide coverage for postprandial plasma glucose in addition to FBG with the same insulin resulting in effective glycemic control. Physicians strive to provide the best treatment to their patients by practicing evidence-based medicine. However, there is little head-to-head data comparing premixed insulin analogs with the addition of prandial insulin in patients inadequately controlled on a basal-only insulin plus OAM regimen. Data from studies involving the use of multiple doses of prandial insulin lispro have been reported [15, 16].

Single-arm or observational studies have shown improvement in glycemic control when treatment was intensified with a premixed insulin regimen from basal insulin regimen with or without OAMs. This improvement in glycemic control did not lead to risk of hypoglycemia or increased weight [17]. Similarly, open-label, randomized crossover studies have shown significant improvement in glycemic control when intensified with LM25 twice daily plus metformin compared to once-daily insulin glargine in combination with metformin [18]. These studies did not show consistency in the definition of failure to previous therapy. This study did not include a run-in period to optimize the insulin glargine dose as it appears that the patients recruited were on optimal doses of basal insulin, as shown by the FPG concentration ≤121 mg/dL. In Indian patients, fasting glucose was well controlled; however, postprandial glucose contributed to the high HbA_{1c} values. Therefore, the optimal strategy would be to add a prandial component rather than increase the basal dose. Despite the increase observed in FBG in the study, overall glycemic control improved with both strategies, all of which indicate that the prandial component was responsible for the improvement.

The data in the present study report on an Indian subgroup post hoc analysis from a recently completed multiregional clinical trial [10]. In this patient population recruited in

Table 2 Reported hypoglycemia with insulin lispro low mixture or basal insulin glargine in patients with type 2 diabetes mellitus

	LM25[a] (*N*=40)		IGL[b] (*N*=41)	
Hypoglycemia	Patients with ≥1 episode (*n* [%])	No. of episodes per patient-year (mean [SD])	Patients with ≥1 episode (*n* [%])	No. of episodes per patient-year (mean [SD])
Overall (≤70 mg/dL)	12 (30.0)	4.3 (10.13)	20 (48.8)	9.4 (22.02)
Documented symptomatic (≤70 mg/dL)	8 (20.0)	2.1 (5.97)	14 (34.1)	3.6 (9.68)
Asymptomatic (≤70 mg/dL)	10 (25.0)	2.0 (5.19)	13 (31.7)	5.6 (17.01)
Nocturnal	6 (15.0)	0.5 (1.49)	5 (12.2)	1.1 (5.05)
Severe	1 (2.5)	0.1 (0.34)	0.0 (0.0)	0.0 (0.0)

IGL insulin glargine plus prandial insulin lispro, *LM25* insulin lispro low mixture, *No.* number

[a] Insulin lispro low mixture (insulin lispro protamine suspension 75 % and insulin lispro solution 25 %)

[b] Basal insulin glargine once-daily and prandial insulin lispro once-daily

India, we observed mean reduction in HbA_{1c} in both groups. These results are consistent with the trial-level results, which showed a decrease in HbA_{1c} after 6 months in patients with T2DM treated with LM25 or IGL. The results from the Indian subpopulations also showed a decrease in mean changes in daily average blood glucose values from 7-point SMBG profile values and a decrease in mean changes in glycemic variability in both treatment groups at weeks 12 and 24, which were consistent with the trial-level results. Mean weight changes in the Indian subpopulation were not clinically relevant, and the study did not demonstrate any obvious differences in overall satisfaction with insulin treatment, although no statistical analysis was done in the Indian subgroup. The overall safety profile was similar between the two groups.

The overall trial-level results of the present study showed glycemic control with LM25 to be noninferior (margin of 0.4 %), and subsequently superior to glycemic control with IGL as measured by change in HbA_{1c} after 24 weeks of treatment. This study did not show any statistically significant differences in mean average daily glucose or glycemic variability. There were no significant treatment differences for the secondary efficacy variables evaluated such as proportion of patients achieving a target HbA_{1c} <7.0 or ≤6.5 % at 24 weeks, change in FPG concentration from baseline to 12 and 24 weeks, and insulin dose (total, basal, and prandial) at 12 and 24 weeks. The mean observed changes in body weight from baseline to week 24 were not clinically relevant [10].

Limitations of this clinical trial included the open-label trial design, dictated by the two regimens', use of insulin with different appearances, dosing requirements, and injection devices. Prandial insulin lispro was administered before the meal that had the highest 2-h postprandial blood glucose concentration and was given with the same meal throughout the study. Flexibility in dose scheduling was not allowed; however, we believe that this would not have affected study results, as the bolus was given with the largest daily meal, irrespective of the country, diet, or time of day. The study was not powered to assess potential differences between the two insulin regimens depending on the timing of the main meal. Therefore, any impact on glycemic profile, glycemic variability, or incidence of hypoglycemia may be further assessed in future studies. Another limitation was study duration, as longer term effects of two insulin regimens were not explored; however, the 24-week duration has been used in previous studies that examined the efficacy of first insulin intensification with premixed insulin or prandial insulin added to basal insulin [10, 15].

This post hoc subgroup analysis adds new information to the consideration of LM25 as a treatment option in Indian patients inadequately controlled with basal-only insulin plus

Table 3 Patient-reported outcomes on the Insulin Treatment Satisfaction Questionnaire and Perceptions About Medications-Diabetes 21

	LM25[a]			IGL[b]		
Patient-reported outcomes	Baseline	End point	Change from baseline to end point	Baseline	End point	Change from baseline to end point
Insulin Treatment Satisfaction Questionnaire, mean (SD)						
Inconvenience of regimen	80.3 (15.17)	84.7 (13.54)	4.3 (14.31)	83.2 (15.09)	88.9 (10.66)	5.8 (10.78)
Lifestyle flexibility	67.9 (27.14)	74.6 (22.54)	6.7 (25.08)	76.8 (16.85)	79.1 (17.38)	2.3 (21.27)
Glycemic control	82.7 (12.65)	83.9 (12.06)	1.2 (11.00)	76.4 (18.60)	82.2 (17.46)	5.8 (14.01)
Hypoglycemic control	79.9 (14.55)	86.1 (10.07)	6.2 (11.06)	80.6 (15.35)	84.8 (13.44)	4.2 (15.24)
Insulin delivery device satisfaction	83.2 (12.85)	87.1 (12.13)	3.9 (9.42)	81.4 (16.82)	84.8 (16.25)	3.0 (11.53)
Total score	79.6 (12.67)	84.2 (10.55)	4.5 (9.93)	80.0 (14.14)	84.6 (11.53)	4.3 (11.32)
Perceptions About Medications-Diabetes 21, mean (SD)						
Convenience/flexibility	75.6 (21.23)	83.6 (16.31)	8.1 (19.66)	78.1 (26.60)	86.2 (20.91)	8.1 (27.90)
Perceived effectiveness	71.9 (19.00)	75.0 (22.18)	3.1 (24.02)	73.4 (28.11)	77.0 (26.00)	3.5 (28.82)
Emotional effects	83.7 (18.97)	85.7 (16.78)	2.0 (15.34)	74.5 (23.47)	88.1 (15.31)	13.7 (25.99)
Physical effects	95.4 (6.75)	96.7 (6.17)	1.3 (4.83)	95.9 (5.96)	96.8 (7.11)	1.0 (8.97)

IGL insulin glargine plus prandial insulin lispro, *LM25* insulin lispro low mixture

[a] Insulin lispro low mixture (insulin lispro protamine suspension 75 % and insulin lispro solution 25 %)

[b] Basal insulin glargine once-daily and prandial insulin lispro once-daily

OAM regimens. Further studies may be required to interpret the best regimen for any specific population/country.

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Conflict of interest Simon Cleall and Shweta Uppal are employees of Eli Lilly and Company, and Steve Chen was an employee of Eli Lilly and Company during the writing of this manuscript. The authors report no conflicts of interest in this work.

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ORIGINAL ARTICLE

Association between single nucleotide polymorphisms (SNPs) in the gene of ADP-ribosylation factor-like 15 (*ARL15*) and type 2 diabetes (T2D) in Korean Chinese population in Yanbian, China

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Abstract This study aimed to investigate the association between single nucleotide polymorphisms (SNPs) in the gene of ADP-ribosylation factor-like 15 (*ARL15*) and morbidity of type 2 diabetes (T2D) in Yanbian Korean Chinese population. A total of 651 subjects including 327 subjects with T2D and 324 subjects with normal glucose tolerance (NGT) were involved. Genotypes and alleles of SNPs rs26770, rs4311394, and rs35941 in *ARL15* were analyzed. The results indicated: (1) frequency of genotype GG for SNP rs26770 in T2D group was higher than that in NGT group and showing as recessive model (P=0.045), (2) frequency of combined genotype AG-GG-CC of the three SNPs rs26770, rs4311394, and rs35941 in *ARL15* in T2D group was higher than that in NGT group (P=0.045), (3) Haplotype G A T for the three SNPs showed positive correlation with T2D development (P= 0.007). The following conclusion was drawn: for SNPs rs26770, rs4311394, and rs35941, genotype GG for rs26770, haplotype G A T, and joint genotype AG-GG-CC may be risk factors for T2D development in Korean Chinese population in Yanbian, China.

Zhengwei Cui and Tianxin Sheng contributed equally to this work.

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Keywords ADP-ribosylation factor-like 15 (*ARL15*) · Single nucleotide polymorphisms (SNPs) · Type 2 diabetes (T2D)

Introduction

Type 2 diabetes (T2D) is a complicated disease with relatively high genetic heterogeneity featured by increased plasma glucose [1, 2]. Advanced diabetics are usually suffered from one or more severe complications of cardiovascular diseases, ocular diseases, kidney diseases, etc. [3, 4]. Even though its pathogenesis is still not completely understood, but the insulin resistance and its relative scarcity are surely primary causes for hyperglycemia. In 2009, a study with genome-wide association studies (GWAS) on the single nucleotide polymorphisms (SNPs) which are located in the gene of ADP-ribosylation factor-like 15 (*ARL15*) by Richards et al. showed that the allele rs4311394G is highly related to hypoadiponectinemia and T2D with coronary artery disease (CAD) on Europeans [5] and in 2014, Li et al. found the same allele is associated with T2D development [6]. These previous studies manifest polymorphisms of *ARL15* may play important roles in T2D development and its complications.

Considering of ethnic specificity, for making sure of the association between *ARL15* and T2D, different races in different places are needed to be in research. The aim of this study was to investigate the association between three SNPs, rs26770, rs4311394, and rs35941, in *ARL15* and T2D in Korean Chinese population in Yanbian, China, and to ascertain that if *ARL15* is a susceptible gene for this population.

Yanbian is a Korean Autonomous Prefecture located in Jilin Province of the northeast of China. A population of 2, 209,646 lived there. Among them 1,341,175 (60.69%) are Han Chinese who largely emigrated from Shandong Province of the east of China, whereas 801,210 (36.26%)

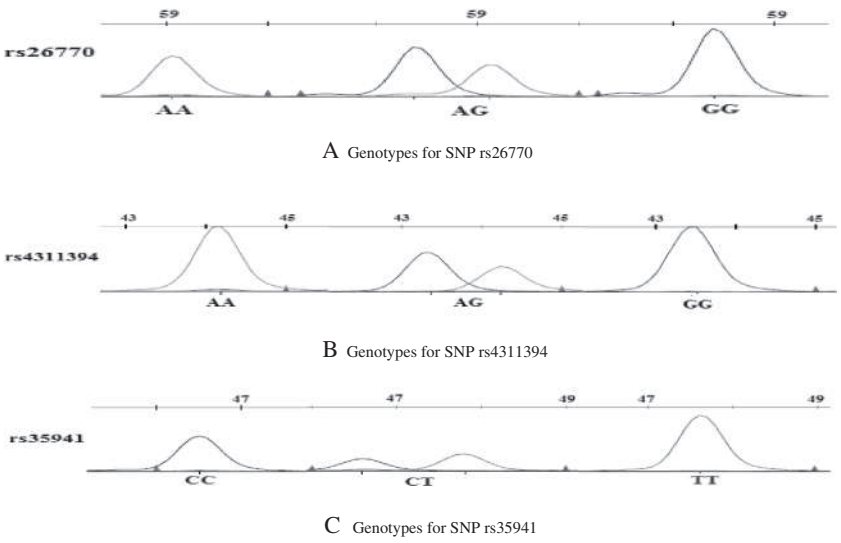

Fig. 1 Genotypes found at the three loci

A Genotypes for SNP rs26770

B Genotypes for SNP rs4311394

C Genotypes for SNP rs35941

are Korean Chinese whose ancestors came from North Korea, and the others of 67,261 (3.05%) are of other races (Fifth national population census of China, 2000).

Subjects and methods

Subjects

The 651 subjects involved in this study were ethnic Koreans with Chinese nationalities and aged 35-year-old or above who do not have genetic relationship with each other and were living in Yanbian for at least 10 years. All of them have signed Informed Consent Forms. These 651 subjects were divided into two groups: 327 subjects in T2D group (153 males and 174 females) and 324 subjects in normal glucose tolerance (NGT) group (144 males and 180 females). T2D patients were diagnosed based on the criteria suggested by the American Diabetes Association (ADA), whereas excluding other types of diabetes [7]. They were diagnosed within the recent 5 years and showing mild complications, e.g., numbness of foots and deterioration of vision. According to the doctor's advice, they injected premixed insulin routinely.

Clinical data measurements

Waist and hip circumferences, height, and weight were measured with CREAJOY flexible rule (Shanghai Creajoy, Inc., Shanghai, China) and RGZ-160 height and weight scale (Jiangsu Suhong Medical Equipments, Inc., Changzhou, Jiangsu, China). Triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and fasting plasma glucose (FPG) were measured on 7600 Clinical Analyzer (Hitachi, Ltd., Chiyoda, Tokyo, Japan) by Yanbian University Hospital (Yanji, Jilin, China). Plasma adiponectin (PA) and fasting plasma insulin (FPI) were tested with Human Adiponectin/Acrp30 Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN, USA) and Human Insulin ELISA Development Kit (PeproTech, Rocky Hill, NJ, USA) by Shanghai Westang Bio-Tech Co., Ltd. (Shanghai, China). Genomic DNA was extracted from whole blood with AxyPrep Blood Genomic DNA Miniprep Kit (Corning Inc., Tewksbury, MA, USA). Waist-to-hip ratio (WHR), body mass index (BMI), and homeostasis model assessment of insulin resistance (HOMA-IR) were calculated with the formulas WHR=waist/hip, BMI=weight/(height)^2, and HOMA-IR= FPG·FPI/22.5, respectively.

PCR amplification

Genotypes were screened with ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems, Inc., Foster City, CA,

Table 1 Clinical data in T2D and NGT groups

Item	T2D group (n=327)	NGT group (n=324)	P
Age (years)	62.12±6.70	60.50±9.33	0.130
WHR	0.88±0.10	0.87±0.06	0.243
BMI (kg/m^2)	24.54±3.26	23.91±3.79	0.080
FPG (mmol/L)	8.39±3.87	5.40±1.59	<0.001
TC (mmol/L)	4.97±1.03	4.85±0.91	0.534
TG (mmol/L)	2.34±1.65	2.13±1.62	0.374
HDL-C (mmol/L)	1.38±0.37	1.61±0.46	<0.001
LDL-C (mmol/L)	2.58±0.76	2.56±0.82	0.851
PA (ng/mL)	3.87±2.19	5.34±3.08	<0.001
FPI (ng/mL)	14.78±11.32	18.24±9.80	0.035
HOMA-IR	5.51±1.95	4.38±0.69	<0.001

Data are expressed as mean±SD. P values were calculated with t test

Table 2 Distribution of frequencies of alleles and genotypes for the SNPs in *ARL15* in T2D and NGT groups

rs26770	T2D group (n=327)	NGT group (n=324)	P	rs4311394	T2D group (n=327)	NGT group (n=324)	P	rs35941	T2D group (n=327)	NGT group (n=324)	P
Allele				Allele				Allele			
G	240 (0.367)	230 (0.355)	0.651	G	288 (0.440)	287 (0.443)	0.927	T	227 (0.347)	213 (0.329)	0.483
A	414 (0.633)	418 (0.645)		A	366 (0.560)	361 (0.557)		C	427 (0.653)	435 (0.671)	
Genotype				Genotype				Genotype			
GG	57 (0.174)	37 (0.114)	0.009	GG	72 (0.217)	60 (0.185)	0.208	TT	40 (0.122)	30 (0.093)	0.463
GA	126 (0.386)	155 (0.482)		GA	147 (0.447)	167 (0.516)		TC	147 (0.450)	152 (0.472)	
AA	144 (0.440)	132 (0.404)		AA	108 (0.336)	97 (0.299)		CC	140 (0.428)	142 (0.435)	
Dominant model				Dominant model				Dominant model			
GG+GA	183 (0.56)	193 (0.596)	0.394	GG+GA	219 (0.664)	227 (0.701)	0.396	TT+TC	187 (0.572)	182 (0.565)	0.794
AA	144 (0.44)	132 (0.404)		AA	108 (0.336)	97 (0.299)		CC	140 (0.428)	142 (0.435)	
Recessive model				Recessive model				Recessive model			
GG	57 (0.174)	37 (0.114)	0.045	GG	72 (0.217)	60 (0.185)	0.266	TT	40 (0.122)	30 (0.093)	0.220
GA+AA	270 (0.826)	287 (0886)		GA+AA	255 (0.783)	264 (0.815)		TC+CC	287 (0.878)	294 (0.907)	

Data are expressed as frequency and its percentage in parentheses. P values were calculated with χ^2 test

Table 3 Distribution of the joint genotypes for the three SNPs in *ARL15* in T2D and NGT groups

rs26770-rs4311394 -rs35941	T2D group (n=327)	NGT group (n=324)	P
AA-AA-CC	11 (0.034)	12 (0.037)	0.814
AA-AA-CT	14 (0.043)	13 (0.040)	0.863
AA-AA-TT	7 (0.021)	3 (0.009)	0.347
AA-AG-CC	29 (0.089)	28 (0.086)	0.919
AA-AG-CT	25 (0.076)	33 (0.102)	0.255
AA-AG-TT	12 (0.037)	6 (0.019)	0.157
AA-GG-CC	21 (0.064)	14 (0.043)	0.235
AA-GG-CT	20 (0.061)	17 (0.052)	0.632
AA-GG-TT	5 (0.015)	6 (0.019)	0.749
AG-AA-CC	15 (0.046)	27 (0.083)	0.052
AG-AA-CT	21 (0.06)	18 (0.056)	0.641
AG-AA-TT	5 (0.015)	4 (0.012)	1.000
AG-AG-CC	30 (0.092)	41 (0.127)	0.154
AG-AG-CT	29 (0.089)	40 (0.123)	0.150
AG-AG-TT	5 (0.015)	7 (0.022)	0.549
AG-GG-CC	12 (0.037)	4 (0.012)	0.045
AG-GG-CT	9 (0.028)	11 (0.034)	0.635
AG-GG-TT	0 (0.000)	3 (0.009)	0.244
GG-AA-CC	16 (0.049)	10 (0.031)	0.239
GG-AA-CT	15 (0.046)	10 (0.031)	0.319
GG-AA-TT	4 (0.012)	0 (0.000)	0.315
GG-AG-CC	6 (0.018)	3 (0.009)	0.511
GG-AG-CT	9 (0.028)	9 (0.028)	0.984
GG-AG-TT	2 (0.006)	0 (0.000)	0.483
GG-GG-CC	0 (0.000)	3 (0.009)	0.244
GG-GG-CT	5 (0.015)	1 (0.003)	0.223
GG-GG-TT	0 (0.000)	1 (0.003)	0.498

Data are expressed as frequency and its percentage in parentheses. P values were calculated with χ^2 test

USA). Primers and probes were designed based on the sequence of *ARL15* (GenBank NC_000005.9).

SNPs screening

DNA sequencing was performed on ABI PRISM 3730XL DNA Analyzer (Applied Biosystems, Inc., Foster City, CA, USA) by Shanghai Invitrogen Biotechnology Co., Ltd. (Shanghai, China).

Statistical analysis

SHEsis (http://202.120.7.14/analysis/) was used for calculating frequencies of alleles and genotypes. Mean, standard deviation (SD), t test, and χ^2 test were calculated with SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium was determined with Hardy-Weinberg

Table 4 Distribution of the frequencies of haplotypes for the three SNPs in *ARL15* in T2D and NGT groups

Haplotype	T2D group (n=327)	NGT group (n=324)	χ^2	P	OR (95 % CI)
A A C	105.76 (0.162)	131.43 (0.203)	3.694	0.055	0.758 [0.571–1.006]
A A T	79.57 (0.122)	61.32 (0.095)	2.465	0.116	1.325 [0.932–1.885]
A G C	159.45 (0.244)	138.29 (0.213)	1.706	0.192	1.188 [0.917–1.540]
A G T	69.22 (0.106)	86.96 (0.134)	2.479	0.115	0.764 [0.546–1.069]
G A C	121.37 (0.186)	134.59 (0.208)	1.008	0.315	0.869 [0.661–1.143]
G A T	59.30 (0.091)	33.66 (0.052)	7.366	0.007	1.820 [1.175–2.820]
G G C	40.42 (0.062)	30.69 (0.047)	1.315	0.251	1.325 [0.818–2.147]
G G T	18.91 (0.029)	31.06 (0.048)	3.192	0.074	0.591 [0.330–1.059]

Data are expressed as frequency and its percentage in parentheses. P values were calculated with χ^2 test

equilibrium calculator (http://www.oege.org/software/hardy-weinberg.html) [8]. Linkage disequilibrium was determined with CubeX (http://www.oege.org/software/cubex/) [9]. P<0.05 was set as the statistically significant threshold.

Results

Sequencing

Nine genotypes were found for three SNPs: AA, AG, and GG for rs26770; AA, AG, and GG for rs4311394; CC, CT, and TT for rs35941 (Fig. 1).

Clinical data in groups

Clinical data in T2D and NGT groups were shown in Table 1. In T2D group, in addition to FPG, TG, and HOMA-IR were higher than that in NGT group (P<0.001); HDL-C and PA were lower than that in NGT group (P<0.001).

Hardy-Weinberg equilibrium and linkage disequilibrium

The distributions of genotypes of the three SNPs were determined in Hardy-Weinberg equilibrium (Ps>0.05). For linkage disequilibrium, the |D'|s are equal to 1.0, so the loci can be considered in completely linkage disequilibrium.

Correlation between the SNPs and T2D

The distribution of frequencies of alleles and genotypes for the three SNPs in *ARL15* in T2D and NGT groups was showed in Table 2. Genotype GG for the SNP rs26770 manifested the trend of recessive inheritance responsible for the development of T2D (P=0.045). So, a subject with genotype GG for SNP rs26770 may have a higher chance of suffering from T2D (OR=1.637).

Correlation between joint genotypes of the three SNPs and T2D

The distribution of the joint genotypes of the three SNPs in *ARL15* in T2D and NGT groups was showed in Table 3. The frequency of the joint genotype AG-GG-CC was higher in T2D group than that in NGT group (P=0.045).

Correlation between haplotypes for the SNPs and T2D

The distribution of the frequencies of haplotypes for the three SNPs in *ARL15* in T2D and NGT groups was showed in Table 4. The frequency of haplotype G A T is higher in T2D group than that in NGT group (P=0.007).

Conclusion

Summarizing the results above, the following conclusion can be drawn: for SNPs rs26770, rs4311394, and rs35941, genotype GG for rs26770, haplotype G A T, and joint genotype AG-GG-CC may be risk factors for T2D development in Korean Chinese population in Yanbian, China.

Discussion

ARL15 is a member of ARLs which belong to the family of ADP-ribosylation factors (ARFs) of Ras superfamily [10]. Even though the function of ARL15 is still unknown, however based on the sequence of the protein, its structure may resemble that of Ras-related GTP-binding proteins (RGPs) which play an important role in intercellular transportation, especially in insulin signal transmission and glucose-stimulating insulin secretion (GSIS) [11]. ARL15 is widely expressed in insulin-sensitive tissues such as fat, liver, and especially in skeletal muscles, which are the primary insulin-mediated and glucose-storing sites [12].

Similarly with previous researches, we found HDL-C and PA in type 2 diabetics were lower than that in normal individuals [13–16]. This study also showed that in Yanbian Korean Chinese population, a subject with genotype GG for SNP rs26770 in *ARL15* would manifest higher risk for T2D development with conspicuous traits of recessive inheritance. But we did not find the polymorphisms of alleles on rs4311394 are related to T2D development as do in Richards et al.'s research on Europeans and Li et al.'s research on Yunnan Han Chinese population [5, 6]. The difference may be due to ethnic specificity. A risk haplotype G A T and a risk joint genotype AG-GG-CC for SNPs rs26770, rs4311394, and rs35941 were found to be related to the development of T2D in Yanbian Korean Chinese population, and so far, we did not find similar studies on the risk factors in other populations.

Even though the study pointed to the risk factors for T2D development in Yanbian Korean Chinese population, but the reason for their different effects in different races and the mechanism of *ARL15* in T2D development are still unclear. Therefore, we are trying to conduct further studies on this gene and hoping to unravel the mysteries.

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Conflict of interest There are no conflicts of interest.

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ORIGINAL ARTICLE

Diabetes foot complication: assessing primary and secondary outcomes of multidisciplinary team versus standard care (a systematic review)

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Abstract About 15 % of all diabetes patients experience foot complication at some stage in their life. The goal of this review is to systematically assess on effectiveness of multidisciplinary teamwork compared to the standard care in risk reduction of diabetes-related foot complications with a primary and secondary outcome. Literatures of only English language were analyzed under strict inclusion criteria from electronic databases search. Result from overall pooled estimate up to 0.65 % reduction, with 95 % CI ($p<0.005$) in foot ulceration and amputation using a multidisciplinary team care as a tool compared to the standard care in primary outcome. Evidence also supports program benefits in overall cost (0.6 % reduction, $p<0.005$), rate of hospitalization (80 % dropped, $p<0.003$), and patient quality of life as secondary outcomes. Study's characteristic differed substantially in term of health care setting, nature of interventions, and outcomes measured reported. Evidently, multidisciplinary team efforts from specialists in diabetes, vascular and infectious disease, along with podiatry expertise and patient educators result in a significant reduction in diabetes-related foot complications compared to the standard care.

Keywords Diabetic foot complication · Ulceration · Amputation · Multidisciplinary team · Hospitalization

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Background and aim of the review

According to World Diabetes Foundation [1], it is estimated that approximately 285 million people have diabetes (6.4 %) of the world's population and around 80 % of these people live in developing countries. Foot problem is among the most serious and costly complication of diabetes that can not only lead to individual disability but also placed an immense cost on the healthcare system [2]. Diabetes-related foot ulceration accounts for 15 % of total diabetes patients [3]. Each year, approximately one million amputations were performed on people with diabetic foot complication [3, 4], meaning every 30 s a lower limb is lost to diabetes somewhere in the world [5]. Further epidemiological reports add up to suggest more than 1 million diabetic foot disease is a progressive condition and the majority of the people affected are in the under developed countries [6, 7]. Up to 70 % of all leg amputations are performed on people with diabetes, and this population has a 25 times greater chance of losing another leg than people without having the condition [4].

The percentage of population of diabetic foot in developing countries is rapidly outpacing than that in the developed countries [8]. In majority of such low socioeconomic countries, diabetes foot care remains a low priority; foot complications do not receive the same level of attention as other diabetes complications [9]. As a result, for example, in India, almost 40,000 legs are amputated every year as a consequence of diabetes [10].

Evidence suggested that up to 85 % of amputation can be prevented, and despite the fact that preventive interventions are often applied in the clinical practices, there is very little scientific evidence demonstrating the effectiveness for such interventions [2]. Existing literatures are mainly focused on the therapeutic management [10–13] but lack on holistic team approach in patient foot care. Based on the available literature

search for interventions, a comprehensive systematic review is therefore needed to assess on the current widely recognized multidisciplinary team approach in preventing foot ulceration or subsequent risk reduction in amputation as a primary outcome compared to the standard care. The review also compared on the cost-effectiveness, hospitalization, and patient overall quality of life as secondary outcomes from this standardized care. Thus, the overall aim from this review is to assess whether multidisciplinary team approach can reduce foot complication and cost, and improve patient quality of life compared to the standard. Also, this study aims to assess whether this approach is suitable for many different cultural and low socioeconomic countries. The outcomes were measured systematically from each study using standardized criteria.

Methodology

A recommended model (*Systematic Review; University of York, 2009*) [14] guidance was adopted for conducting this comprehensive review. The review was originally conducted between 2011 and 2012, and with recent updates, the authors added another set of evidence to support on the final findings from literature published between July 2012 and February 2015. All the relevant information were accessed and crosschecked with literature search from PubMed, The Cochrane Library, Ovid MIDLINE–Ovid EMBASE, EBSCO, and CINAHL. Reports and articles were also accessed electronically from organizations, i.e., International Working Group on Diabetic Foot (IWGDF), International Diabetes Federation (IDF), and World Diabetes Federation (WDF). The following search index terms were used: "diabetic foot," "risk of amputation," "foot ulcer," "prevalence," "multidisciplinary team," "patient health and socioeconomic impact." Further bibliographies of all relevant and retrieved publications were identified through this process. Findings from each study were aggregated to produce a "bottom line" on the clinical effectiveness, feasibility, appropriateness, and meaningfulness of the intervention for risk reduction in diabetes-related foot complication.

Inclusion/exclusion criteria

Patients with risk of foot ulceration or subsequent amputation of age 25 years or above, both male and female gender, and diagnosed with type 1 or type 2 diabetes mellitus are included in the study. Individual article/journal published from July 2000 to February 2015 highlighting either component of primary or secondary outcomes were included for this review. Mixed observational and clinical trial studies of English language or translated were included. Full-text versions were analyzed for any duplication and quality, against the modified version [14, 15] (Table 1). Studies were classified into themes according to their relevance to the review objectives. The quality assessment was done independently by the reviewers and were strictly based on the study design, setting, duration, participants with type of intervention and outcome measures, allocation concealment, baseline comparability of treatment groups for important variables, use of intention to treat analysis, extent of loss to follow-up, and blinded outcome assessment. Any case series, report, or control studies (except where nested as part of a cohort studies) and economic evaluation were excluded.

Quality assessment and data analysis

A rigorous review of literature also allowed allocation of studies that were particularly relevant to the review and eliminated studies that were judged unfit for its purpose using the standardized methodological and qualitative narrative analysis in order to determine a minimum threshold for selection to ensure studies validity and reliability. Quality assessment was carefully done based on the strict inclusion criteria, and the results were used for descriptive purposes to provide an overall evaluation of the included studies.

Methods of synthesizing the studies (Fig. 1) were measured for quality, design outcome measure, and heterogeneity of the selected studies. The heterogeneity explored the strategies for intervention such as:

(i) Health care setting (e.g., podiatry clinics versus general hospitals versus general practice)
(ii) Type of intervention (e.g., content of complex intervention; brief versus intensive programs; education on foot care only versus more comprehensive diabetes care);
(iii) Nature of contrast (e.g., intervention versus control intervention; intervention versus no intervention)
(iv) Incidence of new ulcer, amputation, or previously healed ulcer
(v) Patients quality of life from ulceration/amputation, cost, hospitalization, and socioeconomic impact

Types of outcomes measured

Primary outcomes

Incidence and risk reduction in foot ulceration and subsequent amputation using a multidisciplinary team approach over standard care were measured.

Table 1 A modified approach for review quality assessment and study selection

Abstract/Introduction	Were the abstract/introduction clearly translate the article details and matched the aspiration?
Background	Did the literature review offer a substantive background?
Aim/objective	Are the aims and objectives of the study/paper clearly stated?
Research question/ hypothesis	Are the research question and the hypothesis clearly specified?
Study design/ Methodology	Were the study design clearly based to the outcomes of review in an experimental design were dependent/independent variables clearly stated?
Sample selection	Were sufficient and appropriate participant selected? How much information is there in relation to the sample, ethic standard and consent obtained, size, number dropout and why?
Instrument/tools	What instrument/tools were they appropriate to the review findings? Would this be the norm for the research approach?
Result/finding	Were sufficient and appropriate finding presented based on the review? Was pilot study conducted and are the results explored/ explained?
Data analysis	Was the statistical analysis correct, explained, the data presented to the reader in a friendly way?

Secondary outcomes

Patients' quality of life, cost-effectiveness, rate of hospital admissions, foot care knowledge, and patients' behavior were used as secondary outcomes. Studies were included even if only secondary outcomes were reported to the standard measured in the multidisciplinary team care.

Risk of publication bias

Cochrane publication bias assessment tool was used for individual study selection, performance, detection, attrition, and reporting biases [15]. A checklist table was created and studies were then categorized in low-, moderate-, and high-risk groups. Bias in the final included studies however did not compromise the final outcomes of the review. The information reported in all studies were clearly stated and evidently presented and were found sufficient enough to assess on the main outcomes for interventions.

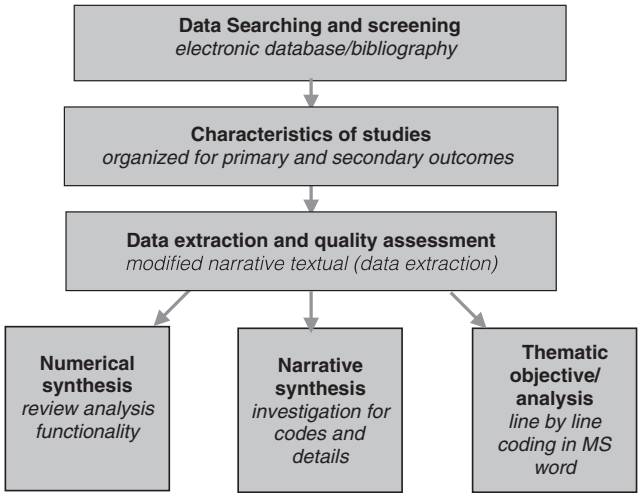

Fig. 1 Data synthesis

Result of review

Total of 1745 articles from about 100 different journals were retrieved. After careful review of title/abstract, 82 % (n=1440) were excluded and 29 % (n=305) did not meet the review inclusion criteria. Another 27 % (n=85) failed on quality assessment leaving 10 % (n=9) of studies for final review (Fig. 2). After an independent assessment by the reviewers, studies were matched to the inclusion, with disagreements being resolved by discussion. Details of the study population, settings, intervention, and outcomes are presented in (Table 2). Twenty-two percent (n=2) of the observational studies (one was prospective cohort design and one was a descriptive retrospective observational analysis) compared multidisciplinary team approach with standard care. Twenty-two percent (n=2) of the studies were retrospective quality analysis based on the model strategies outcomes and one cross-sectional analysis with secondary outcomes on the cost and rate of hospitalization through stage-wise management program. Thirty-three percent (n=3) were RCT including 11 % (n=1) observational blind randomized control trial and 22 % (n=2) non-blind comparison groups with one in control hand and one interventional group and 11 % (n=1) non-randomized retrospective study that compared educational tools as primary outcomes. Eleven percent (n=1) in case–control study evaluated on the effectiveness of risk classification using International Working Group on Diabetes Foot risk classification system.

Literature interpretation and findings

Primary outcomes

Outcomes measured from the included studies are also summarized in Table 2.

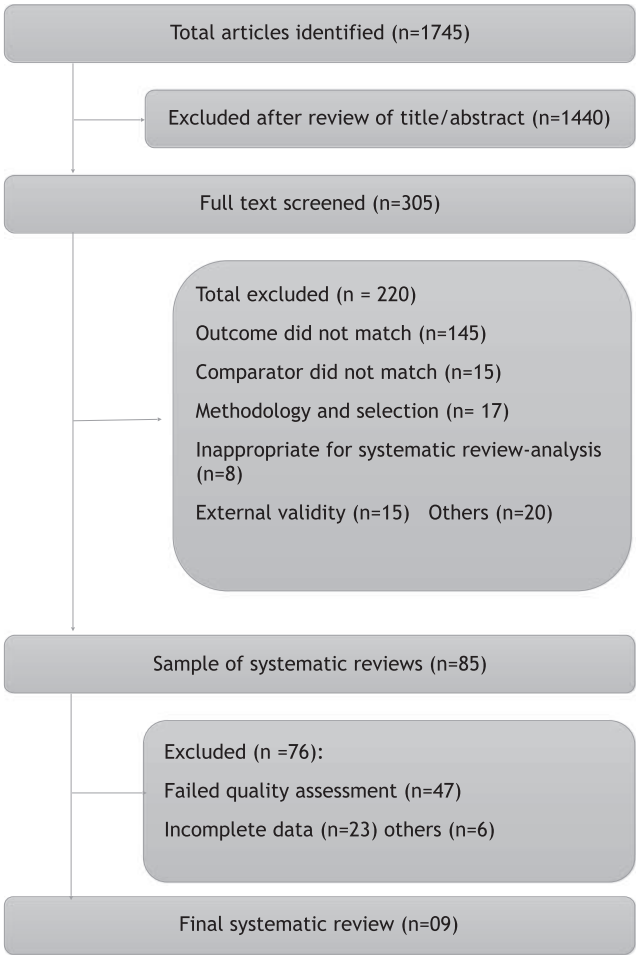

Fig. 2 Review result chart flow

Prevention of foot ulceration and amputation

A number of studies in the inclusion criteria show greater outcomes in incidence and risk reduction of foot ulceration and amputation by using multidisciplinary team approach in a variety of fashion when compared to the standard care. Many of such hospital-based long perspective studies have shown a greater success through coordination with input from specialists in areas including diabetes, orthopedists, plastic and vascular surgery, infectious disease specialist, rehabilitative specialist, diabetic educator, wound care nurses, and foot wear technician in the prevention and management of diabetic foot risk of ulceration and major amputation [7, 16, 17]. Specialists were trained on regular basis with protocols on foot care for patients that at risk of infection or ulceration or have already been affected with wound. This approach significantly show a reduction in incidence of both major and minor amputation rate at 70 and 60 % (4.1 vs. 13.6 %, p=0. 03) comparing with standard protocol [16]. With exception of one long prospective study from Turkey [17], a significant decrease only in major amputation by about 40 % (20.4 vs. 12.6 %, p=0.026) was observed, and the result for overall

Table 2 Tabular summarization of individual study outcomes

Study ID	Primary outcome	Secondary outcome
Ronald et al. (2003)	No primary outcomes reported	Hospitalization(0.09 admission/person vs. 0.50, p=0.0002), lower foot-related inpatients days (0.91 day per person vs. 3.97, p=0.0289), lower foot-inpatient charges ($1321 per person vs. 5411, p=0.0151); fewer amputation hospitalization (0.04 per person vs. 0.19, p=0.0351), fewer emergency visit (0.06 vs. 1.22 visits, p=0.0043), lower emergency charges (104$ vs. 208, p=0.0057), and lower total charges ($4776/ person vs. $9402, p=0.0141)
Boutoille et al. (2008)	No primary outcomes reported	The ulceration group had more physical limitation and pain than the amputees group. Median score was quite low in both groups, reflecting strong social and psychological consequences of diabetic foot. Global prognosis was bad with 56 % of amputees had experienced another major cardiovascular event 20 months following amputation. The later complication reported by the author in this study finding however have not risk outcome to the review primary or secondary outcomes
Lincoln et al. (2008)	No primary outcomes reported	Education for secondary prevention was significant (0.03) in 12 months follow-up patients group but had no significant difference (p>0.05) observed in the intervention group over 6 months
Schmidt et al. (2008)	No primary outcomes reported	Patient who participated three times or more in educational program performed significantly better in self-care

Table 2 (continued)

Study ID	Primary outcome	Secondary outcome
		activities than with single visits or untrained patient on foot care education
Abbas et al. (2011)	Between period of 2004 to 2007, 11,714 patients screened, of which 37 % with high-risk feet, 11 % with ulcer, 9.8 % went under major amputation. Total fall of incidence from 24 in 2005 to 8 % in 2008	No secondary outcomes reported
Anichini et al. (2003)	Overall incidence of foot amputation decreased in 5 years period from 10.7 in 1999 to 6.24 in 2003) and incidence of major amputation decrease from 6.3 in 1999 to 3.1 in 2003	Hospital admission reduced by median of 95 % CI, from 19.5 days in 1999 to 5.5 days in the following years until 2003, $p<0.05$, that decrease the financial burden on the society
Peters and Lavery (2001)	During 3 years follow-up, ulceration occurred in 5.1, 14.3, 18.8, and 55.8 % of the patient in groups 0, 1, 2, and 3 ($p<0.001$). All amputation were found in groups 2 and 3 (3.1 and 20.9 %, $p<0.001$)	No secondary outcomes reported
Rerkasem et al. (2008), Rerkasem et al. (2009)	Incidence of both major and minor amputation rates were significantly decrease by 70 and 60 % (4.1 vs. 13.6 %, $p=0.03$) No primary outcome reported	No secondary outcomes reported 2 years follow-up on cost and quality of life using DFP and (SF-36) in a multidisciplinary setting shows about 34 % reduction in cost compared to the standard care in retrospective 2-year analysis. Also the program shows significant improvement in quality of life
Yesil et al. (2009)	Major amputation decreased (20.4 vs. 12.6 %, $p=0.026$). However overall amputation and minor amputation remained to have no change	No secondary outcomes reported

amputation and minor amputation rate remained similar throughout.

As impacts of the disease severity were more prevalent in less-developed countries, a model study was proposed with the Step-by-Step foot project in Tanzania with support from IWGDG and IWDF which has shown an outstanding achievement in risk reduction considering to the needs of patients and the country's socio-economic environment [18]. A total of 11,714 patients were screened from a large-scale population in this program. Of this, 37 % were found as high-risk feet, 11 % were diagnosed with ulcer, and 9.8 % had major amputation. They then open a number of acute care units and trained health care providers from a multidisciplinary unit using standard protocols [2, 3]. The amputation rate in high-risk population had significantly dropped (from 20 % in 2005 to almost 9 % in 2008). A vast recognition and success of this approach were also adopted in many other less-developed countries such as Egypt, India, and Pakistan.

By using diabetic foot risk classification system based on IWGDF (2009) in a case control group [19] at University Texas Healthcare Center, USA, a total of 225 patients were divided into groups (group 0 without neuropathy, group 1 with neuropathy but no ulceration or peripheral vascular disease (PVD), group 2 with neuropathy and deformity or PVD, and group 3 patients have either history of foot ulceration or a lower extremity amputation). With 3 years of follow-up, foot ulceration occurred in 5.1, 14.3, 18.8, and 55.8 % of the patients in groups 0, 1, 2, and 3 (linear-by-linear association, $p<0.001$). Amputation rate in groups 2 and 3 was 3.1 and 20.9 %, respectively, ($p<0.001$). This assessment not only helps in the prediction and severity of ulceration and amputation among diabetes patients but also it can be used as part of the assessments tools in a multidisciplinary setting to lower the risk of future foot ulceration and subsequent amputation.

Another similar approach by using an International Consensus on Diabetic Foot ICDF (2011) as an instrument in a prospective follow-up over a 5-year period (1999–2003) in a district general hospitals covering a clearly defined and relatively static population shows a significant drop in risk of foot ulceration along with rate of hospitalizations [20]. The incidence of amputation per 100,000 inhabitants decreased from 10.7 in 1999 to 6.24 in 2003. The protocol followed the standard patients' referral to particular diabetic foot clinics with involvement of GPs at initial assessment followed by multidisciplinary team specialists.

Secondary outcomes

Outcomes were measured as an assessment tools that were either measured in part of multidisciplinary team care approach or assessed as an individual tools in secondary prevention of foot ulceration and amputation that met the inclusion criteria.

Assessment on foot care knowledge and patient behavior

Patient behavior assessment and education toward foot care are considered important parts of the multidisciplinary team care. Such assessments were carried in a variety of fashion. In one randomized trial study [21] with multi-centers observer-blinded groups (intervention (n=87) and control (n=85)), patients received either targeted, one to one education or standard care in three specialist clinics in UK. The assessment for patient education on diabetic foot care was based on approved standard care and summarized questionnaires using Nottingham Assessment of Functional Footcare that were followed at 6 and 12 months. This method has shown a positive result in foot care especially at 12 months follow-up group (p=0.03) but did not observe a significant differences (p>0.05) between groups in ulcer incidence at 6 months group patients. On the other hand, in a cross-sectional study, a total of 269 patients with type 1 and type 2 diabetes were divided in different risk groups from a general population in Germany, and data was obtained on patients' self-reported survey [22]. It uses a Frankfurter Catalogue of Self-Foot Care Prevention of the Diabetic Foot Syndrome (FCFSP) [22]. Patients who had participated in more than three educational programs performed better self-care than patients who had not or had received single training program. Also, patient performed better self-care with professional assistance than being untrained on self-foot care.

Hospitalization and cost-effectiveness

Cost is an important factor to measure when proposing an intervention for low socioeconomic population. This review also targeted studies that have shown benefits in cost and hospitalization reduction as an individual or suggested part of multidisciplinary team outcomes in foot care. With one of included non-randomized retrospective study using data from 1998 to 1999 at Louisiana Public Hospital, it showed significant decrease in rate of hospitalization and cost using consistent step wise care protocols in diabetic foot program compare to standard care (0.50 admission/person vs 0.09 p=0. 0002 and $4776 vs. $9402 p=0.0141 per person per year) [23]. This staged-management program uses devices of offload pressure, self-care education, and after healing custom-fabricated orthoses, proper footwear and monitoring progressive ambulation though an organize care in a multidisciplinary level. This program also significantly reduced emergency visits and hospital utilization.

Patients' quality of life

Better understanding of disease severity and consequences secondary to diabetic foot complication is an important part of treatment in high risk population and as well as among primary care physicians. In one of the review, quality of life was assessed by using medical outcome form 36 items health survey (MOS SF-36) [24]. Patients who were experiencing foot ulcer had more physical limitations and pain than the amputation group, whereas median score was quite low in both groups, reflecting in terms of social and psychological consequences. Another 2-year prospective study shows that diabetic foot care under multidisciplinary team care can save not only on the cost but also improve patient overall quality of life when it was compared to the standard care in a retrospective study of similar duration [25]. Psychological evaluation and support is therefore important before and after amputation since it is a traumatic step for patients.

Discussion and data interpretation with updates on final findings

The overall outcomes were measured from literature published between 2001 and 2015. The selection criteria and quality assessment of the studies were consistently assessed for originality, type of interventions, and final outcomes. The review aimed to establish effectiveness of multidisciplinary team care with a primary outcome of risk reduction in foot ulceration and amputation. The review also focused on patient quality of life and cost analysis as secondary outcomes. The result of this review was presented in a study-by-study qualitative analysis. Pooling of the result was precluded by marked clinical heterogeneity, due to type of participants, methodological approach, duration of studies and follow-up period, types of intervention, outcome measures, and assessment and risk of bias that varies widely between studies.

Overall consensus from studies in this review shows multidisciplinary team approach in diabetic foot care considered to be more effective in preventing foot complication and long-term health disabilities when compared to the standard care [16–20]. In addition to review original findings, a set of recent published studies (between 2011 & 2015) were also added to the pool. The evidence from these studies further supported multidisciplinary team approach in reducing the risk and incidence of diabetes-related foot complications when the outcomes were compared to the standard care [26–31]. Forexample included two recent high-outcome studies from Spain and Germany in particular showed a stunning result in reducing overall foot complication by >66 % (p>0.001) and of >75 % (p<0.0001), respectively, with a high mortality/morbidity benefits after the introduction of a structure healthcare program for diabetic foot management [27, 28]. This multidisciplinary setup is organized in variety of fashions: some are managed within hospital care system with inputs from specialists care, others are organised as an emergency or outpatient clinics. Few of such countries, e.g., Germany and Italy, have also adopted the structural approach

of the IWGDF guidelines and showed likewise positive outcomes [26, 27, 32, 33]. The high-outcome results were quite encouraging for some that they now helping others nations on how such program can best be organized and implemented in diabetic foot care [32].

The team expertise and contribution come from various specialties including specialist from endocrinology, family physician, rehabilitative physician, plastic and vascular surgeon, orthopedist, infectious disease specialist, radiologist, and qualified trained nurse who work in coordination toward comprehensive foot care [26, 27, 33]. With wide recognition and establishment of this multidisciplinary teamwork, some healthcare settings have now strictly institutionalized this approach toward diabetes foot care. The setup however varies in many parts of the world in terms of care provided from place to place and from country to country [34]. It is therefore very important to measure the outcomes based on the patients' needs and healthcare system especially when considering the applications of such protocols in less privileged countries.

The underlying causes of risk increment were found to be high in underdeveloped countries compared to that in developed nations perhaps due to number of social, cultural, and political reasons [19]. However despite this we can see some outstanding examples of such structured programs that have been applied successfully in high-risk countries such as Tanzania, where early screening for high-risk diabetic foot was followed by aggressive structured management. This approach was found to be an effective way in risk and morbidity reduction in other similar high risk nations [18]. International organizations (IDF, IWGDF) dedicated to diabetic foot care have also shown good contributory result after successful implementation of their staged-wise structured foot care protocols in many healthcare settings by involving both health professionals and patients.

A part of the multidisciplinary or individual secondary outcomes from studies included also shows a significant benefit in cost, hospitalization, and patient overall quality of life. Multiple patient educational trainings on foot care were found to be more productive than single intervention [21, 22]. Moreover, a regular training and consistent surveillance on the integrated self-care activities received a further emphasis on foot care [21]. The association of quality of life, cost, and hospitalization with foot ulcer and amputation is an important factor that requires consistent evaluation in a structured foot care program [23]. It is also important to have understanding of the disease severity and consequences from diabetic foot complications both by the patients and their primary care physicians in order to have an earlier preventive approach through a multidisciplinary teamwork. Patient psychological evaluation and support is important before and after amputation since it is a highly traumatic event in life for the patient [24].

Wider selections of studies were reviewed based on the current policies and strategies in acute and chronic management of diabetic foot complication. Predictably, studies included in this review share a common set of characteristics in term of participants, intervention, education and behavior assessment, outcomes measure, duration, and follow-up, apart of few shorter duration studies, thereby opening present and future pooling. The use of standardized measures, including ulcer classification and outcome measures, in research studies would have greatly enhance the ability of reviewers to undertake better analysis, including comparison of outcomes of interventions.

Conclusion

Managing risks of diabetes-related foot ulceration and subsequent amputation is certainly the most challenging part of treatment in chronic diabetes population. Patient's lack of awareness on the disease severity along with insufficient training and coordination among clinicians puts patients at high risk from early disability. The evidence from this review suggests that by adopting a multidisciplinary team approach, it can save more legs and prevent early disability or even death when compared to the standard care. The role of individual discipline in multidisciplinary team specialist care also seems to be crucial in preventing long-term foot complications. Furthermore, it is important to note that the care provided under this setup may vary from country to country based on differences in the healthcare system and cultures. Few low socioeconomic countries however have successfully adopted a step-by-step program supported by IWGDF offering comprehensive diabetic foot care courses and training.

Future researchers should consider stratification of treatment and control arms in randomized control trials to ensure equal distribution of male and females, types of diabetes, underlying foot pathology, and comorbidity. Consideration should also be given to ensure that adequate information is given in relation to exclusion and inclusion criteria (i.e., methods of assessment for the "at risk" foot ulcers-associated morbidities and previous amputations).

Compliance with ethical standards We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property and that all ethical obligations and consideration associated with this work were carefully fulfilled and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing, we confirm that we have followed the regulations of our institutions concerning ethical guidelines and intellectual property.

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ORIGINAL ARTICLE

The relationship between the level of mean platelet volume and the carotid artery intima-media thickness in patients with type 2 diabetes mellitus

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Abstract Both of the carotid artery intima-media thickness (IMT) and mean platelet volume (MPV) may add to the risk of atherosclerosis for predicting cardiovascular events. It is not known, however, whether there is an association between them in diabetes patients. The aim of the study was to detect the relationship between the level of mean platelet volume and the carotid artery intima-media thickness in patients with type 2 diabetes mellitus, and to determine the correlation of MPV with body mass index (BMI), duration of diabetes, glycosylated hemoglobin A1 (HbA1C), fasting blood glucose (FBG), C-reactive protein (CRP), triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), serum uric acid (UA), ankle-brachial index (ABI), history of hypertension, and smoking in diabetic patients, respectively. A total of 128 subjects were enrolled into this study, who admitted consecutively to our clinic from January 1, 2013 to January 30, 2014. They were divided into three groups according to their IMT: normal IMT group (A, IMT ≦1.1 mm, n=26), IMT thickness group (B, IMT >1.1 mm, n=45), IMT thickening with plaque group (C, n=57). A significant difference with respect to BMI, HBA1c, TG, HDL-C, CRP, UA, FBG, ABI, and cigarette use between the study groups was not found (P>0.05). MPV was significantly higher in group A as compared to both group B and group C (7.94±0.97 fl, 7.24±0.71 fl, 7.03±0.79 fl, respectively, P 〈0.001), MPV had a high positive correlation with IMT (P 〈0.001) and age, as with TC and LDL-C (P 〈0.05). The level of MPV content decreased as the diabetic macroangiopathy progressed. Level of MPV was correlated with IMT in diabetic patients. MPV might be a useful marker for detecting atherosclerosis risk in diabetic patients.

Keywords Type 2 diabetic mellitus (T2DM) · Carotid intima-media thickness (IMT) · Mean platelet volume (MPV)

Introduction

The increase in prevalence of diabetes is a critical issue worldwide. It should be viewed as a serious threat not just from a public health, but also from a development perspective [1]. Based on a larger number of studies, there will be 439 million adult diabetics by 2030 [2]. The global health expenditure on diabetes is expected to total at least USD 490 billion or ID 561 billion in 2030 [3]. One or more macro-vascular complications were present in 28 % of diabetes patients [4]. Fifty percent of people with diabetes die of cardiovascular disease (primarily heart disease and stroke) [5]. However, atherosclerosis was the independent risk factor of macro-vascular lesion. It has been generally recognized that the degree of atherosclerosis can be predicted by examining the carotid artery intima-media thickness (IMT) [6]. Mean platelet volume (MPV) as one of the basic attribute of normal platelets is a surrogate marker of platelet activation; the normal values in our region is 7.7–13.4 fl. Meanwhile, a growing body of evidence suggested that the MPV level was higher and it would be an independent predictor or might be a risk of vascular complications in patients with T2DM [7–10]. Nevertheless, a study found that MPV may not be a risk factor for coronary artery disease (CAD) among diabetic patients [11]. Therefore, we

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perform a study to determine if there was a positive correlation between IMT and MPV and to validate the research conclusions above.

Materials and methods

Study population The Ethics Committee of Liaoning University of Traditional Chinese Medicine approved this clinical trial, and all the patients signed an informed consent form. We assigned 128 patients from general medical clinics who were already diagnosed to have type 2 DM (According to the WHO type 2 diabetes diagnosis standard in 1999—classic symptom of hyperglycemia plus random plasma glucose ≥11.1 mmol/l or fasting plasma glucose ≥7.0 mmol/l or 2-h plasma glucose ≥11.1 mmol/l during oral glucose tolerance testing. WHO recommended HbA1c ≥6.5 % as one of the diagnostic criteria of DM in 2011, but it was not recommended in China); the subjects were consecutive. Among the participants, there were 70 men and 58 women who had no previous history of coronary heart disease. Patients with insulin-dependent diabetes mellitus patients, acute diabetic complications, serious infection, malignant tumors, anemia, thrombotic disease, and liver and renal failure were excluded. In addition, acute pancreatitis, inflammatory enteropathy, hyperthyroidism, and thrombocytopenia which may affect MPV were eliminated. People who took antiplatelet drugs like aspirin recently were also removed. Clinical characteristics of subjects were recorded: age, sex, body mass index (BMI, weight in kilograms divided by height in square meter), mean platelet volume (MPV), glycosylated hemoglobin A1 (HbA1C), fasting blood glucose (FBG), C-reactive protein (CRP), triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), serum uric acid (UA), ankle-brachial index (ABI, dividing the ankle systolic pressure by the brachial systolic pressure), duration of diabetes, history of hypertension as well as history of smoking. Hypertension was defined as systolic blood pressure of 140 mmHg or above, diastolic blood pressure of 90 mmHg or above, or use of antihypertensive medication. According to the results of carotid color duplex ultrasonography, patients were divided into three groups: normal IMT group (A, IMT <1.1 mm, n=26), IMT thickness group (B, IMT ≥1.1 mm, n=45), and IMT thickening with plaque group (C, IMT≥1.1 and the thickness intimal projectured to the vascular intracavitary, n=57). Carotid atherosclerosis was defined as max-IMT≥1.1 mm according to the criteria of the Japan Academy of Neurosonology [12].

Laboratory analysis The IMT of the carotid artery was measured using ACUSON S2000 ultrasound machine (Siemens Medical Solutions USA, Inc); the mean intima-media thickness of the common carotid artery was measured over a segment of the common carotid artery that was 1 cm long, located approximately 0.5 cm below the carotid artery bulb. The MPV was performed using the ADVIA 2120i, (manufactured by Siemens Healthcare Diagnostics Inc, USA). Venous blood samples were collected in hemogram tubes with dipotassium EDTA and biochemistry tubes, and tested within 1 h of collection to minimize variations due to sample aging. Samples were maintained at room temperature. HbA1c was measured by Automated Glycohemoglobin Analyzer (DCA 2000, Bayer Corporation, Germany). FPG, HDL-C, LDL-C, TG, TC, UA, and CRP were analyzed by Automatic Chemistry Analyzer (Hitachi 7600, Japan). The ABI were measured using Form PWV/ABI, BP-203PRE II (Omron Colin Co, Ltd, Bunkyo, Tokyo, Japan).

Statistical analysis

Statistical evaluation was analyzed by statistical package for the social sciences (SPSS) version 17 (for Windows) using one-way analysis of variance. Correlation of MPV with other parameters was performed by Pearson correlation test (r value as the coefficient). Data were expressed as mean±standard deviation. A P value <0.05 was considered statistically significant.

Results Among the 128 diabetic subjects, there were 70 males and 58 females. Out of 128 DM patients, there were 26 patients in group A, 45 patients in group B, and 57 patients in group C. The mean BMI in group A was 25.97±2.98 kg/m^2 whereas it was 25.07±3.41 kg/m^2 in group B and it was 25.02 ±2.61 kg/m^2 in group C. The mean age of group A was 47± 8.42 years and of the subjects in group B was 57.38± 11.05 years, whereas that of group C was 60.58±9.3 years. The mean DM duration of the diabetics were 5.1±3.72 years. For groups B and C, they were 5.72±4.64 years and 10.68± 7.49 years, respectively. There was no significant difference among the subgroups for demographic characteristics of study participants, including BMI, history of hypertension, and smoking, but the age was younger in people of group A as compared with other groups (P<0.001); the DM duration were more longer in group C than in the other two groups (P<0.001). There was no significant difference in HBA1c, HDL-C, CRP, UA, TC, and TG between the groups. In the patients of group B and group C, MPV was significantly lower (7.24±0.71 fl and 7.03±0.79 fl, respectively) as compared to group A (7.94±0.97 fl) (P=0.000). The mean LDL-C level in group C was 3.28±0.89 mmol/L, while that of the other groups were 2.48±0.67 mmol/L (group A, P<0.001) and 3.06±0.87 mmol/L (group B, P>0.05). No statistically significant differences existed within MPV and BMI, HbA1c, duration of DM, FBG, CRP, ABI, UA, history of hypertension, and history of smoking. MPV was significantly associated

with IMT (r=0.373, P 〈0.001), age (r=0.263, P=0.003), Tc (r=0.178, P=0.044), and LDL-C (r=0.187, P=0.035). After adjustment for age, Tc, and LDL-C, MPV was still correlated inversely with IMT (Tables 1 and 2).

Discussion

The prevalence of diabetes is increasing worldwide, and the prevalence increased significantly from 2001 to 2009, moreover an active registry of youth diagnosed with diabetes at age <20 years [13]. Type 2 diabetes imposed a substantial economic burden on health-care systems; effective interventions that prevent or delay type 2 diabetes and diabetic complications might result in substantial long-term savings in health-care costs [14]. Meanwhile, a large number of persons with type 2 diabetes are suffering from macro-vascular complications; the outcome of them is often fatal, so it is very necessary to find risk factor modification interventions to delay all these changes. MPV is a machine-calculated measurement of the average size of platelets found in the blood, which is considered as the determinants of platelet size. Moreover, the function and activation of the platelet is determined by MPV [15]. Meanwhile, the Changfeng Study draw a conclusion that MPV was related to carotid atherosclerosis in normotensive, euglycemic, and normolipidemic males independently [16]. Since the average platelet size is larger when the body is producing increased numbers of platelets, the MPV test results can be used to make inferences about platelet production in bone marrow or platelet destruction problems. So, a low MPV indicates decreased production of platelets; our study revealed a low mean platelet count for diabetics with IMT and plaque. This was in contrast with the findings of the studies conducted by Ozder A et al. [9, 17–20]. Even if macroscopic thrombi do not form, the repeatedly damaged or diseased vessel walls shortened the survival of the platelet [21]. In diabetic patients, hyperglycemia impaired vascular endothelial cells and NO production which contributes to the formation of atherosclerosis by mediating leukocyte activation and adhesion to the endothelium via platelet P-selectin [22], and because platelet participated in the formation of atherosclerotic plaque, platelet was consumed and the activity was decreased continuously with or after the development of atherosclerosis. So, the level of MPV was low as atherosclerosis progressed in diabetes.

Table 1 Comparison of various parameters between groups A, B, and C

Characteristic	Group A	Group B	Group C
Number of patients	26	45	57
MPV (fl)	7.94±0.97	7.24±0.71*	7.03±0.79*
BMI (kg/m^2)	25.97±2.98	25.07±3.41	25.02±2.61
HbA1c (%)	7.95±1.84	8.69±2.01	8.08±1.73
DM duration (years)	5.1±3.72	5.72±4.64	10.68±7.49
Age (year)	47±8.42	57.38±11.05*	60.58±9.3*
Gender (M/F) (N)	16/10	23/22	31/26
FBG (mmol/L)	9.14±3.05	9.42±2.89	8.71±2.57
CRP (mg/L)	3.09±5.04	3.01±4.17	3.12±10.5
TG (mmol/L)	3.42±2.75	2.2±2.59#	2.12±1.74#
TC (mmol/L)	4.83±0.83	5.18±1.02	5.46±0.91#
LDL-C (mmol/L)	2.48±0.67	3.06±0.87	3.28±0.89
HDL-C (mmol/L)	1.06±0.19	1.17±0.29#	1.16±0.35*
UA (ummol/L)	363±94.3	312±96.6	312±99.1
ABI	1.08±0.12	1.04±0.14	0.97±0.16*
History of smoking	2.04±5.87	6.24±11.5	5.8±11.63
History of hypertension	1.31±2.94	2.76±6.72	5.44±8.06

Results are listed as mean±SD

*P<0.001; #P<0.05

Table 2 Correlation of MPV to the various parameters studied

Factors	R	P
IMT	0.373	<0.001
Age	0.263	0.003
BMI	0.091	0.308
HbA1c (%)	0.076	0.395
TC	0.178	0.044
LDL-C	0.187	0.035
DM duration	0.169	0.056
ABI	0.155	0.081
History of hypertension	0.163	0.066
TG	0.031	0.73
HDL-C	0.094	0.292
CRP	0.026	0.768
UA	0.068	0.444
FBG	0.01	0.914
History of smoking	0.036	0.687

Several studies showed that there is a strong positive correlation between MPV and FPG as well as HbA1c levels [9, 18–20], but it was in contrast to our research; we demonstrated that there was no association between MPV and HBA1c as well as FPG, but MPV was significantly associated with IMT, just like the conclusion of Arslan N et al. [23]. IMT increases in the presence of diabetic macroangiopathy, which is thought to be a relatively easy, non-invasive technique to identify atherosclerosis. Meanwhile, people with diabetes can evaluate the risk of atherosclerosis by measuring IMT [24, 25], and can be a marker in the primary prevention of people with diabetes.

The Edinburgh artery study in 1988 showed ABI could be a risk factor of cardiovascular events, and the lower the worse [26]; in our study, the ABI was lower as the atherosclerosis progressed. The uric acid serves as a radical scavenger and inhibits oxidation, so it did not increase the risk of atherosclerosis [27], and also in Chinese inpatients with type 2 diabetes [28].

Limitations Low numbers of subjects and study restriction to small geographic area are considered as two important limitations of our study.

Conclusions Increased risk of atherosclerosis in regard with type 2 DM may be a result of low MPV, or maybe the low level of MPV was the result of the development of atherosclerosis. Carotid IMT was correlated with MPV in selective DM patients, and MPV may be an indicator in the primary prevention of cardiovascular disease.

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

Disclosure statement The authors have nothing to disclose.

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ORIGINAL ARTICLE

Prevalence and risk factors of pre-diabetes and diabetes among patients with active TB disease attending three RNTCP centres in Odisha

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Abstract Type 2 diabetes mellitus (T2DM) and tuberculosis (TB) often present together and complicate each other at many levels. The Revised National Tuberculosis Control Program (RNTCP) calls for strengthening collaboration between TB and diabetes control programs for better management of diabetic patients with TB. In this study, we determined the prevalence of pre-diabetes and diabetes among 220 TB patients registered in selected tuberculosis units (TUs) of RNTCP in hospitals of three different regions of Odisha namely Bhubaneswar, Cuttack and Nayagarh. Various socio-demographic factors like age, gender, marital status, literacy status, locality, habits, etc. and clinical profile were assessed. Out of 220 TB patients, 24 % patients were having pre-diabetes and 14 % patients were having diabetes. Both the conditions, pre-diabetes or diabetes, were more common among males, married patients, advancing age, having less education, no specific job, sedentary lifestyle, smoking/drinking, living in crowded areas, poor living conditions and unhygienic environment. Majority of pre-diabetic and diabetic patients were having pulmonary TB with 1+ sputum positivity and category (Cat)-I type of treatment. The variations in random blood glucose (RBG) and fasting blood glucose (FBG) levels among people with pre-diabetes are very less whereas those among people with diabetes were very high, thereby indicating that the blood glucose levels show more fluctuations before and after intake of food. Further, our results show that 57 % were not knowing that they were pre-diabetic and 43 % were not aware that they were having diabetes. It is feasible to screen TB patients for diabetes resulting in high rates of T2DM detection. Screening for diabetes mellitus (DM) in TB patients could improve DM case detection and early treatment, indirectly leading to better TB-specific treatment outcomes and prevention of DM complications.

Keywords Prevalence · Pre-diabetes · Diabetes · TB patients · RNTCP centres · Bhubaneswar · Odisha

Introduction

Type 2 diabetes mellitus (T2DM) has assumed pandemic proportions and is indeed the scourge of the modern era. At present, there are 300 million diabetics worldwide and the number is expected to double over the next 5 years [1]. With an annual tuberculosis (TB) incidence of 2.2 million cases (range 2.0–2.5 million) and an estimated 63 million people living with diabetes mellitus (DM), India has the highest TB burden and second highest DM burden in the world. India with its huge diabetic population holds the distinction of being dubbed the diabetic capital of the world, with 63 million diabetics. India has the largest number of TB cases and also the largest number of dually infected individuals, in the world [2, 3]. The association between T2DM and TB and their synergistic role in causing human disease has been recognized for centuries [4–6]. About 10 % of TB cases globally are linked to diabetes. The association between T2DM and TB is a challenge for global TB control. Improved understanding of the bi-directional relationship of the two diseases is necessary for proper planning and collaboration to reduce the dual burden

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of diabetes and TB [7–9]. In people with TB, it may be appropriate to actively screen for DM. Prevention, screening and treatment of both diseases together are more effective [10]. A model similar to the TB-HIV program may be the best approach [11]. Recognizing the serious threat posed by diabetes-TB, The Revised National Tuberculosis Control Program (RNTCP) calls for strengthening collaboration between TB and diabetes control programs for better management of diabetic patients with TB and TB patients with diabetes [12].

In this study, we screened the TB patients attending the three RNTCP centres in Odisha, for estimating the prevalence of pre-diabetes and diabetes and determining the risk factors.

Methodology

Ethical approval The detailed plan of study was submitted to the Ethical Committee as well as the Scientific Advisory Committee (SAC) of the Institute, which approved the assumptions for human research.

Study design and study period This was a descriptive pilot study conducted from Jan 2014 to June 2014 in the Dept. of NCDs, Regional Medical Research Centre, Bhubaneswar. Blood samples for screening were collected from adults willing to participate in the study. All TB patients who have been consecutively diagnosed and registered under RNTCP were screened for diabetes. This included patients with new and previously treated TB and stratified into smear-positive pulmonary TB, smear-negative pulmonary TB and extra-pulmonary TB. Those who have completed treatment were excluded.

Screening intervention and diagnosis of diabetes The screening for DM was followed according to the guidelines stipulated by the Revised National Tuberculosis Control Program (RNTCP) and National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NPCDCS) in India [12, 13]. Those guidelines stipulate that a fasting blood glucose be carried out using a finger prick and glucometer with cut-off thresholds in line with those recommended by the World Health Organization. Screening TB patients for DM should be conducted as early as possible after diagnosis of TB but can be done at any time during the course of TB treatment. Because of the difficulties in getting TB patients to first come to the clinic in a fasting state, TB patients will be initially screened with a random blood glucose (RBG) using a glucometer. If the RBG is less than 110 mg/dl, this is a normal result and no further tests need be carried out. If the RBG is at or greater than 110 mg/dl, this might indicate an abnormal glucose state and there is a possibility of DM. The patient will be asked to return in a fasting state, and a fasting blood glucose (FBG) will be carried out. A FBG at or greater than 126 mg/dl indicates DM. The screening procedure and criteria for diagnosis of DM are summarized in Table 1.

Table 1 Screening for diabetes and making the diagnosis of diabetes

At first visit: time of registration or close to starting TB treatment

Screen first with a random blood glucose (RBG) using a glucometer at any time.

- If the RBG is less than 110 mg/dl, then no further action is needed
- If the RBG is 110 mg/dl or greater, then do a second screen at next visit

Next visit[a]:

Screen second with a fasting blood glucose (FBG) using glucometer.

- If FBG is less than 110 mg/dl, then this is normal
- If FBG is 110–125 mg/dl, then this indicates impaired glucose tolerance
- If FBG is 126 mg/dl or higher, then this indicates diabetes mellitus

[a]For this visit, the patient should eat the last meal in the night before 9 p.m. a day before and should abstain from food and drink except water until the next day morning till the procedure is completed

Methods

In all, 220 patients with active TB disease, registered in RNTCP, attending the OPDs of three primary care hospitals, namely Capital Hospital in Bhubaneswar, City Hospital in Cuttack and B.M. TB Hospital in Nayagarh, were enrolled after taking informed consent. The socio-demographic and clinical profiles like age, gender, marital status, literacy status, profession, lifestyle (sedentary and active), habits (alcohol/smoking), locality, type of TB, index of sputum positivity, status and period of treatment, blood glucose levels (fasting/random), complications at the time of testing, etc. were documented using standardized questionnaires and analyzed in the context of symptoms at the time testing. They were screened for random blood glucose (RBG) levels by finger prick method using a glucometer (Glucocard 01-mini, Blood Glucose Monitoring Kit, Arkay Factory, Inc., Koka-Shi, Shiga, Japan). Only those patients having higher values were selected and advised to return to the clinic the next day and/or next visit in a fasting state, and a repeat test was carried out for fasting blood glucose (FBG) levels. This test was used to differentiate pre-diabetes and diabetes.

Diagnostic criteria for IFG IFG is a state of higher than normal fasting blood (or plasma) glucose concentration, but lower than the diagnostic cut-off for diabetes: FBG ≥110 and ≤126 mg/dl as per WHO 1999 criteria [14].

Diagnostic criteria for pre-diabetes and diabetes Pre-diabetes and diabetes were classified using FBG levels, the diagnostic criteria being 110–125 mg/dl for pre-diabetes and FBG levels ≥126 mg/dl for diabetes or a self-reported history of taking anti-diabetic drugs after diagnosis by a medical professional in accordance with national guidelines [15, 16].

Diagnostic criteria for Cat-I and Cat-II Based on the nature/severity of the disease and the patient's exposure to previous anti-tubercular treatments, RNTCP classifies TB patients into two treatment categories, category (Cat)-I and Cat-II. Cat-1 includes patients with newly diagnosed sputum-positive pulmonary TB, sputum-negative pulmonary TB with extensive parenchymal involvement and severe form of extra-pulmonary TB whereas Cat-2 includes sputum smear-positive treatment failure cases, relapse cases and return after interruption [4]. Details about Cat of treatment, i.e. Cat-I or Cat-II, and sputum status at the time of diagnosis, i.e. sputum-positive, Sputum-negative or extra-pulmonary TB, were noted from the TB treatment card.

Statistical analysis

SPSS version 16.0 was used for statistical analysis. Prevalences are reported with 95 % confidence intervals calculated considering the design effect. Mean and standard deviation for continuous variables and proportions for categorical variables are reported. All variables were described as proportions, and differences between groups were compared for statistical significance using the chi-square (χ^2) test and *t* test, as applicable. *P* values of <0.05 were considered statistically significant.

Results

Out of 220 TB patients, 24 % were having pre-diabetes and 14 % were having diabetes. One percent of TB patients were found to be HIV-positive. Table 1 shows that 96 patients were recruited from Nayagarh, 72 from Bhubaneswar and 52 from Cuttack. Of these, 39 % were having high RBG levels, suggesting impaired hyperglycemia. The number of TB patients showing hyperglycemia varied ranging from 40 % in Bhubaneswar, 48 % in Cuttack to 33 % in Nayagarh and is statistically significant ($p=0.006$). As diabetes and pre-diabetes are outcomes with overlap, Bonferroni adjustment was done for the critical value of the level of significance ($p=0.0125$).

Table 2 shows the socio-demographic profile of TB patients. In all, 74 % male and 25 % female patients were enrolled in the study. Of these, 27 and 16 % of males were having pre-diabetes and diabetes, respectively. Among females, 15 % were having pre-diabetes and 8 % were having diabetes. This shows that more males were having either pre-diabetes or diabetes. Thus, gender appeared to be a significant factor ($p=0.032$) in our study. Thirty-five percent of people in the age group 31–45 years and 20 % in the age group 46–60 years were having pre-diabetes while 12 % of people in the age group 31–45 years and 28 % in the age group 46–60 years were having diabetes. Advancing age is a significant factor ($p<0.001$). Eighty-one percent of TB patients were married, and 18 % were unmarried. Further, 25 and 17 % of married patients were having pre-diabetes and diabetes, respectively. Forty-four percent of TB patients were illiterate, and 45 % had studied up to high school. Among the illiterate TB patients and those having less education, the prevalence of pre-diabetes and diabetes was similar. Occupation wise, the largest proportion consisted of either labourers or those having no specified jobs. Among these, 26 % had pre-diabetes or 11 % had diabetes. Analyzing the risk factors of diabetes among TB patients, 37 % of TB patients were having habits of all types, namely chewing gutka/tobacco, smoking and alcohol. Twenty-five percent of people with pre-diabetes and 17 % with diabetes were having all types of habits whereas 27 % of TB patients denied of having any such addictive habits. More patients with diabetes were found to be addicted to either smoking, drinking alcohol and/or chewing tobacco/gutka. However, it is difficult to say how many people were actually physiologically addicted to smoking and/or drinking.

Fifty-five percent of patients enrolled in our study were sedentary, i.e. those not involved in activity, whereas 44 % were active. Twenty-five and 17 % of sedentary patients were having pre-diabetes and diabetes, respectively. Prevalence of diabetes was comparatively less, i.e. 11 % among those patients having an active lifestyle.

The mean BMI of patients with TB, pre-diabetes and diabetes is 17, 16.6 and 19.15, respectively.

In this study, it was observed that 35 % of patients attending the RNTCP centres were from urban slums, 20 % were from housing colonies, and 43 % patients were from rural areas. Twenty-nine percent of the patients residing in urban slums were having pre-diabetes, and 12 % were having diabetes. The prevalence of both the conditions is high among those living in housing colonies and rural areas. Table 3 shows the clinical profile of TB patients. Ninety percent of patients were having pulmonary TB whereas 10 % were having extra-pulmonary TB. Twenty-four percent of pulmonary TB patients were having pre-diabetes whereas 14 % were having diabetes. With reference to bacillary index, 14 % of patients with diabetes and 23 % with pre-diabetes were having 1+ sputum status. Fifty-five percent were of Cat I type and 22 % were of Cat-II type. Our study indicated that more patients with pre-diabetes (24 %) and diabetes (18 %) were taking Cat I type of treatment. Less patients having Cat II show that they have either not defaulted during and/or discontinued their previous treatment.

Table 1 Number of TB patients from RNTCP centres in hospitals of three different regions

RNTCP centres	TB patients (n=220)	Hyperglycemia n=86, (39.09 %)	Pre-diabetes n=54, (24.55 %)	Diabetes n=32, (14.55 %)	Normal n=48, (21.82 %)
Bhubaneswar	72	29 (40.27)	20 (27.77)	9 (12.50)	14 (19.44)
Cuttack	52	25 (48.07)	15 (28.84)	10 (19.23)	2 (3.84)
Nayagarh	96	32 (33.33)	19 (19.79)	13 (13.54)	32 (33.33)

Chi-square=18.27, p=0.006; Bonferroni adjustment=0.0125. Values in parenthesis are percentage

Figures 1 and 2 depict the FBG and RBG levels of TB patients having pre-diabetes and diabetes, respectively. There are very less variations in RBG and FBG levels among people with pre-diabetes, the range being 40–70 whereas the variations of RBG/FBG were very high, being 100–150, among patients with diabetes. This indicates that the blood glucose levels show more fluctuations before and after intake of food among patients with diabetes. The variations in RBG and FBG levels in both the conditions are highly significant, p<0.0001.

Table 4 depicts the diagnostic status of TB patients having pre-diabetes and diabetes. Our results show that 57 % were not knowing that they were having pre-diabetes and 43 % were not aware that they were having diabetes. Only 42 % were knowing that they were having problems such as excessive thirst, excessive hunger and increased urination along with increased weight, central obesity, fatigue, high blood pressure, etc. but not aware that these could be the signs of pre-diabetes. This is very serious as T2DM is a silent progressive disease, and in the absence of appropriate treatment and lifestyle

Table 2 Socio-demographic profile of TB patients

Parameters		Numbers screened (n=220)	Pre-diabetes [n=54, (24.54 %)]	Diabetes [n=32, (14.54 %)]	Normal [n=134, (60.91 %)]	Chi-square, (p value)
Socio-demographic profile						
Gender	Male	163	45 (27.61)	27 (16.56)	91 (55.83)	6.83, p<0.032
	Female	57	9 (15.79)	5 (8.77)	43 (75.44)	
Age group (in years)	16–30	65	11 (16.92)	1 (1.54)	53 (81.54)	29.30, p<0.001
	31–45	80	28 (35.00)	10 (12.50)	42 (52.50)	
	46–60	75	15 (20.00)	21 (28.0)	39 (52.00)	
Marital status	Married	180	46 (25.56)	31 (17.22)	103 (57.22)	4.50, p<0.024
	Unmarried	40	8 (20.0)	1 (2.50)	31 (77.50)	
Literacy status	Illiterate	97	20 (20.62)	14 (14.43)	63 (64.95)	4.95, p<0.292
	5th - 10th	101	25 (24.75)	14 (13.86)	62 (61.39)	
	Graduation	22	9 (40.91)	4 (18.18)	9 (40.91)	
Occupation	Unemployed	8	2 (25.0)	0 (0.00)	6 (75.0)	31.45, p<0.002
Locality	Regular job	19	3 (15.79)	9 (47.37)	7 (36.84)	
Habits	Labourers	44	15 (34.09)	2 (4.55)	27 (61.36)	
	Business	30	9 (30.0)	7 (23.33)	14 (46.67)	
	Housewife	34	7 (20.59)	2 (5.88)	25 (73.53)	
	No specified job/others/students	53	14 (26.42)	6 (11.32 %)	33 (62.26)	
	Farmer	32	4 (12.50)	6 (18.75 %)	22 (68.75)	
	Urban slums	78	23 (29.49)	10 (12.82)	45 (57.69)	2.97, p<0.560
	Housing colonies	46	11 (23.91)	9 (19.57)	26 (56.52)	
	Rural	96	20 (20.83)	13 (13.54)	63 (65.63)	
	Smoking	21	6 (28.57)	4 (19.05 %)	11 (52.38)	5.37, p<0.710
	Alcohol	13	5 (38.46)	1 (7.69 %)	7 (53.85)	
	Gutka/tobacco	43	10 (23.26)	7 (16.28 %)	26 (60.47)	
	All	82	21 (25.61)	14 (17.07 %)	47 (57.32)	
	None	61	12 (19.67)	6 (9.84 %)	43 (70.49)	
Lifestyle	Sedentary	122	31 (25.41)	21 (17.21 %)	70 (57.38)	1.98, p<0.370
	Active	98	23 (23.47)	11 (11.22)	64 (65.31)	

Values in parenthesis are percentage

Table 3 Clinical profile of TB patients

Clinical profile						
Parameters		Numbers screened (n=220)	Pre-diabetes [n=54, (24.54 %)]	Diabetes [n=32, (14.54 %)]	Normal [n=134, (60.91 %)]	Chi-square, (p value)
Type of TB	Pulmonary	198	48 (24.24)	28 (14.14)	122 (61.62)	0.46, p<0.795
	Extra-pulmonary	22	6 (27.27)	4 (18.18)	12 (54.55)	
Category of treatment	Cat-I	122	30 (24.59)	23 (18.85)	69 (56.56)	5.04, p<0.08
	Cat-II	49	21 (42.86)	9 (18.37)	19 (38.78)	
Bacillary index (sputum positivity)	1+	88	21 (23.86)	13 (14.77)	54 (61.36)	9.32, p<0.156
	2+	53	10 (18.87)	4 (7.55)	39 (73.58)	
	3+	39	13 (33.33)	5 (12.82)	21 (53.85)	
	-ve	40	10 (25.0)	10 (25.0)	20 (50.0)	

Values in parenthesis are percentage

changes, it would lead to debilitating complications. Fifty-six percent were having diabetes, established previously, and were taking treatment for the same. The patients not aware of their pre-diabetes or diabetes condition were referred to the Department of Endocrinology and Diabetes for further treatment, care and management.

Discussion

In this study, we determined the prevalence of pre-diabetes and diabetes among 220 TB patients registered in selected tuberculosis units (TUs) of RNTCP in hospitals of three different regions of Odisha namely Bhubaneswar, Cuttack and Nayagarh. In our study, 24 % patients were having pre-diabetes, 14 % were having diabetes, and 1 % were found to be HIV-positive.

The number of TB patients showing hyperglycemia varied from the three regions. More males, married patients and those having less education were having either pre-diabetes or diabetes. Both the conditions were more common with advancing age and sedentary lifestyle. More TB patients were found to be addicted to either smoking, drinking alcohol and/or chewing tobacco/gutka. It was observed that 12 % (10) of these patients residing in urban slums and 13 % (13) in rural areas were having diabetes. Majority of patients with pre-diabetes and diabetes were having pulmonary TB with 1+ sputum positivity and Cat-I type of treatment. The variations in RBG and FBG levels among patients with pre-diabetes are very less whereas that among patients with diabetes were very high, thereby indicating that the blood glucose levels show more fluctuations before and after intake of food among patients with diabetes. Further, our results show that 57 % (31) were not knowing that they were having pre-diabetes and 43 % (14) were not aware that they were having diabetes. Therefore, advancing age, illiteracy, having no specific job, inactive lifestyle, smoking/drinking, living in crowded areas, poor living conditions and unhygienic environment appeared to be the risk factors for DM among TB patients in this state.

A nationwide INDIAB study conducted in the general population of Tamil Nadu, South India, showed that the prevalence rates of diabetes and pre-diabetes were 10.4 and 8.3 %, respectively [17]. A study by Vishwanathan et al. showed that prevalence rates of DM and pre-diabetes were 25.3 and 24.5 %, respectively, among TB patients registered under RNTCP in South India [18]. In particular, type 2 DM is often unrecognized. In two studies from Tanzania and Indonesia, 73

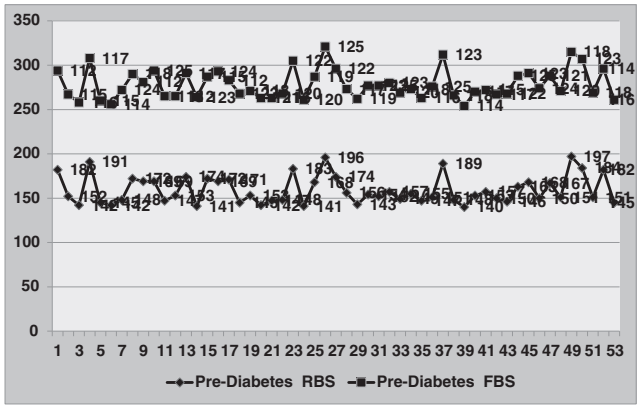

Fig. 1 Variation in FBG and RBG levels of TB patients having pre-diabetes

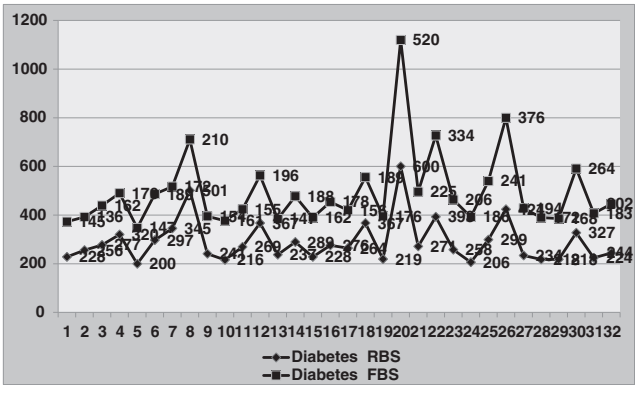

Fig. 2 Variation in FBG and RBG levels of TB patients having diabetes

Table 4 Diagnostic status of TB patients having pre-diabetes and diabetes

		Number (%)
Pre-diabetes (n=54)	Newly diagnosed pre-diabetes	31 (57.40)
	Established pre-diabetes	23 (42.59)
Diabetes (n=32)	Newly diagnosed diabetes	14 (43.75)
	Established diabetes	18 (56.25)

Values in parenthesis are percentage

and 61 % of diabetics, respectively, were newly diagnosed concurrent with active TB [19, 20]. Similar studies have been carried out at China and Uganda [21, 22]. The diabetes has increased the burden of TB, especially in populations where the prevalence of TB infection is high among young adults [23]. In Odisha, the prevalence rate of DM is considered to be around 10 and rising fast and that of TB (i.e. 418 per 100,000 population) is below the national average (i.e. 445 per 100,000 population) [24, 25]. A wide range of DM prevalence from 1.9 to 35 % was reported by screening for DM among patients with TB in different regions of India [26–28]. The highest values were reported from regions with high prevalence of DM. Many of these patients were newly diagnosed as a result of receiving expanded medical attention related to TB treatment [29–31]. While some authors have expressed concern over the feasibility of screening DM at field level as well as the technologies used for assessment of blood glucose and co-management of TB-DM cases, others suggest that high-quality implementation research is needed to assess the value and ways of screening for DM in patients with TB and vice versa and to set up standardized systems of monitoring and evaluation [32–34].

The strengths of this study are that we implemented screening within the routine health system. It is feasible to screen TB patients for diabetes resulting in high rates of T2DM detection. The early identification of patients with co-morbidity, especially among the newly diagnosed cases, helped us to link these patients to appropriate DM care, which could lead to improved TB treatment outcomes.

Limitations are that we could screen small number of patients and analyzed the data over a short period of time. Some patients did not turn up for repeat testing; therefore, they were followed up by DOTS providers. About 15 % did not agree for a fasting blood test. However, the loss to follow-up was less, the primary reasons for the low loss to follow-up being the close proximity of the TB and DM clinics.

Another limitation of our study was that we were not able to ascertain whether a high FBG in patients with TB was indicative of true DM or of infection-induced hyperglycemia. This requires periodic blood glucose testing over the course of TB treatment, which was beyond the scope of the current study. Further research is needed to ascertain this and the optimum timing of DM screening among TB patients. More research is warranted to investigate how the increasing incidence of DM impacts TB control efforts in this state.

Conclusions

Screening for DM in TB patients could improve DM case detection and early treatment, indirectly leading to better TB-specific treatment outcomes and prevention of DM complications.

The diagnosis of pre-diabetes and diabetes among TB patients is important for monitoring disease progression and risk analysis which, in turn, would generate baseline data for planning further research studies and intervention strategies for prevention of several complications. The status of co-morbid conditions would guide the clinicians in deciding the appropriate treatment regimens and comprehensive management of at-risk individuals. An early treatment, if initiated, would help in further deterioration of the diabetes.

We, therefore, feel that screening TB patients, irrespective of their complaints and symptoms, at regular intervals, for RBG levels would go a long way in early detection of the pre-diabetes and diabetes conditions.

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

Authors' contributions ED and SD were involved in recruiting and testing the samples from TB patients. SKK provided support for the study. VSY of the National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR), Tajganj, Agra, did the statistical analysis of data. TH conceptualized the idea, designed the study, compiled the data, wrote and edited the article throughout all stages.

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ORIGINAL ARTICLE

Importance of anthropometry in assessing insulin resistance as a pre-alarming sign before the onset of metabolic syndrome: a study among apparently healthy subjects

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Abstract Insulin resistance (IR) and obesity are inter-related causes of metabolic syndrome. Early identification before the onset of metabolic syndrome will be useful to lead a healthy life. The purpose of the present study was to identify the importance of IR before the onset of metabolic syndrome in apparently healthy, non-diabetics subjects. Data of 227 apparently healthy non-diabetics (20-70 years) who reside in a sub-urban area in Colombo district, Sri Lanka, were recruited for this study. Fasting blood glucose (FBG), fasting serum insulin (FSI), weight, height, waist circumference (WC), hip circumference (HC), and mid-upper arm circumference (MUAC) were measured and homeostatic model assessment for insulin resistance (HOMA-IR) was calculated. Body mass index (BMI), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) were calculated. Data were analyzed using Statistical Package for Social Science (ver.17). Majority were females (61.8 %). Prevalence of IR was 59.9 %. Mean BMI of IR subjects was 28.3±2.7 kg $m^{-2}$where 75.3 % of obese had IR. All anthropometric variables except height had significant positive correlations ($P<0.01$) with IR. Linear regression analysis indicated that BMI is useful in predicting IR while logistic regression analysis showed that BMI and WC are the best predictors of IR in males whereas it was WHtR and WC in females. Even though study subjects were apparently healthy and not diagnosed as diabetes, those with elevated anthropometric parameters had higher prevalence of IR. Best anthropometric predictors of IR for a specific sex should be used as an easy self-monitoring alarming sign before the onset of metabolic syndrome.

Keywords Insulin resistance · Body mass index · Waist circumference · Apparently healthy · Waist-to-height ratio · Waist-to-hip ratio

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Introduction

Insulin resistance (IR) is a state in which normal amounts of serum insulin are not adequate to produce the expected biologic response in target tissues like adipocytes, muscle, and liver [1]. IR is a characteristic feature of type 2 diabetes (T2D) which is mainly linked with metabolic syndrome (MS). IR is considered as one of the major causative factors for MS, a cluster of metabolic abnormalities including diabetes, high blood pressure, and high cholesterol levels along with obesity. Over the past two decades, worldwide prevalence of MS has increased significantly. Approximately 20–25 % of the worlds' adult population accounts for MS, and they are prone to fivefold greater risk of developing T2D [2]. It is estimated that around 90–95 % of diabetes worldwide are diagnosed as T2D with IR. Furthermore, prevalence of MS among South Asians is estimated to be 20–25 %, and early onset of T2D and cardiovascular diseases (CVD) is common among Asians [3]. In 2005, the prevalence of T2D among Sri Lankans was approximately 11 % and one fifth of adults were found to be dysglycemic [4]. Obesity, as one of the major components of MS, has reached epidemic proportions during the last three

decades [5]. A global pandemic of obesity is driving the increased incidence and prevalence of T2D and, on the other hand, increased IR causes some degree of obesity. Overweight and obesity prevalence was at peak in the USA out of all the WHO regions and lowest was reported in South East Asia. Women have higher tendency to be obese than men in the world. Incidence of overweight and obesity among Sri Lankans were at elevated levels. In year 2005/2006, the prevalence of overweight of both genders was 25.2 %, and in 2006/2007, 31.2 % of women were found to be overweight [6].

Studies have shown that increased IR results in failure of suppression of hepatic glucose production and peripheral IR impairs peripheral glucose uptake. Thus, it leads to fasting and postprandial hyperglycemia with high fasting serum insulin (FSI) levels with the progression to T2D. Later in the course of T2D, IR begets IR by reduced non-oxidative glucose metabolism in muscles, further exacerbating hyperglycemia [7]. Yet, many studies consider IR as a diagnostic criterion in overt T2D individuals despite the fact that increase in IR makes a strong predictor for early development of MS and future diabetes [8]. Therefore, assessing IR is a useful indicator to know the state of hyperinsulinemia before the onset of T2D or to alarm the individuals who have the high potential to develop MS [9]. A variety of methods are being used to measure IR, where hyperinsulinemic euglycemic clamp is considered as the gold standard. The homeostasis model assessment of IR (HOMA-IR) was developed based on the above to provide a simple, consistent, and inexpensive method to detect IR in individuals. It involves only measuring FSI value as well as fasting blood glucose (FBG) value of a subject. Thus, it indicates fasting steady state levels of blood glucose and serum insulin for any dynamic function of pancreatic beta cells and insulin sensitivity. HOMA index ≥1 is considered as insulin resistant [10]. Therefore, moderate or large increases in HOMA-IR can have an effect on apparently healthy, non-diabetic, but early MS subjects.

Conventionally, multiple methods of cheap costless anthropometry are being used to assess obesity and body fat distribution. As obesity itself could be used as a marker to predict IR, anthropometric measures and indices such as waist circumference (WC), body mass index (BMI), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) are used to classify general obesity and regional adiposity, and also to define the risk groups who have the tendency to develop metabolic abnormalities. Some studies have correlated different anthropometric parameters with IR [11] in MS subjects.

In genetically predisposed subjects, defects in insulin secretion can lead to impaired fasting glucose (IFG) which can be assessed by the anthropometric measures. Some individuals with obesity might be metabolically healthy and some lean individuals with an increased amount of visceral body fat distribution might have significant IR. Therefore, it is vital to identify which anthropometric parameter has the significant association with IR and which parameter enables optimizing the clinical prediction of IR. Further, detecting insulin-resistant subjects in a general population provides an evaluation of early diagnosis of apparently healthy but early MS individuals. To our knowledge, no study has been carried out to identify the significance anthropometric parameters in measuring IR as an early detection method before the development of MS. Hence, the aim of the present study was to identify the significance of costless, self monitoring anthropometric parameters that would optimize clinical prediction of IR in apparently healthy, non-diabetic, male and female subjects.

Methods

Study design and participants

A descriptive cross-sectional study was carried out at the Family Practice Centre, University of Sri Jayewardenepura, located at Nugegoda, a city exceeding 250,000 of multi-ethnic population. Non-probability, convenience sampling was used and sample size was determined by the equation for estimating mean.

The study was approved by the Ethics Review Committee of University of Sri Jayewardenepura and informed written consent was obtained from all individual participants prior to the study. This study involved 227 study participants between 20 and 70 years of age. The subjects enrolled in this study were fully informed about the study protocol, and informed written consent was obtained from all the subjects, prior to the study. Apparently healthy subjects who were not diagnosed as having diabetes (FBG <6.9 mmol/L) were included in the study, whereas subjects who were pregnant, on steroidal drug, and having severe diseases; who have physical and cognitive impairments; and who dislike to participate in the study were excluded.

Anthropometric measurements and indices

All the anthropometric measurements were taken of the standard protocol according to NHANES [12]. Individuals' height was measured using a stadiometer to the nearest 0.1 cm. Body weight was measured using an electronic digital weighing scale (Chyo, Mu-150 K, Japan) to the nearest 0.1 kg. WC was measured at the approximate midpoint between the lower margin of the last palpable rib and the crest of the ileum (top of the hip bone), placing the non-stretchable tape around the trunk in a horizontal plane. The tape was parallel to the floor and it was without compressing the skin. The measurement was made at the end of a normal expiration to the nearest 0.1 cm. Similarly, HC was measured by placing the non-

stretchable tape around the widest portion of the buttocks, and the sides of it were adjusted to ensure that it is in a horizontal plane. The measurement was taken from the right side of the subject to the nearest 0.1 cm. Mid-upper arm circumference (MUAC) was measured when the subjects were standing upright, shoulders relaxed, and the right arm bent at 90°. The tape was wrapped around the midpoint of the arm between the shoulder (Acromian process) and the tip of the elbow (Olecranon process), and the measurement was taken to the nearest 0.1 cm.

Anthropometric index BMI was calculated dividing body weight in kilograms by the square of the height of the body in meters. Anthropometric ratios such as WHR and WHtR were calculated as the ratio of the circumference of the waist to the hip and the circumference of the waist-to-height, respectively [13, 14].

Blood samples and biochemical analysis

The subjects were instructed to come with an overnight fasting period. Prior to phlebotomy, all the required materials for blood drawing were assembled and fasting blood samples were collected from each individual by a trained phlebotomist in an aseptic environment while the subject was in a seated position preceded by a 10–15-min rest. Blood samples were collected into empty sterile centrifuge tubes without an anticoagulant and allowed to clot for 30–40 min at room temperature, for serum separation. About 150 μL blood was pipetted simultaneously, into an Eppendorf tube with NaF for FBG analysis. Collected samples were taken and analyzed at the Faculty of Medical Sciences, University of Sri Jayewardenepura.

Blood in Eppendorf tubes was centrifuged at 3600 rpm for 10 min and serum was separated for FBG analysis. The rest of the blood in centrifuge tubes was centrifuged at 3600 rpm for 10 min at room temperature to separate the serum. Serum was stored in a deep freezer at −20 °C for batch assay of serum insulin while analysis of FBG was done on the same day.

FBG and FSI levels were analyzed using glucose oxidase (GOD-PAP) kit method and ELISA method, respectively. All the required steps of the procedure were carried out as indicated in the kits (Biolabo reagent, Maizy; DRG International, Inc, USA) according to the manufacturers' guidelines. Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR) defined as

$$HOMA{-}IR = [FSI\ (\mu U/mL)\ x\ FBG\ (mmol/L)]/22.5$$

Definitions of outcome and covariates

Subjects were classified as normal and impaired fasting glucose according to IDF/WHO criteria [15]. WC and WHR were divided into two categories, as metabolic risk and normal based on WHO classification for males and females separately [13]. Obese and non-obese categories were derived from WHO South Asian classification for BMI [16]. MUAC was also classified as metabolically normal and risk, based on cutoff values given in literature [17]. WHtR was classified into metabolic risk and normal groups based on the findings of Ashwell and Hseih [14]. As a measure of IR, subjects having HOMA-IR ≥1 was taken as insulin resistant [10].

Statistical analysis

All data were double entered and cross-checked for consistency. Data were analyzed using the Statistical Analysis Package for Social Sciences version 17 (SPSS Inc, Chicago, IL, USA). Means and standard deviations were computed for anthropometric and biochemical variables. Independent sample *t* - test was done to find out the significant differences among IR and normal groups for anthropometric parameters.

Stepwise linear regression analyses, utilizing continuous anthropometric measures as potential independent predictors, were performed to predict HOMA-IR. The parameters used were weight, height, WC, HC, MUAC, BMI, WHR, and WHtR. A stepwise regression was performed on all participants, using all anthropometric parameters as predictive variables and HOMA-IR as the dependent variable. A second stepwise regression was done, with the same conditions, after dividing the participants into two groups as females and males.

Binary logistic regression analysis was carried out in dichotomous variables, to identify the relative risk factor and the most suitable anthropometric parameter to predict the risk of IR. All the analysis was carried out for the total population as well as for males and females separately. Logarithmic values of HOMA-IR were used in the analysis to normalize its distribution.

Results

The study population had only 40.1 % males and females were the majority. The comparison of the biochemical and anthropometric data with HOMA-IR ≥1 and <1 is given in Table 1. The study indicates that, except age and height, all the other clinical parameters are significantly higher in insulin resistant group. The percentage of subjects with increased IR in this study was 59.9 % (n=136). Among the subjects with increased IR, female population was high with 61.8 %, and 38.2 % were males.

Among the insulin-resistant subjects, 49.3 % of both sexes were obese (BMI ≥25 kg m^{-2}) having a mean BMI of 28.3± 2.7 kg m^{-2} even though the mean BMI of subjects having HOMA-IR ≥1 was 25.1±9.7 kg m^{-2}. This was higher than

Table 1 Baseline parameters of apparently healthy non-diabetic subjects with HOMA-IR values

Characteristics		Non-diabetic, apparently healthy subjects (n=227)		*P* value
		IR (HOMA-IR ≥1) (*n*=136)	Normal (HOMA-IR <1) (*n*=91)	
Age (years)		41±14	41±13	*P*>0.05
Anthropometric data				
	Weight (kg)	64.2±14.2	58.4±11.1	*P*<0.05
	Height (cm)	159.8±9.8	159.5±14.6	*P*>0.05
	WC (cm)	86.1±11.1	79.9±9.9	*P*<0.05
	HC (cm)	98.6±8.8	95.4±7.6	*P*<0.05
	MUAC (cm)	30.3±3.7	28.1±3.4	*P*<0.05
	BMI (kg m^{-}2)	25.1±9.7	22.6±3.7	*P*<0.05
	WHR	0.9±0.1	0.8±0.1	*P*<0.05
	WHtR	0.5±0.1	0.5±0.1	*P*<0.05
Biochemical data				
	FBG (mmol/L)	4.8±0.7	4.5±0.5	*P*<0.05
	FSI (pmol/L)	63.1±1.7	20.9±1.5	*P*<0.05
	HOMA-IR	2.5±1.9	0.6±0.2	*P*<0.05

All the characteristics are described as mean±standard deviation. Means were significant (two-tailed) at $P<0.05$

WC waist circumference, *HC* hip circumference, *MUAC* mid-upper arm circumference, *BMI* body mass index, *WHR* waist-to-hip ratio, *WHtR* waist-to-height ratio, *FBG* fasting blood glucose, *FSI* fasting serum insulin, *HOMA-IR* homeostatic model assessment of insulin resistant

the mean of whole study population which was 24.1± 4.1 kg m^{-2}and much higher than the BMI of subjects who had HOMA-IR less than 1 which was only 22.6± 3.7 kg m^{-2}. But, among the insulin-resistant subjects, 60.8 % were overweight or above (BMI ≥23 kg m^{-2}). The percentage of participants classified as insulin-resistant was highest among obese subjects, which was 75.3 %.

All the continuous, anthropometric parameters were used in a standard regression analysis to predict IR as independent variables. Stepwise multiple linear regression analysis was performed for the continuous variables in the total population to find out significant determinants for HOMA-IR. The raw and standardized regression coefficients of the predictors together with their correlations with IR, their squared semi-partial correlations, and their structure coefficients are shown in Table 2. Even though BMI, WHR, and WC were selected, and both WHtR and MAC were excluded in both males and females, the correlation of the variables, except height, had statistically significant (P=0.000) difference. According to the stepwise regression analysis carried out, the prediction model had two of the eight predictors for the total population. The model was statistically significant, F (227) = 29.269, P<0.01. It suggests that the effect of BMI on HOMA-IR was dominant compared with other parameters (P=0.000) and to a lesser extent by higher WHR levels. In male population, HOMA-IR was primarily predicted by BMI (F=46.128, P<0.01), whereas in females, it was WC (F=23.450, P<0.01).

In binary logistic regression analysis, odds ratios of the anthropometric parameters are given in Table 3. Among the total population, relative risk of BMI and WHtR had a higher association with IR. In males, BMI as well as WC had high relative risk for IR, and in females, WHtR and WC had high relative risk.

Discussion

Variety of anthropometric techniques is being used to identify obesity and body fat distribution, and obesity itself is used as a marker to predict IR. Central obesity identified by WC and IR are considered as underlying causative factors of metabolic syndrome and T2D. Several studies have been carried out to identify the association of one or more anthropometric parameters with the high risk for developing above metabolic abnormalities in different populations with various conditions. Measures of central obesity as well as generalized obesity have been studied for positive predictive value in different populations with varying results. The goal of this prospective study was to identify the link between anthropometric parameters and IR using HOMA-IR index and to identify the most suitable anthropometric parameter which would optimize clinical prediction of IR in apparently healthy, non-diabetic, male and female subjects specifically. To our knowledge, this is the study first to predict IR using anthropometric parameters among apparently healthy subjects, carried out in a Sri Lankan population. It showed that apparently healthy, non-

Table 2 Stepwise regression analysis for predicting IR

Model	b	SE-b	Beta	Pearson correlation	sr^2	Structure co-efficient
Total population (n=227)						
Constant	−0.794	0.125				
BMI*	0.037	0.005	0.431	0.431	0.185	1.010
WHR*	0.674	0.276	0.158	0.303	0.025	0.665
Males (n=91)						
Constant	−1.371	0.219				
BMI*	0.061	0.009	0.584	0.584	0.341	1.000
Females (n=136)						
Constant	−0.882	0.202				
WC*	0.012	0.002	0.386	0.386	0.144	0.999

The dependent variable is insulin resistance. sr^2 is the squared semi-partial correlation

WC waist circumference, *BMI* body mass index, *WHR* waist-to-hip ratio

*Significance at $P<0.05$

diabetic individuals had higher prevalence of IR with elevated anthropometric parameters. Linear and logistic regression analyses revealed that, among all the anthropometric parameters, BMI and WC are considered as the best predictors of IR in males and WHtR and WC in females.

Hyperinsulinemia and IR are two markers that are being used to identify subjects who have high tendency to develop IR-related metabolic abnormalities such as T2D and cardiovascular diseases [7]. Most of the studies related to IR have been carried out among subjects with either T2D or some other disease conditions [18–20]. Only a few studies have used random samples from the general populations [21]. The incidence rate of IR in our apparently healthy study population was 59.9 % which was comparatively higher than the prevalence among Chennai (11.2 %) urban subjects, [22] and the recorded IR prevalence in a Chinese population (7.2 %) [23]. This greater incidence rate of IR among non-diabetic subjects may account for racial and ethnic differences among the populations, and furthermore, studies have also showed that South Asians are more insulin resistant than Caucasian individuals independent of their adiposity and body fat distribution [22]. According to literature, South Asian Indians have a higher tendency to develop MS and T2D, perhaps due to genetic predisposition at smaller body sizes with increased central adiposity in the presence of a lower BMI [16]. Thus, screening of both glucose and serum insulin levels and calculating IR even at a lower level of BMI would be advantageous to minimize the risk of developing metabolic abnormalities.

Overweight and obesity had been positively associated with IR and hyperglycemia especially in subjects with T2D and CVD risk factors [24]. Even in non-diabetic individuals, if they are overweight or obese, there is a high tendency to become IR or hyperinsulinemic [25]. In addition, excess regional adiposity is considered as a major causative factor of IR [26]. The incidence of obesity among IR subjects was 49.3 % with a mean BMI of 28.3±2.7 kg m^{-2}, which was greater than the average BMI of whole study population (24.1± 4.1 kg m^{-2}), and 60.8 % of insulin-resistant subjects were overweight or above. This shows that subjects with overweight have a tendency to have high IR. South Asians with high IR were found to be obese. The occurrence of high percentage of obesity (75.3 %) among the more IR population indicates that Sri Lankans, too, have a high tendency to develop MS. These findings are in line with the several other

Table 3 Odds ratio for the anthropometric risk factors associated with insulin resistance.

Anthropometric parameters	Total (n=227) OR (95 % CI)	Males (n=93) OR (95 % CI)	Females (n=134) OR (95 % CI)
BMI (kg m^{-2})	2.91 (1.668–5.101)*	4.35 (1.740–10.869)*	2.33 (1.140–4.776)*
WHtR	2.87 (1.638–5.045)*	2.86 (1.188–6.870)*	2.88 (1.384–6.009)*
WHR		2.25 (0.942–5.374)	2.52 (1.234–5.160)*
WC (cm)		3.92 (1.309–11.708)*	2.72 (1.307–5.660)*

WC waist circumference, *BMI* body mass index, *WHR* waist-to-hip ratio, *WHtR* waist-to-height ratio, *OR* odds ratio

*Odds ratio significant at $P<0.05$

earlier study findings [27, 28]. Further, the elevated IR level was positively associated with increased anthropometric parameters. Increasing IR has been found to be linked with increased subcutaneous as well as abdominal adipose tissue in Asian Indians [29]. Thus, increasing IR is inclined to give increased anthropometric parameters in non-diabetics. These findings indicate that IR directly effects by causing obesity and it influences individuals to gain weight physiologically [28]. Therefore, the above outcomes ensure that identifying generalized or regional adiposity has a significant relationship with identifying IR related metabolic abnormalities. Since the prevalence of IR was higher in our study, it would be better to carry out further investigations on a larger sample to find out the prevalence among healthy Sri Lankans.

When we assess the IR prevalence as well as the metabolic risk among the two genders, based on WC and WHR, the percentage of female insulin-resistant subjects, who were at the metabolically risk category, was greater than the percentage of males. Deposition of body fat varied among two genders. Females tend to deposit more fat peripherally when compared to central deposition in males. And, these findings were supported by the earlier reports of Alemzadeh and Kichler [30]. Therefore, female subjects have a high tendency to develop IR-related metabolic abnormalities which suggests that lower total lean body mass and greater fat mass may play a strong role in pathogenesis of IR and related obesity among females.

Regression analysis indicates that measures of central adiposity or measures of general obesity alone cannot predict the overall adiposity vis-à-vis IR among the apparently healthy subjects. In contrast, the results indicate that both anthropometric measurements and indices in determining central or generalized adiposity are collectively corporate in predicting IR. Present study found that both WHtR and BMI are better measures to assess the metabolic abnormalities in a general population with both genders. Further, we found BMI and WC as better predictors of IR among males and WHtR and WC for females.

BMI is an index which reflects the measures of relative weight. Further, categorizing of body weight which includes degrees of underweight and overweight is based on BMI. Hence, it is an acceptable alternative for thinness and fatness that have been directly related to health risks and death rates in many populations [31]. It has been used traditionally as an indicator. However, classical cutoffs of BMI among South Asians showed that even at relatively low levels of obesity, subjects are more prone to develop T2D as South Asians have higher amounts of body fat deposition in abdominal areas [16]. In comparison with general obesity, defined by BMI alone, site of excess body fat deposition is a predominant factor of determining the tendency to develop MS and related diseases like T2D and coronary heart diseases as abdominal obesity is significantly linked with MS [19]. Therefore, involvement of a central adiposity measurement adds to a great advantage.

Out of the measures of central obesity, waist circumference (WC) is the most widely used central adiposity measure as it has a good prognostic value in identifying metabolic abnormalities [13]. According to our study findings, even though WC is a primary predictor of IR in females, males with high WC also have a high risk of developing IR. WC has gender specific cutoff points to assess risk of getting co-morbidities of obesity, as males have a greater total lean mass, bone mineral mass, and a comparatively lower fat mass than females. Female subjects have considerably visceral adiposity than males. Hence, WC is attributed to predict IR in males and females separately, using different cutoff values.

However, WC is dependent upon the height measurement of a subject; hence, WHtR provides a better tool for assessing the metabolic risk in a population with short stature such as South Asians [19]. Ashwell and Hseih [14] have shown that WHtR is more sensitive than BMI as an early predictor of health risk. In our study, RR of WHtR in predicting IR among both males and females was higher (more than two times). Thus, our study findings stated that WHtR is a dependable and an effective anthropometric index to identify metabolic risk among Sri Lankan general population, and it was in corroboration with the findings of Jayawardana et al. [19]. Thus, the very high odds ratios of WHtR $\geq$0.5 found for IR in general Sri Lankan adults can be used to detect the risk of developing metabolic abnormalities.

WHtR was found to be a better predictor of IR in females, and such findings were earlier reported in Asian populations, such as Taiwanese and Japanese. This indicates that subjects with short stature and a large waist would have a high risk in developing obesity related co-morbidities and thus proving the concept of "keeping your waist to less than half your height". In contrast to our findings, Jayawardana et al. [19] have shown a higher correlation between WHtR and disease risk in males. Therefore, further studies based on gender-specific WHtR cutoffs to identify the risk of metabolic abnormalities are needed to validate the above findings.

The limitations of the present study included a convenience sample with cross-sectional study design. Furthermore, HOMA-IR assessment has a limitation of being a static measurement of insulin function and requirement of a defined HOMA-IR cutoff for apparently healthy subjects. Comparatively large sample size, representing the majority of the society, and its applicability to the real world are the strengths of this study as the expenses were at low, and evaluation in a community setting. Our emphasis on an apparently healthy, non-diabetic population is the strength of this study, and it would be of advantage if apparently healthy subjects with elevated, abnormal BMI, WC, and WHtR calculate their IR in order to identify the risk of developing IR and metabolic abnormalities prior to onset. These costless, self-monitoring

anthropometric measures are reliable tools to encourage the increased practice of screening for IR, in a community setting with apparently healthy subjects.

Conclusions

This study demonstrates that with elevated anthropometric parameters, higher prevalence of IR is present in apparently healthy individuals. Among the anthropometric parameters, BMI and WC have been found to be the best predictors of IR in males and WHtR and WC in females. IR levels could be used in healthy individuals as a "warning" sign which could be made easier by measuring simple anthropometric measures suitable for different sexes.

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Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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.

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

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ORIGINAL ARTICLE

Effect of glycaemic control on the diurnal blood pressure variation and endogenous secretory receptor for advanced glycation end product (esRAGE) levels in type 1 diabetes mellitus

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Abstract The relationship between plasma endogenous secretory receptor for advanced glycation end product (esRAGE) level and diurnal variation of blood pressure in type 1 diabetic patients based on their glycemic status has not yet been studied. So, the aim of the present study was to see if a correlation exists between plasma esRAGE levels and dipper status in type 1 diabetic patients based on their glycaemic status. Type 1 diabetic patients were divided as good and poor glycaemic controlled groups based on their HbA_{1c} values. Blood glucose levels, insulin, insulin antibodies, homocysteine and esRAGE levels were determined. The hourly daytime and night-time blood pressure was monitored by using a non-invasive blood pressure (NIBP) apparatus. Of the 16 patients in the well-controlled group, most (14/16) were dippers (95 % confidence interval 1.5 to 20.7 vs. $P=0.006$, Fisher's exact test) when compared to those in the poorly controlled group (5/18). There was a significant correlation between plasma esRAGE levels and % decline in nocturnal blood pressure in both the good ($r=0.69$, $P=0.003$) and poorly ($r=0.79$, $P=0.0002$) controlled groups. Most of the poorly controlled patients showed abnormal circadian blood pressure pattern and low esRAGE level and hence were more prone to end organ damage.

Keywords Diabetes mellitus · Dippers · esRAGE

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Introduction

The most deleterious implications of poor glycaemic control in diabetes mellitus is the hyperglycaemia it causes culminating in the acute and chronic complications of diabetes. One of the mechanisms involved in the pathogenesis of diabetic vascular complications is production of advanced glycation end products (AGE). AGE are formed by binding of excess glucose to tissue protein, lipid and DNA [1]. The endogenous secretory receptor for advanced glycation end products (esRAGE) are receptors which bind and neutralize the action of AGE. Hence, esRAGE is an indicator of end organ damage status in diabetic patients [2].

Persons in whom night-time mean blood pressure (BP) decreases by more than 10 % when compared to daytime BP are classified as dippers, and if this decline in nocturnal BP is absent, they are non-dippers [3]. It has been found that diabetics exhibit abnormal circadian blood pressure variation leading to impaired fall of nocturnal BP [4]. Absence of this nocturnal decline in BP is an index of future end organ damage particularly nephropathy [5] and autonomic nervous system disturbances [6].

The implications of this non-dipping phenomenon in relation to the glycaemic status in type 1 diabetes mellitus patients (T1DM) and its role in the pathogenesis of diabetic complications are not yet well established. Hence, the first objective of our study was to see the presence of end organ damage status in the well-controlled and poorly controlled T1DM patients by usual biochemical methods, esRAGE levels and nocturnal dipper status. The next objective was to see if a correlation exists between plasma esRAGE level (invasive technique) and diurnal variation of blood pressure (non-invasive

technique) in the well-controlled and poorly controlled T1DM patients.

Material and methods

Subjects It was a comparative study conducted during May–July 2006 in which normotensive T1DM patients were recruited from the diabetes clinic in JIPMER, Pondicherry, India. The inclusion criteria were as follows: (1) patients in the age group of 18–60 years; (2) non-hypertensive, type 1 diabetic patients with disease duration of more than 3 years; and (3) patients collecting free monthly issue of insulin vials from the JIPMER hospital pharmacy. The exclusion criteria were the following: (1) patients with underlying liver or kidney disease, (2) patients taking oral hypoglycaemic agents, (3) patients taking other drugs which interfere with plasma glucose, (4) patients collecting insulin by proxy and (5) patients with HbA_{1c} level between 7 and 8 %. The study was cleared by the Institute Research Council and Institute Human Ethics Committee, and written informed consent form was obtained from patients who agreed to participate in the study.

Definitions and categorical cut points Fasting blood glucose <130 mg/dl and post-prandial blood glucose <180 mg/dl was considered as good glycaemic control. HbA1c level >7 % was considered as poor glycaemic control [7]. Hypertension was defined by blood pressure >140/90 mmHg [6]. C-peptide level of <0.3 pmol/ml was regarded as negligible endogenous insulin secretion [8]. Normal renal function was defined by GFR≥ 75 ml/min 1.73 m^2 the median value [9]. Ankle brachial pressure index (ABPI) ≥0.9 was considered normal [10].

Methodology On the day of study, the patients were advised to come after an overnight fast. Demographic variables were recorded, anthropometric values were measured and blood was collected from the type 1 diabetic patients to determine lipid profile, liver and renal function tests, pre-prandial blood glucose levels, HbA_{1c}, insulin, insulin antibodies, C-peptide, homocysteine and esRAGE levels. They were asked to void urine which was discarded. The urine was collected for the next 24 h, and this was used for estimating 24-h urinary protein and creatinine. Urine microalbumin level was also measured. Glomerular filtration rate was calculated using serum creatinine levels using the Modification of Diet in Renal Disease (MDRD) formula [11]. Blood for measuring post-prandial blood glucose was withdrawn 2 h after breakfast. ECG, blood pressure and ABPI were measured. ABPI was calculated by dividing the systolic BP in the ankle by the systolic BP in the arm. Each individual was given a regular south Indian breakfast which consisted of four idlis, chutney and half cup of sambar. Blood for measuring post-prandial blood glucose was withdrawn 2 h after breakfast. The patients were taken to the ophthalmology department where retinopathy was assessed by fundus examination after pupillary dilation. The retinopathy was graded as nil, mild, moderate or severe proliferative retinopathy. ECG and 24-h blood pressure monitoring were done using a non-invasive blood pressure (NIBP) apparatus.

Monitoring 24-h blood pressure The hourly daytime (9 am to 9 pm) and night-time (10 pm to 6 am) blood pressure was monitored by using the NIBP apparatus (Life Sciences, USA). Mean blood pressure was used as the blood pressure index for classifying dipper status. The nocturnal reduction rate of mean blood pressure was calculated according to the formula

$$\%\,\text{reduction of nocturnal BP} = \frac{\text{day time mean BP–night time mean BP}}{\text{day time mean}} \times 100$$

$$\text{Mean blood pressure} = \text{diastolic BP} + 1/3\,\text{pulse pressure}.$$

Dippers were defined as patients whose mean sleeping blood pressure is 10 % less than the blood pressure while awake [12].

Patients were divided into two groups: well-controlled group also called as good-control group and poorly controlled group (poor control) based on their HbA_{1c} values. Patients with an HbA_{1c} value less than 7 % were classified as well-controlled (good-control) group and those with a value above 8 % were considered as poorly controlled (poor-control) group. The patients with HbA_{1c} level between 7 and 8 % were ignored since it could be a borderline value. Thus, there were 16 patients in the good glycaemic control group and 18 patients in the poor glycaemic control group. The data of 34 patients were finally analysed.

Estimation of esRAGE One hundred microlitres of esRAGE antibody HRP conjugate was added to antibody-coated wells. Then, 20 μl of assay standards or samples was added to each well and mixed well. The plate was covered securely with a plate sealer and incubated in a refrigerator for 16 h. The reaction mixture was aspirated from each well, and the plate was washed with 350 μl of wash solution over four times and the contents aspirated each time with a microplate washing device (Labsystems Well Wash 4 Mk 2, MTX Labsystems Inc., Vienna, USA). After the final wash, the plate was inverted and tapped firmly against the absorbent paper. One hundred microlitres of substrate was added to each well. The plate was covered with aluminium foil and incubated for 30 min at 25 °C. One hundred microlitres of stop solution was added into each well. The optical density was measured at 450 nm using an ELISA reader (Labsystems Multiscan Elisa reader, MTX Labsystems Inc., Vienna, USA) within 15 min. The calibration curve was plotted, and the concentration of the

unknown values was obtained by interpolation from the calibration curve (B-Bridge International Inc., CA, USA).

Statistical analysis Data is presented as mean±SEM. Variables between the two groups were compared using unpaired Student's *t* test. Pearson's correlation coefficient was used to correlate between esRAGE and nocturnal decline in blood pressure. $P<0.05$ was considered significant. GraphPad InStat version 3.06 was used for all statistical analysis.

Results

Forty patients were recruited for the study of which there was one drop out. Of the remaining 39 patients, 5 patients had HbA_{1c} between 7 and 8 % and hence their data was not analysed. Out of the remaining 34 patients, there were 16 patients in the good glycaemic control group and 18 patients in the poor glycaemic control group. There was no significant difference in the demographic or anthropometric measurements between the two groups. The poorly controlled groups were older, weighed less and had the disease for a longer duration of time (9.3±1.2 vs. 10.7±0.8 years) when compared with the well-controlled group though none of these parameters were statistically significant. Also, there was no significant difference in the lipid profile; liver and renal function tests between the two groups were within normal limits.

Pre-prandial blood glucose, plasma insulin, insulin antibodies and homocysteine did not differ significantly between the two groups. However, there was a significant difference in the post-prandial blood glucose, HbA_{1c}, esRAGE, % nocturnal decline in blood pressure, microalbumin and ABPI between the two groups. Nearly 37 % (6/16) and 83 % (15/18) of patients in the good and poorly controlled groups respectively had their post-prandial blood glucose levels above the normal reference range.

Patients in the well-control group (Table 1) had significantly higher esRAGE level (0.8±0.1) when compared to the poorly controlled (0.4±0.1) group ($P<0.05$ using unpaired *t* test). Plasma esRAGE (ng/ml) was directly associated with % decrease in nocturnal reduction of BP both in the good glycaemic control ($r=0.69$) and poor glycaemic control ($r=0.79$) group ($P<0.05$) using Pearson's correlation coefficient.

There were a significantly higher proportion of dippers (87.5 %, 14/16) in the well-controlled group and non-dippers (72.2 %, 13/18) in the poorly controlled group ($P<0.05$ using Fisher's exact test). There was no significant difference in the daytime, night-time and mean systolic or diastolic blood pressure between the two groups. However, there was a significant difference in the % decrease in nocturnal reduction of mean BP between the good and the poorly controlled groups (i.e. 11.1±0.9 vs. 8.4±0.9, $P<0.05$ using unpaired *t* test).

Table 1 Clinical metabolic findings of the patients in the well-controlled and poorly controlled groups

Variables	Good control ($n=16$)	Poor control ($n=18$)
Pre-prandial blood glucose (mg/dl)	128.3±9.3	157.7±17.6
Post-prandial blood glucose (mg/dl)	184.9±11.2	260.7±24.6*
HbA_{1c} (%)	6.8±0.02	8.8±0.2**
Homocysteine (μmol/l)	6.6±0.73	7.6±0.73
ABPI	1.3±0.03	1.0±0.05*
esRAGE (ng/ml)	0.8±0.1	0.4±0.1*
Microalbumin (mg/l)	10.4±1.8	25.1±6.3*

Data presented as mean±SEM

*$P<0.05$ and **$P<0.0001$ using unpaired *t* test

Nearly 6 % (1/16) in the good-control group and 22 % (4/18) in the poor-control group had abnormal ABPI values. Microalbuminuria was present in 6 % (1/16) and 34 % (6/18) respectively of the patients in the good and poorly controlled groups. Mild retinopathy was found in 6 % (1/16) of the patients in the well-controlled group, while in the poorly controlled group, 17 % (3/18) had mild retinopathy.

Discussion

Our study showed that the well-controlled group had more number of dippers and higher esRAGE levels and the poorly controlled group had more number of non-dippers and low level of esRAGE levels. Also, retinopathy, microalbuminuria and abnormal ABPI were more prevalent in the poorly controlled group. There was a significant difference in the microalbumin level between the two groups, supporting a previous study that microalbuminuria was more prevalent in poorly controlled non-dippers [13]. Even though the poorly controlled group had low ABPI values, it was not reflected in the plasma homocysteine levels. Thus, there was no correlation between these two variables in our study which is consistent with one study in the Indian population which concludes that there was no correlation between plasma homocysteine levels and vascular disease [14].

Levels of esRAGE were low in these diabetic patients when compared to healthy volunteers [15]. There was a significant difference in the esRAGE level and nocturnal decline in blood pressure in both the well-controlled and poorly controlled groups. The high blood glucose levels in the poorly controlled group could have led to decrease in esRAGE levels leading to the increased incidence of diabetic complications and more numbers of non-dippers in the poorly controlled group. Thus, there was a direct association between the esRAGE and nocturnal decline in blood pressure in both the groups. Since AGE causes damage to vascular walls [1, 2],

this can affect the blood vessels supplying the nerve and kidney which could have led to a decrease in the autonomic function (autonomic neuropathy), nephropathy and fluid retention [5, 6], affecting the nocturnal reduction in blood pressure. Thus, the non-dipping state in these patients could be due to diabetes itself. These findings stress the fact that glycaemic control is necessary to prevent any diabetic complication.

Patients were from a poor socioeconomic status, and nearly 43 and 72 % of the patients in the good- and poor-control groups respectively were underweight. But this could also be due to muscle wasting in diabetes. Poorly controlled patients were older (though not to a statistically significant level) when compared to the well-controlled group. Since the process of formation of AGE increases with age, this could have also contributed to the decreased esRAGE level in this group of patients [16].

Strengths of the study Since daytime activity and night-time sleep will affect blood pressure monitoring, the study was done after hospitalizing the patients, allowing better monitoring of blood pressure with respect to physical activity [17].

Limitations of the study We could not account for the causal relationship between dipper status and plasma esRAGE level, though a correlation existed between these two variables. However, further studies with increased sample size are required to establish the causality between the esRAGE levels and dipper status in type 1 diabetes mellitus patients. We could not account for a larger sample size since the study population was restricted to our hospital patients collecting monthly insulin vials free of cost from the hospital pharmacy. Of this, we could get only 16 patients who were glycaemically well controlled. Also, if we had measured autonomic dysfunction which is mostly associated with a non-dipping profile, it could have given us a better insight regarding this issue.

The poor end organ damage status in most of these patients was due to hyperglycaemia and its pathophysiological mechanisms leading to increased AGE level and poor dipper profile in these patients. Since most of the patients with poor glycaemic control showed an abnormal circadian blood pressure pattern and were more prone to end organ damage as reflected by their non-dipping BP profile, low esRAGE levels and other biochemical parameters, this study further reinforces the need for strict glycaemic control.

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Compliance with ethical standards The study was approved by the Institute Research Council and Institute Human Ethics Committee. Written informed consent form was obtained from patients who agreed to participate in the study.

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ORIGINAL ARTICLE

Epidemiological transition in type 2 diabetes mellitus: the role and function of a sub-district health promoting hospital

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Abstract Health authorities in Thailand implemented a policy especially to cope with chronic diseases in that they transformed former health stations into sub-district health promoting hospitals (SHPH). The "top down" measure left the staff of the SHPH somewhat confused and uncertain how to manage their duties effectively. One of the weak points was the questionable outreach of care into the community. In an attempt to demonstrate how to enhance community participation, one SHPH was selected and assisted in improving the care of type 2 diabetes mellitus (T2DM) patients. Patients from another SHPH served as controls. The combined efforts of patients, their families, SHPH staff, and the community with the help of village health workers succeeded in improving the control of T2DM in that a steady decline in capillary blood glucose (CBG) could be observed and the results could also be verified by the determination of glycated hemoglobin (HbA1c).

Keywords District health promotion · Type 2 diabetes mellitus · Thailand

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Introduction

The disease pattern of low and middle income countries like Thailand shifted from infectious towards chronic diseases and linked to demographic changes and nutritional aspects [1]. Among those challenges, type 2 diabetes mellitus (T2DM) turned out to be a major public health problem. A nationwide survey in Thailand estimated the prevalence of T2DM to be 9.6 with 4.8 % new cases in 2000 and 6.7 % in 2005 [2, 3]. The health authorities, in particular, the Ministry of Public Health (MoPH) of Thailand, inaugurated policies to cope with the increasing burden of chronic diseases and that included the attempt to transfer the former primary care units to the now called sub-district (tambon) health promoting hospitals (SHPH). These institutions are located at the lower level of the governmental health delivery system and are supposed to be instrumental in chronic care management [4]. This initiative was executed as a top down approach and left the officials at the lower level of the health delivery system somewhat confused and uncertain how they should perform their duties within the new established units. It was the aim of this investigation to assess in a first phase of the study the perception of the staff of SHPH about the role and functions of their institutions. After identifying misunderstandings and problems faced, a second attempt was undertaken to demonstrate how an SHPH can address a pressing public health problem related to a chronic disease and as an example T2DM was selected.

Subjects and methods

Study area

The project was conducted in two sub-districts of the Warinchamrap District of the Ubonratchathani Province of Thailand. The two sub-districts, namely Nonkasem and Rasamran, were 20 km apart from each other. The SHPHs of both districts served a total population of 3000 to 7000 individuals living in 5 to 10 villages. The sub-districts were selected on purpose and could be considered typical for the rural area of the Ubonratchathani Province and northeastern Thailand as a whole. Nonkasem sub-district was selected as intervention, and the Rasamran sub-district served as the control area.

First phase of the study Investigating the perception of the staff of the SHPH about the role of their institutions.

Selection of participants

A sample of 300 health personnel working in SHPHs was selected following the procedures as explained below: The method employed in stage1 was simple random sampling. The whole province consists of 25 districts with a total number of 325 SHPHs, divided into 4 zones as follows: zone 1, 2, and 3 comprise 6 districts each with 81 SHPHs per zone, and Zone 4 consists of 7 districts with 82 SHPHs. The technique used in stage 2 was probability proportional to size. A total number of 13 SHPHs was allocated as follows: 4 SHPHs for zone 1 and 3 SHPHs for each of the zones 2, 3, and 4. The final step consisted of random selection of 75 health officials from each zone, the total of whom summed up to 300 individuals.

Content of questionnaire

The questionnaire consisted of 80 statements to which the respondent could agree or disagree. For this communication, only 15 statements are selected since they best represent the participant's perception about the role of the institutions they were working with. The most relevant answers to the questions are summarized in the result section.

Second phase of the study: Since the result of the questionnaire revealed substantial deficiencies in the perception of the staff of the SHPH in the role and possible functions especially towards prevention and community outreach, an intervention project had been started to demonstrate the ability of the SHPH to make an inroad into the community and into secondary prevention of T2DM.

Selection of participants

For estimating the number of participants for the intervention, a sample size calculation was done and 90 patients with T2DM were selected, 45 patients from each of the 2 participating SHPHs. Each group of patients had been purposively selected, and one served as intervention and the other as the control group. The patients had been selected by simple random sampling out of a total of 186 patients. Inclusion criteria had been to be an ambulatory patient diagnosed to have T2DM and being under oral medication with metformin. Blood glucose levels of all the patients selected had to be found to exceed 130 mg/dl two times prior to entering the project indicating that the patients have difficulties in keeping their blood glucose levels in an acceptable range. According to the Ministry of Public Health, a normal blood glucose level should not exceed 125 mg/dl. Patients under insulin treatment and diabetes related complications were not taken. None of the socio-demographic characteristics of the participants of the two groups differed statistically significantly. The proportion of females outnumbered those of males. The majority of participants was over 50 years old and married.

Procedures

The quasi-experimental study took place for 12 weeks. Once a week, fasting capillary blood glucose (CBG) levels were measured by glucometer (Accu-chek, Roche Diagnostics). Also, glycated hemoglobin (HbA1c) was assessed at the beginning and the end of the project.

In Table 1, the main activities of the intervention aspect of the project are given. The care for patients of the experimental group was more intensive in comparison to the control group in that they were invited to participate in an introductory meeting at the beginning of the intervention period to be informed about the nature and problems of diabetes mellitus and how to improve self care and control their dietary intake. The care for the patients of the experimental group was assisted within and by the community in that not only the respective health personnel from the SHPH but also the village health volunteers performed the tasks. The control group was exposed to the regular service for T2DM outpatients of the SHPH including occasional health education, assessment of problems according to home health care by health personnel if appearing necessary, and checking for CBG once a month and inspecting the feet of the patients.

Laboratory investigation

CBG was determined using the cut of point <130 mg% by glucometer (Accu-chek, Roche Diagnostics) and HbA1c with the cut of point <7.0 % by Cobas Intregar 400/800, Tina-quant Hemoglobin A1c gen.2 (URIA Co.,Ltd.). Cut of points were

Table 1 Participation of stakeholders in project activities

Topic	Patients and family	SHPH	Community (village health volunteer, VHV)
1. Daily self assessment for discussion with staff from SHPH during home visit	+	0	0
2. Information about the nature and dangers of DM	+	+	+
3. Seeking to solve problems of individuals identified by self assessment with help from SHPH and VHV	+	+	+
4. Monthly meeting to exchange experiences in managing DM disease	+	+	+
5. HbA1c determination every third month	+	+	0
6. CBG determination every week by trained VHV	+	0	+
7. Home visits from staff of SHPH and VHV	0	+	+
9. Villagers informed about prevention of DM	+	+	+
10. Raising of budget for CBG testing and other community activities from various sources	0	+	+

Remark: + = do activity, 0 = no activity

selected according to the diabetes clinical practice guideline of the MoPH [5].

Statistical evaluation

For data evaluation, the statistical software SPSS version 19 was applied. Results of the questionnaire are given as numbers of answers and proportions while statically significantly differences between the intervention- and the control group for CBG and HbA1c the mean ± standard deviation of the independent sample *t* test and the ANCOVA test had been applied [6]. The ANCOVA test was used to assess differences of baseline characteristics between the two groups or control covariates comparing the pre-test with the final assessment.

Ethical consideration

The study had been approved by the Ethics Committee for Research Involving Human Subjects of the Mahasarakham University (Approval no. 0136/2011) in that individual interview results had been kept completely confidential and the consent to participate in the study was entirely voluntary. The comparison group had been treated by the same intervention after 6 months of the research.

Results

The most significant answers of the staff of the SHPH are given in Table 2. The SHPH staff questioned expressed the opinion that, for them, the objectives of the functions of SHPH are clear and that they did understand the goals of the initiative to establish SHPH. The majority of the staff also stated that they were clear about the priorities the SHPH should have in dealing with the objectives of the role of the institutions but they were almost equally divided about whether the role of the key player in the overall scheme was clear or not. Only a minority of about 30 % were of the opinion that an improvement of the quality of the service of the SHPH can be

Table 2 Excerpt of answers from 300 questionnaires about policy, mission, organization, attitude of staff, and resources concerning the sub-district health promotion hospital (SHPH)

Questions	Strongly agree/agree *N*%	Neither-nor/ disagree/disagree strongly *N*%
Policy		
Objectives of SHPH are clear.	250 (83.3)	50 (16.7)
Health personnel understand the goals of the policy.	230 (76.7)	70 (23.3)
Mission		
Priority stages of the policy are clear.	198 (66.2)	101 (33,8)
Roles of agencies related to SHPH are determined.	148 (49.3)	152 (50.7)
Organization		
Coordination inside and outside the SHPH smooth.	129 (43.0)	171(57.0)
Developing of the quality of service is possible.	98 (32.7)	202 (67.3)
SHPH is beneficial for the community	219 (73.0)	81 (27.0)
SHPH gets sufficient budget support from local administration.	105 (35.0)	195 (65.0)
Attitude of officials		
Prevention should be more important than curative services.	266 (88.7)	34 (11.3)
Prevention hampered by work overload.	143(47.7)	157(52.3)
SHPH does not differ much from former PCU.	176 (58.7)	124 (41.3)
Community outreach results in work overload.	110 (36.7)	190 (63.3)
Resources		
Budget of SHPH is sufficient.	81 (27.0)	219 (73.0)
Circumstances for working are adequate.	181(60.3)	119 (39.7)
Knowledge and skills for staff are continuously improved.	56 (18.7)	244 (81.3)
System support from central government sufficient.	88 (29.3)	212 (70.7)

achieved. The overwhelming majority of respondents were certain that the SHPH was beneficial for the communities, but more than 50 % doubted that they got enough support moneywise from the local authorities. Almost 90 % of the staff questioned was convinced that prevention of diseases should be a major item of activities of SHPH, but almost 50 % thought that this effort was hampered by work overload of the staff. When it came to judge about the available resources, the majority of respondents bemoan an insufficient budget but the majority was content with the improvement of knowledge and skills of the staff and the support they received from the central government.

The ultimate achievement of the project is given in Table 3 in that concentration of CBG and percentage of HbA1c significantly declined at the end of the project in comparison to baseline data for the intervention group in contrast to the controls.

Discussion

It is not uncommon for Thailand that such initiatives as the transfer of primary care units (PCU) into SHPH are executed as a top down approach [7]. The somehow hasty launch of such initiatives often leaves the officials on the lower level of the health delivery system confused. The confusion in Thailand was aggravated by a change of the national administration towards decentralization [8]. Down to sub-district level, the administrative management was given to elected representatives of the population who rule the spending of budgets derived partly from the government and partly from locally inaugurated taxes [9]. The Tambon Administrative Organization (TAO) decides about matters concerning the community from road building up to public health initiatives. The role of the TAO and the district health office in connection with the SHPH remained largely unclear since the policy steps were taken without clearly defined responsibilities. No wonder that the SHPH staff were unsure who would be the key player in the health delivery system at the bottom of the system. Therefore, about 70 % were of the opinion that an improvement of the quality of the service cannot be achieved. Although the majority of staff was of the opinion that the SHPH should be of benefit for the community and should concentrate on prevention of diseases, they doubted that they had enough time besides their routine work within the hospital and they were also skeptical about the availability of the necessary amount of resources in order to provide an optimal service to the community.

The second part of this study intended to find out whether the skepticism of the staff themselves counteracted defining their role within the health system and testing their impact on the community. T2DM, as mentioned above, is a major health problem in Thailand. Recently, it was found out that diabetic patients know quite well about the disease and how they should care for themselves, but, contrary to their knowledge and intention, their actual ability to sufficiently control the disease remained very weak [10]. The selection of T2DM patients within the community as a trial group, therefore, seemed to be meaningful. The close involvement of the family of the patient and the village health volunteers within the community under the guidance of the SHPH combined the efforts of the health staff of the curative sector with the community as being suggested by the chronic care model. The village health volunteers as well as the family members of the patients provided resources for the community work and assisted the staff of the SHPH to reduce time for community work. Taking the improvement of CBG and HbA1c for the intervention group as indicators, the project activities including improving knowledge about T2DM, self-efficacy, and social support were successful. This "vertical approach" over the boundaries of responsibilities, in that the supervision of the village health volunteers remains with the district health

Table 3 Capillary blood glucose (CBG) and glycated hemoglobin (Hb1Ac) levels at baseline and post-test of the intervention and control group

Variables	Measuring point	Intervention Mean ± SD	Controls Mean ± SD	Difference: intervention to controls	*p* value
CBG (mg/dl)	Baseline	184.4 ± 7.1	183.7 ± 7.3	0.5	0.005
	Post-test	128 ± 9.9	185.2 ± 6.6	−56.5	0.005
	Difference: baseline to post-test	−55.7	1.4		
	p value	0.000	0.052		
ANCOVA	*p* value	0.000			
HbA1c	Baseline	13.6 ± 1.7	13.4 ± 1.7	0.2	0.908
	Post-test	7.4 ± 1.4	13.7 ± 1.9	−6.3	0.000
	Difference: baseline to post-test	−6.2	0.3		
	p value	0.000	0.164		
ANCOVA	*p* value	0.000			

office, proved to be a meaningful approach and demonstrated to the staff that they can optimize their work for the benefit of the community.

A similar study on this investigation had been conducted on a wider scale in Thailand recently by interviewing the heads of sub-districts health centers [11]. It was found that "core competencies of the heads of THC (sub- district health centers) in chronic disease prevention and control were found at the 'somewhat good' level except for the work skill domain which needed to be developed". By and large in comparing the results of the questionnaire of this communication with those from Leerapan et al., it seems that both are similar. This study, however, intended to go a step further in addressing the actual problem in the field by a quasi-experimental study. The results achieved are meant to be an encouragement for other SHP hospitals in other areas of Thailand and countries in a similar situation.

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Authors' contribution Songkramchai Leethongdee assisted in the layout of the study. Pissamai Homchampa assisted in the layout of the study. FPS assisted in the layout of the study and writing the manuscript.

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ORIGINAL ARTICLE

Effect of reward-based motivation on metabolic control in children and adolescents with type 1 diabetes mellitus

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Abstract The aim of this study was to investigate the impact of reward-based motivation on metabolic control in children and adolescents with type 1 diabetes mellitus (DM). Forty-four children and adolescents (female/male: 21/23 and prepubertal/pubertal: 17/27) with type 1 diabetes mellitus with a mean age of 12.3 ± 2.8 years (8–18 years) and a mean diabetes duration of 4.7 ± 2.7 years (2–11 years) were enrolled in the study. Before the study, patients were informed that three patients who will have the best metabolic control at the end of 1 year would be rewarded. Number of control visits and hypoglycemic episodes, daily insulin requirement and mean hemoglobin A1c (HbA1c) values were compared before and 1 year after study. During the study period, a statistically significant decrease in the mean HbA1c value, number of hypoglycemic attacks, and daily insulin requirement were determined ($p < 0.05$). Decrease in the mean HbA1c value was significant in both sexes and especially in the pubertal group ($p < 0.05$). It was observed that the patients had more frequent control visits during the motivation study. While 56 % of the patients had regular control visits before the motivation activity, during the motivation period, regular follow-up ratio improved and increased up to 81 %. The positive impact of motivation has been maintained throughout 6 months after completion of the study. This study showed that motivating activities might provide significant improvement in the metabolic control of children and adolescents with type 1 DM with a more evident effect in the pubertal group.

Keywords Type 1 diabetes · Reward · Motivation · Metabolic control

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Introduction

Type 1 diabetes mellitus (DM) is a chronic metabolic disease characterized by insulin deficiency and hyperglycemia due to autoimmune damage of the pancreatic beta cells [1]. Type 1 DM may cause microvascular (nephropathy, retinopathy, neuropathy) and macrovascular (peripheral artery disease, coronary artery disease, cerebrovascular events) complications associated with poor metabolic control in the long term. Good metabolic control at younger age is important in prevention and delay of such complications [2]. Current approach for improved metabolic control in type 1 DM includes intensive or flexible insulin treatment models, continuous diabetes education for patients, and close monitoring of blood glucose and HbA1c levels. However, it is well known that psychological disorders such as depression, anxiety, and eating disorders as well as a lack of motivation may negatively affect self-care and metabolic control in subjects with type 1 DM [3]. Therefore, motivational therapy and psychological support can be needed as a part of medical treatment to improve metabolic control in patients with type 1 DM [4]. However, in the literature, there are only a few studies on this issue [5–9].

The purpose of this study was to investigate the effect of reward-based motivation on metabolic control in children and adolescents with type 1 DM.

Materials and methods

This prospective longitudinal clinical trial was conducted in Izmir Tepecik Training and Research Hospital Pediatric Endocrinology Unit between November 2013 and November 2014. After the local ethics committee approved the study protocol, patients with tip 1 diabetes who had been followed-up in our clinic and satisfied the inclusion criteria were invited consecutively by a phone call to participate in the reward- based motivation study. Patients with type 1 DM (8–18 years) and their families were informed that three patients, who would have the best metabolic control at the end of 1 year, would be awarded with tablet computer which will be given in the world diabetes day ceremony. The patients and their parents who were willing to participate in this study signed informed consents. Patients with psychiatric disorders, chronic complications, and diabetes duration less than 1 year were excluded from the study. Patients were evaluated in three monthly visits during the study period by the diabetes team. The diabetes education and dietary compliance of the subjects were assessed in each visit. Demographic characteristics and follow-up data starting from at least 1 year before the beginning of the study and during the study period were recorded: age, sex, pubertal status, diabetes duration, number of control visits (four or more visits per year was considered as regular follow-up), number of hypoglycemic attacks per week, daily insulin requirement (IU/kg/day), and hemoglobin A1c (HbA1c) values. Hypoglycemic attack was defined as symptoms of hypoglycemia with a blood glucose level <70 mg/dL. The number of hypoglycemic episodes was determined by reviewing blood glucose records of patient's glucometers. Throughout 6 months after completion of the study, HbA1c levels of the patients were monitored to assess proceeding effect of the reward motivation.

The patients were divided into two groups based on HbA1c levels: good-moderate metabolic control (HbA1c <9 %) and poor metabolic control (HbA1c ≥9 %). A testis volume of ≥4 mL and a breast development of Tanner stage 2 and higher were considered as the pubertal finding [10].

HbA1c measurements were performed by using Nycocard II Reader (Axis-Shield Diagnostics Ltd, Dundee, UK) that complies with the reference system for International Federation of Clinical Chemistry Working Group.

Statistical analysis

Statistical analysis was performed using SPPS 21.0 (SPSS Inc., Chicago, IL, USA) software. All data were given as mean ± SD. Homogeneity of the data was assessed using Kolmogorov-Smirnov test. Differences in the means of variables before and after motivation activity were tested using paired sample *t* test or Wilcoxon test depending on the distribution of the variables. Chi-square test was used to compare the frequency of the data. A p value less than 0.05 was considered statistically significant.

Results

Forty-four children (<10 years) and adolescents (≥10 years) with type 1 DM with a mean age of 12.3 ± 2.8 years (female/male: 21/23 and prepubertal/pubertal: 17/27) and a mean diabetes duration of 4.7 ± 2.7 years (2–11 years) were enrolled in the study.

Mean HbA1c value, number of hypoglycemic attacks, and daily insulin requirement of the subjects were significantly decreased at the end of the study period (Table 1). A statistically significant decrease in the poor metabolic control rates and a statistically significant increase in the moderate-good metabolic control rates were observed during the study period (52.3 vs. 29.5 and 47.7 vs. 70.5 %, respectively, $p < 0.001$) (Fig. 1).

Before study, the mean HbA1c level was significantly higher in the pubertal group compared to the prepubertal group ($p < 0.05$). Although a decrease was observed in HbA1c levels during motivation activity in both the pubertal and prepubertal groups, it was only statistically significant in the pubertal group ($p < 0.05$). A significant decrease was observed in the mean HbA1c values of both sexes after motivation activity ($p < 0.05$). The mean HbA1c levels of the groups according to sex and pubertal status before and after study are shown in Table 1.

It was observed that while 56 % of the patients had regular control visits before the motivation activity, during the study period, regular follow-up ratio improved and increased up to 81 % (Fig. 2).

Six months after motivation study was completed, it was determined that the mean HbA1c level of the patients was still significantly lower than the level before motivation study (9.29 ± 2.7 and 8.38 ± 1.68, respectively, $p = 0.039$) (Fig. 3).

Discussion

In addition to conventional treatments, psychosocial approaches may be necessary to improve metabolic control in children and adolescents with type 1 DM, particularly if there are difficulties in achieving optimal metabolic control [11]. Previously, it was suggested that lower HbA1c levels, better compliance to treatment, and increase in quality of life are achievable with motivational methods in children with type 1 DM [5–9]. Viner et al. [9] piloted a motivational and solution-focused therapy group intervention to improve glycemic control in adolescents with poorly controlled type 1 DM. They detected a significant improvement in HbA1c in

Table 1 Metabolic status of children and adolescents with type 1 DM before and after study

	Before study ($n=44$)		After study ($n=44$)		p value
HbA1c (%)	9.29 ± 2.7		8.33 ± 1.71		0.032[¥]
Hypoglycemic episode/patient/week	3.90 ± 1.5		2.1 ± 2.07		0.001[¥]
Mean insulin requirement (U/kg/day)	1.16 ± 0.24		1.0 ± 0.12		0.001[¥]
Pubertal status	Prepubertal ($n=17$)	Pubertal ($n=27$)	Prepubertal ($n=17$)	Pubertal ($n=27$)	
HbA1c (%)	8.4 ± 1.2*	10.3 ± 2.2[*,β]	7.9 ± 0.9	8.5 ± 1.9[β]	
Sex	Female ($n=21$)	Male ($n=23$)	Female ($n=21$)	Male ($n=23$)	
HbA1c (%)	9.2 ± 1.8[*,μ]	9.9 ± 2.4[*,β]	8.0 ± 1.8[μ]	8.6 ± 1.4[β]	

Data are given as mean ± standard deviation

[*,μ,β] $p<0.05$, [¥] paired sample t test, *Mann-Whitney U test, [μ,β] Wilcoxon test

cases at 12 months post intervention (9.3 and 8.7 %, $p=0.03$) compared with no change in controls (9.0 and 9.2 %, $p>0.05$). The same study showed that the improvement in HbA1c was partly maintained at 7–12 months post intervention. Ellis et al. [6] investigated the effect of the home-based psychotherapy on 127 poorly controlled type 1 diabetes mellitus with adolescents in their randomized controlled trial and reported that intensive, home-based psychotherapy improved the frequency of blood glucose testing and metabolic control and decreased inpatient admissions among adolescents with chronically poorly controlled type 1 DM. Another study, where the same therapy method (intensive, home-based psychotherapy) was used, reported that the frequency of blood glucose measurement was significantly increased after therapy (1.8 measurement/day vs. 2.6 measurement/day) and HbA1c values significantly decreased from 11.4 to 10.7 % [7]. Stanger et al. [8] showed that family-based risk management and multicomponent motivational intervention increased the frequency of blood glucose measurements, improved metabolic control, and significantly decreased HbA1c levels from 11.6 to 9.1 % in children with diabetes. They also showed that the decrease in HbA1c level was more evident in patients with poor metabolic control (initial HbA1c >9.5 %). Investigators also determined significant improvement in the teen's self-care and observed significant improvement in HbA1c that was maintained 3 months after the end of treatment. Similarly, Channon et al. [5] analyzed the efficacy of motivational interviewing on 66 teenagers with type 1 DM. In this randomized controlled study, teenagers were randomly divided into two groups (intervention group and control group). The intervention group received motivational interviewing and the control group received support visits. At the end of the intervention, the mean HbA1c in the motivational interviewing group was significantly lower than that in the control group. There were differences in psychosocial variables at 12 months, with the motivational interviewing group indicating more positive well-being, improved quality of life, and differences in their personal models of illness. Different from other studies, in the present study, a reward-based motivation method, which resulted in a significant decrease in the mean HbA1c level, number of hypoglycemic attacks, and daily insulin requirement, as well as a significant decrease in the number of patients with poor metabolic control, was used. We suggest that the improvement in metabolic control was probably associated with the improvement in regular follow-up visits, treatment, and dietary compliance. Our results also

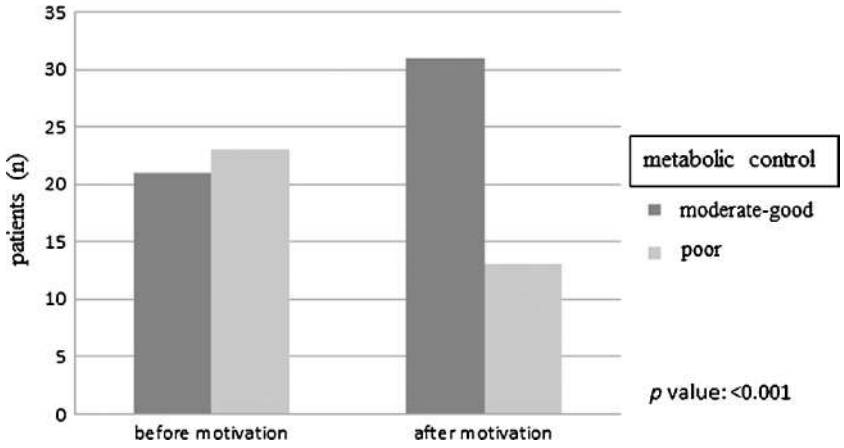

Fig. 1 Metabolic status of patients before and after study

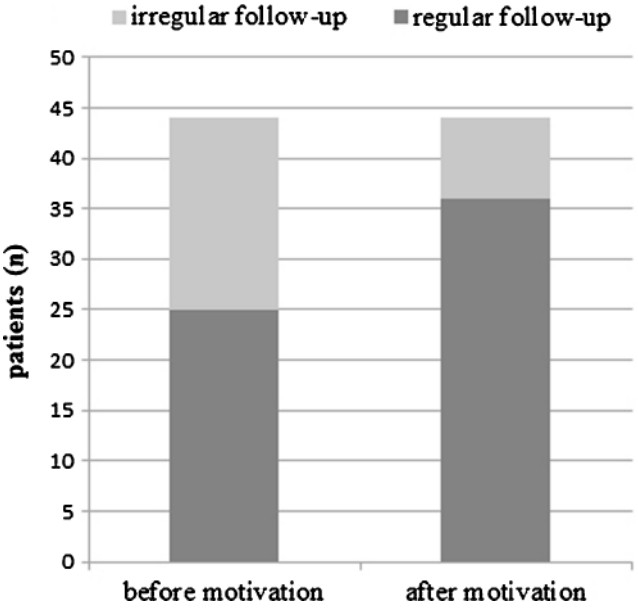

Fig. 2 Regular follow-up ratio of patients before and after study

showed that even after completion of the reward motivation activity, the decrease in HbA1c levels was preserved, which was considered as the maintenance of positive behavior gained with motivation activity.

In many children with type 1 DM, metabolic control may be worsened during puberty as a result of physiological insulin resistance and psychological problems related to adolescence [12–14]. In several studies, HbA1c levels were found to be higher in pubertal children compared to prepubertal children and these differences were explained by hormonal effects, psychosocial problems, and decreased motivation observed in the pubertal period [15]. Similar to the previous reports, our study found significantly higher HbA1c values in the pubertal group when compared to the prepubertal group. Although the HbA1c values of both groups decreased after motivation, the decrease in HbA1c value was found to be statistically significant only in the pubertal group. These findings indicate that motivational therapy can be more effective and helpful to provide optimal metabolic control in patients with type 1 DM during pubertal period. In addition, the mean HbA1c value of boys was higher than that of girls before motivation; however, a significant decrease was observed in the mean HbA1c values in both sexes after motivation. These findings showed that the reward-based motivation improved the HbA1c of all patients regardless of the sex.

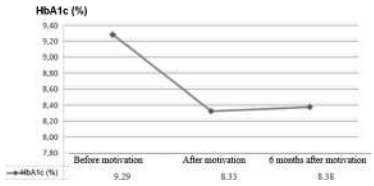

Fig. 3 The impact of reward motivation on mean HbA1c values of the patients

The study group is relatively small and the follow-up period is short to conclude on long-term benefits of motivation. However, despite these limitations, we were able to identify significant benefit of reward-based motivation on metabolic control of children with type 1 diabetes.

Conclusions

According to our knowledge, this is the first study to show that reward-based motivation may be effective and helpful in providing optimal metabolic control in children and adolescents with type 1 DM, especially in the pubertal period and in cases where conventional treatment models are insufficient. Good metabolic control is related to good compliance with insulin, nutritional management, and regular follow-up visits. Reward-based motivation similar to other motivational interventions such as problem-focused psychotherapy, family-based risk management, and motivational interviewing seems to be associated with significant improvement in the metabolic control of children and adolescents with type 1 DM. Finally, we recommend motivational interventions as a part of conventional treatment to improve metabolic control in type 1 DM patients.

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Compliance with ethical standards

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.

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

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

Source of support Nil

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ORIGINAL ARTICLE

Association of RBC count and Hct with MS and its components in China rural population

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Abstract Due to the abnormal hemorheology in patients with metabolic syndrome (MS), some differences may exhibit in erythrocyte parameters. This cross-sectional study was to determine whether the red blood cell (RBC) count and hematocrit (Hct) were associated with MS in China rural area. We selected 11,191 (5170 males and 6021 females) subjects over 35 years. MS was defined according to the revised ATPIII criteria. We used Student's *t* test to compare the erythrocyte parameters between normal and MS groups. We also assessed the erythrocyte parameters for different MS component numbers. A partial correlation coefficient was used to evaluate the relationship between erythrocyte parameters and each MS component. The RBC counts and Hct were generally higher in MS/component groups than normal groups for both genders. The RBC counts and Hct increased with the increasing MS component numbers. The partial correlation coefficients between erythrocyte parameters and waist circumference (WC) were bigger than other components (RBC count: $r = 0.258$ for male and $r = 0.218$ for female, $p < 0.001$; Hct: $r = 0.209$ for male and $r = 0.149$ for female, $p < 0.001$). RBC count and Hct were positively related with MS/component for both genders. They were more associated with WC and diastolic blood pressure (DBP) than with other MS components.

Keywords RBC count · Hct · Metabolic syndrome

Introduction

Metabolic syndrome (MS), a clustering of abdominal obesity, dyslipidemia, hypertension, and impaired fasting glucose, has become increasingly concerned around the world due to the elevated prevalence and the increased risk of cardiovascular disease [1, 2]. The erythrocyte parameters, as a part of blood routine test, are easy to get in clinical works and in routine examinations. The MS patients tend to have a high blood viscosity and erythrocyte aggregation because of the changing hemorheology and the chronic inflammation. So many scientists were interested in finding out whether the erythrocyte parameters such as red blood cell (RBC) count and hematocrit (Hct) were associated with MS and its components [3–5]. A study from Japan determined the positive relationship between RBC count and MS, to contradict with another study in Korea which denied this association for female [4, 6]. One study in China urban area suggested that the erythrocyte parameters could be served as a predictor for MS while another study in Taiwan reported that no association was found between RBC count and insulin resistance as well as glycemic metabolism [3, 7].

However, many studies used body mass index (BMI) instead of waist circumference (WC) [3, 8], and most studies selected subjects from urban where people often work in office sedentarily. In our study, we investigated whether there was a positive relationship between erythrocyte parameters and MS with a large sample size from China rural area where labor work was the main working style. We measured WC according to ATPIII criteria. We also evaluated the relationship between erythrocyte parameters and each MS component for both genders.

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Methods

Subjects

Liaoning Province is located in Northeast China. The study was conducted from January 2012 to August 2013. To ensure that the social and natural environment of this area indeed have an impact on the subjects, a representative sample of participants aged ≥35 years was selected to characterize the prevalence, incidence, and natural history of cardiovascular risk factors in rural areas of Liaoning Province. The study adopted a multi-stage, stratified, random-cluster sampling scheme.

Participants who were pregnant or had malignant tumors or mental disorders were excluded from the study. All the eligible permanent residents aged ≥35 years from each village were invited to attend the study (a total of 14,016 participants). Of those, 11,956 participants agreed and completed the study to give a response rate of 85.3 %. The study was approved by the Ethics Committee of China Medical University (Shenyang, China). All procedures were in accordance with ethical standards. Written consent was obtained from all participants after being informed of the objectives, benefits, medical items, and confidentiality agreement regarding their personal information. In this article, we used only the data from participants whose relevant study data was completed and credible, which provided a final sample size of 11,191 (5170 males and 6021 females).

Data collection

According to the American Heart Association protocol, blood pressure (BP) was measured three times at 2-min intervals after at least 5 min of rest using a standardized automatic electronic sphygmomanometer (HEM-907; Omron), which had been validated according to the British Hypertension Society protocol. The participants were advised to avoid caffeinated beverages and exercise for at least 30 min before the measurement. During the measurement, the participants were seated with their arms supported at the level of the heart. The mean of three BP measurements was calculated and used in all analyses. WC was measured at the umbilicus using a non-elastic tape (to the nearest 0.1 cm), with the participants standing at the end of normal expiration. Fasting blood samples were collected in the morning after at least 12 h of fasting. Blood samples were obtained from an antecubital vein into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA). Fasting plasma glucose (FPG), high-density lipoprotein cholesterol (HDL), triglyceride (TG), and other routine blood indexes were analyzed enzymatically using an auto-analyzer. To maintain the uniformity of the data, all the laboratory measurements were performed and analyzed in the same centralized clinical laboratory. To minimize the lab error, all laboratory equipment was calibrated, and blinded duplicate samples were used for these analyses.

Definition

According to the revised criteria of the Adult Treatment Panel (ATPIII) report, MS was defined by the fulfillment of three or more of the following items: (1) waist circumference ≥90 cm in males and ≥80 cm in females for Asian population according to the recommendation by International Diabetes Federation; (2) TG levels ≥150 mg/dl (1.7 mmol/l); (3) HDL-c levels <40 mg/dl (<1.04 mmol/l) in men or <50 mg/dl (<1.30 mmol/l) in women; (4) SBP ≥130 mmHg or DBP ≥85 mmHg or taking antihypertensive medications; (5) FPG ≥100 mg/dl (≥5.6 mmol/l) or taking diabetes medications.

Statistical analysis

Descriptive statistics were calculated for all variables. Continuous variables were reported as mean values and standard deviations between study groups using the Student's *t* test. RBC counts and Hct were present across the numbers of metabolic syndrome components. Pearson's partial correlation coefficients were calculated to characterize the associations between the MS components and hematological parameters adjusted by age. Data were analyzed using SPSS version 17.0 and a p value less than 0.05 was considered to be significant.

Results

Table 1 showed the baseline characteristics of subjects with or without MS according to gender. The subjects comprised 5170 males and 6021 females. WC, BMI, TG, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), white blood cell (WBC), RBC, hemoglobin (HGB), Hct, mean corpuscular volume (MCV), and low-density lipoprotein (LDL) were significantly higher ($P < 0.001$), whereas HDL level was significantly lower ($P < 0.001$) in subjects with MS than those without MS for both genders. There was no significant difference in MCHC and PLT.

Subjects were divided into six groups according to the MS component number. RBC counts and Hct roughly increased as the MS component number increased for both genders (Fig. 1).

Table 2 showed that the mean RBC counts and Hct were higher in most abnormal MS component groups than normal groups for both genders.

As shown in Table 3, after adjusting for age, RBC count was significantly associated with all the MS components for male and female. RBC count was more associated with WC

Table 1 Baseline characteristics of participants

	Male (N = 5170)			Female (N = 6021)		
	Normal	MS	P value	Normal	MS	P value
Number	3544	1626		3284	2737	
Age (year)	54.44 ± 10.92	54.32 ± 10.58	0.711	51.09 ± 10.08	56.16 ± 9.92	<0.001
WC (cm)	80.18 ± 8.05	91.63 ± 8.40	<0.001	76.66 ± 8.39	86.72 ± 8.26	<0.001
BMI (kg/m^2)	23.59 ± 3.06	27.20 ± 3.27	<0.001	23.38 ± 3.35	26.60 ± 3.46	<0.001
TG (mmol/L)	1.20 ± 0.98	2.67 ± 2.31	<0.001	1.10 ± 0.53	2.23 ± 1.71	<0.001
HDL (mmol/L)	1,50 ± 0.43	1.19 ± 0.32	<0.001	1.53 ± 0.33	1.26 ± 0.29	<0.001
SBP (mmHg)	139.58 ± 21.95	152.26 ± 21.57	<0.001	131.99 ± 21.38	149.62 ± 23.32	<0.001
DBP (mmHg)	81.32 ± 11.18	88.94 ± 11.53	<0.001	77.29 ± 10.59	84.37 ± 11.39	<0.001
FPG (mmol/L)	5.63 ± 1.19	6.66 ± 2.28	<0.001	5.38 ± 0.96	6.44 ± 1.99	<0.001
WBC (×10^9/L)	6.20 ± 1.74	6.71 ± 1.74	<0.001	5.81 ± 1.64	6.31 ± 1.67	<0.001
RBC (×10^{12}/L)	4.78 ± 0.43	4.95 ± 0.43	<0.001	4.33 ± 0.37	4.49 ± 0.40	<0.001
HGB (g/L)	147.07 ± 13.45	151.06 ± 13.26	<0.001	128.38 ± 13.06	132.05 ± 12.96	<0.001
HCT (%)	44.20 ± 3.58	45.36 ± 3.56	<0.001	39.18 ± 3.39	40.20 ± 3.46	<0.001
MCV (fL)	92.78 ± 5.88	91.76 ± 5.38	<0.001	90.70 ± 6.07	89.85 ± 5.77	<0.001
MCHC (g/L)	332.46 ± 18.49	333.03 ± 19.10	0.306	327.37 ± 18.85	328.08 ± 19.35	0.149
PLT (×10^9/L)	201.61 ± 76.65	200.63 ± 53.95	0.644	221.16 ± 59.88	223.56 ± 61.61	0.127
LDL (mmol/L)	2.79 ± 0.74	3.06 ± 0.85	<0.001	2.81 ± 0.76	3.16 ± 0.90	<0.001

WC waist circumference, *BMI* body mass index, *TG* triglyceride, *HDL* high-density lipoprotein, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *LDL* low-density lipoprotein cholesterol, *FPG* fasting plasma glucose, *WBC* white blood cell, *RBC* red blood cell, *HGB* hemoglobin, *HCT* hematocrit, *MCV* mean corpuscular volume, *MCHC* mean corpuscular hemoglobin concentration, *PLT* platelet

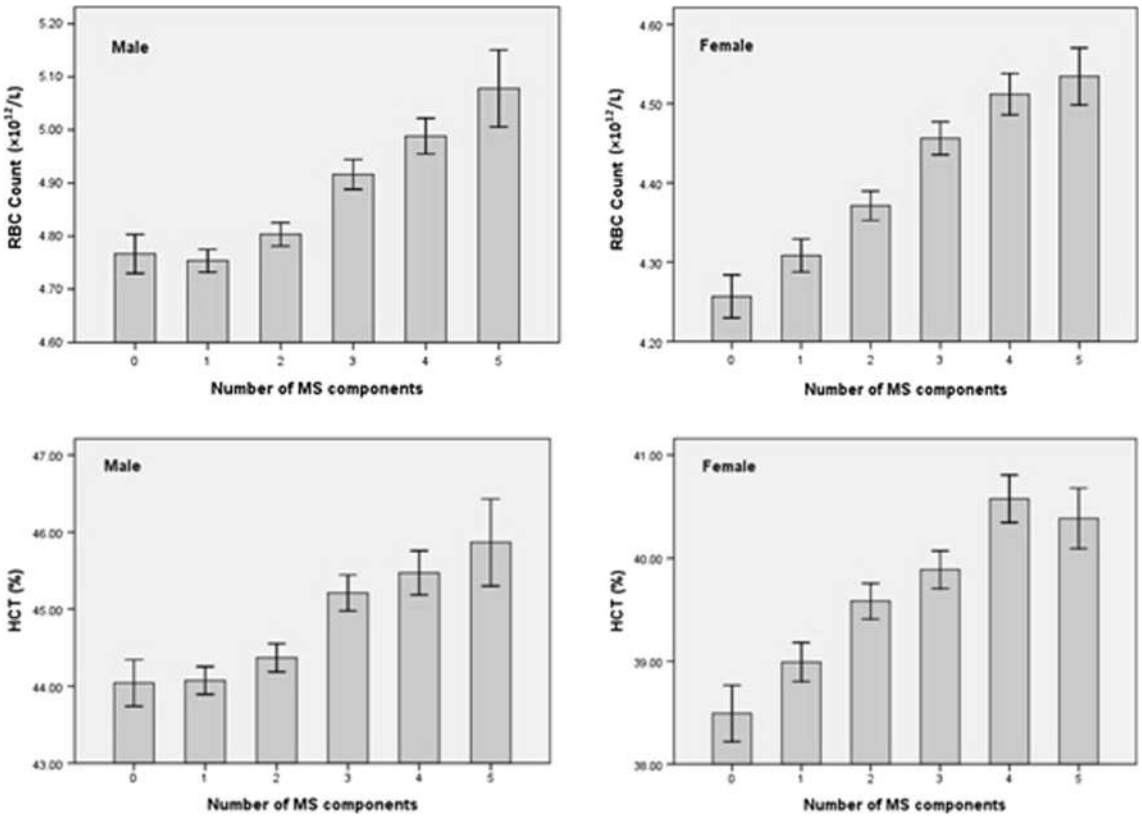

Fig. 1 The relationship between RBC count, Hct, and components of MS according to gender

Table 2 RBC count and Hct for individual components of metabolic syndrome for male and female

Components of MS	Male (N = 5170)				Female (N = 6021)			
	RBC count	P value	Hct	P value	RBC count	P value	Hct	P value
WC								
Elevated	4.98 ± 0.40	<0.001	45.44 ± 3.34	<0.001	4.47 ± 0.39	<0.001	40.08 ± 3.46	<0.001
Normal	4.78 ± 0.44		44.23 ± 3.66		4.32 ± 0.37		39.09 ± 3.38	
TG								
Elevated	4.96 ± 0.42	<0.001	45.47 ± 3.52	<0.001	4.49 ± 0.40	<0.001	40.40 ± 3.31	<0.001
Normal	4.78 ± 0.43		44.16 ± 3.58		4.36 ± 0.38		39.31 ± 3.47	
HDL-c								
Descended	4.94 ± 0.46	<0.001	44.80 ± 3.74	<0.05	4.42 ± 0.41	<0.05	39.61 ± 3.66	0.472
Normal	4.81 ± 0.43		44.51 ± 3.59		4.39 ± 0.37		39.67 ± 3.31	
SBP								
Elevated	4.84 ± 0.44	0.191	44.63 ± 3.62	<0.05	4.44 ± 0.38	<0.001	39.98 ± 3.34	<0.001
Normal	4.82 ± 0.44		44.61 ± 3.60		4.33 ± 0.38		39.10 ± 3.57	
DBP								
Elevated	4.90 ± 0.43	<0.001	45.12 ± 3.53	<0.001	4.51 ± 0.38	<0.001	40.47 ± 3.22	<0.001
Normal	4.79 ± 0.44		44.16 ± 3.62		4.35 ± 0.38		39.25 ± 3.50	
FPG								
Elevated	4.84 ± 0.44	0.083	44.68 ± 3.64	<0.05	4.45 ± 0.39	<0.001	40.02 ± 3.47	<0.001
Normal	4.82 ± 0.44		44.45 ± 3.58		4.36 ± 0.38		39.35 ± 3.42	

(r = 0.258 for male and 0.218 for female) and DBP (r = 0.181 for male and 0.233 for female) than other components. Except for HDL in female, Hct was associated with all other components. Hct was more associated with WC (r = 0.209 for male and 0.149 for female) and DBP (r = 0.182 for male and 0.202 for female) than others. But generally speaking, the association of Hct with MS components was weaker than that of RBC count.

Discussion

The present study confirmed the positive association between RBC count, Hct, and MS with a large sample size in China rural population. RBC count and Hct were associated with WC and DBP more than other MS components.

MS always predisposes to the coronary artery embolism and cerebral embolism. Besides the endothelial injury, another important reason is the reduced blood flow which may lead to altered hemorheology. And this can be reflected in the erythrocyte parameters. Therefore, MS patients may have elevated RBC count and Hct [9]. In consistence with ours, many previous studies from other countries have determined this positive association between RBC count, Hct, and MS [8, 10, 11]. A Brazilian study reported significant correlations between RBC, Hct, and insulin resistance [12]. A cohort study in Ethiopia determined the positive association between hematologic parameters (hemoglobin, hematocrit, and RBC) and MS components [11]. Mardi et al. found a positive association

Table 3 Partial correlation coefficients (r) between individual components of MS and RBC count, Hct adjusted by age for male and female

Components of MS	Male (N = 5167)				Female (N = 6018)			
	RBC count		Hct		RBC count		Hct	
	r	P value	r	P value	r	P value	r	P value
WC	0.258	<0.001	0.209	<0.001	0.218	<0.001	0.149	<0.001
TG	0.146	<0.001	0.129	<0.001	0.147	<0.001	0.111	<0.001
HDL	−0.151	<0.001	−0.034	<0.001	−0.038	<0.05	0.023	0.075
SBP	0.101	<0.001	0.08	<0.001	0.182	<0.001	0.121	<0.001
DBP	0.181	<0.001	0.182	<0.001	0.233	<0.001	0.202	<0.001
FPG	0.064	<0.001	0.030	<0.05	0.166	<0.001	0.09	<0.001

between the number of MS components and erythropoiesis [13].

The mechanism underlying the increase in RBC count for MS patients remains unclear. The insulin resistance which produces excess insulin like growth factors may enhance the erythropoiesis [14, 15]. It has been suggested that the patients with MS present to be systemic chronic inflammation [13]. It is generally thought that the chronic inflammation might have a suppressive effect on erythropoiesis. But the chronic inflammation in MS results in the production of erythrocyte [6, 13]. The specific mechanism was unclear. That might be due to the dual regulation of proinflammatory cytokines. This dual function mainly depends on other physical environments including immunomodulation or anti-inflammatory regulations [9, 16–20]. The inflammatory stimulation of insulin and adipokines such as leptin and adiponectin may be the main factor for the erythrocyte metabolism [21–23].

Elevated blood viscosity decreases microvascular blood flow which may be unable to deliver sufficient insulin and oxygen to insulin-sensitive tissues. This results in flow related insulin resistance and inhibited oxidative action which may in turn increase the Hct level [9].

Our study found that among those MS components, RBC and Hct connected most with WC and DBP.

Abdominal obesity is the main disorder of MS. According to ATPIII, instead of BMI, we used WC which may reflect the truly characteristic of MS. Our data confirmed that RBC count and Hct associated stronger with WC than other MS components, especially for male. There were also another study finding the close relationship of WC and viscosity [10]. Obesity is the most important factor among the MS components. The obesity in MS patients tends to have insulin resistance. The extra subcutaneous white adipose tissue in obesity can produce more adipokines and insulin-related inflammation cytokines. Subjects with obesity show higher plasma viscosity and erythrocyte aggregation and lower erythrocyte deformability [24]. All these factors may contribute to the increase in RBC count.

Besides WC, we found RBC count and Hct also correlated with DBP tightly, especially for female. This was consistent with K Nebeck who determined the association between RBC count and DBP for both genders [11]. Vitool Lohsoonthorn also confirmed the association between Hct and DBP [8]. Subjects with MS tend to have a high viscosity and increased RBC count. And DBP mainly reflects the peripheral arterial resistance which depends on the small arterial rheology. This may lead to the close relationship between the erythrocyte parameters and DBP.

Although some studies determined that Hct was a risk factor for type 2 diabetes [25, 26], we did not find the close relationship between Hct and FPG, which was consistent with some previous studies [8, 27].

About the gender differences, some studies found that elevated RBC count and Hct associated obviously for female than male and contributed this to the estrogenic action [8, 11, 27]. In our data, we could not see many differences between both genders. More evidence needs to clarify this conclusion.

There were some limitations in our study. For the economic restrictions, we did not measure reticulocyte count and HbA1c. So it cannot fully reflect the erythrocyte metabolism especially the erythropoiesis in bone marrow. And we did not exclude the subjects with anemia which may partially influence the results. Finally, as our research was a cross-sectional study, there might be restricted interpretation of the observed associations in terms of cause and effect. Longitudinal studies are needed for further investigation.

In conclusion, this cross-sectional study confirmed that RBC count and Hct generally associated with MS/its components in China rural area. RBC count and Hct connected more to WC and DBP than other MS components for both genders, and this association with FPG was very weak. There was no association found between HCT and HDL for female. Further studies need to clarify these conclusions.

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Compliance with ethical standards The study was approved by the Ethics Committee of China Medical University (Shenyang, China). All procedures were in accordance with ethical standards. Written consent was obtained from all participants after being informed of the objectives, benefits, medical items, and confidentiality agreement regarding their personal information.

Conflict of interest None.

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ORIGINAL ARTICLE

Prevalence of the metabolic syndrome in the middle-aged and older Chinese population

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Abstract Prevalence of the metabolic syndrome (MetS) is rapidly increasing in developing countries. The aim of the study was to provide the latest nationwide estimate on the prevalence of MetS in china. Using a complex, multistage, probability sampling design, a cross-sectional study was performed in a nationally representative sample of 17,708 adults aged 45 years and older from 28 provinces in 2011–2012. MetS was defined by the "Harmonizing the Metabolic Syndrome (HMS)," the guidelines from the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (modified ATP III) and the International Diabetes Federation (IDF) definition, respectively. Overall, the age-standardized prevalence of MetS defined by the modified ATP III criteria was 33.7 %, but the prevalence defined by the new HMS and IDF definition significantly increased to 43.4 and 36.2 %, respectively. And prevalence of central obesity was considerably higher (52.1 vs. 24.0 %) with the HMS (or IDF) criteria than with the modified ATP III criteria. The age-standardized prevalence of high blood pressure, hyperglycemia, hypertriglyceridemia, and low HDL cholesterol was 54.1, 57.7, 27.6, and 43.1 %, respectively. Prevalence of the metabolic syndrome was rapidly increasing in the middle-aged and older Chinese population. We may bear a higher MetS-related burden and underscore the need for strategies aimed at the prevention, detection, and treatment of MetS and special attention should be paid to elderly women population.

Keywords Metabolic syndrome · Prevalence · Cross-sectional studies · China

Introduction

The metabolic syndrome (MetS) is a multiplex risk factor of diabetes [1], cardiovascular disease (CVD) [2], and all-cause mortality [3], and prevalence of the MetS is rapidly increasing in developing countries [4–6]. According to previous national surveys, the prevalence of the MetS was 9·8 % in Chinese adults aged 35–74 years from the 2000 to 2001 International Collaborative Study of Cardiovascular Disease in ASIA (InterASIA) [7], and was 8.5 % from 2002 National Health and Nutrition Survey (NHANS) [8] both defined by the National Cholesterol Education Program Adult Treatment Panel III (ATP III, and modified ATP III) criteria [9, 10].

The definitions of MetS proposed by the ATP III and the International Diabetes Federation (IDF) [11] are often used in publications. Recently, the experts, who come from the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute, have built a unify criteria—Harmonizing the Metabolic Syndrome (HMS) [12]. The main differences among the modified ATP III, IDF, and HMS definitions are that the IDF makes a threshold value for waist circumference as obligatory, and the HMS makes population- and country-specific cut points for elevated waist circumference and three abnormal findings out of five would qualify a person for the metabolic syndrome. However, the previous national surveys rarely provided the prevalence of MetS defined by the HMS and IDF criteria. Therefore, we provide the latest nationwide estimate on the prevalence of MetS.

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Methods

All data collected in the China Health and Retirement Longitudinal Study (CHARLS) are maintained at the China Center for Economic Research (CCER), part of the National School of Development of Peking University, Beijing, China. The project team filed an ethical review application to Ethical Review Committee (IRB) at Peking University in January 2011. After a revision of the Informed Consent section, the survey obtained approval. We only used data collected in CHARLS of Chinese people aged 45 years or older [13, 14].

The detailed sampling design has been published previously [13, 14]. Briefly, the CHARLS used multistage probability sampling method to select representative of people aged 45 and over in china. In the first stage, all county-level units was stratified by region and within region by urban districts or rural counties and per capita statistics on gross domestic product (GDP); 150 county-level units within 28 provinces were randomly selected with the probability-proportional-to-size (PPS) sampling technique. In the second stage, neighborhoods (shequ or juweihui) in urban areas and administrative villages (cun) in rural areas were used as primary sampling units (PSUs), which were the lowest level of government organization, and 3 PSUs within each county-level unit were selected using PPS sampling. In the third stage, all of the dwellings in each selected primary sampling unit were selected from the frame based on maps, which constructed by a mapping/listing software named CHARLS-GIS on Google Earth maps with the support of local informants. Finally, one resident aged 45 years in each sampled household within each PSU was randomly selected as a participant in the survey. If the chosen household had more than one age-eligible member, one such member was randomly selected, and his or her spouse who was also aged 45 years was also included in the survey. All stages of the sampling were conducted by computer to avoid human manipulation.

The CHARLS is a nationally representative longitudinal survey of persons in China 45 years of age or older, which obtained information on demographic background, health status and functioning, health care and insurance, work, retirement, and pension. The interviewers were trained at Peking University by CHARLS staff members, and the interviews took place in respondents' households using a face-to-face computer-assisted personal interview (CAPI) technology. The interviewers also carried equipments to measure the health functioning and performance in respondents' homes. After completing the household interviews, respondents were invited to the local office of China Center for Disease Prevention and Control (CDC) or to township hospitals, where trained nurses drew 8-mL samples of fasting blood. The CHARLS survey was conducted from May 2011 to March 2012 in 28 provinces of China. Overall, out of the total estimated number (12,740) of age-eligible households, CAPI interviews were conducted on a total of 17,708 individuals aged 45 years living in 10,287 households with the overall response rate of 80 %. Among all study participants, 13,978 individuals (78.9 %) provided anthropometric and physical performance measures. The target sample for taking blood samples was the entire group of 17,708 main respondents and spouses from the main CHARLS national baseline. Out of this, we collected blood samples for 11,847 individuals, a response rate of 67 % (women 69 % vs. men 65 %) [13–15].

Data collection was conducted in examination centers at local health stations or community clinics in the participants' residential areas by trained staff according to the standard protocol [14]. Waist circumference was measured at the level of their navel by the Soft measure tape (Manufacturer: Krell Precision Co. Ltd., Yangzhou, China). Respondents were asked to remove any bulky clothing and place the tape measure around their waist at the level of their navel. The respondents were instructed to stand up, inhale and slowly exhale, and hold their breath at the end of the exhale. Blood pressure was measured on the respondent's left arm three times at 45-s intervals by Omron™ HEM-7112 Monitor (Manufacturer: Omron Co., Ltd., Dalian, China). Respondents were instructed to sit down with both feet on the floor and their left arm comfortably supported with the palm facing up. Respondents were asked to roll their sleeve up unless they had on a short sleeve shirt or a thin shirt. The bottom of the cuff was approximately half an inch above the elbow and the air tube ran down the middle of the respondent's arm.

The overnight fasting blood specimens were collected from each respondent by medically trained staff from the China CDC at centralized locations, based on a standard protocol [14, 15]. First, a 2-mL tube of blood was used for a complete blood count (CBC) test, which was performed at county CDC stations or town/village health centers. Second, a 4-mL tube of whole blood was processed and divided into plasma and buffy coat within the same timeframe as the CBC measurement (the CBC was measured within 141 min of collection and the median time from collection to CBC assay was 97 min) and during shipment at 4 °C. After that, the plasma was stored in three 0.5-mL cryovials and the buffy coat in a separate cryovial, which were immediately stored frozen at −20 °C and then transported to the China CDC in Beijing within 2 weeks where they were placed at −80 °C in a deep freezer.

The study measured glucose, uric acid, high-sensitivity CRP, and a lipid panel (total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides) from frozen plasma or whole blood samples, which assays were performed at the Youanmen Center for Clinical Laboratory of Capital Medical University that has regular external quality assessment organized by the Chinese Ministry of Health. The assay method of glucose, total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) was all Enzymatic colormetric test; the coefficient of variation (CV) of within-

assay was 0.90, 0.80, and 1.00 %, respectively; the CV of between-assay was 1.80, 1.70, and 1.30 %, respectively [15].

According to the modified ATP III criteria [8, 10], the MetS was defined as the presence of three or more of the following risk factors: (1) central obesity: waist circumference ≥88 cm in women, ≥102 cm in men; (2) hypertriglyceridemia: serum triglyceride concentration ≥1.7 mmol/L; (3) low HDL: HDL-cholesterol concentration <1.3 mmol/L in women or <1.0 mmol/L in men; (4) high blood pressure: blood pressure ≥130/85 mmHg or known treatment for hypertension; (5) hyperglycemia: serum glucose concentration ≥5.6 mmol/L or known treatment for diabetes. According to the IDF criteria [11], MetS was defined as the presence of central obesity (waist circumference ≥80 cm in women, ≥90 cm in men.) plus two or more of the following factors: the definition for hypertriglyceridemia, low HDL, high blood pressure, and hyperglycemia were the same as those of the modified ATP III. According to the HMS criteria [12], MetS was defined as the presence of three or more of the following risk factors: the definition for central obesity, hypertriglyceridemia, low HDL, high blood pressure, and hyperglycemia were the same as those of the IDF (according to the 2005 IDF recommended the best cut-off point value of WC to screen DM for Chinese adults, central obesity was defined as WC of 90 cm or more in men and 80 cm or more in women [16]).

This data was analyzed to provide precise estimates on the prevalence of MetS. All calculations were weighted to represent the overall Chinese adult population aged 45 years or older on the basis of 2010 Chinese population census data [17]. Weight coefficients were derived from the Chinese population census data, the complex survey design, and non-response rate of the current survey to obtain national estimates; and for the analysis of individual biomarkers, the different set of weights were needed because just over 20 % of the respondents did not get biomarkers taken, so the CHARLS did the same type of inverse probability weighting adjustment [15, 18]. The age-standardized prevalences were also calculated with the use of data on the population distribution in China in 2010 [17]. Standard errors were calculated with the Taylor-linearization method. *P* values were two-sided, and *P* values <0.05 were considered significant. All data analyses involved the use of SAS system, version 9.1 (SAS Institute Inc.).

Results

Baseline characteristics of the subjects are given in Table 1.

The age-standardized prevalence of high blood pressure, hyperglycemia, hypertriglyceridemia, and low HDL cholesterol was 54.1, 57.7, 27.6, and 43.1 %, respectively. Prevalence of central obesity with the HMS criteria (or IDF criteria) was considerably higher than with the modified ATP III criteria (52.1 vs. 24.0 %); the prevalence of one subject having ≥1, ≥2, ≥3, ≥4, and 5 components of the MetS was 86.9, 60.8, 33.7, 15.7, and 5.0 % based on the modified ATP III criteria, respectively, and was 89.7, 67.5, 43.3, 21.6, and 8.0 % based on the HMS criteria (or IDF criteria), respectively. Prevalence of MetS based on the HMS criteria was 43.4 %, which was much higher than based on the IDF and modified ATP III criteria (33.7 and 36.2 %, respectively). The prevalence of hypertriglyceridemia, low HDL cholesterol, components number of MetS, and MetS was higher among women than among men based on these three criteria. The higher prevalence of individual components (except hyperglycemia), components number of MetS, and MetS was in urban areas than in rural areas based on these three criteria (Table 2).

Among women, based on the modified ATP III criteria, the prevalence of hyperglycemia, high blood pressure, and central obesity increased with age. The prevalence of MetS components number and MetS increased with age based on the HMS, modified ATP III, and IDF criteria, respectively, but the prevalence of one subject having ≥4 and 5 components of MetS and MetS increased with age until 75 years of age, when the prevalence began to lower. Among men, the prevalence of hypertension, one subject having ≥1 and ≥2 components of MetS increased with age, whereas the prevalence of hypertriglyceridemia and one subject having 5 components of MetS decreased with age (Table 3).

Discussion

In this national representative sample of Chinese adults, the prevalence of the metabolic syndrome was 43.4, 33.7, and 36.2 % based on the HMS, IDF, and modified ATP III criteria, respectively. In terms of absolute numbers, it represents approximately 190.0 million (with 71.0 million men and 119.1 million women), 161.8 million (with 52.9 million men and 109.0 million women), and 152.7 million (with 55.3 million men and 97.4 million women) Chinese adults aged 45 years or older affected with metabolic syndrome based on the HMS, IDF, and modified ATP III criteria, respectively.

This nationwide survey provides the latest and reliable nationwide estimate of the prevalence of MetS. Furthermore, to our knowledge, this study is the first large-scale national representative epidemiological data in China that are available to compare the prevalence of MetS defined by the HMS, modified ATP III, and IDF criteria, respectively. To avoid introducing bias during the estimation, standard protocols [14] of the measurement along with strict training processes for data collection were used.

Several previous national studies [7, 8, 19, 20] have documented the prevalence of the metabolic syndrome in China. The International Collaborative Study of Cardiovascular Disease in ASIA (InterASIA) [7], which conducted a cross-sectional survey in a nationally representative sample of 15,

Table 1 Demographic, anthropometric, and plasma biochemical characteristics of participants according to gender, 2011–2012

Characteristic	Men	Women	*P* value
Mean age (95 % CI, year)	60.4 (60.1, 60.7)	59.1 (58.8, 59.3)	<0.0001
Urban residence (95 % CI, %)	33.9 (32.5, 35.3)	36.0 (34.7, 37.3)	0.1747
High school or higher level of education (95 % CI, %)	38.7 (37.2, 40.1)	21.2 (20.1, 22.3)	<0.0001
Mean fasting glucose (95 % CI, mmol/L)	6.1 (6.1, 6.2)	6.1 (6.1, 6.2)	0.0360
Mean waist circumference (95 % CI,– cm)	83.9 (83.6, 84.3)	84.6 (84.3, 85.0)	0.0241
Mean systolic blood pressure (95 % CI, mmHg)	130.7 (130.0, 131.3)	131.0 (130.4, 131.6)	0.1654
Mean diastolic blood pressure (95 % CI, mmHg)	76.2 (75.9, 76.6)	75.5 (75.1, 75.8)	0.0027
Mean triglycerides (95 % CI, mmol/L)	2.1 (2.1, 2.2)	2.4 (2.3, 2.4)	<0.0001
Mean HDL cholesterol (95 % CI, mmol/L)	1.3 (1.3, 1.3)	1.3 (1.3, 1.3)	0.5377

Data are mean (SE) or percentage (SE)

540 Chinese adults aged 35–74 years in 2000–2001, provided the best comparison data for our study. In the InterASIA study, the age-standardized prevalence of MetS by the modified ATP III criteria was 9·8 % in men and 17·8 % in women. Compared to the InterASIA study, the prevalences of the MetS among men with age 45–54, 55–64, and 65–74 increased more than fold (the trend was 10.5–23.2, 11.3–24.4, and 10.4–23.7 %, respectively); the prevalence among women with the 45–54,

Table 2 The age-standardized prevalence of individual components and the number of components of MetS based on the criteria of HMS, modified ATP III, and IDF, 2011–2012

	Men			Women			Total
	Urban	Rural	Total	Urban	Rural	Total	
MetS components							
High blood pressure	62.2 (3.1)	49.5 (1.5)	55.1 (1.8)	57.2 (2.0)	50.2 (1.3)	53.4 (1.2)	54.1 (1.3)
Hyperglycemia	58.2 (2.9)	57.1 (1.5)	57.8 (1.6)	57.4 (2.4)	57.9 (1.6)	57.7 (1.5)	57.7 (1.3)
Hypertriglyceridemia	28.1 (2.8)	22.7 (1.1)	25.4 (1.5)	30.7 (1.5)	28.9 (1.5)	29.8 (1.1)	27.6 (1.1)
Low HDL cholesterol	38.4 (2.4)	25.6 (1.5)	31.8 (1.6)	59.5 (3.1)	48.4 (1.7)	53.9 (1.9)	43.1 (1.6)
Central obesity modified ATP III*	7.3 (1.0)	4.1 (0.5)	5.5 (0.5)	44.6 (2.8)	37.7 (1.4)	40.8 (1.6)	24.0 (1.0)
Central obesity HMS (or IDF) †	40.7 (2.3)	24.5 (1.4)	31.5 (1.4)	76.4 (2.1)	65.9 (1.5)	70.7 (1.5)	52.1 (1.3)
Number of components of MetS by modified ATP III							
One or more	87.4 (1.6)	81.2 (0.9)	84.0 (1.0)	91.1 (1.0)	88.3 (0.7)	89.6 (0.7)	86.9 (0.7)
Two or more	56.7 (3.1)	47.2 (1.4)	51.3 (1.5)	74.6 (2.2)	65.3 (1.3)	69.5 (1.4)	60.8 (1.0)
Three or more	29.2 (2.0)	19.2 (1.1)	23.6 (1.1)	46.0 (3.0)	40.8 (1.5)	43.1 (1.6)	33.7 (1.1)
Four or more	10.9 (1.2)	6.7 (0.7)	8.5 (0.7)	25.6 (2.0)	19.8 (1.1)	22.4 (1.1)	15.7 (0.8)
Five	2.0 (0.4)	1.0 (0.3)	1.5 (0.2)	10.6 (1.2)	6.5 (0.6)	8.4 (0.6)	5.0 (0.4)
MetS by NCEP	29.2 (2.0)	19.2 (1.1)	23.6 (1.1)	46.0 (3.0)	40.8 (1.5)	43.1 (1.6)	33.7 (1.1)
Numbers of components of MetS by HMS (or IDF)							
One or more	89.1 (1.5)	82.9 (0.9)	85.7 (0.9)	94.5 (0.7)	92.6 (0.6)	93.5 (0.5)	89.7 (0.6)
Two or more	63.1 (3.3)	52.6 (1.4)	57.2 (1.5)	81.3 (1.7)	73.4 (1.2)	77.0 (1.2)	67.5 (1.0)
Three or more	39.9 (2.6)	25.8 (1.2)	31.9 (1.3)	60.0 (3.3)	48.8 (1.5)	53.9 (2.0)	43.3 (1.3)
Four or more	20.3 (1.6)	10.7 (0.8)	14.8 (1.0)	31.1 (2.5)	25.3 (1.3)	27.9 (1.4)	21.6 (0.9)
Five	6.9 (1.0)	3.6 (0.5)	5.0 (0.5)	13.1 (1.4)	9.0 (0.7)	10.9 (0.7)	8.0 (0.6)
MetS by HMS	39.9 (2.6)	25.8 (1.2)	31.9 (1.3)	60.0 (3.3)	48.8 (1.5)	53.9 (2.0)	43.3 (1.3)
MetS by IDF	31.0 (2.2)	16.6 (1.0)	22.8 (1.3)	55.7 (3.6)	42.8 (1.5)	48.7 (2.1)	36.2 (1.5)

Data are adjusted percentage (SE); *waist circumference ≥88 cm for women and ≥102 cm for men; †waist circumference ≥80 cm for women and ≥90 cm for men

MetS metabolic syndrome, *HMS* Harmonizing the Metabolic Syndrome; *modified ATP III* U.S. National Cholesterol Education Program Adult Treatment Panel III, *IDF* International Diabetes Federation

Table 3 Age- and sex-specific prevalence of individual components and the number of components of MetS based on the criteria of HMS, modified ATP III, and IDF, 2011–2012

	45–54		55–64		65–74		75+	
	Men	Women	Men	Women	Men	Women	Men	Women
MetS components								
High blood pressure	47.7 (2.8)	40.6 (3.2)	54.6 (2.5)	54.0 (1.6)	63.5 (2.5)	67.8 (4.1)	72.7 (2.9)	79.9 (2.7)
Hyperglycemia	54.1 (3.6)	51.5 (2.3)	58.2 (2.9)	63.3 (1.7)	58.2 (2.4)	60.5 (3.2)	70.8 (4.8)	61.2 (3.7)
Hypertriglyceridemia	29.7 (2.2)	26.3 (1.6)	25.7 (1.6)	31.3 (1.4)	18.7 (1.6)	36.1 (3.4)	17.5 (4.1)	29.6 (3.2)
Low HDL cholesterol	33.9 (1.8)	54.8 (2.3)	30.0 (1.8)	52.5 (1.9)	30.4 (3.4)	56.8 (2.9)	31.1 (4.2)	49.8 (3.9)
Central obesity modified ATP III*	6.2 (0.9)	35.7 (2.1)	5.1 (0.7)	43.2 (1.5)	5.3 (0.9)	44.9 (3.0)	4.0 (1.3)	47.3 (4.5)
Central obesity HMS (or IDF)†	33.6 (2.4)	69.9 (2.1)	30.1 (2.3)	70.5 (1.5)	34.5 (3.6)	73.9 (2.0)	22.7 (3.4)	69.6 (3.2)
Number of components of MetS by modified ATP III								
One or more	82.4 (1.5)	85.7 (1.2)	84.5 (1.3)	90.3 (0.8)	84.7 (1.7)	94.5 (0.9)	87.9 (1.9)	95.6 (1.2)
Two or more	47.6 (2.9)	63.5 (2.4)	50.6 (2.8)	69.1 (1.6)	57.5 (2.7)	78.2 (2.0)	58.3 (3.6)	81.6 (2.2)
Three or more	23.2 (2.0)	35.0 (2.1)	24.4 (1.7)	46.0 (1.6)	23.7 (3.9)	51.5 (3.4)	22.1 (3.5)	53.7 (3.7)
Four or more	9.8 (1.2)	17.3 (1.5)	8.1 (0.9)	25.1 (1.4)	7.2 (1.0)	28.9 (2.1)	7.0 (1.6)	24.4 (3.2)
Five	2.1 (0.5)	5.5 (0.7)	0.9 (0.2)	10.2 (0.8)	1.5 (0.5)	12.6 (1.5)	0.7 (0.4)	7.5 (1.6)
MetS by NCEP	23.2 (2.0)	35.0 (2.1)	24.4 (1.7)	46.0 (1.6)	23.7 (3.9)	51.5 (3.4)	22.1 (3.5)	53.7 (3.7)
Numbers of components of MetS by HMS (or IDF)								
One or more	84.5 (1.4)	91.7 (0.9)	86.2 (1.2)	93.1 (0.6)	85.7 (1.7)	96.0 (0.7)	88.9 (1.9)	97.6 (0.6)
Two or more	54.6 (3.0)	71.8 (2.0)	56.0 (3.1)	76.9 (1.3)	63.7 (2.4)	85.0 (1.5)	60.5 (3.5)	85.5 (1.9)
Three or more	31.3 (2.3)	47.6 (3.1)	32.4 (2.2)	55.0 (1.7)	34.5 (3.7)	63.8 (2.8)	28.8 (3.7)	59.9 (3.4)
Four or more	15.3 (1.6)	22.2 (1.6)	14.6 (1.2)	30.8 (1.5)	15.0 (4.2)	34.5 (2.6)	12.9 (3.1)	31.5 (3.5)
Five	6.4 (0.9)	8.0 (1.1)	4.4 (0.7)	12.4 (0.9)	4.2 (0.8)	15.5 (1.6)	3.1 (1.1)	10.6 (1.8)
MetS by HMS	31.3 (2.3)	47.6 (3.1)	32.4 (2.2)	55.0 (1.7)	34.5 (3.7)	63.8 (2.8)	28.8 (3.7)	59.9 (3.4)
MetS by IDF	23.4 (2.0)	43.2 (3.2)	21.8 (1.9)	50.3 (1.7)	25.9 (4.0)	57.5 (3.1)	18.6 (3.4)	51.8 (3.4)

Data are adjusted percentage (SE); *waist circumference ≥88 cm for women and ≥102 cm for men; †waist circumference ≥80 cm for women and ≥90 cm for men

MetS metabolic syndrome, *HMS* Harmonizing the Metabolic Syndrome, *modified ATP III* U.S. National Cholesterol Education Program Adult Treatment Panel III, *IDF* International Diabetes Federation

55–64, and 65–74 year age group all increased almost two times (the trend was 17.5–35.0, 28.0–46.0, and 28.6–51.5 %, respectively), and which increased more or less 20 percentage points in elderly women.

However, the InterASIA study did not provide the prevalence of MetS based on the HMS and IDF criteria. The growth in the prevalence of MetS may be greater if the HMS or IDF definition was used, because the IDF definition requires central obesity as a requisite for diagnosis of the MetS, and the HMS and IDF definition proposes lower cut-off points for waist circumferences than the modified ATP III in Chinese population (90 vs. 102 cm in men, 80 vs. 88 cm in women). The China Health and Nutrition Survey [19], which included a total of 52,621 Chinese adults from 1993 to 2009, showed that the prevalence of central obesity increased 3.9 percentage points from 9.1 to 13.0 % according to the modified ATP III criteria during the period of 2000–2009, but the prevalence of central obesity increased 8.6 percentage points from 28.8 to 37.4 % according to the HMS or IDF criteria. Therefore, the burden of MetS rapidly increased in 2000–2012, especially in the elderly women. The rapid aging of the population, urbanization, and changes in lifestyle and diet, with the consequent epidemic of individual components, such as central obesity [21] and hypertension [22], have probably contributed to this rapid increase.

In the USA, data from the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2010 [5] indicated that the prevalence of the MetS defined by the ATPIII criteria was 22.90 % in the US adult population aged 20 years or older, and data from the combined 1999–2006 NHANES [23] showed that the age-adjusted prevalence of MetS defined by the ATP III criteria was 34.2 %. In the Republic of Korea, data from the 2007 Korean National Health and Nutrition Examination Surveys [24] showed that the age-adjusted prevalence of MetS defined by the ATPIII criteria was 31.3 % in Koreans over 20 years of age, more than 40 % in Koreans age 50–59, and nearly 65 % in Koreans age 60–69, respectively. Comparing with prevalence in the corresponding age group, the prevalence of MetS in China was much less than the

prevalence in the USA and Republic of Korea, but the prevalence of MetS among the elderly women was only slightly lower than the prevalence in the developed countries.

In our study, the prevalence of MetS in China was lower in men than in women, in rural areas than in urban areas, and in younger than in older women, which are consistent with those studies [7, 8, 19]. According to previous epidemiological studies, the individual components of MetS (hypertension, central obesity, diabetes, and dyslipidemia) were higher in urban areas than rural areas due to the great differences in socioeconomic status, lifestyle, and medical services [25]. For example, the prevalence and the treatment of hypertension were substantially lower in rural areas than in urban areas [26], and the prevalence of diabetes was also higher in urban than in rural residents from the 2010 China Noncommunicable Disease Surveillance [27]. Higher intake in dietary lipids and alcohol and lower physical activity [28] are a few of the lifestyle behaviors [29] that are associated with urbanization might help explain this disparity. As our results showed, the major differences of individual components of MetS between the gender were the prevalence of abdominal obesity (5.5 % men vs. 40.8 % women based on the ATPIII definition; 31.8 % men vs. 70.7 % women based on the HMS or IDF definition) and low HDL cholesterol (31.8 % men vs. 53.9 women), which were primarily attributable to the gender difference in prevalence of MetS, especially among the elderly. And the MetS was also more prevalent in older women than in younger, which might be due their postmenopausal status that could increase central obesity [30]. The elderly women were the high-risk population of MetS, so we need to pay special attention to this high-risk population on precaution of the MetS.

This study indicates that the metabolic syndrome was a rapidly increasing health burden in the Chinese middle-aged and elderly population. We may bear a higher MetS-related burden and underscore the need for strategies aimed at the prevention, detection, and treatment of MetS and special attention should be paid to elderly women population.

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Compliance with ethical standards

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Conflict of interest The author declares that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Medical Ethics Committee of Peking University 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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ORIGINAL ARTICLE

Effect of square aerobic exercise on cardiovascular risk factors and health-related quality of life in Chinese women with type 2 diabetes

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Abstract The study aimed to determine whether square aerobic exercise (SAE), the most popular group-based activity in China, is effective in improving cardiovascular risk factors and quality of life in Chinese women with type 2 diabetes (T2D). In total, 60 women with T2D (50–65 years) were randomly and equally divided into an SAE group and a control group treated with usual care. The body weight, glucose metabolism, and lipid metabolism at both 3 and 6 months were compared between groups. The Short-Form Health Survey Questionnaire (SF-36) was completed at the start and end of the study. No participant in the two groups was shed. During the 6-month follow-up, the body mass index, glucose metabolic indexes (except 2-h postprandial insulin), low-density lipoprotein cholesterol, and scores of SF-36 domains (except pain and vitality) in the SAE group were significantly improved from baseline. The body weight, SF-36 scores, or glucose and lipid metabolism were not significantly improved in the control group. SAE is associated with improved cardiovascular risk factors and quality of life in Chinese T2D women. The participants also adhered well to exercise. Therefore, SAE can be recommended as a daily fitness program for T2D patients.

Keywords Diabetes mellitus · Physical activity · Quality of life

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Introduction

Sixteen million people die every year because of noncommunicable diseases (NCDs), according to the World Health Organization (WHO) *Global Status Report on NCDs* 2014 [1]. Type 2 diabetes (T2D), a major risk factor for stroke and coronary heart disease, may largely contribute to this figure. In China, the prevalence of T2D was estimated to be 11.6 % (12.1 % in males, 11.0 % in females), and the prevalence of prediabetes was 50.1 % in adults; these are equivalent to 113.9 million adults with T2D and 493.4 million with prediabetes [2]. T2D is a public health problem that severely impacts the quality of life (QoL) and life expectancy of people worldwide and intensifies the economic burden in developed and developing countries.

With the emergence of new hypoglycemic agents, such as glucagon-like peptide-1 (GLP-1) receptor agonists or dipeptidyl peptidase-4 inhibitors (DPP-4i), pharmacological interventions can effectively control glycemic and reduce T2D-associated complications [3]. In front of the powerful pharmacological intervention, lifestyle interventions are not significantly effective, which explains why the Look AHEAD trial was halted. In addition, bariatric surgery also showed the exact effect in treatment of obesity and diabetes [4]. However, integrated management of multidisciplinary and lifestyle intervention is still the basis for treatment of T2D, even in the use of surgical treatment [5].

Several studies show positive effects of lifestyle intervention on T2D [6]. A major part of lifestyle intervention is physical activity and exercise, which were proven effective in controlling blood glucose and improving the QoL of T2D patients [7]. Thus, exercise should be strongly recommended. However, the main forms of physical activity for T2D patients are walking and domestic chores, and most of the patients are not motivated to change their lifestyles [8, 9]. Therefore, prescription of appropriate modalities and exercise intensity is very important to T2D patients.

However, because of an unbalanced development between economic and medical systems in most countries, particularly in developing countries, gym-based or tailored exercises are only applicable to a small number of T2D patients. In addition, due to the pathophysiological nature of T2D in the elderly, many physicians in clinical practice cannot grasp the modality, intensity, or frequency of exercise. Therefore, it is particularly important to find out some economical, moderately intense, and long-standing forms of voluntary leisure-time exercises that the patients prefer and easily grasp.

In recent years, square aerobic exercise (SAE) has become the most popular group-based exercise in China. In the morning or after dinner every day, people spontaneously gather in squares and do aerobics or dancing to music together. Several participants first learned some dance moves through the Internet and then volunteered as a coach to teach others. The intensity and time of SAE can be adjusted according to participants' personal feelings and without too much supervision. Almost 90 % of the participants are women aged 50–65 years. If SAE can improve metabolism and QoL of diabetic patients, it will be a desirable option for exercise prescription that is easy to do and maintain by T2D patients, especially for middle or early elderly women who are a high-risk population of T2D. Therefore, this study was conducted to assess whether SAE can improve the cardiovascular risk factors and health-related QoL in Chinese women with T2D.

Materials and methods

Patients

A total of 60 T2D women were recruited, who were treated at our outpatient clinics between January 2013 and February 2014. All patients were informed of the study design and purposes and signed a consent form. This study was approved by the Ethics Committee at our hospital. The inclusion criteria include (1) age between 50 and 65 years, (2) at least 2 years after diagnosis of T2D, (3) usually without regular exercise, and (4) willingness to learn SAE. Exclusion criteria were the conditions that may restrict physical activity, such as severe cardiopulmonary disease and diabetic complications.

Methods

The 60 patients were randomly divided into an SAE group and a control group (each $n = 30$) by a random lottery approach. The control group received standard diabetes treatment, and the medication was adjusted according to changes in blood glucose. The participants were also recommended to maintain their diet and exercise patterns. The SAE group was free to choose the location and form of SAE. Before the study, they could perform 1–2 weeks of adaptive training and learning. The participants were advised to choose a pair of medium-size, comfortable athletic shoes. Each participant had a home blood glucose meter. During the study, the timing of exercise was in 1–2 h after dinner, 30–60 min each time, more than 3 times a week, but exercise intensity was not required. The intensity and time of exercise were adjusted according to personal feelings. In case of discomfort, a participant could immediately stop the exercise. It was recommended that chocolate or candy and an Emergency Card (with a description that said "I am a T2D patient, using hypoglycemic agents, prone to hypoglycemia") were taken during exercise, which would prevent the occurrence of hypoglycemia.

The diet history questionnaire was conducted separately at baseline, 3 months, and 6 months, and the results were analyzed by nutritionists. We encouraged all participants to adhere to the standard recommendations for diabetes medical nutrition therapy (carbohydrates, proteins, and total fats account for 50–60, 10–15, and <30 % of average energy intake, respectively). However, to exclude the interference of diet, we did not intervene with the dietary habits of all participants and recommended them to maintain the baseline energy intake.

Demographic data were collected at baseline. Body weight and metabolic parameters were measured separately at baseline, 3 months, and 6 months. Overweight or obesity was defined by Asian standards as a body mass index (BMI) ≥ 23 kg/m^2 [10]. GLP-1 receptor agonists or DPP-4i were used as new oral hypoglycemic medications. Before any medication, blood was sampled by registered nurses between 7:30 and 8:30 a.m. after at least 8–10 h of overnight fasting. Then, 75-g oral glucose tolerance test was conducted.

Glycemic metabolic parameters included fasting plasma glucose (FPG), fasting C-peptide (FC-P), fasting insulin (FINS), glycated hemoglobin (HbA1c), 2-h post-load plasma glucose (2hPG), 2-h postprandial insulin (2hINS), and 2-h postprandial C-peptide (2hC-P). HbA1c was converted between the National Glycohemoglobin Standardization Program (NGSP) units and the International Federation of Clinical Chemistry (IFCC) units as follows: NGSP-HbA1c = 0.0915 × (IFCC-HbA1c) [mmol/mol] + 2.15 % ($r^2 = 0.998$). Insulin resistance index (HOMA-IR) was calculated as [fast insulin (mU/L) × fast glucose (mmol/L)]/22.5 [11]. Lipid metabolic parameters included triglycerides (TG), total cholesterol (TC), high-density lipoprotein

cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). All the laboratory tests were conducted at the physical examination center of our hospital.

Health-related QoL was evaluated with the Short-Form Health Survey Questionnaire (SF-36), which comprises 36 questions with an eight-dimension profile [12]. The eight dimensions included functional capacity, physical limitation, pain, general health state, vitality, social aspects, emotional limitations, and mental health. SF-36 has been demonstrated to be valid and reliable for diabetes patients [13].

Statistical analysis

All data were expressed as number (percentage) or mean ± standard deviation (SD). The quantitative data of metabolic parameters were compared between groups by the independent samples *t*-test, and the qualitative data were compared by Pearson's chi-square test. Data were analyzed on SPSS 18.0 (IBM Software, Armonk, NY, USA) with significance level at $P < 0.05$.

Results

Baseline characteristics of the participants are presented in Table 1. The average ages of the SAE and control groups were 58.1 and 57.4 years old, respectively, and the durations of diabetes mellitus (DM) are 7.8 and 6.9 years, respectively. The mean BMIs of the SAE and control groups were 25.3 ± 3.5 and 24.1 ± 4.2 kg/m^2, respectively, and 9 (30 %) and 10 (33.3 %) participants were overweight or obese, respectively. There were fewer current smokers (6.7 % and 3.3 %) in the two groups, and all participants had a relatively low level of education (only 3.3 and 6.7 % with college and above).

Other concomitant chronic diseases and their medication are detailed in Table 2. Half of the participants had hypertension or hyperlipidemia and were taking blood pressure-lowering or lipid-lowering medication. More than 20 % of the patients used insulin, and approximately 15 % took new antidiabetic drugs. All 60 patients completed the 6-month follow-up.

Changes in BMI, dietary energy intake, and glucose and lipid metabolic parameters of the two groups are detailed in Table 3. The mean BMIs of the two groups were not significantly different at baseline (25.3 vs. 24.1 kg/m^2, $P = 0.216$), and both exceeded the standard of overweight. At 3 months, the mean BMI of the SAE group was not significantly reduced from baseline (24.9 kg/m^2, $P = 0.623$) and was still not significantly different from the control group (23.4 kg/m^2, $P = 0.114$). At 6 months, the mean BMI of the SAE group was 22.5 kg/m^2 and was significantly reduced from those at the baseline and 3 months ($P = 0.001$ and 0.006), indicating the participants were away from the ranks of overweight. The mean BMI of the SAE group was also significantly different from that of the control group (24.7 kg/m^2, $P = 0.033$) (Fig. 1a).

Table 1 Baseline demographic characteristics for participants

Variable	SAE group	Control group
Number	30	30
Age(years)	58.1 ± 3.6	57.4 ± 4.1
BMI (kg/m^2)	25.3 ± 3.5	24.1 ± 4.2
Overweight	9(30)	10(33.3)
Duration of DM (years)	7.8 ± 1.9	6.9 ± 2.2
Current smoker	2(6.7)	1(3.3)
Educational level		
None/elementary	11(36.7)	10(33.3)
Middle school	10(33.3)	11(36.7)
High school	8(26.7)	7(23.3)
College and above	1(3.3)	2(6.7)

SAE square aerobic exercise, *BMI* body mass index, *DM* diabetes mellitus

Values are expressed as number (percentage) or mean ± SD; overweight was defined as a BMI ≥ 23 kg/m^2

The dietary energy intakes at baseline were not significantly different between groups (6.9×10^3 vs. 7.0×10^3 kJ/day). To exclude the interference of diets, we recommended participants to maintain the baseline energy intake and dietary habits. Thus, the two groups showed no significant changes in energy intake at either 3 or 6 months (Table 3).

During the 6-month follow-up, the glycemic indicators of the SAE group were significantly improved. The mean FPG dropped insignificantly from 7.5 at baseline to 7.2 mmol/L at 3 months ($P = 0.188$) but decreased significantly to 7.1 mmol/

Table 2 Other concomitant chronic diseases and the situation of medication used

Variable	SAE group	Control group
Number	30	30
Hypertension	15(50)	17(56.7)
Hyperlipidaemia	12(40)	14(46.7)
Ischaemic heart disease	1(3.3)	2(6.7)
Musculoskeletal condition	5(16.7)	5(16.7)
Traditional OHA	18(60)	19(63.3)
OHA + insulin	8(26.7)	6 (20)
Newer agents	4(13.3)	5(16.7)
Blood pressure lowering medications	15(50)	17(56.7)
Lipid-lowering medication	10(33.3)	14(46.7)
Aspirin	9(30)	11(36.7)

SAE square aerobic exercise, *OHA* oral hypoglycemic medications, *New agents* glucagon-like peptide-1(GLP-1) receptor agonists or dipeptidyl peptidase-4 inhibitors (DPP-4i)

Values were expressed as number (percentage)

Table 3 Changes in BMI and glucose and lipid metabolic parameters of two groups

Outcome	SAE group			Control group		
	Baseline	3 months	6 months	Baseline	3 months	6 months
BMI (kg/m^2)	25.3 ± 3.5	24.9 ± 3.4	22.5 ± 2.6*	24.1 ± 4.2	23.4 ± 3.5	24.7 ± 4.7#
Energy intake (×10^3, kJ/day)	6.9 ± 1.9	7.2 ± 2.0	7.1 ± 2.2	7.0 ± 2.2	6.9 ± 2.1	7.1 ± 1.9
FPG (mmol/L)	7.5 ± 0.7	7.2 ± 0.6	7.1 ± 0.6*	7.8 ± 0.8	7.7 ± 0.7#	7.6 ± 0.7#
FC-P (ng/mL)	2.7 ± 0.9	2.3 ± 0.9	1.7 ± 1.0*	2.4 ± 1.2	2.5 ± 1.3	2.4 ± 1.1#
FINS (mU/L)	13.2 ± 4.4	11.8 ± 3.1	11.0 ± 2.7*	12.3 ± 2.7	12.8 ± 2.6	13.0 ± 2.4#
2hPG (mmol/L)	11.1 ± 1.9	9.4 ± 2.5	8.6 ± 3.3*	10.1 ± 1.7#	9.9 ± 2.8	10.3 ± 2.5#
2hINS (mU/L)	50.1 ± 14.7	48.9 ± 10.0	45.8 ± 13.5	58.6 ± 16.1#	55.4 ± 16.5	56.6 ± 14.9#
2hC-P (ng/mL)	7.3 ± 3.4	5.8 ± 3.6	5.2 ± 3.5*	7.7 ± 3.7	7.3 ± 3.6	7.0 ± 3.2#
HOMA-IR	3.8 ± 2.8	2.8 ± 2.5	2.4 ± 2.4*	3.4 ± 2.1	4.5 ± 3.6#	4.1 ± 3.0#
NGSP-HbA1c (%)	7.6 ± 0.7	7.1 ± 0.8	6.7 ± 0.9*	7.4 ± 0.7	7.3 ± 0.6	7.2 ± 0.8#
IFCC-HbA1c (mmol/mol)	60 ± 8	55 ± 10	51 ± 10*	57 ± 8	57 ± 8	56 ± 9#
TC (mmol/L)	5.2 ± 1.0	5.0 ± 0.9	4.8 ± 1.6	5.0 ± 0.9	5.0 ± 1.4	4.7 ± 1.5
TG (mmol/L)	1.5 ± 1.1	1.4 ± 1.0	1.3 ± 0.9	1.3 ± 0.8	1.6 ± 1.1	1.4 ± 1.1
HDL-C (mmol/L)	1.1 ± 0.2	1.3 ± 0.1	1.2 ± 0.4	1.2 ± 0.3	1.3 ± 0.2	1.3 ± 0.1
LDL-C (mmol/L)	3.3 ± 0.8	2.9 ± 0.9	2.4 ± 0.8*	3.4 ± 0.9	3.0 ± 0.9	3.1 ± 1.1#

FPG fasting plasma glucose, *FC-P* fasting C-peptide, *FINS* fasting insulin, *HbA1c* glycated haemoglobin, *NGSP* National Glycohemoglobin Standardization Program units, *IFCC* International Federation of Clinical Chemistry units, *2hPG* 2-h post-load plasma glucose, *2hINS* 2-h postprandial insulin, 2hC-*P* 2-h postprandial C-peptide, *HOMA-IR* insulin resistance index, *TG* triglycerides, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol. Values were expressed as mean ± SD

*$p < 0.05$, compared with baseline within each group

#$p < 0.05$, comparison at same time between two groups

L at 6 months (Fig. 1b). Like FPG, the HbA1c decreased insignificantly at 3 months but declined significantly from 7.6 % (60 mmol/mol) at baseline to 6.7 % (51 mmol/mol) at 6 months ($P < 0.001$) (Fig. 1d). The glycemic indicators were significantly improved at 6 months, including FINS, FC-P, 2hPG, 2hC-P, and HOMA-IR (Fig. 1c). The 2hINS at 6 months declined slightly but not significantly from baseline. Two participants who used insulin suffered hypoglycemia at the beginning. They continued to participate in the trial through adjusting the exercise intensity and food intake.

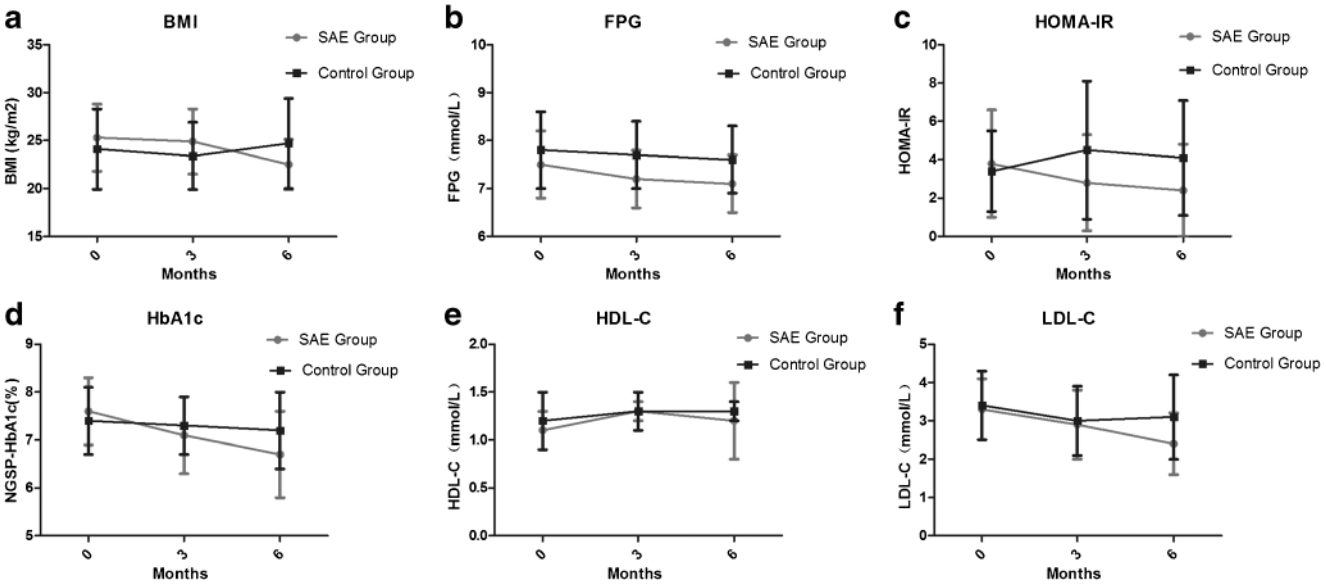

Fig. 1 Changes in BMI, FPG, HbA1c, HOMA-IR, HDL-C, and LDL-C of the two groups. *FPG* fasting plasma glucose, *HbA1c* glycated hemoglobin, *NGSP* National Glycohemoglobin Standardization Program units, *HOMA-IR* insulin resistance index, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

In the control group, the majority of glycemic indicators were relatively stable and were not significantly improved from baseline. The control versus SAE group had significantly higher FPG ($P = 0.009$) and HOMA-IR ($P = 0.042$) at 3 months and also had significantly higher HbA1c ($P = 0.032$), 2hPG ($P = 0.033$), FINS ($P = 0.005$), 2hINS ($P = 0.005$), FC-P ($P = 0.031$), and 2hC-P ($P = 0.046$) at 6 months.

TC, TG, and HDL-C of the two groups and the LDL-C of the control group were relatively stable during the 6-month follow-up, and none of these indices was significantly improved from baseline. In the SAE group, the mean LDL-C at 3 months was not significantly reduced from baseline, but the mean LDL-C at 6 months significantly dropped from the levels at baseline and at 3 months ($P < 0.001$ and 0.038). The mean LDL-Cs at 6 months was significantly different between groups ($P = 0.023$) (Fig. 1e, f).

In the SAE group, the scores of functional capacity, physical limitation, general health state, social aspects, emotional limitations, and mental health at 6 months were all significantly improved from baseline and from the control group. However, there was no significant improvement in the scores of pain or vitality. In the control group, none of the SF-36 domains was significantly improved from baseline (Table 4).

Discussion

This study demonstrates that SAE can improve glucose metabolism and reduce the LDL-C level and BMI of women with T2D. The benefits of exercise for diabetes patients have been reported [14], but we report the benefits of SAE for the first time, which is the most popular form of public exercise in China.

The prevalence of overweight and obesity is a serious public health problem like DM [15]. Overweight and obesity play a crucial role in the occurrence and development of T2D and cardio-cerebral vascular diseases, and the risk of stroke is increased by 5 % with each unit increase in BMI [16]. Compared with Western populations, the Chinese have different body size and fat distribution, and thus, with the same BMI, the Chinese T2D patients tend to have higher cardiovascular risk. In this study, though the overweight or obesity rate was only 30 %, approximately half of the participants suffered hypertension or hyperlipidemia. With economic development, improved standard of material life and excessive energy intake, a substantial increase in the prevalence of obesity and T2D has been observed. Consequently, weight loss is even more important in the Chinese population. This study reveals a significant decrease of BMI in the SAE group. The mean BMI dropped from 25.3 to 22.5 kg/m^2 at 6 months, which means the SAE group was away from the rank of overweight. The decrease of BMI was, in turn, associated with the improvement of glucose and lipid metabolism. Therefore, overweight or obese T2D patients can benefit from SAE.

Glucose metabolism is also affected by skeletal muscles, and during exercise, approximately 80 % of blood glucose is absorbed by them. The glucose entering cells is mainly mediated by glucose transporter isoform 4 (GLUT4), which can be triggered by insulin. Through exercise, the translocation of GLUT4 from intracellular sites to cell membranes is enhanced, thereby promoting glucose disposal. In addition, long-term regular exercise can also upregulate the GLUT4 expression, which would enhance the insulin sensitivity of skeletal muscles [17, 18]. In the present study, FINS, FC-P, 2hPG, 2hC-P, and HOMA-IR were improved to some degree in the SAE group, which means SAE can enhance insulin sensitivity in skeletal muscles and reduce insulin resistance.

HbA1c, which reflects the average blood glucose over 2–3 months, has high predictive value for complications of DM and is a major observed indicator during diabetic treatment [19]. In this study, mean decline in HbA1c following SAE is

Table 4 Comparison between SF-36 domains in two groups at the start and end of the study

SF-36 domains	SAE group		Control group	
	Baseline	6 months	Baseline	6 months
Functional capacity	52.8 ± 22.1	72.3 ± 13.2*	51.9 ± 18.6	53.5 ± 19.1#
Physical limitation	62.7 ± 36.5	81.8 ± 20.4*	61.4 ± 26.3	60.2 ± 31.1#
Pain	82.3 ± 28.1	80.0 ± 19.6	81.5 ± 31.7	80.4 ± 22.0
General health state	55.6 ± 20.7	68.9 ± 18.2*	51.7 ± 34.1	54.5 ± 30.8#
Vitality	63.5 ± 29.5	67.4 ± 30.6	60.2 ± 29.4	64.2 ± 34.8
Social aspects	69.7 ± 19.2	80.6 ± 15.8*	72.3 ± 21.3	70.4 ± 28.6#
Emotional limitations	71.2 ± 30.4	83.6 ± 23.5*	70.5 ± 26.2	71.0 ± 33.5#
Mental health	68.6 ± 29.8	85.0 ± 22.4*	70.2 ± 32.1	73.5 ± 33.5#

SF-36 Short-Form Health Survey. Values were expressed as mean ± SD

*$p < 0.05$, compared with baseline within each group

# $p < 0.05$, comparison at same time between two groups

0.9 %, which is consistent with a systematic review (−0.73 %) about the association between structured aerobic exercise and glucose control [6]. Although physical activity is an indispensable part of diabetic treatment, its effects on HbA1c remain controversial in Asians, who have relatively smaller BMI. As reported, HbA1c and leisure-time physical activity are negatively correlated in non-obese Japanese people but not in obese participants [20]. Physical activity can improve glycemic control only in Korean people with BMI ≥ 25 kg/m^2 [21]. These contradictory findings may be caused by the differences of research design. Moreover, the effects of exercise intensity on HbA1c are also controversial. As reported, the frequency but not intensity of physical activity is associated with HbA1c reduction [22]. However, we find an intensity-dependent relationship between physical activity and HbA1c. The exercise intensity in this study, though not predetermined, should belong to a low-to-moderate level. Along with the progress of the trial and the participants' adaptation to SAE, the exercise intensity may gradually increase to a moderate level. Such an intensity-dependent relationship might explain why HbA1c significantly declined until the 6 months and is consistent with a previous study [20]. Therefore, the intensity of SAE falls within the beneficial range for glycemic control.

There are also controversial views about the effects of physical activity on serum lipids, which are often associated with the form and intensity of exercise. Preliminary data show that resistance training can reduce TC and LDL-C and increase HDL-C [23]. Aerobic exercise may have different effects on lipid metabolism. A meta-analysis suggests that aerobic exercise can reduce LDL-C but not TC, TG, or HDL-C [24]. Here, we observed a similar result. The American College of Sports Medicine and American Diabetes Association jointly declare that exercise may reduce LDL-C but not TC, TG, and HDL-C [25]. In addition to the form of exercise, its dose–effect relationship with lipid metabolism was also reported [20, 26]. These inconsistent findings may be attributed to the difference in experimental designs, but the combination of various exercise forms and intensity is still advocated.

In addition to better metabolism and weight loss outcomes, the SAE group also showed good adherence because all participants followed through and expressed their willingness to continue SAE after the trial. They found a long-term adherent form of exercise, which is exactly what we wanted. One advantage of SAE is that the diversity of exercises includes various dance and aerobics. We did not limit the form, intensity, or duration of SAE, so participants were free to choose according to personal interests and actual situation. The adherence to exercise was improved by increasing the autonomy of the patient [27].

Another advantage of SAE is the improvement of health-related QoL. The scores of social aspects, emotional limitations, and mental health were significantly increased. With the expansion of the elderly population, especially in large- and medium-size cities, SAE has become the most popular form of public exercise in China. Because the average life expectancy is short (during the 1960s and 1970s), the retirement age of the Chinese population is the earliest in the world. The average retirement age is less than 55 years old, and some female retirement age is 50 years old. In the first few years after retirement, they may lose the focus of life. They need to get out of their homes to make friends and cultivate new interests. These social factors contribute to the prevalence of SAE, especially for middle or early elderly women. Through the practice of SAE, they can find a new focus in life and make new friends. In other words, in addition to the fitness value, SAE also provides participants with psychological and social support.

This study has a few limitations. First, the sample size was small. However, this was the first study on the benefits of SAE, which was economical, easy to learn, preferred by patients, and of appropriate intensity. Nevertheless, SAE is indeed effective in improving metabolic parameters and QoL in women with T2D. Second, the patients were selected from a small age span and did not cover all age groups. Third, the patients' energy intake was calculated based on self-reported questionnaire, which may cause some discrepancies.

Conclusions

This study demonstrates for the first time that SAE, the most popular group-based public exercise in China, is effective in improving cardiovascular risk factors and QoL in Chinese women with T2D. The participants also adhered well to exercise. Therefore, SAE can be recommended as a daily fitness program for Chinese women with T2D. However, scientific, large-size, and long-term randomized controlled trials are needed to further assess the effects of SAE on diabetes complications.

Compliance with ethical standards 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.

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

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

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ORIGINAL ARTICLE

Oxidative stress markers in coronary artery disease patients with diabetes mellitus

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Abstract Coronary artery disease (CAD) is the major cause of morbidity and mortality. Diabetes is one of the powerful and independent risk factor for CAD. Hyperglycemia and hypercholesterolemia initiate the oxidative stress and complications like atherosclerosis which induces poor prognosis in diabetic CAD patients. The aim of the present study was to assess oxidative stress by comparing the levels of malondialdehyde and comet tail length in diabetic CAD patients, non-diabetic CAD patients and healthy controls. The study included 400 subjects of which 200 were healthy controls, 100 were diabetic CAD patients, and 100 were non-diabetic CAD patients. Fasting and postprandial glucose levels, glycosylated hemoglobin, serum lipid levels, malondialdehyde, and DNA damage were estimated in all subjects by using commercially available kits and standard protocols. FBS (185.60 ± 6.0 mg/dL), PPG (250 ± 7.06 mg/dL), HbA1c (10.65 ± 2.01 %), TC (280.72 ± 5.25 mg/dL), TG (195.11 ± 5.99 mg/dL), LDL (163.28 ± 5.68 mg/dL), MDA (9.74 ± 2.33 n moles/mL), and comet tail length (21.60 ± 5.69 μm) were significantly high in diabetic CAD patients ($p < 0.05$) compared to non-diabetic CAD patients and controls. Fasting and postprandial blood sugar levels significantly correlated with oxidative stress markers like MDA ($r = 0.553$, $r = 0.557$, $p < 0.01$) and comet tail length ($r = 0.489$, $r = 0.626$, $p < 0.01$) in diabetic CAD patients compared to non-diabetic CAD patients. Our study showed that diabetic CAD patients with increased levels of oxidative stress markers (MDA and DNA damage) might have the poor prognosis than non-diabetic CAD patients.

Keywords Coronary artery disease · Diabetes · Oxidative stress · MDA · DNA damage

Introduction

Coronary artery disease (CAD) is one of the major contributor of morbidity and mortality throughout the world [1]. Among classical risk factors, diabetes mellitus is an independent risk factor for the development and progression of the CAD [2]. It has also been found that risk of CAD is twice and also increases the chance of mortality by four times in diabetics compared to non-diabetic population [3]. Therefore, there is a compelling need to undertake a study to evaluate the effects of hyperglycemia in CAD patients.

Hyperlipidemia and hyperglycemia causes the hardening of blood vessels and accelerates the atherosclerotic process and induces poor prognosis in diabetic CAD patients [4, 5]. Oxidative stress occurs due to the imbalance between antioxidants and reactive oxygen species, induces oxidation of lipids, DNA damage, etc.

Malondialdehyde (MDA), a stable end product of lipid peroxidation, stimulates the various physiological consequences such as changes in the structural integrity of membranes, inactivation of membrane bound enzymes, and surface receptors leading to pathogenesis of disease.

Single cell gel electrophoresis (SCGE) or comet assay is a sensitive, simple, inexpensive, and a rapid method used to detect DNA damage of individual cells and reveals the presence of double-strand breaks, single-strand breaks, and

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alkali-labile sites. Levels of plasma MDA and comet tail length (DNA damage) have been reported in type 2 diabetes and CAD, but there is a scarcity of literature on plasma MDA levels and DNA damage in CAD patients with type 2 diabetes [1, 6–13]. The aim of this study is to compare the levels of malondialdehyde and comet tail length in diabetic CAD patients with non-diabetic CAD patients and healthy controls.

Materials and methods

Study subjects

The present study is designed to compare the oxidative stress markers (MDA, DNA damage) in diabetic CAD patients, non-diabetic CAD patients, and healthy controls. The study included angiographically documented 100 Type 2 diabetic CAD patients and 100 non-diabetic CAD patients from Department of Cardiology, Durgabai Deshmukh Hospital and Research Centre, Hyderabad. Patients with concomitant valvular heart disease, cardiomyopathy, acute renal failures, acute and chronic viral or bacterial infections, tumors or connective tissue diseases, and diabetics on dialysis were excluded from the study. Two hundred healthy age and sex matched individuals were also included in the study as control subjects. The study protocol was approved by the ethical committee of the institution, and written consent was obtained from all the subjects. Detailed socioeconomic, demographic, and other relevant clinical information was recorded using a special consent form. Five milliliter venous blood sample was drawn from all subjects following an overnight fasting period into vacutainers.

Fasting and postprandial blood glucose

Serum samples were used to estimate the fasting and postprandial blood glucose levels as per manufacturer's instructions (ERBA, Transasia, Germany).

Measurement of glycosylated hemoglobin

Glycosylated hemoglobin (HbA1c) levels were measured by modified Fluckiger and Winterhalter colorimetric procedure [14].

Estimation of lipid profile

Estimation of lipid profile (total cholesterol, triglycerides, HDL) was carried out by commercially available kits (ERBA, Transasia, Germany), and low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald's equation: LDL-C = total cholesterol − (HDL − triglycerides/5) mg/dL.

Estimation of MDA

Estimation of plasma MDA was carried out according to the method of Gavino et al. [15].

DNA damage by comet assay

The single-cell gel electrophoresis technique proposed by Singh et al., [16] with alterations suggested by Ahuja and Saran [17] was followed in the present study, with the exception of silver staining of comets, as described by Gandhi [18]. All the procedures for the comet assay were done at low temperature to minimize spontaneous DNA damage. DNA damage was calculated as the difference between the length of the comet and the diameter of the comet head.

Statistical analysis

The analysis were performed with online statistical software tools such as Open Epi version 3.03 to examine the differences in demographic, clinical, and oxidative variables between diabetic CAD, non-diabetic CAD patients, and healthy controls, and p value ≤ 0.05 was used as the criterion of statistical significance.

All numeric values are expressed as the mean ± SD. Pearson's correlation coefficient (r) was calculated by using SPSS statistical software (version 20) for the comparison of hyperglycemia (fasting and postprandial) with oxidative stress markers.

Results

There were 200 healthy controls, 100 non-diabetic CAD patients, and 100 diabetic CAD patients with mean duration of diabetes for 3.75 ± 1.47 years. The demographics and clinical data of the patients and controls are presented in Tables 1 and 2. There were more male subjects than females in the patient population. The risk factors for coronary atherosclerosis, including BP, obesity, and smoking were higher among the diabetic CAD patients compared to the non-diabetic CAD patients and healthy controls.

Total cholesterol, triglyceride, and glycosylated hemoglobin values were significantly high in diabetic CAD patients followed by non-diabetic CAD patients and control group. While HDL cholesterol levels were lower in patients than healthy controls. LDL levels were significantly high in the diabetic CAD patients compared with non-diabetic CAD patients and control group ($p < 0.05$) as shown in Table 2.

Plasma MDA levels and comet tail length (oxidative DNA damage) were found to be significantly high in the diabetic CAD patients compared to the non-diabetic CAD and control group ($p < 0.05$) as shown in Table 3. Hyperglycemia (fasting

Table 1 Demographic data of the Controls, non- diabetic CAD and diabetic CAD patients

S No	Variables	Controls $n = 200$	Non-diabetic CAD patients $n = 100$	Diabetic CAD patients $n = 100$	p value
1.	Female/male	85/115	35/65	28/72	–
2.	Age (years)	54.55 ± 14.39	55.22 ± 13.61	59.35 ± 12.78	0.39
3.	BMI	23.8 ± 3.27	24.96 ± 3.05	26.12 ± 3.08	0.657
4.	SBP (mmHg)	128.23 ± 5.86	140 ± 22.2	160 ± 19.91	<0.05*
5.	DBP (mmHg)	80.89 ± 3.22	82.9 ± 9.91	83.5 ± 10	<0.05*
6.	Smokers/non-smokers	45/155	40/60	48/52	<0.05*
7.	Family history	Nil	40/60	68/32	–
8.	Diabetes mellitus (mean duration in years)	Nil	Nil	3.75 ± 1.47	–

Data represented as mean ± standard deviation

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

*Statistically significant ($p < 0.05$)

and postprandial blood glucose) was found to be significantly correlated with oxidative stress markers such as MDA ($r = 0.553$, $r = 0.557$, $p < 0.01$) and comet tail length ($r = 0.489$, $r = 0.626$, $p < 0.01$) in diabetic CAD patients compared to non-diabetic CAD patients as shown in Table 4.

Discussion

Coronary artery disease (CAD) is a common multifactorial disorder with the clinical events unstable angina and myocardial infarction [19]. Genetic and environmental risk factors such as physical inactivity, positive family history, obesity, hypertension, hyperlipidemia, diabetes mellitus, etc., are involved in the pathology of CAD [20].

Elevated lipid profiles increases the rate of atherosclerosis and lipid peroxidation. Lipid peroxidation is involved in the oxidative modification of LDL; several studies suggest that oxidized lipids (ox-LDL) involved in the development of CAD, and the process is enhanced by reactive oxygen species (ROS). The level of MDA content indicates the state of free radicals and oxidative stress. Therefore, the present study is carried out to assess the oxidative stress markers by measuring MDA, DNA damage along with lipid profile in diabetic CAD patients. Hyperglycemia in diabetes increases the prevalence of advanced glycation end products such as modified proteins or lipids, promotes the production of reactive oxygen/nitrogen species (ROS/RNS). It has been documented that excess production of ROS/RNS causes oxidative stress which causes damage of cellular structures, DNA, lipids, and proteins [21]. Oxidative stress has also been implicated as a contributor for insulin resistance and complication like CAD in diabetes mellitus [22].

Dyslipidemia is another metabolic disorder characterized by high serum triglycerides, low HDL cholesterol, and increased concentration of small dense LDL cholesterol particles [23, 24]. In the present study, we have also observed that increased levels of cholesterol, triglycerides, LDL, and decreased levels of HDL in diabetic CAD patients compared to non-diabetic CAD patients and controls.

Elevated LDL cholesterol levels under oxidative stress get modified into Ox-LDL by free radicals. Ox-LDL has adverse

Table 2 Comparison of clinical data in controls, non-diabetic CAD, and diabetic CAD patients

S No	Variables	Controls ($n = 200$)	Non-diabetic CAD patients ($n = 100$)	Diabetic CAD patients ($n = 100$)	p value
1.	FBS (mg/dL)	97.0 ± 5.86	100 ± 3.25	185 ± 6.0	<0.05*
2.	PPG (mg/dL)	125.7 ± 5.36	140.58 ± 6.68	250 ± 7.06	0.002*
3.	HbA1c (%)	4.56 ± 3.34	6.5 ± 3.39	10.65 ± 4.01	0.082
4.	Total cholesterol (mg/dL)	162.51 ± 27.56	265.28 ± 41.56	280.72 ± 5.25	< 0.05*
5.	Triglyceride (mg/dL)	147.76 ± 34.57	181.11 ± 51.59	195.11 ± 5.99	<0.05*
6.	LDL (mg/dL)	85.55 ± 24.34	145.47 ± 41.7	163.28 ± 5.68	<0.05*
7.	HDL (mg/dL)	45.89 ± 10.8	30.73 ± 9.12	28.2 ± 12.25	0.013*

FBS fasting blood sugar, *PPG* postprandial blood glucose, *HbA1c* glycosylated hemoglobin, *LDL* low density lipoprotein, *HDL* high density lipoprotein

*Statistically significant ($p < 0.05$)

Table 3 Oxidative stress markers (MDA and comet tail length) of controls, non-diabetic CAD and diabetic CAD patients

Variable	Controls $n = 200$	Non-diabetic CAD patients $n = 100$	Diabetic CAD patients $n = 100$	p value
MDA (n moles/mL)	1.73 ± 0.67	7.88 ± 2.22	9.74 ± 2.33	<0.05*
Comet tail length (μm)	10.29 ± 0.277	17.88 ± 3.18	21.60 ± 5.69	<0.05*

Data represented as mean ± standard deviation

*Statistically significant (p value is <0.05)

effects on vascular function resulting in the endothelial cell apoptosis, increase in smooth muscle cell proliferation and synthesis of pro-inflammatory molecules, etc. [25]. MDA is considered as a reliable marker of oxidative damage. Belch et al. (1995) and Serdar et al. (2007) suggested the interrelationship between the oxidative stress and atherosclerosis by measuring the MDA levels [26, 27]. Our previous results have shown that higher MDA levels in CAD patients compared to controls ($p < 0.01$) [6]. Therefore, the present study made an attempt to compare the levels of MDA in diabetic and non-diabetic CAD patients and found significantly higher MDA levels in diabetic CAD patients compared to the non-diabetic CAD patients and healthy controls.

Hyperglycemia also induces the production of free radicals and promotes the oxidative DNA damage, leading to genomic instability. These DNA damages are responsible for cell cycle arrest, apoptosis, senescence, DNA repair of various cells, and subsequently changes the cardiovascular reactivity [28–30]. Hyperglycemia also have correlation with oxidative stress markers such as MDA and DNA damage (comet tail length) in diabetic CAD patients compared to non-diabetic CAD patients indicating its severity on development and progression of CAD.

Cervelli et al. (2012) reported that prolonged exposure to risk factors (e.g., dyslipidemia, hyperglycemia, etc) results in the production of ROS, major stimuli for DNA damage [31]. Maria et al. (2002) showed the presence of DNA damage and its consequences in CAD patients by using the micronucleus test and comet assay [32]. Mohammad et al. (2006) has studied the somatic DNA damage and its contribution to the promotion of phenotypic changes like cell senescence, cell death in coronary artery disease [30]. In the present study, we have assessed the DNA damage using the comet assay and found that there is a significant increase in DNA damage in diabetic CAD patients followed by non-diabetic CAD patients compared to controls.

Therefore, it might be suggested that hyperglycemia in diabetics increases the oxidative stress and causes more DNA damage in diabetic CAD patients than non-diabetic CAD and healthy controls. Knowledge of oxidative stress markers as prognostic predictors may help in the development of better therapeutic strategies for the management of diabetic coronary artery disease.

Table 4 Pearson's correlation between hyperglycemia and oxidative stress markers in diabetic CAD and non-diabetic CAD patients

	Diabetic CAD patients ($n = 100$)		Non-diabetic CAD patients ($n = 100$)	
	MDA (r)	Comet tail length (r)	MDA (r)	Comet tail length (r)
FBS	0.553**	0.489**	0.469**	0.410**
PPG	0.557**	0.626**	0.506**	0.520**

FBS fasting blood sugar, *PPG* postprandial glucose

**Correlation is significant at the 0.01 level (2-tailed) ($p < 0.01$). r = Pearson's correlation

Conclusion

Thus, from this study, we conclude that hyperglycemia has a significant role on development and progression of CAD. Therefore, our study provides greater insight to the role of hyperglycemia inducing oxidative stress in diabetic coronary artery disease and the need to use antioxidants along with statin therapy as a prophylactic step for better disease management such as reduction of cholesterol and control of lipid peroxidation and blood glucose levels in diabetic coronary artery disease patients.

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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 research committee.

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

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ORIGINAL ARTICLE

Does white blood cell count predict diabetes incidence in the general Chinese population over time?

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Abstract Type 2 diabetes is a major global health concern. Recent evidence suggests that inflammation may play a role in the development of this disease. Therefore, immune system markers could serve as prognostic biomarkers for diabetes. The aim of the study was to examine whether white blood cell (WBC) count could predict diabetes incidence in the general Chinese population during a 15-year follow-up. Data were collected in 1992 and again in 2007 from 687 individuals. Questionnaire, physical examination, and laboratory tests were performed using a standardized protocol. To assess the effects of baseline WBC count on the onset of diabetes, Cox's proportional hazards regression models were used to estimate hazard ratios, and the area under the receiver-operating curve assessed the discriminatory power of anthropometric measures for diabetes. Seventy-four individuals were diagnosed with diabetes during the 15-year follow-up period (incidence: 10.8 %). Time of onset was 11.2 ± 3.8 years. Increased WBC count increased diabetes risk during the follow-up after adjusting for other potential risk factors ($P = 0.041$). The areas under the receiver-operating curves for WBC count did not significantly predict incident diabetes better than traditional risk factors such as body mass index (BMI) in the general population cohort both at 7–8-year (area under curve (AUC) = 0.06, 95 % CI −0.162–0.282, $P = 0.597$) and 15-year follow-up (AUC = 0.1, 95 % CI 0.006–0.205, $P = 0.065$). An increasing WBC count increases the risk of type 2 diabetes incidence. Yet, it was an inappropriate predictor of diabetes in a middle-aged Chinese population compared to traditional risk factors such as BMI.

Keywords White blood cell count · Type 2 diabetes · General population · Predictor · Chinese

Introduction

In recent years, there has been increasing evidence to support the idea that chronic low-grade inflammation is a key component related to the pathogenesis of type 2 diabetes mellitus (T2DM) [1]. Changes in cytokines during the inflammation process can impair insulin signaling and pancreatic beta-cells [2, 3]. Chronic inflammation has also been associated with an increased incidence of diabetes. White blood cell (WBC) count, a nonspecific marker of inflammation, showed a positive association with diabetes from epidemiological studies [4, 5], but these observations have not been consistent [6, 7]. A recent meta-analysis demonstrated a positive correlation between increased WBC levels and diabetes risk [8]. However, most of the studies enrolled in this meta-analysis were cross-sectional. Also, these findings cannot be generalized since most of them came from studies on occidental populations, and they may not be true for the Chinese Han population. China has the largest number of people with diabetes, but little prospective data have been reported from China [9]. In addition, McNeely et al. have found that the validity of this clinical model to predict incident diabetes was inconsistent between 5 and 6 years and 10 years [10], suggesting that the follow-up time for these types of studies can influence the results. Whether WBC count can predict the risk for diabetes is not yet clear, especially in Chinese Han nationals. Studies using WBC counts to predict diabetes in Chinese Han populations have had less than 15-year follow-up. The aim of our study was to examine the relationship between WBC count

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and diabetes risk, and compare the predictive ability of WBC count to traditional factors for diabetes incidence based on a 15-year prospective study in Chinese adults.

Materials and methods

Study population

As a collaborating center (Sichuan, China) in Chinese Multi-provincial Cohort Study (CMCS), there were 711 individuals in an urban community located in Chengdu, Sichuan, China, included for a cardiovascular (CVD) risk factor survey according to the multinational monitoring of trends and determinants in cardiovascular disease (MONICA) protocol [11]. This survey involved a standardized questionnaire, physical examination, and laboratory tests. The questionnaire contained the subjects' demographic characteristics and CVD risk factors, including smoking status, alcohol consumption levels, physical activity, and family history of CVD. The physical examination consisted of measuring blood pressure, height, weight, and other characteristics. Fasting plasma glucose (FPG), fasting serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were included in the laboratory tests. The same cohort also accepted a health examination in 2007 with the same methods. The detailed information of these participants has been reported elsewhere [12–14]. Among the 711 enrolled participants, 24 were diagnosed with diabetes in 1992, and their data were excluded from subsequent analyses. Therefore, 687 individuals with complete clinical datasets were available. The Ethics Committee of West China Hospital of Sichuan University and the Ministry of Health of China approved this study. All participants provided written informed consent.

Related definitions

Subjects who were receiving antihypertensive medication or had systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg were considered to have hypertension. Diabetes mellitus (DM) was defined by self-reported history or a fasting plasma glucose ≥7.0 mmol/L or current use of insulin or oral hypoglycemic agents. Smoking was classified as average cigarette consumption of at least one per day. Alcohol intake was defined as average intake of alcohol of at least 50 g/day. Physical activity was defined as exercise one or more times per week of at least 20 min [15, 16].

Physical examination and laboratory test

Anthropometric measurements including height and weight were measured while the patients were lightly clothed and without shoes. Measurements were conducted using a calibrated scale, wall-mounted stadiometer, and tape measure. Body mass index (BMI) was calculated using the following formula: weight/height^2 (kg/m^2). Blood pressure (BP) was measured in the right arm with subjects in a sitting position using a regular mercury sphygmomanometer after resting for 15 min. Three consecutive blood pressure readings were used to calculate a mean BP value. Blood was drawn from the antecubital vein in the morning following a 12-h fast. White blood cell count and biochemistry parameters were measured at the laboratory of West China Hospital (Chengdu, China). The detailed information has been reported elsewhere [17–19].

Statistical analyses

Continuous variables were expressed as the mean ± SD or median (interquartile range) as appropriate. Differences of baseline characteristics between participants with and without diabetes were tested by Student's *t* test for normally distributed variables and by the nonparametric Mann-Whitney or Wilcoxon test for skewed variables. Frequency analysis was performed with the Chi-square test. To assess the effects of baseline WBC count on the onset of diabetes, Cox's proportional hazards regression models were used to estimate the hazard ratios (HRs), and the discriminatory power of WBC counts for diabetes was assessed by the area under the receiver-operating curve (ROC). Subjects were divided into three categories according to WBC count (10^9 cells/L) as follows: 4–4.9 (category 1), 5.0–6.9 (category 2), and 7.0–10.0 (category 3). HRs were computed for the combination of WBC count categories 2 and 3 as compared with the category 1 in different Cox's proportional hazards regression models. Covariates including age, BMI, TG, HDL-C, and FPG were fitted as continuous variables in the multivariate analyses, and alcohol intake, smoking, regular physical exercise, and family history of diabetes were fitted as categorical variables. The point representing the largest sum of sensitivity and specificity on the ROC was chosen to obtain a metric for WBC count in predicting diabetes. The difference between the areas under ROCs was assessed using the nonparametric approach algorithm developed by DeLong. SPSS 13.0, and MedCalc 11.0 software were used. Statistical significance was defined as $P < 0.05$.

Results

Basic clinical characteristics

There were 687 eligible subjects studied at baseline who completed the 7–8-year and the 15-year follow-up. The incidence of diabetes was 2.8 % ($n = 19$) at 7–8 years and 10.8 % ($n = 74$) at 15 years. About 67 % (50/74) of the patients that developed diabetes were self-reported during the 15-year follow-up. Among the self-reported cohorts, 37 individuals were on anti-diabetic drugs. The average FPG of the other 24 individuals

Table 1 Baseline characteristics of the population according to diabetes status at 7–8- and 15-year follow-up

Variable	Diabetes status at 7–8-year follow-up			Diabetes status at 15-year follow-up		
	Subsequent diabetic patients (n = 19)	Subsequent non-diabetic patients (n = 668)	P value	Subsequent diabetic patients (n = 74)	Subsequent non-diabetic patients (n = 613)	P value
Ages	50.6 ± 6.6	48.1 ± 6.2	0.082	49.8 ± 5.7	47.9 ± 6.2	0.013
Gender (male)	12 (63.1)	387(57.9)	0.650	48 (64.9)	351 (57.3)	0.211
BMI (kg/m^2)	25.4 ± 3.6	23.3 ± 2.8	0.002	25.1 ± 3.3	23.2 ± 2.6	<0.001
SBP (mmHg)	119.1 ± 18.7	114.4 ± 15.2	0.186	118.9 ± 18.2	114.0 ± 14.9	0.021
DBP (mmHg)	75.8 ± 10.1	73.6 ± 9.1	0.298	75.7 ± 9.6	73.4 ± 9.0	0.095
WBC count (*10^9 cells/L)	6.3 ± 1.5	5.7 ± 1.1	0.024	6.0 ± 1.3	5.7 ± 1.1	0.013
FPG (mmol/L)	4.7 ± 0.8	4.3 ± 0.7	0.007	4.6 ± 0.8	4.2 ± 0.7	<0.001
TC (mmol/L)	4.6 ± 0.7	4.5 ± 0.8	0.377	4.7 ± 0.7	4.5 ± 0.8	0.023
TG (mmol/L)	2.97 ± 1.51	2.08 ± 0.96	<0.001	2.6 ± 1.2	2.1 ± 0.9	<0.001
LDL-C (mmol/L)	2.0 ± 1.0	2.3 ± 0.8	0.119	2.3 ± 0.9	2.3 ± 0.8	0.776
HDL-C (mmol/L)	1.29 ± 0.33	1.24 ± 0.23	0.413	1.18 ± 0.24	1.25 ± 0.24	0.007
Smoking	8 (42.1)	240 (35.9)	0.581	32 (43.2)	216 (35.2)	0.176
Alcohol intake	3 (15.8)	84 (12.6)	0.678	12 (16.2)	75 (12.2)	0.331
Physical activity	2 (10.5)	144 (21.6)	0.247	14 (18.9)	132 (21.5)	0.604
Hypertension	3 (15.8)	101 (15.1)	0.936	16 (21.6)	88 (14.4)	0.099
Family history of diabetes	4 (21.1)	22 (3.3)	<0.001	6 (8.1)	20 (3.3)	0.039

diagnosed with DM was 9.5 mmol/L. The median was 9.1 mmol/L, and the maximum and minimum were 16.2 and 7.1 mmol/L, respectively. Table 1 shows the clinical characteristics of the participants at baseline. Compared with the non-diabetic subjects, the demographic data from 1992 showed that the subjects who would go on to develop DM had a higher WBC count, BMI, fasting plasma glucose, and triglycerides at 7–8-year and 15-year follow-up (all $P < 0.001$; Table 1).

Table 2 Univariate and multivariate Cox regression models for prediction of diabetes in different models

	Categories of WBC count			
	C1 n = 209	C2 n = 390	C3 n = 88	Total n = 687
WBC count(*10^9 cells/L)	(4,5)	(5,7)	(7,10)	
New cases of diabetes, n	16	43	15	74
Univariate regression				
HR	1	1.452	2.322	
95 % CI		0.818–2.577	1.148–4.697	
P		0.203	0.019	0.063
Model 1: adjusted for age				
HR	1	1.417	2.329	
95 % CI		0.798–2.515	1.151–4.711	
P		0.234	0.019	0.061
Model 2: adjusted for age and BMI				
HR	1	1.397	2.448	
95 % CI		0.787–2.482	1.207–4.963	
P		0.254	0.013	0.042
Model 3: adjusted for age, BMI, gender, smoking, alcohol intake, regular physical exercise, family history of diabetes, SBP, HDL, TG, and FPG.				
HR	1	1.409	2.538	
95 % CI		0.786–2.523	1.219–5.238	
P		0.24	0.013	0.041

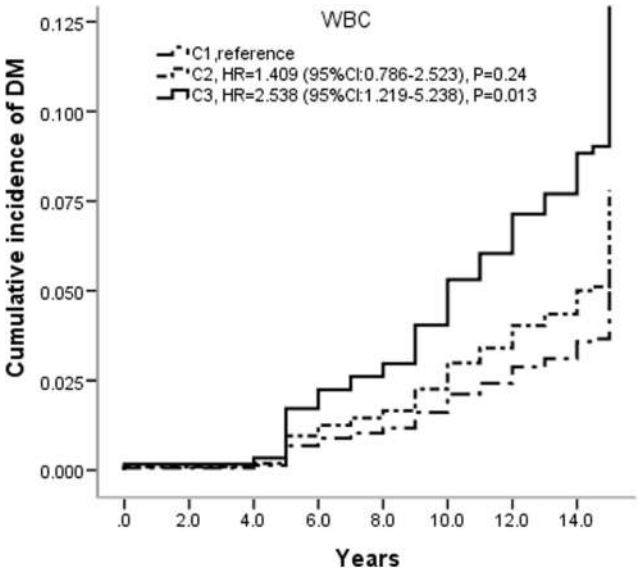

Fig. 1 Cumulative incidences of diabetes mellitus (DM) in different categories of white blood cell (WBC) count in multivariate Cox regression in Model 3

Cox's proportional hazards regression models for prediction of DM

The univariate Cox's proportional hazards regression analysis showed that increased WBC count did not statistically increase the risk for the onset of diabetes with a 15-year follow-up (Table 2). However, in the multivariate Cox's proportional hazards regression models, the WBC count was significantly associated with risk of diabetes after adjusting for potential risk factors including age, gender, smoking, alcohol intake, regular physical exercise, family history of diabetes, BMI, SBP, HDL, TG, and FPG (Table 2). The time of diabetes onset was 11.2 ± 3.8 years. Figure 1 shows the cumulative incidence of DM in the different WBC count categories in the multivariate Cox regression from Model 3.

ROC curve analyses

The areas under the ROC curves were 0.608 (95 % CI 0.570–0.644, $P = 0.154$) for WBC count and 0.668(95 % CI 0.631–0.703, $P = 0.026$) for BMI at 7–8-year follow-up, while at 15-year follow-up, these values were 0.568(95 % CI 0.530–0.606, $P = 0.059$) for WBC and 0.668 (95 % CI 0.601–0.734, $P < 0.001$) for BMI (Table 3). Table 3 indicates that BMI was better than WBC at predicting incident diabetes both at 7–8-year and 15-year follow-up in a Chinese Han population from Chengdu region.

Discussion

In this prospective study, we demonstrate that total WBC count was significantly associated with T2DM, after adjusting for age, gender, smoking, alcohol intake, regular physical exercise, family history of diabetes, BMI, SBP, HDL, TG, and FPG. However, total WBC count, a nonspecific marker of inflammation, was not a better predictor of diabetes in a middle-aged Han Chinese population at 7–8-year or 15-year follow-up, compared with BMI.

We found that an increased WBC count increases the risk of diabetes, which is consistent with the most recent studies [20, 21]. In a cross-sectional study design, elevated WBC count is independently associated with worsening glucose metabolism in middle-aged and elderly Chinese people [21]. The data from our prospective study on the association between WBC count and diabetes risk in the Chinese population suggests that T2DM is a disease of inflammatory origin. Therefore, WBC counts, as a marker of subclinical inflammation, can be used as a biomarker for increased risk of diabetes. Several mechanisms may explain the link between WBC count and diabetes. Several studies have shown that WBC count is directly associated with insulin resistance and inversely associated with insulin secretion. It is possible that chronic inflammation results in the persistent release of cytokines, such as IL-6, which is produced by WBCs [22, 23]. Insulin signaling in the liver has also been shown to be interrupted by inflammatory molecules suggesting there may be a pro-inflammatory effect on insulin or insulin resistance [24, 25]. In addition, as Vozarova et al. suggested, hormones are another possible link between WBC count and insulin sensitivity. Several hormone receptors are expressed on the surface of WBCs and play a role in their production and maturation [5].

Table 3 Areas under the ROC curve for various measurements used to predict diabetes incidence

Variables	AUC (95 % CI)	*P* value	Sensitivity	Specificity	▵AUC (95 % CI)	*P* value compared to BMI
Diabetes incidence after 7–8 years						
BMI	0.668(0.631–0.703)	0.026	0.579	0.796	–	–
WBC	0.608(0.570–0.644)	0.154	0.579	0.657	0.06(−0.162–0.282)	0.597
Diabetes incidence after 15 years						
BMI	0.668(0.601–0.734)	<0.001	0.581	0.697	–	–
WBC	0.568(0.530–0.606)	0.059	0.956	0.176	0.1(−0.006–0.205)	0.065

▵AUC: difference between areas

Our results showed the areas under the ROC curves for WBC count did not significantly predict incident diabetes better than that in traditional risk factors such as BMI in our patient cohort. A potential explanation for the lack of predictive value of WBC counts for diabetes incidence is that a WBC count is a cumulative estimation of the whole-body inflammatory burden, reflecting chronic as well as acute inflammatory processes [26]. Adipose tissue inflammation has mainly been attributed to inflammatory processes observed in obesity, and BMI in our study cohort at baseline was relatively low. Therefore, in the absence of recognized chronic inflammatory processes such as adipose tissue inflammation, an elevated WBC count may represent acute, intercurrent inflammation that is not associated with a higher rate of diabetes incidence [27]. This is similar to the Atherosclerosis Risk in Communities cohort study, which also failed to replicate the contributions of WBC counts or alanine amino transferase to an improvement in the areas under the ROC curves [28]. It is important to note that although novel risk factors including the WBC count may be associated with T2DM, it does not mean that they will therefore contribute to risk prediction [29].

Several limitations of this study warrant consideration. The first is that this study had a relatively small sample size. This limited the study, as we could not analyze the association between WBC count and diabetes risk classified by gender. The results of our study may have limited statistical power, but still provide meaningful insights into this complex biological problem. The second limitation regards the way to diagnose DM. During the investigation, taking into account financing and feasibility, we did not measure glycosylated hemoglobin levels or perform glucose-tolerance tests. Some individuals with diabetes examined by these assays may not have detectable changes in fasting glucose alone or aclinical history, leading to an underestimation of the number of diabetic patients in the study. A third limitation was the availably of only one baseline WBC count. The fourth is the lack of measurements of inflammatory markers like CRP and IL-6, which may better represent the biological processes that lead to T2D. WBC counts are crude measures of the immune system, and may not accurately represent the true inflammatory burden of the individual. Not having specific measurements for inflammation makes it impossible to compare the ability to predict the development of diabetes between WBC count and these inflammatory markers. Finally, all the participants came from Chengdu province, China, so we cannot extend our findings to the general global population.

In summary, our findings showed that increased WBC count increases the risk of diabetes incidence. Yet, it was an inappropriate predictor of diabetes in the middle-aged community compared with traditional risk factors such as BMI. Future research is warranted to assess whether this phenomenon exists in a larger population.

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Author contributions Qi Liu and Ying Xu equally contributed to the article.

Compliance with ethical standards

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

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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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ORIGINAL ARTICLE

The analysis of the cost and amputation rates of hospitalized diabetic foot infection patients

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Abstract Direct and indirect costs of diabetes and its complications figure prominently in health care expenditure globally. The diabetic foot is one of the most common complications of diabetes and is usually associated with neurological and peripheral vascular problems, yielding foot ulcers and infection. The aim of this study was to analyze hospitalized diabetic foot patient costs and amputation rate changes over time. Hospitalized patients with infected diabetic foot ulcerations within a 4-year span, starting in January 2012, were retrospectively evaluated to analyze cost and amputation rate changes over time. One hundred thirty-eight diabetic patients were hospitalized and treated. Major amputation rates tended to increase; however, minor amputation rates tended to decrease over time. Mean cost per patient was $2880. The distribution of the costs according to the years was not significant. Treatment of infected diabetic foot ulceration is challenging and incurs high healthcare costs. Through intensive foot care and multidisciplinary team approaches, major amputation rates have gradually increased in recent years.

Keywords Amputation · Cost · Diabetic foot · Infection · Ulceration

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Introduction

According to the International Diabetes Federation report from 2013, nearly 400 million people have diabetes and a further almost 300 million have impaired glucose tolerance. Global prevalence estimation stands at 8.3 %. With this, the high proportion of people with diabetes is expected to rise to nearly 600 million over the next 25 years [1].

Diabetic foot is one of the most common complications of diabetes and is usually associated with neurological and peripheral vascular problems yielding foot ulcers and infection [2]. Management of diabetic foot may require hospitalization because of the complications. The reported number of discharges with diabetic foot having peripheral arterial disease, foot ulcer, inflammation, infection, or neuropathy doubled from 445,000 in 1988 to 890,000 in 2007 [3]. Peripheral vascular and neurological complications, both closely linked to diabetic foot, accounted for 30 and 28 % of the expenses, respectively [4].

As a one of the most common non-communicable diseases, the direct and indirect costs of diabetes and its complications figure prominently in healthcare expenditure globally [5]. The latest American Diabetes Association cost report from 2012 for diabetes in the USA estimated the direct cost of diabetes to be $176 billion. This rises to a total cost of $245 billion when $69 billion in indirect costs are included. The main component of the total direct cost is inpatient care, a high percentage of 43 %, followed by prescriptions for the complications (antidiabetic drugs and supplies), outpatient visits, and non-hospital care facility stays [4]. This retrospective study was conducted in University of Health Sciences Umraniye Training and Research Hospital Department of Infectious Diseases to analyze the cost of infected diabetic foot ulcer treatment and amputation rate changes over time from 2012 to 2015.

Methods

The patients who were hospitalized with a diagnosis of diabetic foot infection between January 1, 2012 and December 31, 2015 at Ümraniye Training and Research Hospital were investigated retrospectively. The Institutional Ethics Committee provided ethical approval. The main outcomes analyzed in this study were the direct costs and amputation rates among the hospitalized patients with diabetic foot infection.

Infection statuses of the patients were described according to the perfusion, extent, depth, infection, and sensation (PEDIS) classification system guideline [6]. Grade 1 represents no symptoms or signs of infection, grade 2 local infection, grade 3 local infection deeper than subcutaneous tissue without systemic inflammatory response symptoms (SIRS), and grade 4 infection with SIRS. Patients with infected diabetic foot ulcer [local infection involving the skin and the subcutaneous tissue with erythema >2 cm, or involving structures deeper than skin and subcutaneous tissues (e.g., abscess, osteomyelitis, septic arthritis, fasciitis), with or without systemic inflammatory response signs] were included to the study. Infections were also confirmed with tissue culture after hospitalization. The patients with other relevant comorbid conditions requiring hospitalization (nephropathy, diabetic acidosis, etc.) were also excluded from the study.

The data of the patients' demographic characteristics (age, sex), length of hospital stay, diagnostic laboratory tests (blood tests, microbiologic sampling, radiological tests), and in-service interventions (visits, drugs, endovascular revascularization, amputation surgery, etc.) were collected. All data were obtained from three different databases—the hospital admission system, the hospital information system, and revolving fund management. Inpatient care cost was subcategorized as facility, medical, and laboratory. All costs for in-service interventions were included in the medical cost section.

Major amputation was defined as below-knee amputation, knee disarticulation, or above-knee amputation. Minor amputation was defined as forefoot amputations. The number of amputations per year was calculated by counting every single amputation, regardless of whether they took place in the same patient. The rate was formulated as amputation number per year/hospitalized patient per year. The decision between amputation or debridement and level of amputation were evaluated by the hospital diabetic foot council (DFC).

All costs data were calculated in Turkish lira (TRY) then exchanged to US dollars using the Central Bank of the Republic of Turkey Exchange Rates-Banknotes (Converted to TRY) per year through 2012–2015 [7]. The exchange rate of US dollars to TRY for 2012, 2013, 2014, and 2015 were 1.78, 2.06, 2.29, and 2.92, respectively.

The amputation rate and the cost results were compared and discussed with other reports from different national centers. Statistical analysis was performed using Chi-squared for linear trends, and p values <0.05 were considered to be significant.

Results

One hundred thirty-eight adult diabetic patients with a diagnosis of third and fourth category diabetic foot infection according to the PEDIS classification system were hospitalized between January 1, 2012 and December 31, 2015. The DFC in the same hospital evaluated patients' PEDIS scores. Among them, 99 of the 138 patients (66 %) were male and 51 were female, and the mean age of the patients was 61.6 (32–85). Osteomyelitis was present in 106 patients (76 %); however, the remaining 32 patients (24 %) had only soft tissue infection. During this study period, the overall amputation rate was 57 %. Forty-one patients had minor and 39 patients had major amputations. Details of the amputations within the years are shown in Table 1. The trend for major amputations was significantly increased (slope 1, $p < 0.001$); however, minor amputation rates were significantly decreased over time (slope −0.075, $p = 0.0373$).

The mean facility, medical, laboratory, and total costs were $38,926, $49,074, $10,583, and $98,584, respectively. Mean cost per patient was $2880. Distribution of the direct cost according to years is displayed in Table 2.

Discussion

Two main issues for diabetic foot care—amputation rate and direct cost—were investigated in the current study. Amputation rates for diabetic foot are usually reported as a predictor of management of diabetic foot. The decrease in minor amputation rates and increase in major amputation rates were attributed to two main potential reasons. Firstly, our DFC was established in 2011 and is one of the limited numbers of

Table 1 The distribution of the major and minor amputations and its' rates according to the years (rate: amputation number/total number of patients)

Year		2012	2013	2014	2015
Major Amputation	Number	3	7	13	16
	Rate	10	16	38	50
Minor Amputation	Number	10	17	9	5
	Rate	34	39	26	15
Total	Number	13	24	22	21
	Rate	44	55	64	65

Major amputations were defined as those including the toe and proximal parts of the extremity and minor as those distal to the toe

Table 2 The distribution of the cost according to the years

Years		2012	2013	2014	2015	Mean
Patient number		29	43	34	32	35
Cost ($)	Facility	48,948	47,013	28,433	31,310	38,926
	Medical	43,521	64,034	42,686	46,054	49,074
	Laboratory	10,177	11,928	10,653	9572	10,583
	Total	102,647	122,977	81,774	86,937	98,584
Cost per patient ($)		3539	2859	2404	2716	2880

DFC in the most crowded city in the country. The number of patients with more complex needs and high-risk foot has increased by referral from other hospitals in time. Secondly, besides to that, our council opted for a more conservative therapy than prompt minor amputation decision for diabetic foot osteomyelitis to function as "limb-salvage team." This tendency decreased minor amputation rates; however, worse clinical progression in some cases resulted in higher number of major amputation rate. We had no major changes in terms of antibiotic therapy, surgical and endovascular interventions, or patient education throughout the study period, 2012–2015.

Over the past three decades, many studies have reported an incidence of lower extremity amputations becoming decreased in diabetics with high variability globally [8]. The rates also varied from 18 to 51 % in our country [9–14]. The major amputation rate was 28.5 %, and the minor amputation rate was the same. The overall total rate was 57 %. In general, the patient population is not homogeneous compared to other studies. Infection statuses of the feet were reported insufficiently in previous investigations. The authors' here used the PEDIS classification system which is a versatile tool for predicting the ulcer outcome in diabetic foot patients [6]. All the patients in this study had a PEDIS 3 or 4 diabetic foot. The comparison of the amputation rates of the centers in our country were summarized in Table 3. The Diseases Society of America (IDSA) and the International Working Group on the Diabetic Foot (IWGDF) put forth a classification system, IDSA-IWDGF in 2004 [15]. They evaluated amputation rates for diabetic foot with IDSA-IWDGF, which is very similar to PEDIS classification. The rates were 46 and 78 % in patients with IDSA-IWDGF 3 and 4, respectively, in parallel with our results.

Table 3 The comparison of amputation rates with other Turkish centers

Authors	Amputation rate (%)
Karakoc et al. [9]	18
Tabur et al. [10]	18
Yesil et al. [11]	36.2
Aksoy et al. [12]	39.4
Akcay et al. [13]	50
Gonen et al. [14]	51
Current study	57

Amputation rate in diabetic foot may vary from country to country, although in same countries, these rates have been reported with large variation [16]. Such variation has also been seen from developed countries, like England. Holman et al. found high regional variations in lower extremity amputation rates in diabetic foot [17]. For the purposes of this study, when reviewing national reports, huge variation was again observed. For Turkey, as a developing country, even if the rate is declining, high amputation rates are still a problem, especially for high-risk populations (PEDIS 3–4).

A second outcome of this study is the cost of diabetic foot infection inpatient treatment. Here, the average cost of a patient with diabetic foot infection was found to be $2880. National variation in direct costs from diabetic foot is the result of heterogeneity of selection of patients in studies and types of insurance of the patients. In this case, it is preferable to discuss cost outcomes with national cost studies and international cost separately. Gonen et al. analyzed the cost of 80 patients that had diabetic foot and reported the average cost per patient to be $2573, similar to these results [14]. Another cost analysis from Turkey, by Altuntas et al., found the average cost per patient with Wagner 3 and Wagner 4 diabetic foot was $1215 and $2190, respectively [18]. These results seem high compared to the present results as the mean exchange rate for TRY to USD was nearly 0.2 during the study period of the mentioned study [19]. A recent study by Keskek and colleagues calculated the average cost per diabetic foot patient at $976 [20]. The authors did not report infection status and severity of diabetic foot of the patients from that study. Infection is the main risk factor for hospitalization and amputation of a patient with diabetic foot. It is also associated with longer hospital stays and higher direct costs, especially in moderate and severe infections [21]. Arslan et al. evaluated the costs of hospitalization in 14 different clinics in a state hospital and found average cost of inpatient care $1169 (adjusted to 2015). Hospitalization of diabetic foot infection cost 2.5-fold more than average cost of inpatient care in Turkey [22].

Direct costs for diabetic foot management also have global variability as well as national, the same being true for lower extremity amputation rates. Global variability is mainly associated with the cost of healthcare, different in every country. There are several reasons for variations in the cost of diabetic foot care across different countries. Such as large variations in medical practice, differences in prices paid for the medical care, laboratory tests, and medical devices. Here, we found US $2880 for average cost of diabetic foot infection. Hicks et al. reported the inpatient cost of diabetic foot ulcer (DFU) $13.258 in the USA in 2014 [23]. Very recent study evaluating annual cost per prevalent case of DFU in Canada has found CAD $21,371/per case [24]. The major reason for that huge

variation between the costs can be mostly attributed to purchasing power of the currency of each countries and cost of health care. Even after balancing purchasing power, this variation may still continue. Cavanagh et al. studied economic burden of diabetic foot in five different countries (Chile, China, India, Tanzania, and USA) by using a value "international dollar" which represents same purchasing power in each country. The USA has the highest cost of DFU treatment with Int$188.645 [25]. On contrary to our country (5.1 %), the USA and Canada spent 16.4 % of gross domestic product and 10.2 % on medical care, respectively. According to an OECD report from 2013, the cost of healthcare in Turkey is lower than any EU country and the USA [26]. Besides to the gross domestic product, cost-effectiveness, merits of treatment options, and the reimbursement practices and quality-adjusted life years are also major determinants in effective analysis of cost of DFU. The primary factor of high cost of hospitalization for diabetic foot is deep foot infection which requires longer duration of hospitalization and the higher number of surgical procedures. And also, effort of limb salvage by avoiding early amputation may have a role in high cost diabetic foot care due to extended hospitalization.

Prevention is the main key factor in diabetic foot care. Preventive measures for foot ulcers and amputations include patient education, adequate footwear, and home setting foot care especially in patients at risk. These measures are the most cost-effective ways to reduce the cost of diabetic foot care. In clinical settings, diabetic foot care should manage according to the international guidelines which are established by evidenced-based practice to avoid unnecessary expensive test and treatments consequently higher costs. The cost-effectiveness of interventions in diabetic foot care should be the main priority especially for developing countries which have limited health care resources.

Conclusion

Treatment of diabetic foot infection is challenging and incurs high healthcare costs. Amputation rate is an important parameter for evaluating efficacy of treatment. Cost analysis studies are also important for estimating the magnitude of expenditure. They also assist in managing resource utilization. Further studies with well-standardized patient populations and treatment protocols will help establish more precise amputation rates and costs in diabetic foot infection treatment.

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 performed by any of the authors.

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ORIGINAL ARTICLE

A CT-based comparative study of radiological patterns of pulmonary tuberculosis in patients with type 2 diabetes versus non-diabetics

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Abstract The diabetic epidemic, although apparent across the world, has been most pronounced in non-European populations. Type II diabetes in children, teenagers and adolescents is a serious aspect to the epidemic and is an emerging public health problem. Patients with diabetes mellitus are also at higher risk of tuberculosis. We aim to study the differences in radiological pattern of pulmonary tuberculosis using high-resolution and contrast-enhanced CT scans in patients with concomitant diabetes mellitus as compared to those without diabetes and study the atypical radiological manifestations of pulmonary tuberculosis in diabetics. All the patients included in this study were scanned using VCT LIGHT SPEED 64-slice multidetector CT scanner of GE. A non-contrast scan was acquired followed by a contrast-enhanced scan. Brief history of patients was taken with regard to possibility of adverse reaction to the iodinated contrast media. Features like cavity, consolidation, centrilobular nodules, tree-in-bud pattern, pleural effusion and lymphadenopathy were noted. The patients of tuberculosis (50) with diabetes tended to be older than the patients without diabetes (50). No significant difference was seen in the frequency of upper lobe involvement between the two groups. Consolidation in lower lobes is more common in patients with diabetes. Cavitary lesions are also more common in lower lobes in patients with diabetes. No significant difference was seen in terms of bilateral involvement between the two groups. No significant difference was also seen in terms of pleural effusion and lymphadenopathy. Patients of tuberculosis with diabetes showed a significantly higher prevalence of endobronchial spread (51%) compared to non-diabetics (30%). Diabetes mellitus alters the radiological appearance of pulmonary tuberculosis. In a patient with tuberculosis, a radiological appearance of lower lobe consolidation and lower lobe cavitation should alert the clinician towards the presence of concomitant diabetes mellitus.

Keywords Diabetic complications · Pulmonary tuberculosis · Computerized tomography · Epidemiology

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Introduction

The World Health Organization estimates that approximately three million people die due to tuberculosis every year. Out of these people in the productive age group are mostly affected, thus badly affecting the social and economic development of a country [1]. The seriousness of the association of pulmonary tuberculosis and diabetes mellitus was first noted by the Arab physician Avicenna nearly 1000 years ago. The link between diabetes mellitus and pulmonary tuberculosis has been debated earlier but has never occupied the centre stage of discussions. Based on compilation of studies from different parts of the globe, the WHO has projected that the maximum increase in diabetes would occur in India [2]. With an estimated 23 million today and the numbers set to increase to 57 million by 2025, the increasing prevalence of diabetes reflects the sedentary lifestyle, excessive energy intake, reduced physical activity and obesity. Studies conducted in India in the

last decade have highlighted that not only is the prevalence of type II diabetes high but also that it is increasing rapidly in the urban population. There is also a wide urban-rural difference in the prevalence of diabetes pointing to the major role urbanization may be playing in the causation of the disease [3]. Early diagnosis of the combination is rare. At the time of diagnosis, a large majority of cases have severe diabetes and far-advanced pulmonary tuberculosis. In a recent study from the regional institute of medical sciences, Imphal, the prevalence of pulmonary tuberculosis in diabetics was found to be 27% by radiological diagnosis and 6% by sputum positivity [4]. Irrespective of the triggering mechanism(s), the fact remains that an epidemic of diabetes mellitus is sweeping the country. The recent prevalence data has propelled the estimates for India upwards—32 million in 2000 and 80 million in 2030. India is also the home to the largest number of tuberculosis patients in any one country. And there is growing amount of evidence of one disease fueling the other. The interest in diabetes mellitus and tuberculosis is mounting rapidly, and it promises to be an exciting time for researchers involved in the study of dual diseases [5]. The advent of high-resolution computed tomography has significantly improved the evaluation of pulmonary tuberculosis. A study by Torres-Cruz A et al. [6] revealed that CT scan can show evidence of tuberculosis (e.g. primary complexes, mediastinal or hilar lymphadenopathy) in up to 20% of patients with unremarkable chest X-rays. The imaging features of pulmonary tuberculosis in diabetes mellitus have been described as "atypical" in various comparative studies [7, 8]. Usually, PTB is found predominantly in the upper lobes. Lower lung field tuberculosis occurs but is often misdiagnosed as pneumonia, carcinoma or lung abscesses [9]. With TB-diabetes mellitus comorbidity on the rise, the evaluation of the radiological features of pulmonary tuberculosis in diabetes seems prudent.

Table 1 Age distribution in patients (diabetic) and controls (non-diabetic)

Age group	Number and percentage of cases			
	Diabetic		Non-diabetic	
	Number	Percentage	Number	Percentage
15–24	0	0	18	36
25–34	9	7.6	4	8
35–44	14	27.5	4	8
45–54	11	21.6	9	18
55–64	8	15.7	8	16
65–74	5	10	6	12
75–84	3	5.9	1	2
Total	50	100	50	100

Table 2 Sex distribution in patients (diabetic) and controls (non-diabetic)

Sex	Number and percentage of cases			
	Diabetic		Non-diabetic	
	Number	Percentage	Number	Percentage
Male	28	56	29	58
Female	22	44	21	42
Total	50	100	50	100

Material and methods

This was an observational cross-sectional comparative study conducted at Sir Sunderlal Hospital, Department of Radiodiagnosis and Imaging, Institute of Medical Sciences, Banaras Hindu University, from July 2012 to December 2014 in diabetic and non-diabetic patients with pulmonary tuberculosis. We selected 50 patients of pulmonary tuberculosis (cases) with diabetes (TB-DM) and 50 patients without diabetes (control) and subjected them to a CT scan of thorax with contrast enhancement, and the radiological features were compared. Subjects were patients selected from outpatient and inpatient departments attending for Sir Sunderlal Hospital for the Department of Tuberculosis and Chest Diseases, Department of Medicine I.M.S., BHU, Varanasi, and patients referred to the Department of Radiodiagnosis and Imaging I.M.S., B.H.U. A total of 100 cases of pulmonary tuberculosis were subjected to contrast-enhanced computed tomography (CECT) of the thorax at the time of diagnosis, and their imaging features were evaluated. The patients were informed about the procedure and the use of intravenous contrast.

Table 3 Level of glycaemic control in diabetic population as shown by HbA1c level

HbA1c	Number and percentage of cases	
	Number	Percentage
7.1–8.0	11	22
8.1–9.0	13	26
9.1–10.0	8	16
10.0–11.0	8	16
>11.0	10	20
Total	50	100

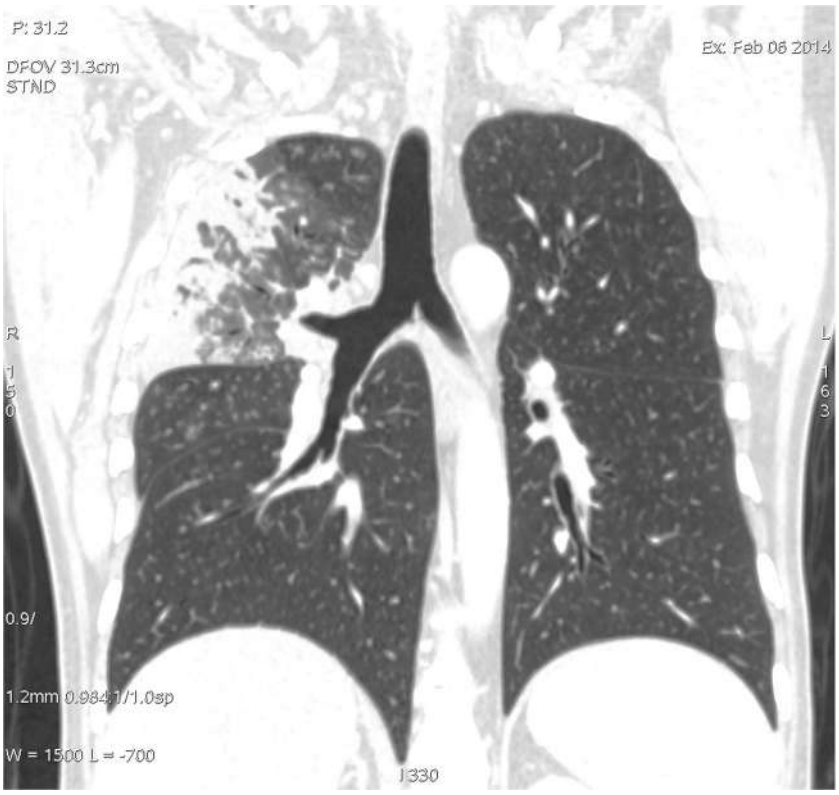

Fig. 1 Upper lung field tuberculosis: coronal section of the thorax at level of the trachea showing consolidation in the right upper lobe. Such an appearance was found to be less common in patients of tuberculosis with diabetes mellitus

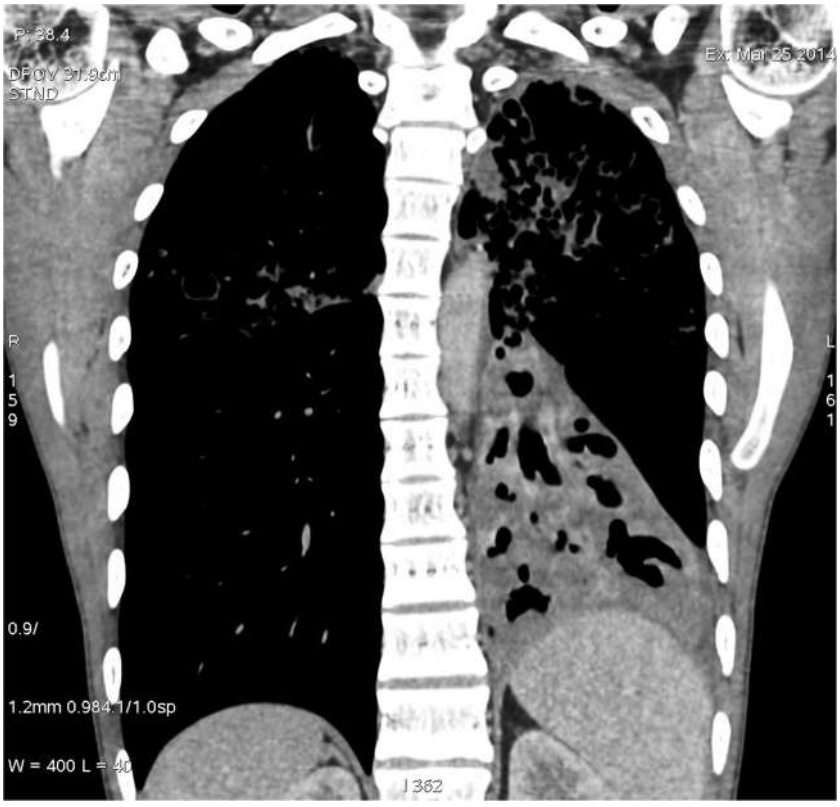

Fig. 2 Lower lung field tuberculosis: coronal section of the thorax showing consolidation with cystic bronchiectasis in the lower lobe of the left lung in a 42-year-old male diabetic with tuberculosis

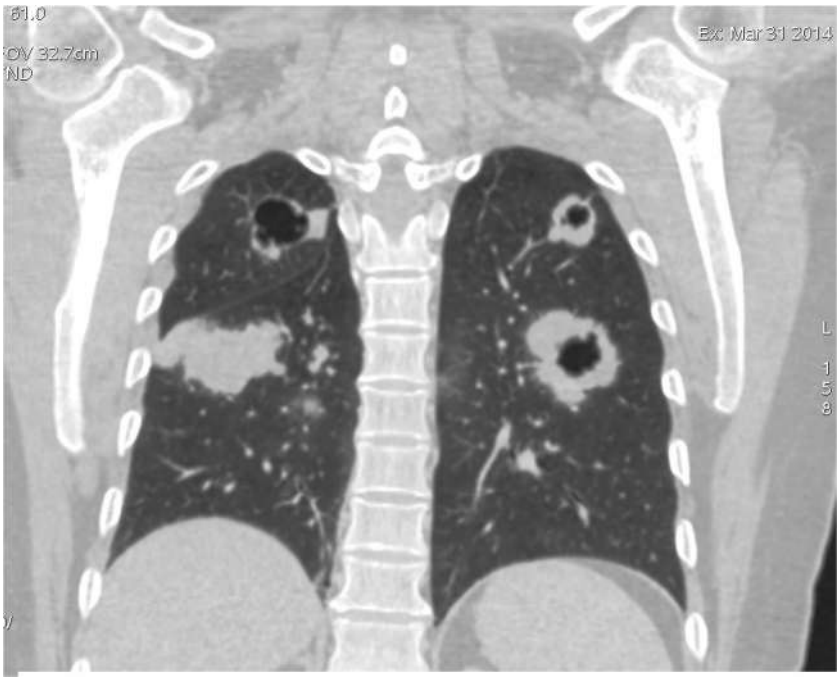

Fig. 3 Bilateral involvement: CECT coronal section of the thorax of a 55-year-old female patient of tuberculosis without diabetes showing bilateral cavitary lesions. Both diabetic and non-diabetic patients showed bilateral involvement in similar frequency

Written informed consent was taken before the procedure. The study protocol was approved by the ethical committee of our institution. A patient was considered to have pulmonary tuberculosis if he/she met any of the following criteria of having positive acid-fast bacilli in sputum, positive culture of *Mycobacterium tuberculosis* in sputum or radiographic features typical of pulmonary tuberculosis at the time of presentation. HIV-positive patients were excluded from the study. Diagnosis of diabetes mellitus was made according to the National Diabetes Data Group and WHO diagnostic criteria [10, 11]. In addition, the HbA1c levels of the patients were also obtained to better evaluate the glycaemic status of the patients. Many patients were unaware of their diabetic status and were informed of their disease for the first time. They were appropriately put on medications and standard diabetic control and protocol. All the patients included in this study were scanned using VCT LIGHT SPEED 64-slice multidetector CT scanner of GE. A non-contrast scan was acquired using

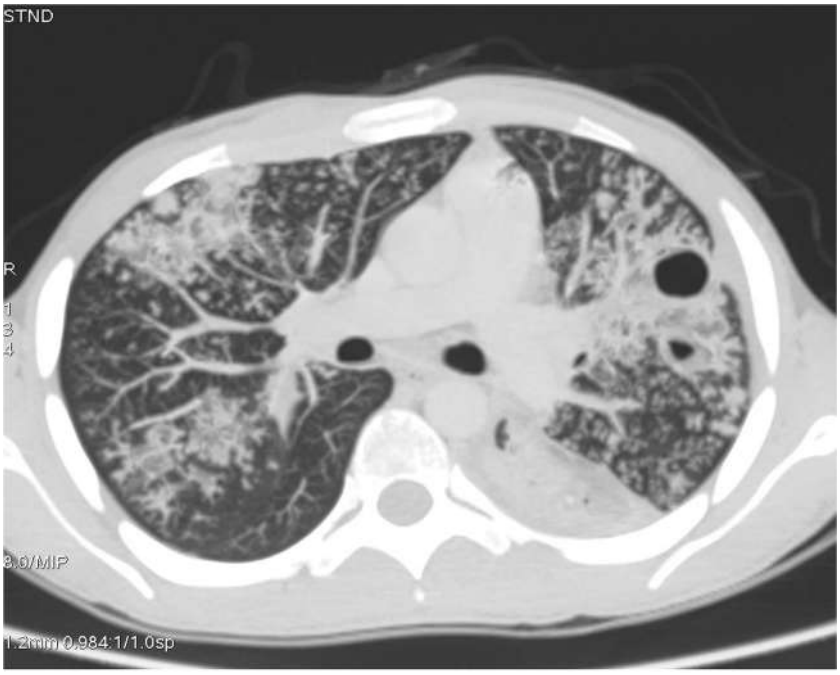

Fig. 4 Endobronchial spread: axial section of the thorax showing a cavitary lesion in the left upper lobe with extensive "tree-in-bud" lesions in both adjacent and opposite lobes of the lung. This feature was observed to be more common in diabetic patients with TB

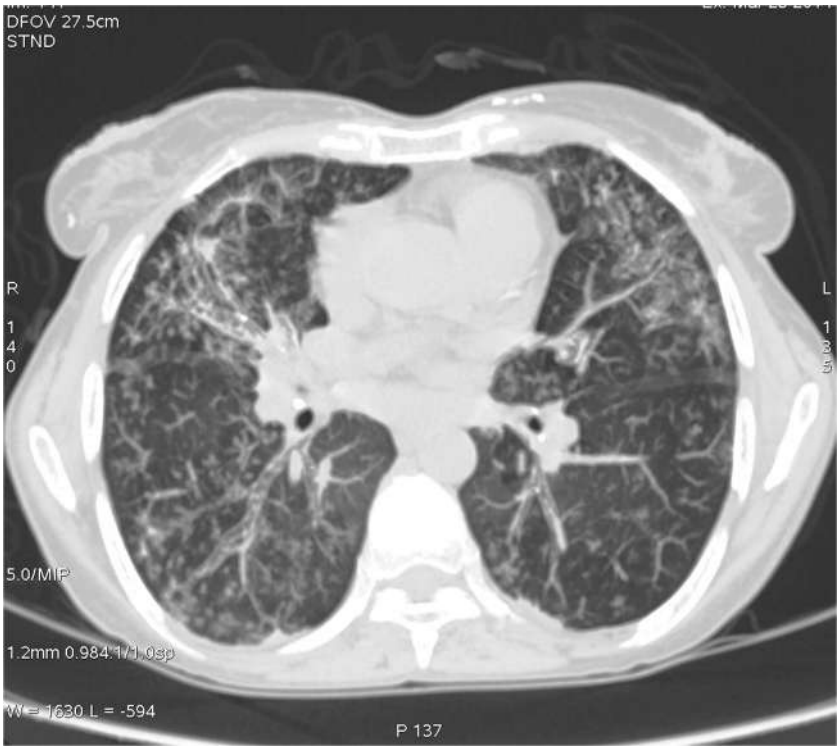

Fig. 5 Endobronchial spread: axial section of the thorax in a 60-year-old female diabetic with smear-positive tuberculosis with normal X-ray chest. Multiple tiny tree-in-bud lesions are seen in the lower lobe of both lungs

standard protocol and technical parameters followed by contrast scan. Acquisition was made using slice thickness 5 mm with reconstruction interval of 1.25 mm. After imaging, reconstruction was done in all three planes at working console using high spatial resolution algorithm. Each image was independently evaluated by two radiologists. There was concurrence in all cases but one which was excluded from the study. The frequency of various features was calculated in both case

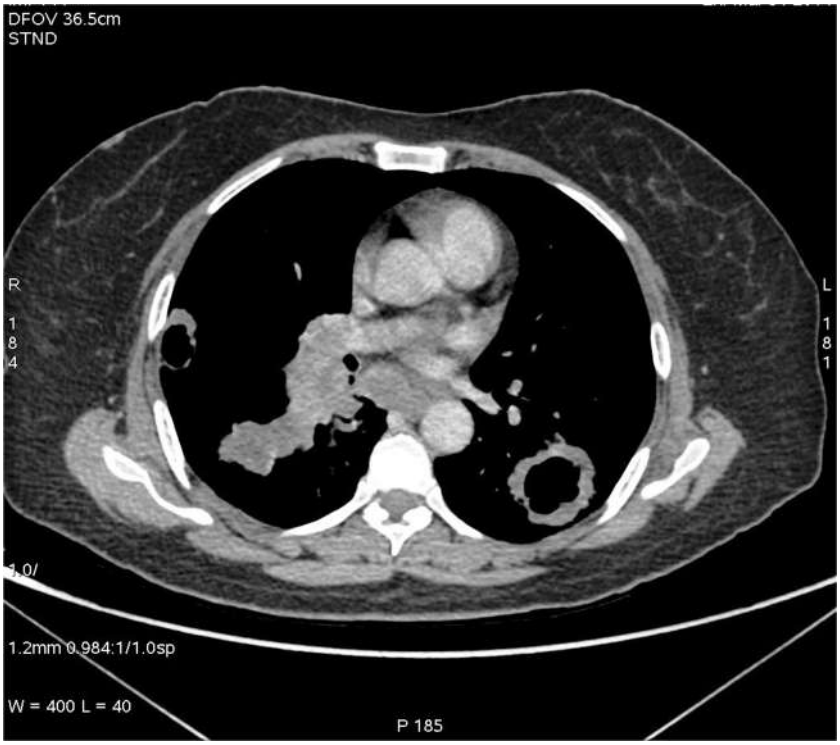

Fig. 6 Lymphadenopathy: axial section of the thorax showing mediastinal and right hilar lymphadenopathy in a 55-year-old female diabetic

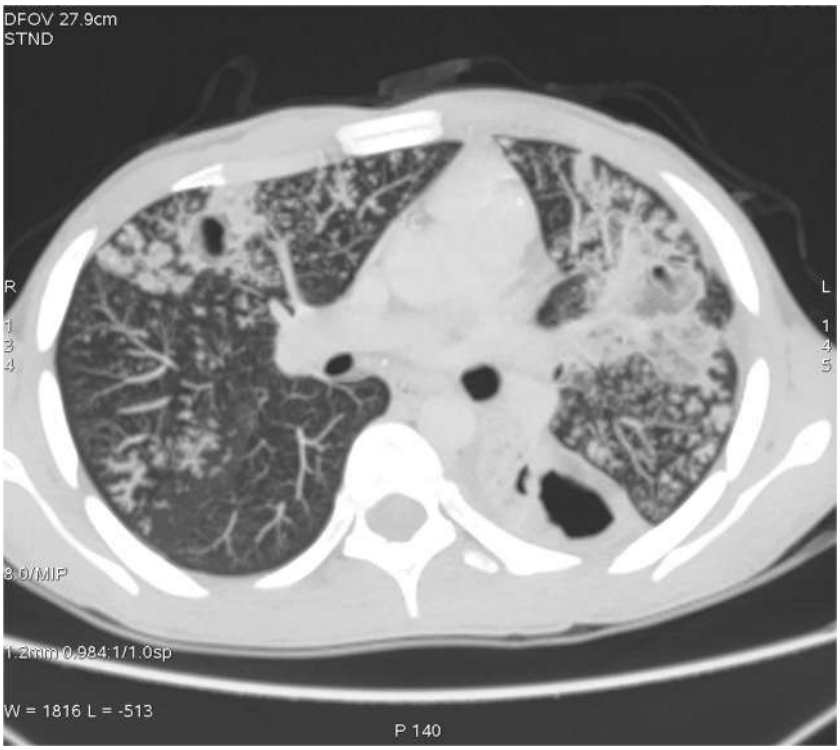

Fig. 7 Patterns of TB in patients with poor glycaemic control: axial scan of the thorax at level of the D6 vertebra in a male diabetic with HbA1c of 10.6% showing multiple cavitations with endobronchial spread

and control groups. The statistical significance of their differences was evaluated by applying the chi-square test using the SPSS 16 system.

Observations and results

In our study, we found that patients of pulmonary tuberculosis with diabetes were significantly older than those without diabetes with as high as 92.2% of TB-DM cases above 30 years of age whereas 40% of non-diabetics were below 30 years (Table 1). In this present study, males represented 56.9% TB-DM cases and females 43.1% which was similar to the non-diabetic population (58.0% males and 42.0% females) (Table 2). The number of diabetic patients and their HbA1c level were recorded (Table 3). In our present study, upper lobe involvement was found in 62.7% of diabetics as compared to 72% of non-diabetics (Fig. 1). This involvement, though, was not statistically significant (p value = 0.077). We found lower lobe cavitations in 31.4% of diabetics as compared to 14.0% of non-diabetics (p value = 0.032). Presence of consolidation in lower lobes (Fig. 2) was also higher in diabetics with 51% patients showing consolidation in middle and lower lobes as compared to 28% of non-diabetics (p value = 0.022). Both the above findings were statistically significant. The present study also found no statistically significant difference in prevalence of total patients with cavities (Fig. 3) (56.9% in diabetics compared to 43.1% in non-diabetics p = 0.196). However, a significant difference was found in prevalence of cavitation in middle and lower lobes (31.4% in diabetics against 14.0% in non-diabetics p = 0.037). With reference to multiple cavities, no statistically significant difference was seen between the

Table 4 Correlation of occurrence of cavitations with HbA1c level is shown along with p values

HbA1c	Cavity		Multiple cavities		Lower lobe involvement		Bilateral involvement	
	No.	%	No.	%	No.	%	No.	%
7.1–8.0	3	5.9	0	0	0	0	0	0
8.1–9.0	7	13.7	1	2.0	1	2	2	3.9
9.1–10.0	6	11.8	4	7.8	3	5.9	0	0
10.1–11.0	5	9.8	4	7.8	5	9.8	3	5.9
>11.0	8	15.7	7	13.7	3	5.9	5	9.8
Statistical significance	p = 0.004		p < 0.001		p < 0.001		p = 0.003	

Table 5 Correlation of occurrence of consolidation with HbA1c level is shown along with *p* values

HbA1c	Lower lobe consolidation		Bilateral involvement		Confluent consolidation	
	No.	%	No.	%	No.	%
7.1–8.0	0	0.0	4	7.8	10	19.6
8.1–9.0	1	2.0	7	13.7	14	27.5
9.1–10.0	3	5.9	4	7.8	7	13.7
10.1–11.0	5	9.8	2	3.9	5	9.8
>11.0	7	13.7	4	7.8	8	15.7
Statistical significance	$p = 0.032$		$p = 0.751$		$p = 0.194$	

two groups. Multiple cavities were seen in 27.5% of diabetics compared to 18% of the non-diabetic group ($p = 0.257$). The present study detected no statistical significance between the two groups with centrilobular nodules present in 64.7% of diabetics as compared to 62% of non-diabetics. Endobronchial spread (Fig. 4) was more common in diabetic patients as compared to non-diabetics ($p = 0.032$). Tree-in-bud patterns (Fig. 5) were seen in 51% of diabetics as compared to 30% in non-diabetics. No significant difference was in presence of pleural effusion in diabetics and non-diabetics. Of the 51 patients of tuberculosis with diabetes, 8 (15.7%) showed pleural effusion compared to 11 (22.0%) out of 50 non-diabetics. In the present study, similar finding was seen with lymph node enlargement in 28% of both diabetic and non-diabetic groups. Lymph node necrosis was found in 23.5% of diabetics as compared to 18.0% of non-diabetics ($p = 0.494$). This difference was not statistically significant (Fig. 6). It was observed that with increasing levels of HbA1c, the presence of multiple cavities, lower lobe involvement and bilateral involvement is more common in patients with uncontrolled diabetes (Fig. 7) representing 13.7, 5.9 and 9.8% of total in patients with HbA1c >11.0 as compared to 2.0, 2.0 and 3.9% in patients with HbA1c between 8.1 and 9.0 (Tables 4, 5). Endobronchial spread was more common in patients with uncontrolled diabetes (13.7% at HbA1c level of >11.0 compared to 9.8% at HbA1c level 7.1 to 8.0) (Table 6). Logistic regression analysis was done to find out the risk of higher HbA1c (independent variable) on cavitation, lower lobe consolidation and consolidation bilateral involvement (dependent variables) (Tables 7, 8). Risk of cavitation in category 2 and category 4 was found statistically significant whereas for other categories the risk (odds ratio) was higher but not significant. The risk of lower lobe consolidation was higher in categories 3 and 4 but not statistically significant. However, for categories 1 and 2, the chance of consolidation was 25 and 83%, respectively, less as compared to reference category. The risk of bilateral consolidation was higher in categories 1, 2 and 4 but not significant. It was observed that the chance of consolidation in category 3 was 11% less than the reference category.

Discussion

Our patients of pulmonary tuberculosis with diabetes were significantly older than those without diabetes. These findings are in concordance with the study done by C. Perez-Guzman et al. [7]. The probable reason for this difference may be that majority of patients of diabetes are of type 2 diabetes which usually manifests above the age of 30. The same study by C. Perez-Guzman et al. also found the ratio of males and females with TB-DM was nearly 1:1. Patel et al. [9] as well as C. Perez-Guzman et al. [7] found a lower prevalence of upper lung field involvement, with Patel et al. reporting 16% upper lobe involvement as compared to 84% lower lobe involvement whereas C. Perez-Guzman reporting 17% upper lobe involvement in diabetics as compared to 56% in non-diabetics. A study by Anand K. Patel et al. [9] found lower lung field involvement to be 84% which was significantly higher as compared to upper lung field 16% ($p < 0.01$) although no comparison was made with the non-diabetic population. C. Perez-Guzman et al. also found significantly higher lower lung field involvement (19%) as compared to non-diabetics which was 7%. The study by C. Perez-Guzman et al. [7] found overall prevalence of cavities to be higher in diabetics (82%)

Table 6 Comparison of prevalence of endobronchial spread with HbA1c level

HbA1c	Endobronchial spread	
	No.	%
7.1–8.0	5	9.8
8.1–9.0	5	9.8
9.1–10.0	4	7.8
10.1–11.0	5	9.8
>11.0	7	13.7
Statistical significance	$p = 0.094$	

Table 7 Logistic regression analysis showing risk and statistical significance of cavitation with various categories of HbA1c categories

Cavitation (dependent variable)	*B*	S.E.	Wald	df	Sig(*p* value)	Exp(*B*) OR	95.0% C.I. for exp(*B*)	
							Lower	Upper
HbA1c_ (7.1–8.0)			9.319	4	0.054			
HbA1c_(1) 8.1–9.0	0.511	0.885	0.333	1	0.564	1.667	0.294	9.445
HbA1c_(2) 9.1–10.0	2.079	1.061	3.844	1	0.049	8.000	1.001	63.963
HbA1c_(3) 10.1–11.0	1.492	0.996	2.244	1	0.134	4.444	0.631	31.294
HbA1c_(4) >11.0	3.178	1.253	6.435	1	0.011	24.000	2.060	279.624
Constant	−0.981	0.677	2.099	1	0.147	0.375		

compared to non-diabetics (59%). The diabetic group had a lower frequency of cavities in the upper lung fields than the non-diabetic group (71 vs. 97%, respectively, $p < 0.01$). In the same way, there was a higher frequency of lower cavities in the diabetic group than in the non-diabetic group (34 vs. 12%, $p < 0.001$). Multiple cavities were seen less in diabetics compared to non-diabetics. However, a CT-based study by Ikezoe et al. [12] found no statistical significance between the two groups, with cavities in 31% of patients in the TB-DM group while 46% of patients in the non-diabetic group had cavities. The study by Ikezoe et al. [12] also found no significant difference between the diabetic and non-diabetic patients (38% in diabetics against 62% in non-diabetics). Thus, the present study further supports the previous finding as described by Ikezoe et al. [12]. The differences in endobronchial spread were not described in any previous study. We evaluated endobronchial spread of tuberculosis as "tree-in-bud" opacities at a site separate from the bronchovascular segment housing the tubercular lesion. Maximum intensity projection (MIP) technique was used to further enhance the visibility of these lesions. Previous studies have not evaluated the differences in prevalence of pleural effusion between diabetics and non-diabetics. Very few studies have made a comparison of lymphadenopathy in post primary tuberculosis especially between diabetic and non-diabetic patients. In the study by Ikezoe et al. [12], lymph node enlargement was seen in 21% of diabetics compared to 13% in the non-diabetic group and was considered not significant. No previous study has mentioned a comparison on the basis of lymph node necrosis. Thus, we can infer that diabetes mellitus does not significantly alter the lymph node status in post-primary pulmonary tuberculosis. The morbidity and the clinical outcome of the patient with diabetes mellitus vary with the level of glycaemic control. Uncontrolled diabetics have a poorer outcome with greater morbidity [13]. HbA1c is the standard method of assessing long-term glycaemic control. It reflects the glycaemic history over preceding 2–3 months. In this study, we made an attempt to evaluate the pattern of tuberculosis with increasing levels of HbA1c. According to the American Diabetes

Table 8 Logistic regression analysis showing risk and statistical significance of lower lobe and bilateral consolidation with various categories of HbA1c categories

	B	S.E.	Wald	df	Sig(*p* value)	Exp(*B*) OR	95.0% C.I. for exp(*B*)	
							Lower	Upper
Lower lobe consolidation (dependent variable)								
HbA1c_ (7.1–8.0)			8.864	4	0.065			
HbA1c_(1) 8.1–9.0	−0.288	0.832	0.120	1	0.729	0.750	0.147	3.828
HbA1c_(2) 9.1–10.0	−1.764	1.229	2.060	1	0.151	0.171	0.015	1.905
HbA1c_(3) 10.1–11.0	0.693	0.949	0.534	1	0.465	2.000	0.312	12.840
HbA1c_(4) >11.0	2.380	1.216	3.832	1	0.050	10.800	0.997	116.998
Constant	−0.182	0.606	0.091	1	0.763	0.833		
Consolidation bilateral involvement (dependent variable)								
HbA1c_ (7.1–8.0)			3.038	4	0.552			
HbA1c_(1) 8.1–9.0	1.135	0.876	1.678	1	0.195	3.111	0.559	17.330
HbA1c_(2) 9.1–10.0	0.981	0.979	1.004	1	0.316	2.667	0.391	18.166
HbA1c_(3) 10.1–11.0	−0.118	1.061	0.012	1	0.912	0.889	0.111	7.107
HbA1c_(4) >11.0	0.981	0.926	1.121	1	0.290	2.667	0.434	16.390
Constant	−0.981	0.677	2.099	1	0.147	0.375		

Association recommendations [14], HbA1c level of >7.0% was taken as uncontrolled diabetes.

Conclusion

Diabetes mellitus alters the radiological appearance of pulmonary tuberculosis with greater prevalence of lower lobe lung field involvement and endobronchial spread. In a patient with tuberculosis, a radiological appearance of lower lobe consolidation and lower lobe cavitation should alert the clinician to the presence of concomitant diabetes mellitus. Also, in a patient of diabetes, the extent of pulmonary tuberculosis may be more than evidenced by X-ray owing to higher prevalence of endobronchial spread. Multidetector computerized tomography gives us valuable insights regarding the extent of tuberculosis in patients of type II diabetes as compared to non-diabetic population of tuberculosis. Uncontrolled diabetics and patients with poor glycaemic control tend to have more extensive involvement. With increasing levels of HbA1c, a higher prevalence of multiple cavities, bilateral involvement and endobronchial spread was observed.

Compliance with ethical standards

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

Research involving human participants The study protocol was approved by the ethical committee of our institution and was in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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

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ORIGINAL ARTICLE

The relationship between neutrophil to lymphocyte ratio and metabolic syndrome in patients with type 2 diabetes

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Abstract Metabolic syndrome (MetS) has been shown to be associated with inflammation. However, in diabetic patients with MetS, its relationship with the neutrophil to lymphocyte ratio (NLR) as a novel inflammation marker is unclear. The aim of the study was to investigate the association between NLR and MetS in patients with type 2 diabetes mellitus. It was a cross-sectional study which included 261 consecutive patients (mean age 56.7 ± 10.5 years, 56.7% female) with type 2 diabetes. NLR and other clinical and laboratory parameters of patients with and without MetS were evaluated. The prevalence of MetS was 85.8%. The NLR was higher in patients with MetS than without MetS ($p = 0.001$). There was a significant correlation between the NLR and the number of MetS components ($r = 0.147$, $p = 0.017$). Logistic regression analysis showed that an elevated NLR value was an independent predictor of MetS. The receiver operating curve analysis suggested that the optimum NLR cut-off point for MetS was 1.50 with a sensitivity of 74.6% and specificity of 52%. There is a significant relationship between NLR and MetS prevalence in patients with type 2 diabetes mellitus. NLR seems as an independent predictor of MetS in diabetic patients.

Keywords Inflammation · Obesity · Diabetes mellitus · Metabolic syndrome

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Introduction

Metabolic syndrome (MetS) is a significant public health problem and is composed of high blood pressure, abdominal obesity, dyslipidemia, and impaired fasting glucose [1, 2]. The risk of cardiovascular disease, stroke, and type 2 diabetes mellitus (T2DM) substantially increases in individuals with MetS [1, 3]. The pathophysiology of MetS is complex, and the underlying pathogenic mechanisms are not yet to be fully understood [1]. Besides this, there are findings implicating that chronic low-grade inflammation has an important role in the pathophysiology of MetS [4, 5]. In previous studies, it has been reported that there is an association between inflammatory markers such as C-reactive protein (CRP), white blood cell count, interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-a) with MetS and components of MetS [5, 6].

The neutrophil to lymphocyte ratio (NLR) is a novel marker of systemic inflammation [7]. The NLR can easily be calculated from the complete blood count, and it has been shown that it is a predictor and marker of prognosis in cardiovascular diseases [8, 9]. Moreover, it has been reported that high NLR is associated with development of atherosclerotic events even when the white blood cell count is within normal ranges [10]. There is limited data regarding the relationship between NLR and MetS. A study of 70 patients with MetS showed a significant correlation between the MetS components and NLR [11]. However, the association between NLR and MetS in patients with T2DM remains unclear. The aim of this study was to evaluate the association between NLR and MetS in patients with T2DM.

Materials and methods

This cross-sectional study included 261 consecutive patients with T2DM who had been following at our university

Table 1 Clinical and biochemical characteristics of study subjects

Characteristic	MetS (+) (n = 224)	MetS (−) (n = 37)	p value
Female, *n* (%)	139 (62.1)	9 (24.3)	<0.001
Age (years)	57.2 ± 10.6	53.6 ± 9.3	0.020
Duration of diabetes (years)	7.0 (3.0–13.9)	6.0 (3.5–10.7)	0.449
HT, *n* (%)	151 (67.4)	4 (10.8)	<0.001
CAD, *n* (%)	35.0 (15.6)	2.0 (5.4)	0.073
CVA, *n* (%)	4 (1.8)	0 (0)	0.540
Current smoker, *n* (%)	38 (17.0)	6 (16.2)	0.564
OAD treatment, *n* (%)	103 (46)	20 (54.1)	0.231
OAD + insulin treatment, *n* (%)	78 (34.8)	4 (10.8)	0.002
Creatinine (mg/dL)	0.8 ± 0.2	0.8 ± 0.1	0.221
HbA1c (%)	7.9 ± 2	7.7 ± 1.9	0.568
Weight (kg)	83.1 ± 15.7	73.4 ± 14	<0.001
BMI ($kg/m^{2)}$	32.4 ± 5.5	27.1 ± 4.7	<0.001
Waist circumference (cm)	106.4 ± 10.6	94.1 ± 13	<0.001
Sum of MetS components	4.0 ± 0.7	1.8 ± 0.3	<0.001
SBP (mmHg)	134 ± 19	118 ± 14	<0.001
DBP (mmHg)	75 ± 9	72 ± 9.7	0.118
Total cholesterol (mg/dL)	202 ± 44	202 ± 48	0.955
LDL-cholesterol (mg/dL)	116 ± 33	114 ± 24	0.667
HDL-cholesterol (mg/dL)	41 ± 8.4	48 ± 11	<0.001
Triglyceride (mg/dL)	163 (120–232)	110 (91–148)	<0.001
FBG (mg/dL)	160 (130–211)	151 (136–221)	0.775
Hemoglobin (g/dL)	14 ± 1.5	14.4 ± 1.1	0.115
Platelet (K/μL)	256 ± 67	247 ± 56	0.645
White blood cells (K/μL)	7.8 ± 1.8	7.0 ± 1.9	0.012
Neutrophil (K/μL)	4.6 ± 1.3	3.8 ± 1.3	0.002
Lymphocyte (K/μL)	2.3 ± 0.6	2.4 ± 0.8	0.872
NLR	2.1 ± 0.7	1.7 ± 0.6	0.001
CRP	3.4 ± 2.1	2.6 ± 1.6	0.026

MetS metabolic syndrome, *HT* hypertension, *CAD* coronary artery disease, *CVA* cerebrovascular accident, *OAD* oral antidiabetic drugs, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *DM* diabetes mellitus, *FBG* fasting blood glucose, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *FBG* fasting blood glucose, *PPG* post prandial glucose, *NLR* neutrophil to lymphocyte ratio, *CRP* C-reactive protein. Variables are expressed as means ± standard deviation, medians (percentiles 25–75), or number (percentage)

hospital's diabetic outpatient clinic between 01 January and 30 January 2016. Patients with any autoimmune disease, acute infection, chronic symptomatic cardiopulmonary disease, malignancy, hematological disease, hepatic or renal disease, chronic inflammatory disease, history of recent (less than 3 months) acute coronary syndrome or stroke, leukocyte count higher or lower than reference values (>10.2 or <4.6 K/μL), and using anti-inflammatory drugs or systemic corticosteroids were excluded from the study.

The demographic and clinical data of the patients were recorded including age, gender, duration of diabetes, smoking status, comorbidities, and antidiabetic medications. T2DM was diagnosed according to the World Health Organization criteria [12]. MetS was defined according to the criteria suggested by National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP 3) [2]. As per this guideline, T2DM patients who met at least two of the following four criteria were diagnosed with MetS: (a) abdominal obesity (waist circumference >102 cm in men and >88 cm in women); (b) high triglyceride levels (>150 mg/dL) or receiving treatment for dyslipidemia; (c) low high-density lipoprotein cholesterol level (<40 mg/dL for men and <50 mg/dL for women); and (d) high blood pressure (systolic

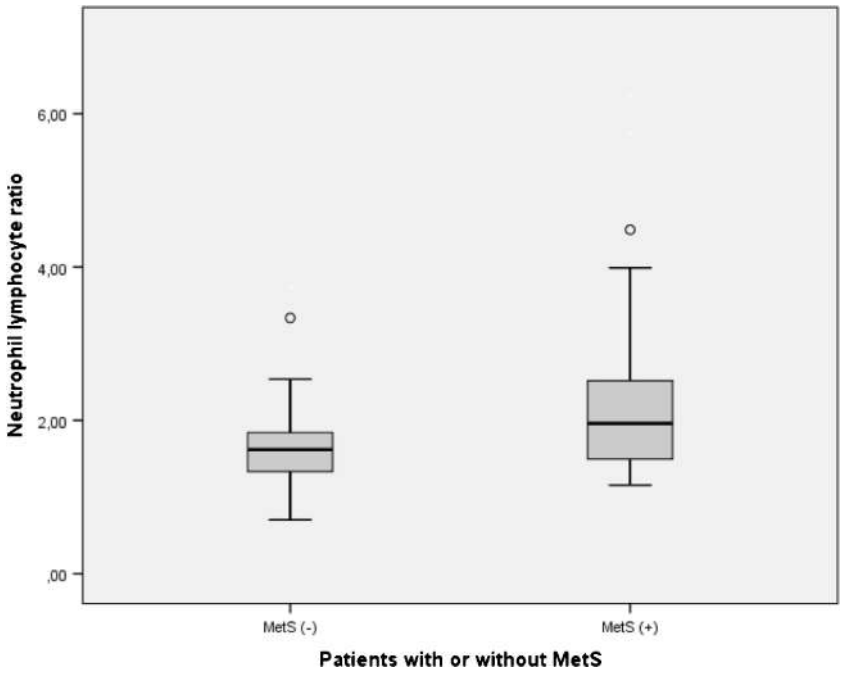

Fig. 1 *Boxplot* showing the neutrophil to lymphocyte ratio in patients with and without metabolic syndrome (MetS) ($p = 0.001$)

>130 mmHg and/or diastolic >85 mmHg), or using antihypertensive medications.

The height and weight of the patients were measured while standing in light clothing. The body mass index (BMI) was calculated by dividing the weight (in kilograms) by the square of height (in meters). The waist circumference was measured in standing position at the midpoint between the lower rib margins and the iliac crest with a tape. Waist circumferences of patients were measured twice and the average of the two values was recorded. Systolic and diastolic blood pressures (mmHg) were measured after 10 min of resting in a sitting position with arm supported at the level of the heart. Three consecutive readings were taken with 5-min intervals of from the right arm. The average of the readings was recorded as the blood pressure of the patient.

Venous blood samples were taken from the patients in the morning after overnight fasting. The glucose, creatinine, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, CRP, and glycosylated hemoglobin (HbA1c) levels were assessed by standard methods. Additionally, the complete blood count with differential blood counts was performed using an automatic blood counter (Abbott CELL-DYN 3700 System, Ramsey, Minnesota, 55,303, USA). NLR was calculated in all patients by dividing the total neutrophil count by the lymphocyte count [7, 8]. NLR, CRP, and other clinical and laboratory parameters of patients with and without MetS were evaluated. This study was approved by the ethical committee of our university review board, and informed consent was obtained from all participants.

Table 2 Multivariate logistic regression analysis of clinical predictors for developing metabolic syndrome in diabetic patients

Variable	OR (95% CI)	*p* values
Age	1.02 (0.98–1.07)	0.185
Gender	0.26 (0.10–0.68)	0.006
Duration of diabetes	0.98 (0.92–1.05)	0.625
BMI	1.26 (1.13–1.41)	<0.001
Total cholesterol	0.99 (0.97–1.01)	0.538
LDL	1.0 (0.97–1.02)	0.934
HbA1c	1.07 (0.87–1.32)	0.494
CRP	1.04 (0.92–1.18)	0.480
NLR	2.44 (1.14–5.18)	0.020

OR odds ratio, *BMI* body mass index, *LDL* low-density lipoprotein, *CRP* C-reactive protein, *NLR* neutrophil to lymphocyte ratio

Statistical analysis

The Kolmogorov–Smirnov test was used to control the data distribution. Continuous data with normal distribution were reported as mean ± standard deviation. Non-normally distributed continuous data were reported as median and percentiles 25–75. Parametric and non-parametric tests were used according to variable distribution. The chi-square test was used to analyze qualitative variables. Spearman's correlation tests were used for the

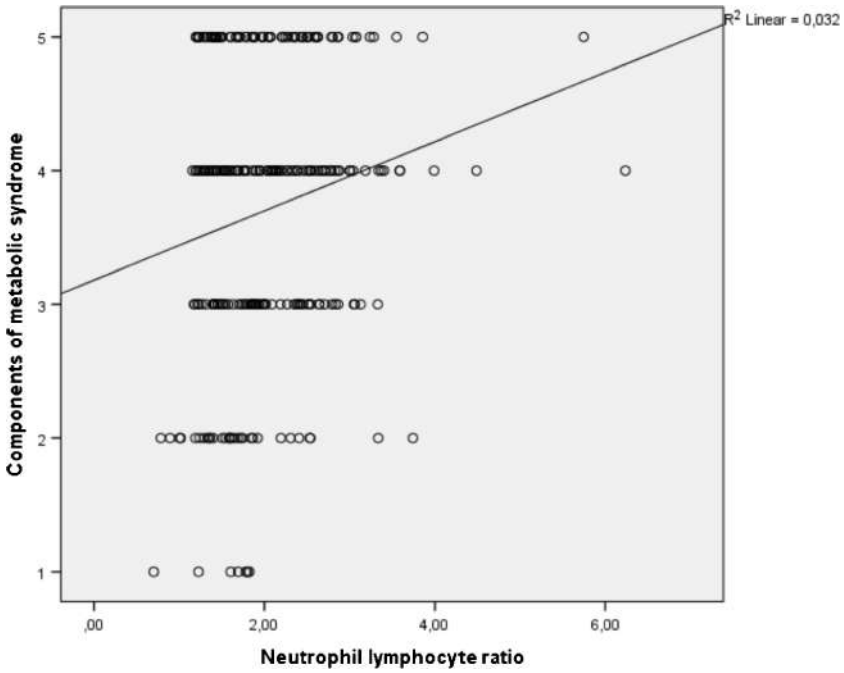

Fig. 2 Correlation between the neutrophil to lymphocyte ratio and sum of metabolic syndrome components in diabetic patients ($r = 0.147$, $p = 0.017$)

correlation analysis. Logistic regression analysis was used to assess the associations between MetS and other studied parameters. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off value of NLR with maximum sensitivity and specificity for MetS. *P* values less than 0.05 were considered significant. Data were analyzed using SPSS for Windows 17.0 (Statistical Package for Social Science; SPSS Inc., Chicago, IL, USA).

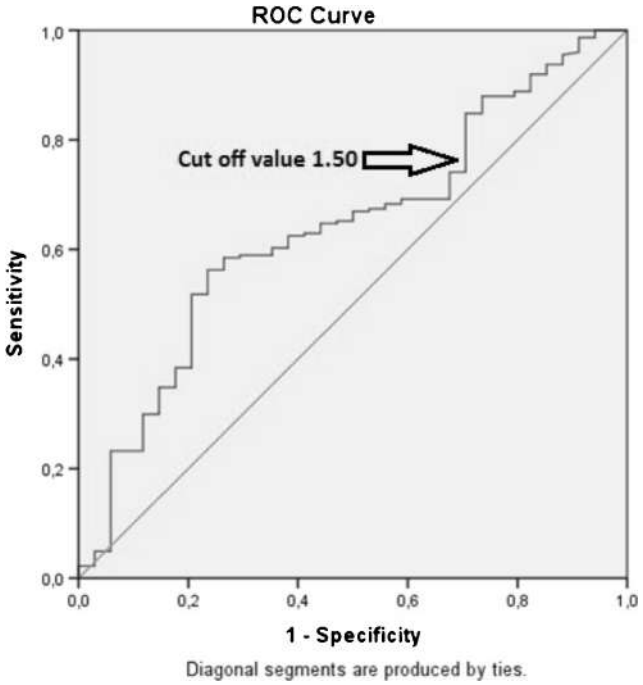

Fig. 3 Receiver operating characteristic (ROC) curve of neutrophil to lymphocyte ratio for predicting metabolic syndrome in patients with type 2 diabetes

Results

Of the 261 patients included in the study, 148 were women (56.7%), and mean age was 56.7 ± 10.5 years. The clinical and biochemical characteristics of the patients are summarized in Table 1. The prevalence of MetS among the study population was 85.8%. MetS prevalence was higher in women than it was in men (93.9 vs. 75.2%) ($p < 0.001$). In addition, the mean age of patients with MetS was higher ($p = 0.020$). The NLR, CRP, and white blood cell count were higher in patients with MetS compared with counterparts in patients without MetS ($p = 0.001$, $p = 0.026$, and $p = 0.012$, respectively) (Table 1, Fig. 1).

Age, gender, duration of diabetes, BMI, total cholesterol, LDL-C, HbA1c, CRP, and NLR were used as covariates for multivariate regression analysis. The multiple logistic regression analysis revealed that NLR was an independent predictor of MetS (OR 2.44, 95% CI 1.14–5.18, $p = 0.020$) together with gender and BMI in type 2 diabetic patients (Table 2). Spearman's correlation analysis indicated a significant correlation of NLR with the number of MetS components ($r = 0.147$, $p = 0.017$) (Fig. 2), CRP ($r = 0.130$, $p = 0.036$), white blood cells ($r = 0.247$, $p < 0.001$), waist circumferences ($r = 0.125$, $p = 0.043$), and HDL-C ($r = -0.141$, $p = 0.022$). The ROC analysis revealed that the optimum NLR cut-off

point for MetS was 1.50 with a sensitivity of 74.6% and specificity of 52% (area under the curve 0.638 (0.543–0.734), (p = 0.009) (Fig. 3).

Discussion

Our results showed that the NLR was significantly higher in diabetic patients with MetS than that of diabetic patients without MetS. Moreover, there was a significant positive correlation between the components of MetS and the NLR. NLR was an independent predictor for presence of MetS in patients with T2DM. To the best of our knowledge, this is the first study that has been conducted to evaluate the association between the NLR and MetS in patients with T2DM. The findings of our study are in line with previous data linking MetS and inflammation [4, 6].

There are many aspects of the pathophysiology of MetS that have not been elucidated yet [1–3]. Previous studies have reported that all of the individual components that constitute the diagnostic criteria of MetS are associated with inflammation [5, 13]. Also, Tsai et al. have shown that, in diabetic patients, there was a relationship between differential leukocyte counts and components of MetS and the frequency of coronary heart disease [14]. In patients with MetS, the increase of adipose tissue leads to an increase in the levels of pro-inflammatory cytokines such as TNF-α and IL-6 through activation of macrophages [5, 6]. It is suggested that these cytokines secreted mainly from the adipocytes result in a systemic subclinical acute phase response [5, 6]. NLR can easily be calculated from the complete blood count differential that is an almost universal laboratory test. In the present study, NLR, as a novel systemic inflammation marker, was also higher in MetS patients with diabetes.

It has been reported that CRP, IL-6, and the plasminogen activator inhibitor generate a pro-inflammatory and prothrombotic state in MetS [15, 16]. The increase of inflammatory and pro-thrombotic molecules increases the risk of the development of cardiovascular disease [3, 16]. Moreover, all components of MetS may propagate the development of atherosclerosis [16]. It is known that inflammation plays an important role in the development and progression of atherosclerosis by interacting with vascular endothelial cells [17]. Previous studies have shown that each type of white blood cells participates in chronic inflammation and contributes to the development of atherosclerosis [14, 18]. Kalay et al. demonstrated that the progression rate of atherosclerosis was higher in subjects with high NLR, and that the NLR may be used as a predictor for progression of atherosclerosis [19]. It has been reported that the NLR was a predictor for all-cause mortality and cardiovascular events in patients who were undergoing angiography or revascularization [20]. NLR was associated with poor glycemic control and glucose intolerance in some recent investigations [21, 22]. Similar to our result, in a cohort that was not entirely diabetic, Buyukkaya et al. demonstrated a higher NLR in patients with MetS in comparison to patients without MetS [11]. Surendar et al. was also found a strong association of NLR with the number of metabolic abnormalities [23]. In addition, the results of a cross-sectional study showed a positive correlation between the white blood cell count and the components of MetS [24].

We found a high prevalence rate of 85.5% for MetS in our study population consisting of prevalent T2DM patients. The frequency of MetS was higher in women, and the mean age of patients with MetS was higher. Shim et al. reported the prevalence of MetS as 76.5% in their study consisting of 822 T2DM patients [25]. Most of the previous studies reported higher rates of MetS in women [25, 26]. It is also known that the frequency of MetS usually increases with aging [25, 26]. In a study using 36 cohorts, the prevalence of MetS was found to be increased according to age groups with a fivefold increase in female from ages 19–39 to 60–78 years and a twofold increase in males [26]. Sedentary lifestyle, weight gain, higher number of comorbidities, physical inactivity, estrogen depletion in postmenopausal women, and lifestyle changes were proposed as reasons for the increased MetS frequency with aging [26].

The present study revealed a negative correlation between NLR and serum HDL-C levels. This result is similar to the findings of the study by Varol et al. in subjects without cardiovascular disease that investigated the association between NLR and HDL-C [27]. In the same study, it was reported that NLR was significantly higher in subjects with low HDL-C than subjects with normal HDL-C [27]. It is known that HDL-C has anti-inflammatory and anti-atherosclerotic activity, and that lower level of HDL-C is associated with coronary heart disease [28].

In conclusion, the results of our study showed a significant relationship between NLR and MetS in patients with T2DM. NLR was higher in diabetic patients with MetS, and there was a significant correlation between the NLR and the components of MetS. The limitations of the current study were the lack of controls and the small number of patients. Further studies are needed to confirm the results of the current study and to investigate the underlying pathophysiological mechanisms of the association between MetS and the NLR in diabetic patients.

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CASE REPORT

Ultrasound-guided femoral and sciatic nerve block in supine position for surgical management of diabetic foot in critical patients: pilot study of 25 cases

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Abstract Twenty-five patients ASA II-IV suffering from diabetic foot that were previously scheduled for debridement or amputation (lower than the ankle) were recruited to receive the sciatic and femoral nerve block. All of the patients were placed supine, with the head of bed at an elevation of 30–45°. Sciatic and femoral nerves were blocked by the injection of 10 mL of 0.5 % ropivacaine into two sites using ultrasound guidance. The first site was 7–9 cm above the popliteal fossa, and the second site was in the middle of the groin. All 25 cases obtained a satisfactory analgesia effect. The mean total procedure time of the sciatic and femoral nerve block was less than 10 min (8.3 ± 2.7 min). The sensory onset time was 16.3 ± 6.5 min, and the duration of sensory block was 586 ± 144 min. The motor onset time was 28.6 ± 13.7 min, and the duration of motor block was 498 ± 255 min. None of the patients required additional analgesics. No remarkable circulatory or respiratory changes related to anesthesia were observed. Four of the patients exhibited nausea and vomiting and were treated effectively with 4 mg ondansetron i.v. One patient complained of weakness in the affected lower limb 2 days after surgery yet fully recovered the next day without any treatment. Our pilot study indicated that ultrasound-guided femoral and sciatic nerve block in supine position for surgical management of diabetic foot in critical patients is a safe and efficacious approach.

Keywords Ultrasound guided · Femoral and sciatic nerve · Supine position · Diabetic foot

Introduction

There is a concern that diabetes may become an epidemic in China. Yang [1] has revealed that almost 92.4 million adults are living with diabetes and 148.2 million adults are prediabetic. Many patients cannot receive effective medical care due to poor economic, educational, and sanitary conditions [2].

Diabetic foot ulcers can occur and are one of the leading causes of morbidity contributing to infection and ultimately amputation. These cases can be treated by repeated operative debridement and resection of distal osseous and soft-tissue structures, daily dressings, strict glycemic control, and intravenous antibiotic therapy for eradication of infection [3, 4].

General anesthesia with inhalational or intravenous agents can both be used effectively in these patients. Spinal anesthesia and peripheral nerve block can be used in lower limb amputation or foot debridement [5]. Due to the diabetic neuropathy and the influence of anesthetics, maintenance of respiratory and circulatory stability during the anaesthetic process is a huge challenge for anesthesiologists [6]. Moreover, in these diabetic patients, the needle-nerve contact electrical stimulation, which can improve the success rate of blocking, is not a reliable method [7]. When a critical ill patient (insufficient in respiratory and/or circulatory function) is difficult to change positions, a safe, acute, and efficacious approach is needed.

Guo-cai Li and Yan-sheng Chen contributed equally to the article.

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Aim

In this pilot study, we aimed to test the suitability using ultrasound-guided femoral and sciatic nerve block in supine position for surgical management of diabetic foot in critical patients.

Material and methods

This study which enrolled 25 patients met the American Society of Anesthesiologists' physical status II-IV (ASA II-IV: Coexisting diseases, the organ function part to severe decompensation). They were diagnosed with diabetes mellitus (DM) with the help of the ADA/WHO 2013 criteria. The duration of diabetes in them was between 3 and 26 years. All of the patients suffering from diabetic foot were previously scheduled for debridement or amputation (lower than the ankle) in Guangdong provincial hospital of Traditional Chinese Medicine (TCM) from February 2010 to May 2014. They were all recruited to receive the sciatic and femoral nerve block.

Most of the patients were critically ill because all of them exhibited more than two of the following complications: sepsis, hypertension, heart failure, pneumonia, respiratory failure, diabetic nephropathy, diabetic retinopathy, and diabetic encephalopathy, with part to severe decompensation in the organs function. The typical surgical procedures included debridement, drainage, and/or amputation below the ankle.

Methods

Because of the observational of the study and non-invasive nature of ultrasound guiding, the local medical ethics board waived the need for the clinical trial registration.

After the written consents were obtained, patients received midazolam (0.015–0.03 mg/kg) and fentanyl (0.8–2.0 μg/kg) titrated to provide conscious sedation before nerve block insertion. Supplemental oxygen (5 L/min) was administered by facemask. When the patient was critically ill, dobutamine, adrenaline, or BiPAP noninvasive ventilation were used if necessary.

Positioning and monitoring

All of the patients were placed supine, with the head of bed at an elevation of 30–45°. Electrocardiogram (ECG), saturation of pulse oxygen (SpO2), and noninvasive blood pressure (NIBP, IBP if necessary) were monitored.

Ultrasound-guided nerve block

Ultrasound-guided nerve block was undertaken using an in-plane (IP) approach with a high-frequency (6–13 MHz) linear array probe (HITACHI-Avius TM, Japan). The puncture areas were adequately disinfected with Betadine, and the probe was covered with an aseptic dressing.

Sciatic nerve block

In order to initiate the sciatic nerve block, the affected limb was elevated by leg support (Fig. 1). The view of the sciatic nerve was located 7–9 cm above the posterior knee wrinkle of the popliteal fossa, medial of the biceps femoris, above the semimembranosus and semitendinosus (By sliding down the probe, the bifurcation point of tibialis and peroneus communis nerves was visualized. Figs. 2a and 3a). Using a 22G, 10-cm needle, the location, either medial or lateral, of the popliteal fossa was used as the injection site. Local anesthetic (0.5 % Ropivacaine, 10 mL) was injected in order to completely immerse the nerve.

Femoral nerve block

In order to initiate the femoral nerve block, the affected limb rotated to an abduction of 30°. The injection site was at the middle of the groin, and by placing the probe parallel to the groin, from outside to inside, the femoral nerve, artery, and vein can be visualized (Figs. 2b and 4a). Puncture and injection were performed with the guidance of the probe. As mentioned for the sciatic nerve block, local anesthetic (0.5 % ropivacaine, 10 mL) was injected to completely immerse the nerve. (Fig. 4b).

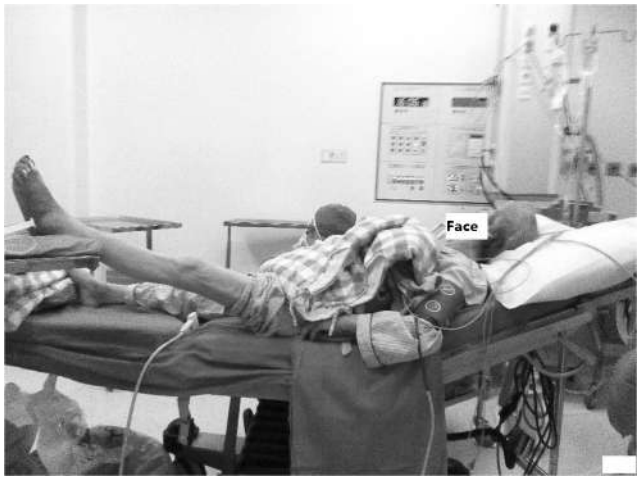

Fig. 1 The affected limb was elevated by leg support

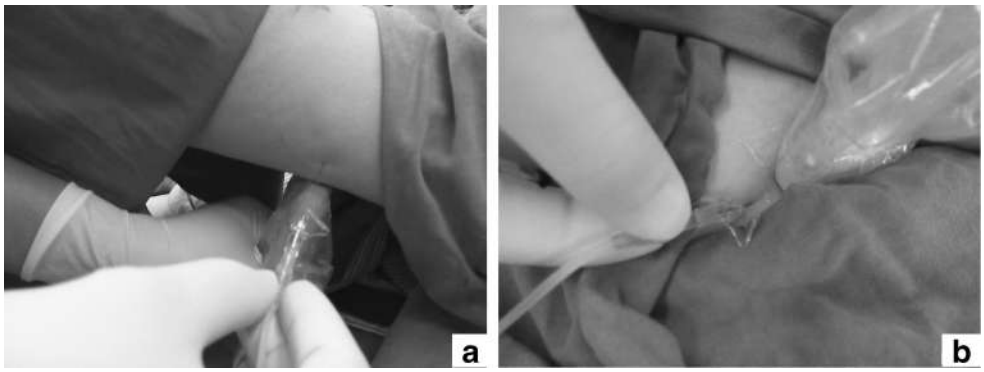

Fig. 2 Using a 22G, 10-cm needle, the location, either medial or lateral, of the popliteal fossa was used as the injection site

Evaluation of the block effects [9]

Sensory block effects

Sensory onset time was defined as the time elapsed from initiation of nerve block until complete sensory loss in affected limb (evaluated by pinprick test on the dorsal and plantar areas of the foot). Duration of sensory block was defined as the amount of time it took the affected limb to regain sensory pain.

Motor block effects

Motor onset time was defined as the time elapsed between nerve block initiation until disruption of normal flexion and extension of the foot. Duration of motor block was defined as the time elapsed between the motor onset time and full recovery of foot movement.

Results

All of the 25 cases (Table 1) that received sciatic and femoral nerve block obtained satisfactory analgesia effect. The mean total procedure time of the sciatic and femoral nerves block was less than 10 min (8.3 ± 2.7 min). The sensory onset time was 16.3 ± 6.5 min, and duration of sensory block was 586 ± 144 min. The motor onset time was 28.6 ± 13.7 min, and the duration of motor block was 498 ± 255 min. None of the patients required additional analgesics during the surgery. No remarkable circulatory or respiratory changes related to anesthesia were observed. Four of the patients exhibited nausea and vomiting and were treated effectively with 4 mg ondansetron i.v. A 56-year-old male patient complained of weakness in the affected lower limb 2 days after surgery. He fully recovered the next day without any treatment (Table 2).

Discussion

Our experience showed that ultrasound-guided, supine position, femoral, and sciatic nerves block was a safe and efficacious approach for surgical management of diabetic foot in critical patients.

The complications of diabetes mellitus (DM) are major perioperative causes of mortality and morbidity. Macroangiopathies and microangiopathies derived from DM are the major causative factors of complications in important organs, including the heart, brain, kidney, and peripheral and autonomic nervous system. It is also a challenge to anesthesiologists [10].

Approaches to anesthesia for such patients mainly include general anesthesia (GA), spinal or epidural anesthesia, and peripheral nerve block. Spinal anesthesia (SA) is the most commonly used method of regional anesthesia (RA) in lower extremity operations and seems to be more advantageous compared to epidural and GA. Whether these methods can

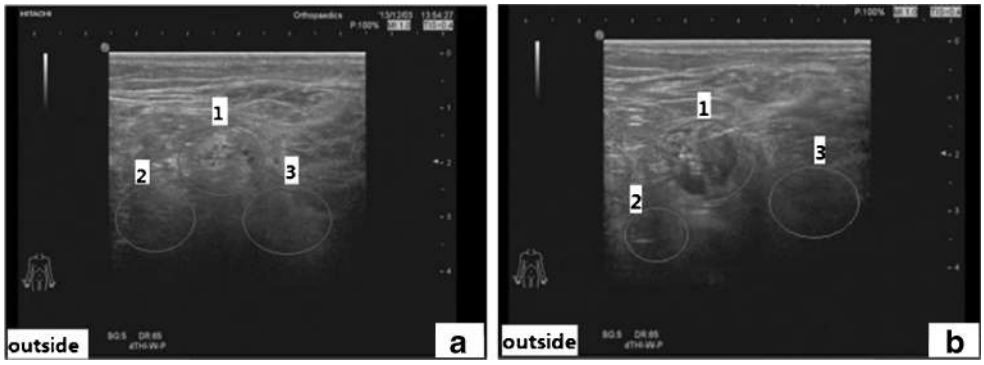

Fig. 3 The bifurcation point of tibialis and peroneus communis nerves was visualized

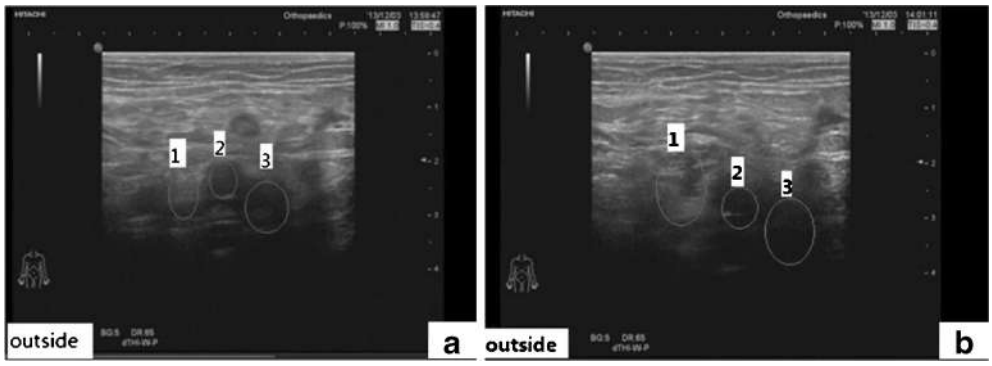

Fig. 4 The femoral nerve, artery, and vein were visualized

maintain stable hemodynamics depends on sympathetic block induced by anesthesia, preoperative cardiac performance, and the condition of intravascular volume of the patient [11].

Peripheral nerve block techniques have limited hemodynamic effects compared to central techniques such as GA and SA due to the decreased effect on sympathetic nerves [12].

As the surgical procedure was limited to below the ankle, a tourniquet was not required above the knee since the blockade of sciatic and femoral nerves would be adequate for surgical anesthesia [13]. In our 25 cases, all the patients felt no pain during the operations. No remarkable circulatory or respiratory changes related to anesthesia were observed. We believe that the method is effective and more suitable for the surgery. The supine position can avoid the potential hemodynamic fluctuations due to the intravertebral anesthesia operation.

The use of ultrasound while performing nerve block procedures is increasing over the more traditional technique of peripheral nerve stimulator. Compared to neurostimulation, ultrasound has been shown to improve the success rate and to reduce the block performance time [14–16]. Due to the special neuropathy of diabetic patients, the needle-nerve contact electrical stimulation is not a reliable method [7, 17]. Ultrasound can provide better nerve visualization resulting in a more accurate block, with lower incidences of neuropathy or accidental vessel puncture [18, 19].

From our experience, we also believe that the ultrasound-guided method is worthy of recommendation. In our approach to nerve blocking, all the performances were completed successfully in less than 10 min in the supine position. Under the guidance of ultrasound, the targeted blockage sites on the sciatic and femoral nerves can be identified quicker and with greater precision.

Table 1 Subject characteristics, ASA grades, complications, and surgical procedures (n = 25)

Age (year)	68.7(53–92)
Gender (male/female)	17/8(68/32 %)
Weight (kg)	57.3(39–76)
Height (cm)	144–176
BMI	18.5–27.7
ASA (II-IV)	
II	6(24 %)
III	12(48 %)
IV	7(28 %)
Diabetes type	
1	4(16 %)
2	21(84 %)
Complications[a]	
Sepsis	12(48 %)
Hypertension	11(44 %)
Heart failure	16(64 %)
Pneumonia	6(24 %)
Respiratory failure	21(84 %)
Diabetic nephropathy	9(36 %)
Diabetic retinopathy	14(56 %)
Diabetic encephalopathy	2(8 %)
Surgical procedures (below the ankle)	
Debridement and drainage	7(28 %)
Amputation	5(20 %)
Combined above	13(52 %)

[a] All of the patients exhibited more than two of the complications

Table 2 Intraoperative: time of nerve block procedure, onset of local anesthetic, surgery, and fluid administered; postoperative: duration of block and complications (n = 25, mean ± SD)

Intraoperation	
Procedure time of nerve block (minutes)	8.3 ± 2.7
Onset time of local anesthetic (minutes)	6.3 ± 2.5
Surgery time (minutes)	43.7 ± 14.6
Fluids administered (mL)	340 ± 58
Postoperation	
Duration of the block (feel pain, minutes)	586 ± 144
Nausea and vomiting	4(16 %)
Anesthesia related complication	1(4 %)[a]

[a] A 56-year male patient complained of weakness in the affected lower limb 2 days after surgery yet fully recovered the next day without any treatment

The limitation of this study lies in its small sample size and absence of a control group.

In conclusion, ultrasound-guided femoral and sciatic nerve block in supine position for surgical management of diabetic foot in critical patients is a safe and efficacious approach.

Compliance with ethical standards Ethical approval for this study (Ethical Committee No. 08W04) was provided by the Ethical Committee of Guangdong Provincial Hospital of TCM, Guangzhou, China (Chairman Prof. Xiao-yun Wang) on March 28, 2009 and performed in accord with the ethical principles of the Declaration of Helsinki [8].

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

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ERRATUM

Erratum to: Diabetes and data in many forms

G. R. Sridhar[1]

Published online: 6 January 2017

Erratum to: International Journal of Diabetes in Developing Countries 37(4): 381–384
DOI: 10.1007/s13410-016-0540-3

Reference 28 in the original version of this article was incorrect. Correct reference is:
Yang P, Heredia VO, Beltramo DM, et al. Pharmacogenetics and personalized treatment of type 2 diabetes mellitus. Int J Diabetes Dev Ctries. 2016. doi:10.1007/s13410-016-0517-2.

The online version of the original article can be found at doi:10.1007/s13410-016-0540-3.

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- 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.

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3. Newsletter: Latest information for diabetes educators
4. Journal of Diabetes Education Quarterly journal is uploaded
5. News and Announcements:
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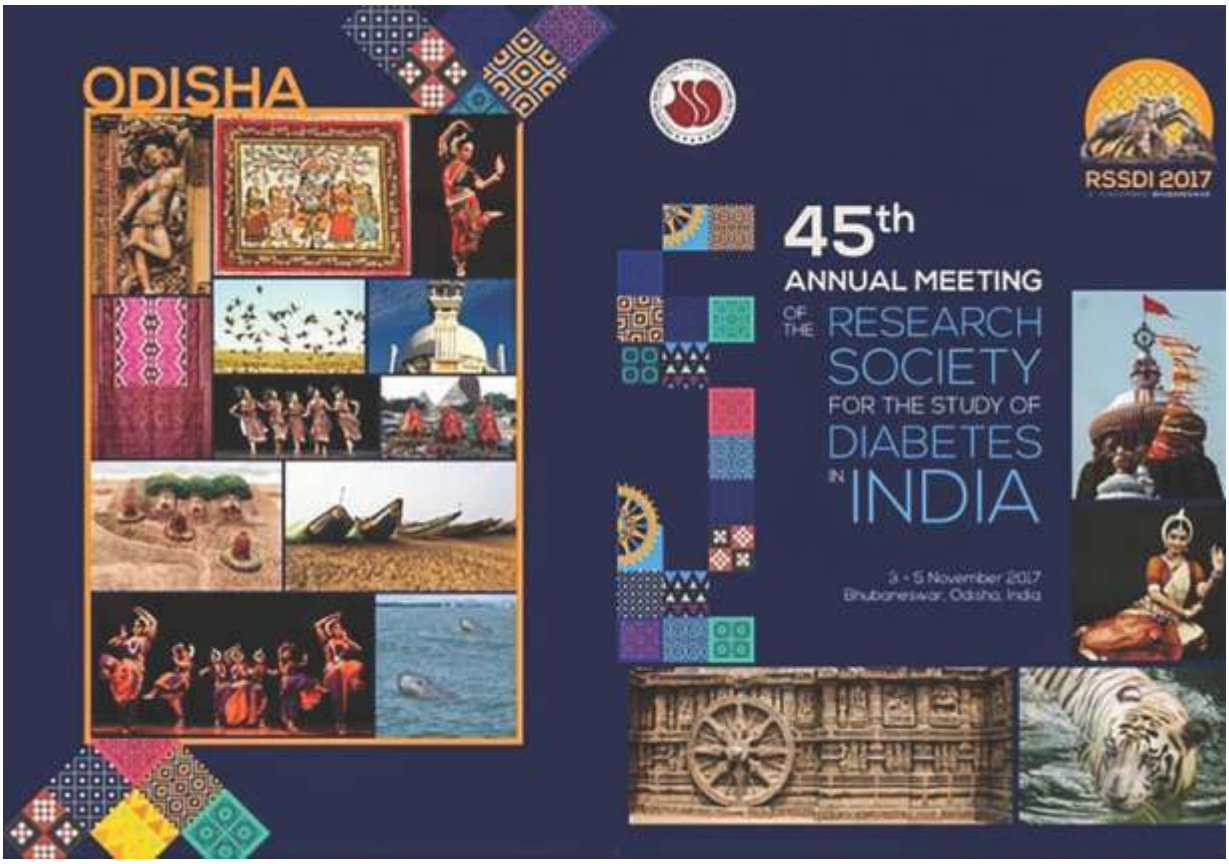
7. Membership directory- The whole membership directory is listed. Each and every member has an email id and password to view the membership directory

Invitation to the RSSDI,2017 Conference



Dear Friends,

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Wishing you a very happy. bright and prosperous new year ahead.

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