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ORIGINAL ARTICLE

How useful is clinical scoring in reducing the need for gestational diabetes screening?

P. K. Gill, W. Y. Choo, A. M. Bulgiba

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Abstract Predicting gestational diabetes by clinical scoring is meant to improve the efficiency of screening. The aim of this study was to determine the usefulness of clinical scoring in a Malaysian population. Using a retrospective cohort of 1997 women, a predictive model was developed and validated on a separate set of 1,000 patients. The efficiency of screening with risk scores was then compared with universal screening. The model derived was well calibrated (p0 0.80) with an AUC 0.74 (95 % CI 0.71–0.76). Different combinations of thresholds for the risk score produced a screening reduction between 26 % and 29 % (Sensitivity- 86 % compared to 97 % from universal screening) in Strategy 1. Strategy 2 had no screening reduction but had a higher sensitivity of 95 %. The performance of the risk score was moderate and the screening reduction minimal. Therefore the usefulness of clinical scoring in our population is of limited value.

Keywords Gestational diabetes · Prediction model · Risk score · Screening

Introduction

Gestational diabetes mellitus (GDM) is well known for its effects on maternal and foetal health when uncontrolled [1–3]. Hence screening for it during pregnancy has become a norm for all pregnant women. The screening tool used is the Glucose Challenge Test (GCT) which is performed on women at their first antenatal visit or at 24 weeks of gestation. Other tools used are fasting blood glucose and glycosylated haemoglobin.

Some studies have shown that applying a clinical risk score can be just as effective or even better at weeding out women who are more likely to develop the disease [4–7]. Risk scoring estimates the probability of developing GDM by applying known risk factors during model building where each significant factor is given a score point. The final model is used to discriminate women at high or low risk for GDM thus effectively reducing the burden of screening among women at low risk. This would also ensure that women at high risk would be closely monitored throughout their pregnancy.

The usual threshold for the GCT can be either 7.2 or 7.8 mmol/L, with the former having 90 % and the latter 80 % sensitivity. The high sensitivity is accompanied by high false positive rates from 65 % to 80 % [8, 9]. Hence many pregnant women have to make an unnecessary extra visit, some requiring half day or a full day leave. Moreover, the cost of screening is high as shown in a UK based study which found that each OGTT requires 25 min of nursing time and costs £12.13 [10]. In that study, 1,385 women with one or more risk factors were tested, of which 90 % had normal results. This amounted to £15,165 in expenditure and 520 h of nursing time.

Previous studies on risk scoring mainly used 100 g Oral Glucose Challenge Test (OGTT) to establish the diagnosis of GDM. However in Malaysia, GDM is more often diagnosed using the 75 g OGTT instead of the 100 g OGTT. This is based on the Malaysian Ministry of Health Clinical Practice Guidelines for Type 2 Diabetes Mellitus [11]. In addition,

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the racial mix and threshold levels used for GCT screening here differs from previous studies. In view of the high false positive rates and costs of screening, this study was carried to test the usefulness of risk scoring in reducing GDM screening in a Malaysian population.

Methods

The study was conducted in a semi-private teaching hospital located in Kuala Lumpur primarily serving a diverse population of middle class income bracket. Other than walk-in patients, the antenatal clinic also receives patients who are referred from nearby government maternal and child health centres as well as general practitioners in the surrounding areas of the Klang Valley. The study protocol was approved by the Institutional Review Board of the hospital. A list of consecutive patient names and registration numbers were retrieved from the labour room Delivery Book from October 2007 until December 2008. All women above 20 years with singleton pregnancies were included; however those with pre-gestational diabetes, foreigners and those who did not have screening done were excluded. The data was derived from a retrospective review of patient records obtained from the hospital record office. In routine practice, all pregnant women are screened by trained nurses using a standardized questionnaire to identify high risk patients. Established clinical factors like family history of DM in first degree relatives (parents/siblings), previous history of GDM, previous macrosomic infant, adverse obstetric history like previous unexplained stillbirth; abnormal infant; or more than two spontaneous abortions, weight above 80 kg at booking, age, gestational age, ethnicity and glycosuria were the determinants used. In this hospital, women presenting at their first antenatal visit are subjected to the glucose challenge test. A GCT value of 7.2 mmol/L and above is used as the threshold for proceeding with the diagnostic 75 g OGTT. In women who have had two GCT tests (where the first is normal) the result from the second test was used for analysis. The outcome variable was the presence or absence of GDM as determined by the OGTT. A fasting value above 6.0 mmol/L or/and post-prandial value above 7.8 mmol/L was taken to be diagnostic of gestational diabetes [12]. In current practice, GCT negative women are treated as having normal glycemic status but are closely monitored till delivery. Presence of sign or symptoms of GDM at anytime during gestation was duly investigated. Because the diagnostic test was not performed on most of these GCT negative women, they were clinically followed up to see if GDM was diagnosed by the end of pregnancy.

Statistical analysis was performed using SPSS for Windows version 15. Age was categorised into three groups: <25, 25–34 and ≥35 years. Ethnicity had three groups: Malay, Chinese and Indians. Interactions between potential predictors were found to be insignificant (data not shown here).

Next multivariate analysis using all the determinants was performed. A liberal p-value of 0.20 was used in the backward stepwise elimination from the full model. A second elimination using a p value of 0.15 was then applied [13]. The significance of the equation each time a variable was removed was checked using the difference in the −2 log likelihood ratios. The reduced model eventually had seven diagnostic determinants: age, weight above 80 kg, family history of gestational diabetes, a previous history of GDM, glycosuria and a previous history of macrosomia. Then a shrinkage factor, derived from the formula model [(chi sq-df)/model chi sq], was multiplied to each regression coefficient to obtain the shrunken model. This was then computed to determine the risk of GDM for each patient. The regression coefficients of the final model were converted into easy to use numbers. Calibration and discrimination of the model was estimated by the Hosmer-Lemeshow test and Receiver Operating Characteristic (ROC) curve respectively, to evaluate its diagnostic accuracy. Finally the sum scores were compared with their corresponding risk of developing GDM.

A temporal validation was conducted on a separate set of 1,000 patients who delivered between March and September 2007. The ROC was used to compare the performance of the risk score in the derivation and validation sets.

Results

There were 429 women with gestational diabetes from the total of 1997 studied, which amounted to 21.5 % of the study population. They were significantly older than women without GDM, 30.7±5.0 years versus 28.5±4.3 years ($p < 0.001$). Ethnicity and multiparity were not significantly associated with the outcome. In this study population, weight above 80 kg, advanced pregnancy (beyond 28 weeks) and glycosuria were clearly associated with GDM (Table 1).

The multivariate analysis resulted in seven significant parameters as shown in Table 2. A previous history of GDM significantly increased the probability of GDM in the current pregnancy by nine times (OR 9.1), while a positive urine analysis for glucose increased the chances by four times (OR 3.9). The model was well calibrated with Hosmer-Lemeshow p value of 0.80, indicating that the predicted probability of GDM presence was similar to the observed probability. The discriminative ability however was moderate, AUC of 0.74 (95 % CI 0.71–0.76) (Fig. 1).

Women who were older, beyond their seventh month of pregnancy, heavier, with family history of diabetes, history

Table 1 Characteristics of study population

Variable	Gestational diabetes mellitus		P value
	No (%)	Yes (%)	
No of patients	1568 (78.5)	429 (21.5)	
Age in years, mean (SD)	28.5 (4.3)	30.7 (5.0)	<0.001
Race			
Malay	1046 (66.7)	275 (64.1)	0.61
Chinese	247 (15.8)	72 (16.8)	
Indian	250 (15.9)	77 (17.9)	
Others	25 (1.6)	5 (1.2)	
Parity			
≤4	1542 (98.3)	421 (98.1)	0.83
≥5	26 (1.7)	8 (1.9)	
POA			
≤27 week	862 (55.0)	212 (49.4)	0.04
≥28 week	706 (45.0)	217 (50.6)	
Booking weight			
<80 kg	1393 (88.8)	344 (80.2)	<0.001
≥80 kg	175 (11.2)	85 (19.8)	
Glycosuria			
No	1557 (99.3)	416 (97.0)	<0.001
Yes	11 (0.7)	13 (3.0)	
GCT (mmol/L)			
Negative	1178 (75.1)	11 (2.6)	<0.001
Positive	390 (24.9)	418 (97.8)	
Family history			
No	1182 (75.4)	212 (49.4)	<0.001
Yes	386 (24.6)	217 (50.6)	
Previous GDM			
No	1543 (98.4)	362 (84.4)	<0.001
Yes	25 (1.6)	67 (15.6)	
Adverse perinatal history			
No	1489 (95.0)	394 (91.8)	0.02
Yes	79 (5.0)	35 (8.2)	
Spontaneous abortion			
No	1502 (95.8)	404 (94.2)	0.15
Yes	66 (4.2)	25 (5.8)	
Stillbirth			
No	1561 (99.6)	423 (98.6)	0.04
Yes	7 (0.4)	6 (1.4)	
Anomaly			
No	1560 (99.5)	425 (99.1)	0.30
Yes	8 (0.5)	4 (0.9)	

SD Standard Deviation; GCT Glucose Challenge Test; POA Period Of Amenorrhoea

of GDM in a previous pregnancy, ever delivered a large baby or had glycosuria were assigned higher marks. A score chart was designed to correlate the risk of GDM against the sum score (Table 2). The performance of this model was then assessed in the validation set. The AUC obtained was 0.71 (95 % CI 0.67–0.75) (Table 2).

Screening strategy

We attempted to analyse the effect of different combinations of thresholds for the risk score to determine the best detection rate. Two strategies were applied and compared against the usual screening practiced in this hospital. The strategies were based on the criteria that the detection rate and false positive rate should not be less than that of usual care. Naylor's strategy of not screening those with score zero and using different thresholds for those at intermediate and high risk was used for this study population. A second strategy was developed whereby those with score less than or equal to 0.5 were screened using 7.8 mmol/L threshold and the rest were screened with a lower cutoff point of 7.2 mmol/L. The results of these are presented in Table 3. Selective screening according to established risk factors as practiced in government-run clinics and hospitals were also estimated to provide comparison of screening strategies. McNemar's test was used to test significance for percentages (Table 3).

With Strategy 1 there was a significant drop in the total number to be screened as well as the number of false positives ($p<0.001$). However, it resulted in a lower detection rate of 86 %. Although Strategy 2 had a higher sensitivity of 95 % for detecting GDM, it was still lower than universal screening, while selective screening by risk factors produced the lowest sensitivity (77 %).

The same model was tested on the validation set (Table 4). Similar findings were noted for both strategies. Although Strategy 1 was able to greatly reduce the number of women who require screening, it would also miss a significant number of GDM cases. On the other hand, Strategy 2 had a slightly lower detection rate but nonetheless was successful in reducing the number of diagnostic tests and the number of false positives ($p<0.001$) (Table 4).

Discussion

This study was conducted to review the usefulness of clinical scoring in GDM screening in our population. The motive of this risk score was to reduce patient burden in terms of eliminating an extra visit for those less likely to have GDM, and healthcare expenses from unnecessary testing. Because of the differences in ethnic composition, threshold level and type of diagnostic testing of previous studies, it was necessary for us to develop a new risk score suitable for the Malaysian population.

Table 2 Final model of factors associated with GDM with corresponding scores

Characteristic		Adjusted OR (95 %CI)	Regression coefficients	Score
Age	<25	1.0		0
	25–35	1.1 (0.8, 1.5)	0.1	0
	≥35	3.0 (2.0, 4.4)	1.1	1
Gestational age (weeks)	<28	1.0		0
	≥28	1.6 (1.3, 2.0)	0.47	0.5
Weight >80 kg	No	1.0		0
	Yes	1.6 (1.2, 2.2)	0.48	0.5
Family Hx DM	No	1.0		0
	Yes	3.1 (2.5, 4.0)	1.12	1
Previous GDM	No	1.0		0
	Yes	9.1 (5.5, 15.1)	2.13	2
Previous macrosomia	No	1.0		0
	Yes	2.5 (1.0, 6.1)	1.32	1
Glycosuria	No	1.0		0
	Yes	3.9 (1.6, 9.5)	1.32	1

Score chart	0	0.5	1.0	1.5	2.0	>2.5
Risk (%)	9	13–14	20–25	30	45	>55

Risk is determined from the formula $1=1 \beta \ EXP^{\delta \ lp\beta}$, where lp is the linear predictor

The high incidence of GDM can be attributed to the fact that this is a tertiary referral centre. However it is consistent with other local and Asian studies where the quoted prevalence was 18.3 % and 16.5 % respectively [14, 15]. The predictors selected in the final model were well established risk factors of GDM. The strongest predictors were history of previous GDM, presence of glycosuria, family history of diabetes and age above 35. The risk model demonstrated reliability as seen from its comparable performances in the derivation and validation sets; however its diagnostic accuracy differed from other studies. This may be due to the differences mentioned above (ethnicity, screening threshold and the gold standard used) as well as the use of regression coefficients to produce the scores instead of the Odds Ratio (OR). Several authors recommended that the calculation of the scores be based on the regression coefficients as applying the OR could reduce the discriminative ability of the scoring rule [16, 17].

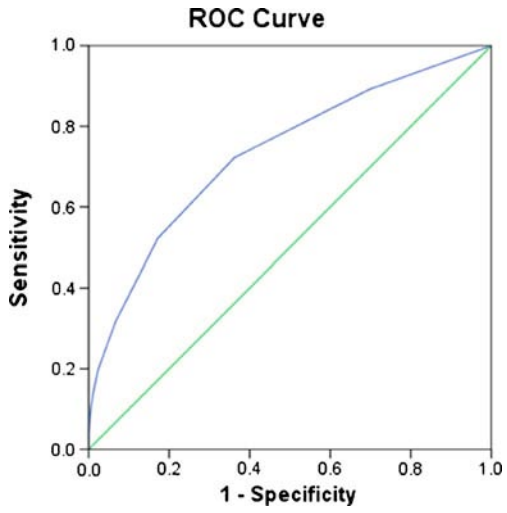

Fig. 1 Receiver operating characteristic curve of the derivation set

Previous studies have shown the usefulness of risk scoring in reducing their screening numbers. For example, an external validation study was conducted by Leeuwen et al. [18] using Naylor's original model. The AUC obtained was 0.64 which was not very different from that of Naylor's study which was 0.68 (statistical significance not mentioned in that paper). However there was poor calibration due to the differences in sample characteristics. Nevertheless, they were still able to reduce the number of screenings by 25 %. Caliskan et al. [6] did a population based study using five predictors: maternal age <25 years, BMI <25 kg/m2, family history of diabetes, macrosomia and adverse obstetric outcome. A simple screening strategy was employed by a blinded researcher, whereby those with a score ≥1 should proceed with GCT. The steps in deriving this score were not stated. However using this method, they were able to reduce the number of screenings by 30 % while maintaining 100 % detection rate. A more recent study by C. Phaloprakarn was aimed at predicting women who were more likely to have abnormal GCT results and thus reduce the number requiring this screening test [5]. Unlike the other studies, here the GCT value was used as the dependent variable and multivariable analysis was conducted to obtain predictors of abnormal GCT. It had both a derivation and validation set. Their screening strategy was to exempt women who had risk

Table 3 Comparison between screening strategies

	GCT N	OGTT N (%)	False positive (%)	GDM (%)	p value*
Current practice UMMC (universal screening)	1997	897 (45)	25	97	
Risk score (strategy 1)	1480 (26 % reduction)	597 (40)	15	86	<0.001[a]
Risk score (strategy 2)	1997	734 (37)	21	95	<0.001[b]
Govt. clinics (selective screening)	Not done	1015 (51)	43	77	

Strategy 1: Score 0 0, no screening; Score 0.5–1.0, screen with 7.5 mmol/L; Score >1.0, screen with 7.2 mmol/L

Strategy 2: Score 0–0.5, screen with 7.8 mmol/L; Score ≥1.0, screen with 7.2 mmol/L

a: Comparison between universal screening and strategy 1

b: Comparison between universal screening and strategy 2

*: McNemar's test using matched case control

scores of less than 380. Based on this method, 41.3 % of the women did not require screening but missed 3.8 % of GDM cases. However, in our study, the usefulness of risk score and its consequent screening reduction was found to be minimal. Although it was possible to reduce the number of diagnostic testing and false positive results, it was difficult to reduce the number of GCT screenings without affecting the detection rate in this research. Exempting women with score zero from screening would leave many cases undetected. Furthermore, the score chart in Table 3 clearly showed that the associated risk with score zero began at 9 % and quickly rose to 14 % at score 0.5, which was higher compared to other studies [4, 6, 19]. This means that even at a minimal score of zero, the predicted probability of developing GDM was relatively high indicating that clinical risk factors were poor predictors of GDM, even when combined. A possible reason could be that Asians are generally at higher risk for GDM and thus more prone to developing it than Caucasians [20–22]. Hence, it becomes more difficult to predict GDM based on risk scores. Moreover the majority (51 %) of the study population had one or more risk factors while among the remaining 982 "low risk" women, 10 % developed GDM. Hence, an important highlight in the present study is the need for universal screening in our high risk population. In government practices, selective risk factor based screening is employed which would inadvertently miss those GDM cases that have no known risk factors. Previous authors have reported that this approach would miss 10–40 % of women with GDM [3, 8, 23, 24].

In terms of practicality or user-friendliness of risk scoring, a simple checklist of the determinants with scores can be employed at booking. The small score range of 0–0.5 makes it easy to differentiate between women who require 7.8 or 7.2 mmol/L as their screening threshold. Having mentioned this, it is important to note that although clinical scoring was able to significantly reduce the number of diagnostic testing, it was not greatly so. Applying Strategy 2, we were unable to reduce the number of GCT screenings unlike other related studies where the reduction ranged between 25 and 41 %. With this strategy the reductions were a mere 8 % of the required OGTTs. On the other hand, while Strategy 1 reduced the screenings by 29 %, it resulted in an unacceptably poorer sensitivity. Hence the applicability of this scoring rule in our study population is of minimal value.

There are several limitations that need to be considered when interpreting the result of this study. Pre-pregnancy BMI has been shown to be associated with development of gestational diabetes [25] which would probably have increased the predictive power of the model. Unfortunately, as this was a retrospective study, missing values of self

Table 4 Validation of the risk score

	GCT N	OGTT N (%)	False positive (%)	GDM (%)	p value*
Current practice UMMC (universal screening)	1000	414 (41)	21	96	
Risk score (strategy 1)	708 (29 % reduction)	283 (40)	13	82	<0.001[a]
Risk score (strategy 2)	1000	329 (33)	16	93	<0.001[b]

Strategy 1: Score 0 0, no screening; Score 0.5–1.0, screen with 7.5 mmol/L; Score >1.0, screen with 7.2 mmol/L

Strategy 2: Score 0–0.5, screen with 7.8 mmol/L; Score >1.0, screen with 7.2 mmol/L

a: Comparison between universal screening and strategy 1

b: Comparison between universal screening and strategy 2

*: McNemar's test using matched case control

reported pre-pregnancy weight could not be overcome. Although it is possible to estimate pre-pregnancy weight [26], the formula is only applicable for pregnancies up to the 28th week. This would have led to a large number of missing values so therefore this variable was excluded. Another reason for the minimal reduction in risk score based screening is the difference in gestational age of the study population. Previous studies have focussed on women between 24 and 28 weeks of gestation as most of the GDM cases develop during this period. Screening at this teaching hospital is usually performed on women in the same gestational age range. However, it may also be conducted earlier or later as indicated by the obstetrician. It was also noted that almost 49 % of the women registered late, after their 28th week of gestation. Hence, in order to ensure better coverage of the risk score, women at any stage of gestation were included in this study. Lastly future studies should consider the issue of generalisability of this scoring rule which needs external validation in Asian communities with similar ethnic composition.

In conclusion, clinical scoring is meant to provide an alternative option to GDM screening without incurring added costs. It should be able to reduce the burden of screening on the health system and at the same time maintain a detection rate comparable to universal screening of pregnant women. However, this study revealed that the discriminative performance of the risk score was moderate and the screening reduction obtained from risk scoring was minimal. Therefore, the usefulness of clinical scoring in our population is of limited value. It would be worthwhile to consider a new risk model with the inclusion of BMI as one of the determinants which would likely improve the predictive accuracy of risk scoring. As noted from the poor performance of selective screening, the two step screening process (universal screening) should be advocated in all public hospitals to improve our detection rates.

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ORIGINAL ARTICLE

Potential effect of insulin resistance and cardiovascular risk factors on metabolic syndrome in subjects with normal fasting plasma glucose levels

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Abstract The prevalence of metabolic syndrome has progressively increased with increasing fasting plasma glucose (FPG) levels. This study aimed to investigate the influence of insulin resistance and cardiovascular risk factors on metabolic syndrome in individuals with normal FPG. Study subjects with FPG levels below 100 mg/dL were divided into 5 groups depending on the exact FPG levels. We then evaluated the association of metabolic syndrome with insulin resistance and total cholesterol/ high density lipoprotein-cholesterol ratio (TC/HDL ratio). The odds ratio of insulin resistance in the level of HOMA-IR above 2.34 group [3.483(95 % CI, 1.110 ~ 10.932)] was significantly increased in the group of FPG level from 93 mg/dL to 99 mg/dL compared to the group below 80 mg/dL. The odds ratio of metabolic syndrome in the group of FPG level from 89 mg/dL to 92 mg/dL [2.459, (95%CI, 1.275 ~ 4.741)] and 93 mg/dL to 99 mg/dL [2.079, (95%CI, 1.052 ~ 4.110)] was significantly increased compared to the group below 80 mg/dL after adjusting age, sex, smoking status, physical activity, heavy drinking, TC/HDL ratio. Higher FPG levels within the normoglycemic range may constitute a risk of insulin resistance and is associated more strongly with the risks of metabolic syndrome.

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Keywords Blood glucose · Metabolic syndrome · Insulin resistance · Cholesterol · Glucose intolerance

Introduction

During 1997–2003, the American Diabetes Association (ADA) proposed Fasting Plasma Glucose (FPG) levels ranging between 110 and 125 mg/dL as indicative of impaired fasting glucose [1]. In 2003, the ADA further modified diagnostic criteria defining FPG levels <100–109 mg/dL as being abnormal [2]. Although there have been several counter arguments against this criteria [3–5], these lower FPG levels were defined because disorder in FPG levels are associated with a high prevalence of diabetes [6]. Further, disorders in FPG are closely related to a high occurrence of risk factors of cardiovascular disease, such as dyslipidemia and hypertension [7, 8]. Recently, metabolic syndrome has been introduced as a multifaceted syndrome responsible for hypertension, abnormalities of glycometabolism,

dyslipidemia and obesity. New diagnostic criteria for metabolic syndrome, using the new criteria [2] of disorders in FPG, have been proposed by the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) [9]. Thus, FPG levels that were once regarded to be within the normal range are now considered abnormal. Such higher FPG levels are considered to indicate impaired fasting glucose (IFG). Many studies have revealed that metabolic risk factors increase with elevations in FPG levels within the normal range and that such patients are prone to developing insulin resistance, which leads to type 2 diabetes [10, 11]. That is, although FPG levels are within the normal range, the risk of metabolic syndrome increases if the FPG level is even slightly elevated. However, few studies have assessed the effects of insulin resistance and the risk factors of cardiovascular disease on metabolic syndrome in subjects with normal FBG level. In this study, therefore, we investigated the influence of insulin resistance on metabolic syndrome by using Homeostatic model assessment-insulin resistance (HOMA-IR) [12, 13] and Quantitative insulin sensitivity check index (QUICKI) [14] as also evaluating cardiovascular risk factors in individuals by measuring total cholesterol, HDL-cholesterol, and total cholesterol/ HDL-cholesterol ratio (TC/HDL ratio) [15].

Materials and methods

Subjects

Our study sample comprised individuals who visited the Health Promotion Center of the University hospitals in Pusan for medical examination from January to December 2007. Among them, 3,207 participated in this study. Our exclusion criteria were as follows: AST and ALT levels exceeding more than twice of the normal value (i.e., 80 units per liter), abnormal values of FT_4 and TSH, individuals currently being treated with diabetes medication, individuals taking medication for dyslipidemia, individuals whose level of insulin could not be measured, and individuals whose habits, such as exercise, smoking, and alcohol consumption, were not examined. These subjects were divided into four groups based on gender and age (30–39, 40–49, 50–59, and 60–69 years old) according to the 2005 census conducted by the National Statistical Office [16]. Then, we randomly selected 1,505 individuals with regard to the population proportions from the census for appropriate calibration. Eventually, 1,307 individuals whose blood FPG levels were normal (lower than 99 mg/dL) were included in this study. Individuals in the age group of 15–29 years and over 70 years were excluded because of very low frequency of visits. All participants gave informed consent and this study was approved by the Institutional Review Board at Pusan National University Hospital.

Measurements

The subjects were examined in terms of the present illness, past history, and habits of drinking alcohol and smoking. Height and weight were measured to 0.1 cm and 0.1 kg, respectively, by electronic medical instruments, HM-300 (Fanics Co. Ltd., Busan, South Korea) while the subjects wore a light dressing gown. Body mass index (BMI, kg/m^2) was calculated based upon the measured height and weight. Systolic and diastolic blood pressure was recorded once using an automatic blood pressure machine (BP-203 RVII Colin Corp., Aichi, Japan). According to the guidelines of the World Health Organization (WHO), the abdominal circumference was directly measured at slimmest section between the lowest ribs and the iliac crest and recorded to 0.1 cm accuracy in the inspiration phase. We performed the following laboratory blood tests after 8 h of fasting. Total Cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C). The liver enzyme GGT were measured by enzymatic colorimetric method with Hitachi 7600 chemical analyzer (Hitachi co., Ltd, Tokyo, Japan). The mean intra-assay and inter-assay coefficients of variation (CV) values were as follows; (Total cholesterol, 0.8 and 0.7 %), (LDL-C, 1.4 and 0.6 %), (HDL-C, 1.2 and 0.4 %), respectively. Triglycerides were measured by using lipase, glycerol kinase (GK), glycerol phosphate oxidase (GPO), peroxidase (POD) with glycerol blank. The mean intra-assay and inter-assay CV values were 0.9 and 1.1 %, respectively. FPG was measured by the glucose oxidase method (LX-20, Beckman Coulter, USA). The mean intra-assay and inter-assay CV values were 1.3 and 0.6 %, respectively. Plasma insulin level was measured by solid-phase ^{125}I radioimmunoassay with Coat-A-Count® Insulin. The mean intra-assay and inter-assay CV values were 4.2 and 6.3 %, respectively. Thyroid stimulating hormone (TSH) was measured by Coat-A-Count TSH IRMA (Siemens Los Angeles, CA, USA), while Free T4 (FT_4) was measured by Coat-A-Count Free T4 (Siemens Los Angeles, CA, USA).

Using a medical questionnaire, we examined lifestyle factors such as drinking, smoking, and exercise. For alcohol consumption, (1 drink 0 14 g of alcohol), excessive drinking was defined as follows: for males ≥14 drinks/week (alcohol, 196 g) and for females and elderly individuals ≥7 drinks or more/week (alcohol, 98 g). This included the consumption of beer, whiskey, and/or rice wine based on the guidelines of the National Institute Alcohol Abuse and Alcoholism [14]. For smoking, we categorized non-smokers as those who had never smoked as well as those who had now quit smoking. Smokers were individuals who smoked currently. With regard to exercise, the high-exercise group comprised individuals who exercised for more than 20 minutes at a time, three times a week or more. This was determined after observing the time and frequency of exercise for a week.

Cardiovascular risk factor

Total blood cholesterol, HDL-cholesterol and TC/HDL ratio [17, 18] values were measured and compared. The TC/HDL ratio is highly correlated to coronary heart disease if the ratio exceeds 4.5. The American Heart Association (AHA) recommends maintaining this ratio ≤3.5 [19]. Therefore, a ratio greater than 3.5 was considered abnormal in this study.

Insulin resistance

HOMA-IR- a well known index of insulin resistance-and QUICKI-a quantitative standard for insulin sensitivity-were calculated by using the following formulae: [HOMA-IR 0 fasting plasma insulin (μU/mL) × FPG (mg/dL) / 22.5× 18.182] [13], QUICKI 0 1/[log fasting insulin(μU/mL) + log FPG(mg/dL)] [14]. The cutoff values for defining insulin resistance was HOMA-IR 0 2.34 and QUICKI 0 0.33 [20].

Definition of metabolic syndrome

We used the 2005 American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria for the diagnosis of metabolic syndrome [9, 21]. We defined central obesity as a waist circumference ≥90 cm in males and ≥85 cm in females, according to geography-specific cut points for waist circumference [22].

Of the following 5 criteria, metabolic syndrome is diagnosed if at least three criteria are satisfied.

(1) Waist measurement ≥90 cm (men), ≥85 cm (women)
(2) Blood pressure ≥130/85 mmHg or individuals taking antihypertensive drugs
(3) FPG ≥100 mg/dL or individuals being treated for diabetes mellitus
(4) Triglycerides ≥150 mg/dL or individuals being treated for dyslipidemia
(5) HDL Cholesterol <40 mg/dL (men), <50 mg/dL (women) or individuals being treated for dyslipidemia

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, USA) version 12.0 for windows. The general and biochemical characteristics of the subjects according to gender were compared using an independent sample T-test. Study subjects were evenly divided into five quintiles (Q1 ≤80 mg/dL, 81 ≦ Q2 ≦ 84 mg/dL, 85 ≦ Q3 ≦ 88 mg/dL, 89 ≦ Q4 ≦ 92 mg/dL, 93 ≦ Q5 ≦ 99 mg/dL) depending on the percentile for FPG <100 mg/dL. In five multivariate models according to the FPG subgroup, we performed linear and linear trend analysis using the chi-square test for abdominal obesity, high triglyceride, LDL-cholesterol, high blood pressure, obesity based on the BMI, and multiple life factors, such as smoking status, alcohol consumption, and exercise. In the multivariate model, a cross ratio of each FPG sub-type and 95 % confidence intervals was compared by using logistic regression analysis for metabolic syndrome, insulin resistance, decreased insulin sensitivity, and high cardiovascular risk factors. Assessments were performed after adjusting for age and gender, multiple life factors such as smoking, drinking and exercise status. Insulin resistance was analyzed by calibrating abdominal circumference and BMI. Cardiovascular risk factors were eventually analyzed by calibrating insulin resistance. A P-value less than 0.05 was deemed statistically significant. All statistical tests were two-sided.

Results

Characteristics of study subjects

Our study sample comprised 678 men and 629 women. There were no significant differences for age and total cholesterol based on gender. However, significant differences were noted for FPG levels, BMI, systolic blood pressure, diastolic blood pressure, triglycerides, LDL-cholesterol, HDL-cholesterol, TC/ HDL-cholesterol ratio, HOMA-IR, and QUICKI values between men and women (p<0.001) (Table 1).

Table 1 Baseline characteristics of study subjects by gender[a]

Variables	Men (N0 678)	Women (N0 629)	p-value
Age (years)	45.7±9.9	46.3±10.0	0.226
Fasting plasma glucose (mg/dL)	87±7	85±7	<0.001
BMI (kg/m^2)	24.5±2.6	23.2±2.7	<0.001
Systolic BP (mmHg)	123±16	116±16	<0.001
Diastolic BP (mmHg)	76±10	71±10	<0.001
Total cholesterol (mg/dL)	194±33	191±34	0.068
Triglyceride (mg/dL)	140±81	98±78	<0.001
HDL cholesterol (mg/dL)	51±13	63±15	<0.001
LDL cholesterol (mg/dL)	125±29	117±31	<0.001
TC/HDL ratio	3.97±1.08	3.21±0.96	<0.001
HOMA-IR	1.17±0.63	0.98±0.61	<0.001
QUICKI	0.39±0.04	0.40±0.06	<0.001

[a] Plus-minus values are represented as mean ± SD. p value by two sample t-test between men and women. BMI Body Mass Index, BP Blood Pressure, HDL High-Density Lipoprotein, LDL Low-Density Lipoprotein, TC/HDL Total Cholesterol/High-Density Lipoprotein cho- lesterol, HOMA-IR Homeostatic Model Assessment-Insulin Resis- tance, QUICKI Quantitative Insulin Sensitivity Check Index

Relationship between metabolic risk factors and lifestyle factors based on FPG levels

Regarding metabolic risk factors and lifestyle factors for the five normal FPG level groups statistically significant increases were noted for the prevalence rate of abdominal obesity, hypertriglyceridemia, high blood pressure, obesity based on BMI and excessive drinking from Q1 to Q5 ($p< 0.001$); moreover, the prevalence rate for non-smoking and smoking was also significantly increased (p00.001). However, no statistically significant difference was noted for LDL-cholesterol levels and exercise status (Table 2).

Total cholesterol and TC/HDL ratio increased with increasing FPG levels

On comparing the lipid profiles for the increasing quintiles of FPG levels, significant increases in the total cholesterol level were noted ($P<0.001$). Further, the prevalence rate for cardiovascular risk (TC/HDL ratio >3.5) also demonstrated a statistically significant increase from Q1 to Q5 ($P<0.001$, p00.008) (Table 2).

Insulin resistance increased with increasing FPG levels

The prevalence rate of insulin resistance for the five groups of FPG levels was evaluated using both the parameters of HOMA-IR >2.34 and QUICKI <0.33; a statistically significant increase was noted from Q1 to Q5 ($p<0.001$). Further, HOMA-IR showed a tendency to increased odds ratio values with FPG levels as compared to group Q1. The values for groups Q4 and Q5 were statistically significant, and the odds ratio remained high after adjusting for age, gender, and lifestyle factors. However, after adjusting BMI and abdominal circumference, only group Q5 showed a significantly high odds ratio of 3.483(95 % CI, 1.110–10.932) (Table 3). QUICKI showed tendencies similar to those noted for HOMA-IR; only the odds ratio for group Q5 [5.374, (95 % CI, 1.146–25.202)] was statistically significant after adjusting for age, gender, lifestyle factor, BMI, and abdominal circumference (Table 3).

Increasing prevalence of metabolic syndrome with increasing FPG levels

The prevalence rate of metabolic syndrome showed a tendency to increase as follows: 5.2 %, 8.3 %, 9.4 %, 16.2 %, and 13.1 % from Q1 to Q5, and this was statistically significant ($p<0.001$). The odds ratios for Q2, Q3, Q4, and Q5 groups were all high as compared to group Q1 for metabolic syndrome; however, Q4 (OR02.935, 95 %CI 1.557–5.534, p00.001) and Q5 (OR02.282, 95 %CI 1.188–4.383, p00.013) were statistically significant. Q4 (OR02.899, 95 %CI 1.531–5.490, p00.001) and Q5 (OR02.485, 95 %CI 1.128–4.233, p00.021) were statistically significant even after adjusting for gender, age, and lifestyle factors. However, the odds ratio of other groups, except Q4 (OR0 2.507, 95 %CI 1.310–4.799, p00.006) were not statistically significant after adjusting for insulin resistance (Table 3).

Table 2 Metabolic and lifestyle factors according to the quintiles of normal fasting plasma glucose levels

Variables	Fasting Plasma Glucose (FPG) level					p-value[a]
	Q1	Q2	Q3	Q4	Q5	
N (numbers)	286	264	286	235	236	
Mean values of FPG (mg/dL)	76.6	82.5	86.5	90.4	95.6	
Range of FPG (mg/dL)	≤80	81–84	85–88	89–92	93–99	
Male gender (%)	42.3	46.2	49.7	61.3	63.1	
Abdominal obesity (%)[c]	18.9	24.2	28.0	34.9	32.6	<0.001
Triglyceride ≥150 mg/dL (%)	15.0	17.0	22.4	31.9	31.8	<0.001
Low HDL (%)[d]	17.5	17.4	14.7	19.6	17.4	0.826
BP ≥130/85 mmHg (%)	16.8	22.3	29.0	31.5	37.7	<0.001
BMI ≥25 kg/m^2 (%)	23.4	27.3	36.4	40.9	45.8	<0.01
Mean values of TC (mg/dL)	190.0	191.0	191.6	194.5	196.6	<0.001[b]
Mean values of HDL-C (mg/dL)	58.9	57.9	57.9	52.8	55.6	<0.001[b]
TC/HDL ratio ≥3.5 (%)	41.3	42.4	45.1	59.6	52.5	<0.001
Smoking status						
Past (%)	13.3	18.2	22.4	26.4	30.5	<0.01
Current (%)	25.9	20.1	20.3	24.7	27.5	<0.01
Heavy drinking (%)[e]	15.4	17.0	22.1	26.4	35.7	<0.001
High physical activity (%)[f]	81.8	81.8	82.2	83.4	82.2	0.753

[a]By linear association, [b]By One way ANOVA, [c]The Asia Pacific abdominal obesity criterion (waist circumference >90 cm in men, >85 cm in women) was used, [d]Low high-density lipoprotein cholesterol <40 mg/dL for men, <50 mg/dL for women, TC/HDL: Total Cholesterol/ High-Density Lipoprotein, BP: Blood Pressure, BMI: Body Mass Index, [e]Heavy drinking denotes consumption of 14 or more drinks per week for men and 7 or more drinks per week for women, [f]physical activity denotes engagement in physical activity for a minimum of 20 min at least three times per week

Table 3 Prevalence and odds ratio for insulin resistance according to the quintiles of normal fasting plasma glucose levels

Variables	Fasting plasma glucose level				
	Q1	Q2	Q3	Q4	Q5
FPG (mg/dL)	≤80	81–84	85–88	89–92	93–99
Insulin resistance (%)[a]	1.4	1.5	3.5	6.8	7.6
OR (95 % CI)	1[Reference]	1.09(0.27–4.38)	2.55(0.79–8.24)	5.15(1.70–15.63)	5.82(1.94–17.45)
Adjusted OR (95 % CI)					
Model 1[b]	1[Reference]	1.06(0.26–4.30)	2.40(0.74–7.77)	4.54(1.48–13.91)	5.15(1.70–15.58)
Model 2[c]	1[Reference]	1.04(0.26–4.20)	2.38(0.73–7.76)	4.50(1.46–13.86)	5.17(1.69–15.84)
Model 3[d]	1[Reference]	0.87(0.21–3.61)	1.72(0.52–5.75)	3.08(0.98–9.69)	3.48(1.11–10.93)
Low Insulin sensitivity(%)[e]	0.7	1.1	2.8	4.3	5.1
OR(95 % CI)	1[Reference]	1.63(0.27–9.85)	4.09(0.86–19.41)	6.31(1.37–29.09)	7.61(1.69–34.34)
Adjusted OR (95 % CI)					
Model 1[b]	1[Reference]	1.62(0.27–9.76)	3.90(0.82–18.60)	5.77(1.24–26.86)	7.05(1.55–32.14)
Model 2[c]	1[Reference]	1.56(0.26–9.76)	3.90(0.81–18.71)	5.66(1.21–26.54)	7.41(1.60–34.26)
Model 3[d]	1[Reference]	1.33(0.22–8.16)	3.01(0.61–14.69)	3.99(0.90–20.83)	5.37(1.15–25.20)
Metabolic syndrome(%)[f]	5.2	8.3	9.4	16.2	13.1
OR(95 % CI)	1[Reference]	1.64(0.83–3.24)	1.88(0.98–3.62)	3.49(1.87–6.51)	2.73(1.44–5.20)
Adjusted OR (95 % CI)					
Model 1[b]	1[Reference]	1.61(0.82–3.20)	1.75(0.91–3.39)	2.94(1.56–5.53)	2.28(1.19–4.38)
Model 2[c]	1[Reference]	1.62(0.81–3.20)	1.74(0.90–3.38)	2.90(1.53–5.49)	2.49(1.13–4.23)
Model 4[g]	1[Reference]	1.62(0.81–3.23)	1.63(0.83–3.19)	2.51(1.31–4.80)	1.80(0.91–3.54)

CI: Confidence Interval, FPG: Fasting Plasma Glucose, OR: Odds Ratio, [a] $p<0.001$ by likelihood test for trend, Insulin resistance: Homeostatic model assessment-insulin resistance ≥2.34, [e] $p<0.001$ by likelihood test for trend, Low insulin sensitivity; Quantitative insulin sensitivity check index ≤0.33, [f] $P<0.001$ by likelihood test for trend, metabolic syndrome is defined by 2005 American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria

[b] Model 1 is adjusted for age and sex

[c] Model 2 is adjusted for age, sex, smoking status (never smoker, past smoker, current smoker), physical activity (a minimum of 20 min at least three times per week), and heavy drinking(14 or more drinks per week for men and 7 or more drinks per week for women)

[d] Model 3 is adjusted for age, sex, smoking status, physical activity, heavy drinking, body mass index, and waist circumference

[g] Model 4 is adjusted for age, sex, smoking status, physical activity, heavy drinking, and insulin resistance

Discussion

In this study, adults without diabetes and with normal FPG levels showed a tendency toward increased insulin resistance and TC/HDL ratio with increasing FPG levels, along with an increasing odds ratio. In insulin-resistance, HOMA-IR and QUICKI both showed statistically significant increases in groups for which the FPG level was the highest as compared to groups for which the odds ratio is the lowest. This supports a recent study that specified that FPG criteria could be set to less than 100 mg/dL for predicting the occurrence of type 2 diabetes.

One prospective study revealed that insulin resistance demonstrated an increasing tendency in people with normal FPG levels but with higher metabolic risk factors [18]. Further, in a recent research higher fasting plasma glucose levels within the normoglycemic range constitute an independent risk factor for type 2 diabetes among young men [10]. For our research, the prevalence of metabolic syndrome increased according to FPG levels. But 4th quintile has more abdominal obesity and highest prevalence of metabolic syndrome. We calculated the odds ratio of metabolic syndrome according to increased FPG levels and complemented this calculation with insulin resistance and evaluated its effect on metabolic syndrome. As a result, the odds ratio showed a statistically significant increase only in group Q4, with no statistical significance for other groups, suggesting that insulin resistance has a significant effect in causing metabolic syndrome even in individuals with normal FPG levels.

Meanwhile, TC/HDL ratio does not have a definite effect on calibrated odds ratio of metabolic syndrome, insulin resistance may plays more important role on metabolic syndrome than lipid profile. Q4 showed a higher value of the TC/HDL ratio than the other groups, may have contributed to the statistically high odds ratio of metabolic syndrome for calibrated insulin resistance. However, the differences between the five groups with normal FPG levels suggests that further research is required to determine the normal FPG range. It has been mentioned that the criteria need to be lowered to below

100 mg/dL in order to predict type 2 diabetes, although recently, the lower limit of impaired fasting glucose has been changed to 100 mg/dL [23, 24]. Higher FPG level is well known to be a continuous risk for diabetes and cardiovascular disease. Dysglycemia refers to this continuous risk just like serum lipid levels [25]. Another report also observed that a higher but normal FPG level was related to a high-sensitivity C-reactive protein (hsCRP); this report corresponded with all of our other findings [26]. Insulin resistance has been reported as an important risk factor for BMI and abdominal circumfer- ence in adults [27]. We consider that greater attention is required for treating insulin resistance, which is critical in causing metabolic syndrome.

This study has several limitations. It is not a prospective but a cross-sectional study, which does not reflect the laboratory data of the age group of 20's and 70's; it is therefore difficult to generalize the results of this research. Further, because the normal FPG group was classified by only one laboratory result, there is a possibility that the group may include patients of diabetes or impaired glucose tolerance; these can be eliminated by oral glucose tolerance test or HbA1c levels. The third is that among several cardiovascular risk factors, only the TC/HDL ratio was considered, again limiting the generaliza- tion of our results. The fourth is that blood pressure was recorded only once. Lastly, it is yet to be seen whether our results can be generalized to other ethnic groups because the present study was conducted exclusively in the Korean adults.

The advantage of this study is that unlike the other studies, we significantly lowered the error because the selection of samples was based on the population proportion from the census; moreover, due to the exclusion of data of patients with diabetes, the bias caused by patients with diabetes under treatment could be significantly calibrated. Also, another strength of this research is that information regarding metabolic syndrome in normal FPG level individuals and the relationship between cardiovascular risk factors and metabolic syndrome is not yet sufficient.

In conclusion, by studying the effect of insulin resistance on the prevalence of metabolic syndrome and the effect of management of insulin resistance through further research, the criteria for treatment need to be determined in order to clarify whether the treatment for people with insulin resistance within the normal FPG range is beneficial.

Conflict of interest There are no conflicts of interest.

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ORIGINAL ARTICLE

Investigation on the effects of experimental STZ-induced diabetic rat model on basal membrane structures and gap junctions of skin

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Abstract This study was designed to determine the impairment of the skin structure in experimentally-induced diabetes with injection of streptozotocin (STZ). Experimental groups consisted of controls (group 1, N010) and diabetes groups (group 2, N010). Dorsal skin was removed for routine histological tissue procedures. Hematoxylene and Eosin (HE), Masson's Trichrome and Periodic Acid Schiff (PAS) stainings, immunohistochemical connexin 43 (Cx43) and type IV collagen stainings were applied. Morphometry of epidermal thickness were also determined. Group 2 revealed decrease in epidermal thickness with disintegration of epithelium and decrease of dermal collagen fibers. Stratum spinosum were morphologically abnormal for group 2. Measurements of epidermal thickness revealed statistically significant decrease (P00.000). PAS staining for group 2 revealed disruption of the basement membrane. Epithelial scar formation, deterioration of transformation in the polyhedral cells, degradation of epidermis and decrease in PAS staining for vascular structures were observed, whereas the reticular dermis and hair follicles were normal. Collagen fiber density in group 2 were found to be prominently decreased in dermis with Masson's Trichrome staining. Evident decrease in immunostaining of Cx43 and type IV collagen were also shown in diabetic group in comparison to the controls. In conclusion, diabetes not only induced impairment of the epidermal integrity and deterioration in the epidermis via loss of gap junctions (the most prominent cellular junctional complex), but also caused dramatically negative impact on the dermal collagen content, and integrity of the basement membrane.

Keywords Diabetes · Skin · Basement membrane · Type IV collagen · Connexin 43 · Epidermal thickness

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Introduction

Diabetes Mellitus (DM) is a disease with increasing prevalence and incidence and a major cause of morbidity and mortality in many populations. Acute and chronic complications of DM are strongly associated with cardiovascular dysfunction, dysregulation of nervous system, nephropathy and retinopathy [1–3]. Streptozotocin (STZ) is a commonly employed compound to produce diabetes mellitus and its complications [4, 5]. The skin is the widest immunologically active organ in the body. Because it interfaces with the environment, the skin plays a key role against pathogens and damage caused due to internal and external environment in bodily defence. It prevents excessive water loss as well as absorption and blockage of UV radiation and thermoregulation [6–9]. Almost 30 % of the diabetic patients display skin lesions, epidermal and dermal stiffness and delay in wound healing. For further understanding the effects of diabetes over skin, molecular aspects of the cell and cellular junctions need to be evaluated in detail. Skin has other important functions not only in cellular transport and communication but also tissue and organ homeostasis. Cellular communication is provided with chemical and electrical stimulation of cells via gap

junctions within the neighbouring cells. Gap junctions consist of internal membrane proteins, that are called 'connexins' [10, 11]. Connexin 43 (Cx43) is the predominant type of connexin type in the epidermal skin. Cx43 is crucial in the maintenance of the tissue homeostasis and angiogenesis as well as cellular communication and it is expressed especially in the keratinocytes, dermal fibroblasts, vascular system and skin appendages, macrophages, neutrophils and mast cells. Other crucial roles of Cx43 is in the wound healing process [12–16]

The most important structure in the cell is the basal membrane which anchors down the epithelium on to its loose connective tissue underneath. The basal membrane acts as a functional unit of mechanical barrier in protecting the tissue integrity and it is essential in cellular communication. The basal membrane structurally expresses laminin, fibronectin, proteoglycans of the heparan sulfate family, entactin and type IV collagen [17–20]. The main purpose of this study is to investigate the possible effects of experimentally induced diabetes immuno-histochemically and demonstrate the alterations of cellular junction complexes, especially gap junctions and alterations in the integrity of the basal membrane structures.

Material and methods

Animals and experimental design

The study protocol complies with the European Community guidelines for the use of experimental animals. All experiments were approved by the Local Animal Care Ethics Committee at Ege University (2009\139). Twenty adult male Rattus albinus weighing 190–230 g were selected for this study, followed for 1 month and studied in 2 equally distributed groups: Group 1, healthy controls; group 2, diabetic rats. Healthy controls (N 0 10) received no medication while the other group was injected with a single dose of intraperitoneal STZ 55 mg/kg body weight; dissolved in 0.1 mol/L sodium citrate buffer (pH: 4.7) for the induction of diabetes [4, 21]. At 48 h after administration of STZ, the tail vein blood glucose levels were measured. Two rats died in group 2 during the experiment. Blood glucose levels of 250 mg/dL and above were considered diabetic and measured at the end of the first month. All the rats were weighed before the commencement of the study (day 0) and also after the diabetes induction at 48, 96 and 120 h. At the end of the observational and experimental period of 2 months, all subjects were sacrificed and 2×2 cm dorsal skin were removed.

Histologic and immuno-histochemical procedures

Tissues were fixed by overnight immersion in 4 % paraformaldehyde (Merck), then dehydrated, embedded in paraffin and sectioned at a thickness of 5 μm (Leica RM 2145). Sections were stained with standard protocols of Hematoxylene and Eosin (HE), Periodic Acid Schiff (PAS) and Masson's Trichrome.

For immunohistochemical analyses, 5 μm thick sections were used for the primary antibodies; connexin 43 (Santa Cruz), type IV collagen (Santa Cruz) all diluted at

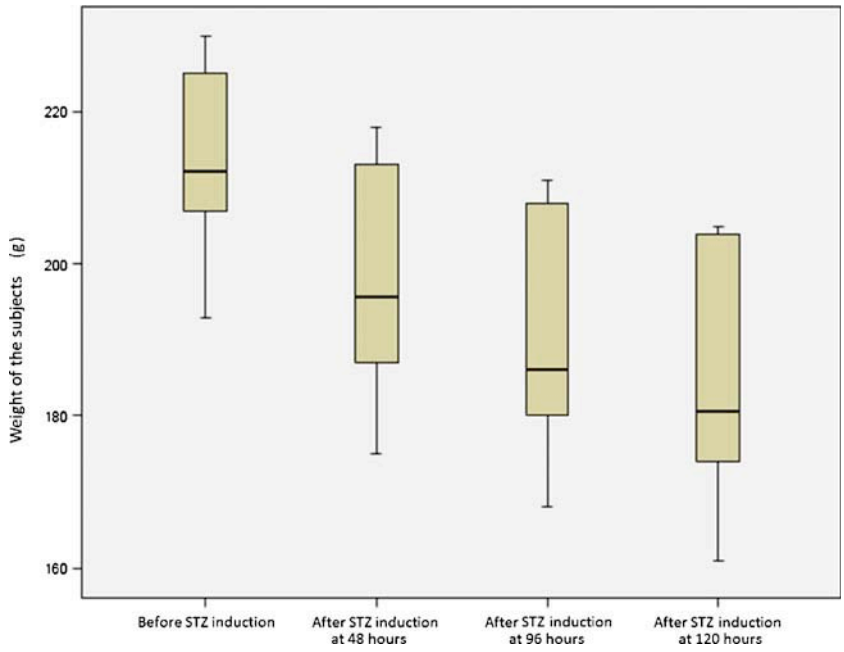

Fig. 1 Distribution of the weight of the rats group 2 before STZ and at 48, 96 and 120 h after STZ induction

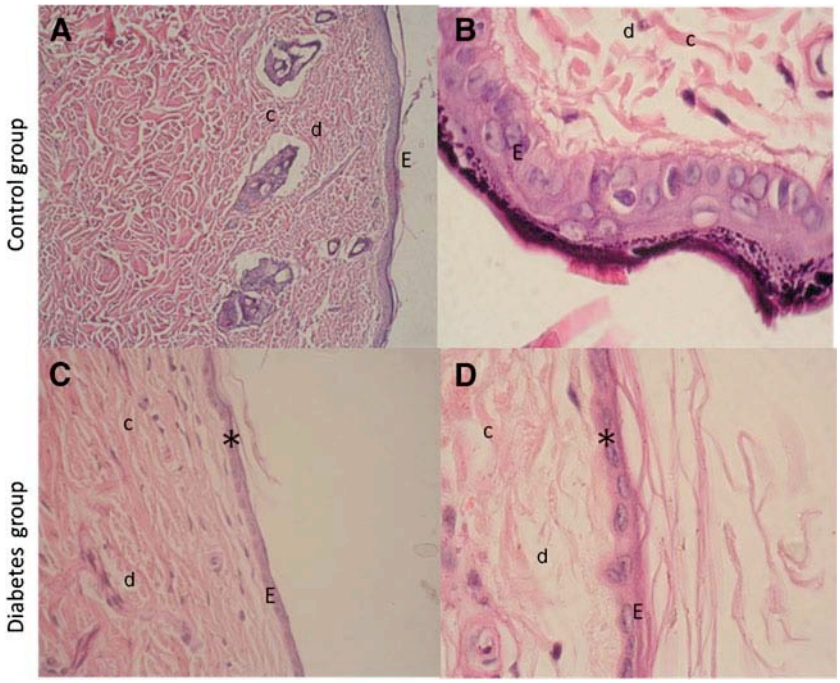

Fig. 2 HE staining. Please note the decrease in stratification of the epidermis (E) as well as the collagen fibers (c) of the dermis (d) in the STZ induced diabetes group C, D. Epithelial thickness was decreased (*) in the diabetes group in comparison to the controls A, B. Magnification 10× A, C and 100× B and D. (HE; Hematoxylene and Eosin, STZ; Streptozotocin)

1/150. In brief, the deparaffinization procedure was accomplished in xylene for 1 h. Sections were then rehydrated in sequential descending alcohol series for 2 min each. After leaving in distilled water for 5 min, the tissues were delineated on the object slide, washed in Phosphate Buffered Saline (PBS) for 10 min, and than left in trypsin for 15 min. The primary antibody was then applied in an incubator at 57°C. Thereafter, the biotinylated secondary antibody was applied and washed with PBS before incubating with the enzyme conjugate and 3,3-diaminobenzidine tetrahydrochloride (DAB). Then sections were counterstained with Mayer's Hematoxylene (Zymed Laboratories) and mounted with entellan. All the sections were examined and photographed with Olympus C-5050 digital camera integrated Olympus BX51 light microscope. Both investigators, blinded to the group distinctions of the specimens, obtained five images from 10 different sections. The intensity of immuno-histochemical staining was graded semi-quantitatively according to the nuclear and cytoplasmic immunoreaction of the skin

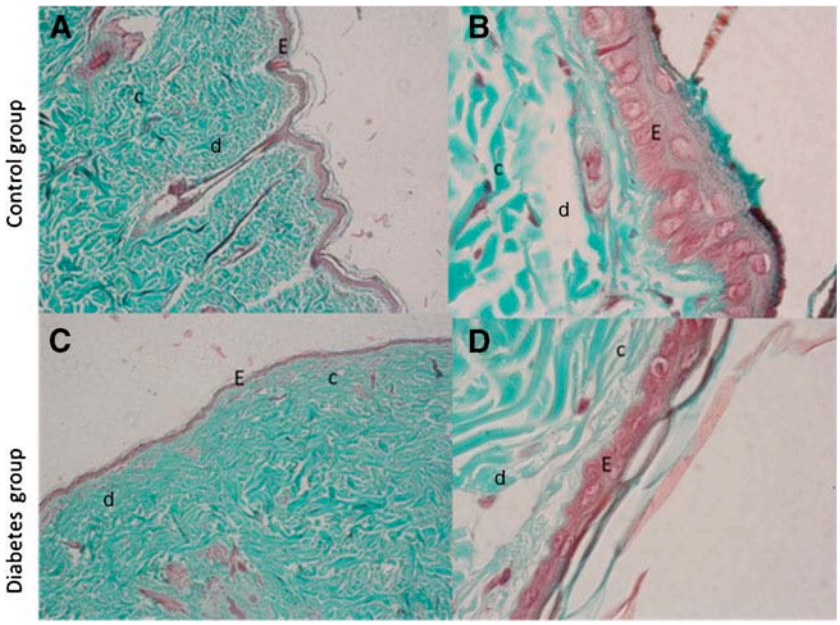

Fig. 3 Masson's Trichrome staining of the control and STZ-induced diabetes groups. Normal morphology of the skin were determined in the control sections A, B while the STZ-induced diabetes group revealed decrease in the epithelial thickness with epithelial scar formation, deterioration of transformation in the polyhedral cells and degradation of epidermis (E). Decrease in the collagen fiber content (c) and irregularity of these fibers were also determined A, B. Collagen fiber density in the diabetes group were found to be prominently decreased in dermis (d) C, D. Magnification 10× A, C and 100× B and D

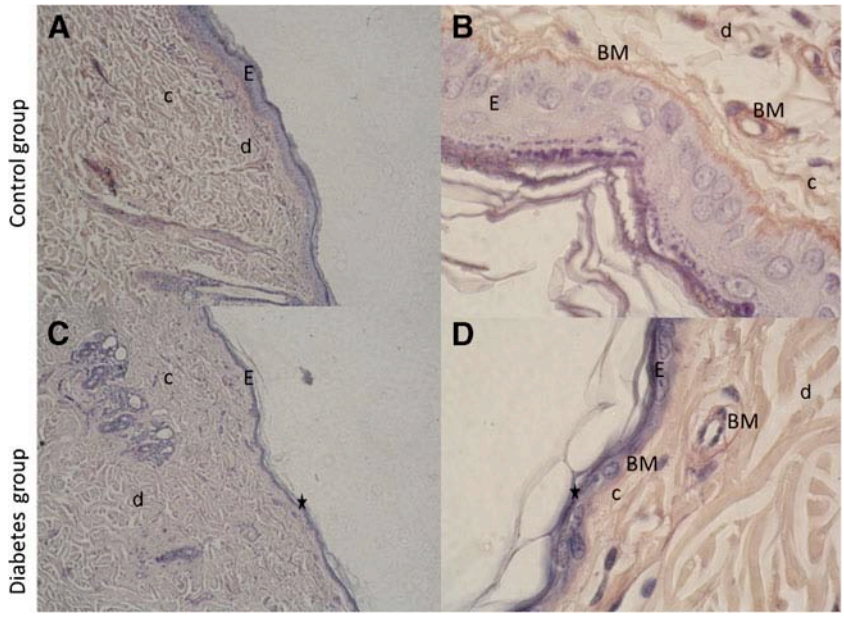

Fig. 4 PAS staining of the control and STZ-induced diabetes groups. Basement membranes (BM) was intact in the controls A, B, while the diabetes group displayed loss of integrity and decrease in the thickness (*) of the corneum and spinosum layers of the epithelium (E). The consistent lining of the basement membrane was also disrupted D. Vascular basement membranes were PAS (+) in the control group B and increased stainings were seen only in this group. Decrease in PAS staining for vascular structures prominently distinguished for the diabetes group C, D. Increased collagen fibers (c) were seen in the dermis (d) for the diabetes group C, D. Magnification 10× A, C and 100× B and D

sections as follows: (−) no immunostaining, (+) weak staining, (++) moderate staining, (+++) strong staining.

Measurement of epidermal thickness

For morphometric analysis, randomly chosen microscopic areas were captured via light microscope integrated colour digital camera (Olympus C-5050) for both experimental groups by two investigators. Acquired images saved onto Image-Pro Express (Media-Cybernetics,
2002, USA) image analysis software loaded computer were used. Both investigators, blinded to the group distinctions of the specimens, performed measurements under x100 magnification. Epidermal thickness was measured from the top of cornified epithelium to the inferior of the basal cell layer. Means of detected morphometric parameters were analyzed statistically and significance was defined as a P value of <0.05.

Results

Clinical evaluation of the subjects after the diabetes induction revealed that all rats displayed symptoms like polyurea and polyphagia. Weight alterations of the subjects were recorded and analyzed (Fig. 1).

Results of blood glucose levels within subjects at 48, 96 and 120 h were found to be statistically significant with multivariate tests (P00.000). Pairwise comparisons of the blood glucose levels at 48, 96 and 120 h with Bonferroni test revealed statistically significant differences (P00.000 for all samples).

Histochemical results

HE staining In the control group (Fig. 2a, b), dermoepidermal junctions were consistent and regular normal morphology of the papillary dermis and reticular dermis was seen.

Table 1 Distribution of Cx43 immunoreactivity in the groups

Groups/Structures	Stratum spinosum	Stratum basale	Stromal vessels and capillary areas	Hair follicles
STZ induced diabetes group	+	+	+	++
Control group	+++	+++	+++	+++

The intensity of immunostaining is grouped in the following categories: - no staining; + weak staining; ++ moderate staining; +++ strong staining. (STZ; Streptozotocin)

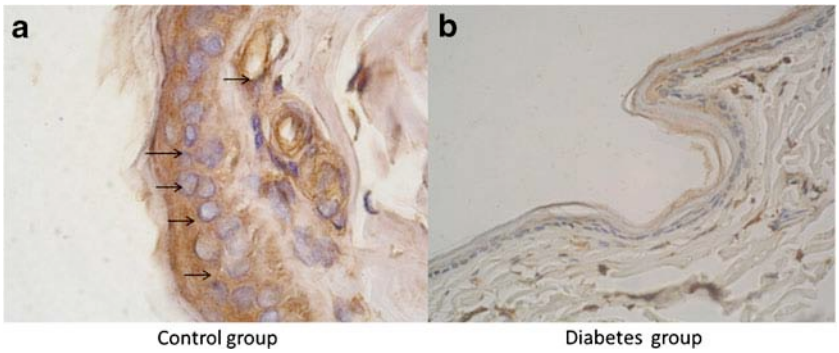

Fig. 5 Cx43 staining of the control and STZ-induced diabetes groups. Polyhedral cell groups in the stratum spinosum layer of the control group epidermis showed Cx43 expression (arrows) a. Cx43 immunostaining of skin appendages and capillary structures in the diabetes group b were decreased in comparison to the control sections. Protrusion of the basal cells in the stratum basale of the control group a showed abundant Cx43 immunostaining while the experimental diabetes group b showed findings of decreased immunostaining for these areas. Magnification 100×

Regular morphology for both the epidermis and the hypodermal collagen and stromal elements was seen. Hairy structures and the glandula sebacea appeared to be normal. The cellular lamination of the skin displayed classical stratified and cornified squamous epithelium.

Histopathological examination of the experimental diabetes group displayed altered epithelial thickness where the epithelial structural integrity was seen to be lost in comparison to the control sections. While stratum corneum was not any different, morphological abnormalities were recorded for the stratum spinosum of group 2 (Fig. 2c, d). Stratified morphology of the skin was also diminished and a decrease in epidermal thickness and loss of epidermal integrity with disintegration of the stratified epithelium was seen. Dermal collagen content was altered and a decrease in the density of dermal collagen fibers was also observed.

Masson's Trichrome In this staining, epidermis was found to be normal where the green areas showed normal fiber content. Epidermis, dermis, all the other skin appendages like hair follicles and glandula sebacea showed normal morphology (Fig. 3a, b). The experimentally induced diabetes group revealed decrease in the epithelial thickness. Epithelial scar formation, deterioration of transformation in the polyhedral cells and degradation of epidermis due to the loss in the stratified appearance of the skin were noted. Decrease in the collagen fiber content and irregularity of these fibers were also seen (Fig. 3c, d). While the reticular dermis and hair follicles revealed normal morphology, collagen fiber density in the diabetes group were found to be prominently decreased in dermis with Masson's Trichrome (Fig. 3d).

PAS staining Integrity of the basal membrane structures were evaluated in this staining. While basal membrane structures were determined to be intact for the controls (Fig. 4a, b), the diabetes group displayed loss of integrity and decrease in the thickness of the corneum and spinosum layers of the epithelium. The consistent lining of the basement membrane were also thin and disrupted (Fig. 4c, d). Vascular basement membranes were PAS (+) for the controls (Fig. 4b) and increased stainings were seen only in this group, while decrease in staining for vascular structures was prominently distinguished in the diabetes group. Increased collagen fibers were seen in the dermis in the diabetic group. (Fig. 4c, d).

Immunohistochemical evaluation

Cx43 staining Distribution of Cx43 immunoreactivity in the groups is shown at Table 1. Protrusion of the basal cells in the stratum basale of the control group showed abundant Cx43 immunostaining (Fig. 5a) while the experimental diabetes group showed findings of decreased immunostaining for these areas (Fig. 5b). Polyhedral cell groups in the stratum spinosum layer of the control group epidermis showed Cx43 expression (Fig. 5b). Cx43 immunostaining of skin appendages and capillary structures in the diabetes group were decreased (Fig. 5a) in comparison to the control sections.

Type IV collagen staining Distribution of Type IV collagen immunoreactivity in the groups is shown at Table 2. The experimental diabetes group displayed an evident decrease

Table 2 Distribution of type IV collagen immunoreactivity in the groups

Groups/ Structures	Stratum spinosum	Stratum basale	Stromal vessels and capillary areas	Hair follicles
STZ induced diabetes group	+	+	+	++
Control group	+	+++	+++	+++

The intensity of immunostaining is grouped in the following categories: - no staining; + weak staining; ++ moderate staining; +++ strong staining. (STZ; Streptozotocin)

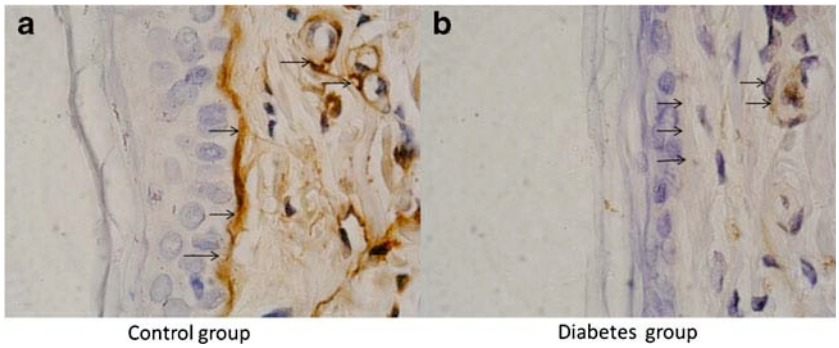

Fig. 6 Type IV collagen staining of the control and STZ-induced diabetes groups. While the basement membrane structures and the vascular elements in the control group a revealed increased immunostaining pattern for type IV collagen (arrows), the experimental diabetes group displayed evident decrease of staining b in these structures. Magnification 100×

of immunostaining pattern for type IV collagen (Fig. 6a) in the basal membrane structures and the vascular elements, while the control group (Fig. 6b) revealed increased immunostaining pattern for these structures.

Morphometry of epidermal thickness

Distribution of epithelial thickness for both control and STZ induced diabetes groups is shown at Table 3. Morphometric quantitative assessment of epidermal thickness revealed alterations for both groups and analysis of the means of detected morphometric parameters were analyzed statistically by Mann Whitney test (significant differences between group 1 and group 2, P0 0.000) (Fig. 7).

Discussion

The incidence of diabetes, is rapidly escalating in every country in the world [22]. The skin is one of the most effected organ in diabetes. Almost 30–70 % of the affected people display skin symptoms accompanying other disturbances [1, 4, 23, 24]. The main purpose in this study was to investigate effects of STZ induced diabetes over skin histology via molecular evaluation of gap junctions and basement membrane structures. Administered STZ for the experimental induction of Type 1 DM in rats is known to display its effects via decreasing insulin receptors on target cells and thereby inhibiting both pancreatic insulin release and tyrosine kinase activity [2, 4, 21, 25, 26]. In organized tissues like the skin and the ligaments, collagen contributes to the molecular structure in protection of morphological and mechanistic properties.

Table 3 Distribution of epithelial thickness (μm) for both control and STZ-induced diabetes groups

GROUPS	MEDIAN (μm)	MIN (μm)	MAX (μm)
Control group	149,7836	114,46	304,68
STZ induced diabetes group	37,8604	20,38	55,57

Groups compared with Mann Whitney test (P0 0.000)

Structural longevity and integrity of the dermis depends on the collagen content. There are many previous studies regarding collagen catabolism in diabetic rats describing the increase in collagen catabolism in the skin specimen [27, 28]. Thickened dermal vessel sheaths, loss of integrity and laminated structure for the basal membrane with degenerated collagen fibers were other observations in this study which correlated with previous studies [29, 30]. In the STZ-induced rat model by Chen et al., the loss of stratified epithelium, degeneration of the collagen fibers, inflammation and infiltration of inflammatory cell groups, and increase in glucose levels and other glycation compounds were reported [31]. Our study agreed with previous literature as much as the epidermal thickness was altered and decreased.

Basal membrane in the skin is crucial in tissue organization, stability and differentiation during development. A study of the structure of the basement membrane elucidates many normal and pathological mechanisms. There are many studies on the capillary basal membrane structure where the main components are stated to be collagen (mostly type IV), chondroitin, heparan sulfate and laminin. The most affected parameter among these are collagen type IV and laminins

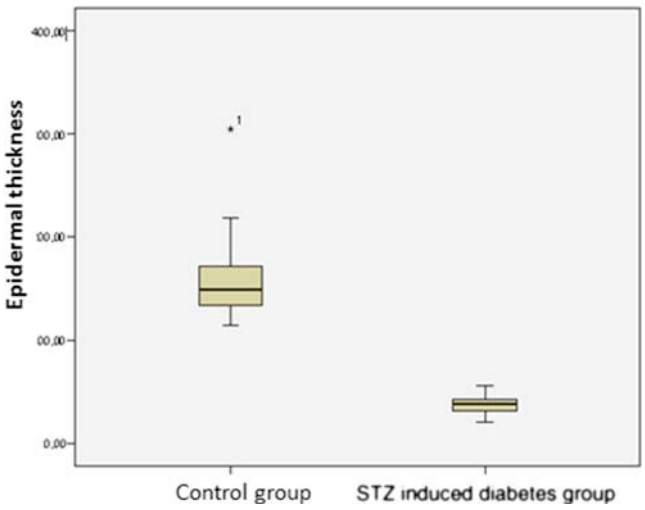

Fig. 7 Graph of epidermal thickness alterations for control and STZ-induced diabetes mellitus groups. Groups compared with Mann Whitney test (P0 0.000)

[32, 33]. Results of our study are all in accordance with the previous literature, as collagen type IV was more irregular and decreased in the diabetes group in comparison to the controls. Diabetes has certainly demonstrated a negative impact on basement membrane structures. The other most important result of this study is related to the structural integrity of the gap junctions and its association with diabetic skin. Gap junctions have gained worldwide importance lately. Gap junctions are known as gates of communication between cells in the skin and they are important in maintaining homeostasis [15, 34]. The intercellular communication and connection during keratinocyte development and differentiation depends on the proper function of gap junctions. Solan et al. previously described critical roles of vertebrate gap-junctions in tissue homoeostasis, normal cell growth and differentiation as well as embryonic development and co-ordinated contraction of excitable cells [35]. 9 connexin genes among which Cx43 is also prominent, have been shown to be expressed during keratinocyte differentiation. While being the most dominant protein in the human epidermis and keratinocytes, Cx43 has many other roles in the embryonic development and tissue homeostasis, normal cellular development and differentiation [11, 36–38]. Wang et al. stated that Cx43 immunostaining in the diabetic skin was determined especially in the basal layer of epidermis, dermal fibroblasts, hair follicles, and vessel structures [38]. Interactions considering cellular junctions and basal membrane integrity of the skin due to diabetic disruption had not been evaluated in detail previously. In this study, immunohistochemical expression of Cx43 were evaluated in diabetic skin and results were interesting especially in the diabetic group; showing decreased expression in the polyhedral cells of the stratum spinosum of the epidermis. The skin appendages and the capillary in the dermis also revealed decreased expression of Cx43 in comparison to the control immuno-histochemistry. Cellular connections of the basal layer of the cells in the epidermis revealed apparent Cx43 expression while the diabetic skin basal layer showed specific decrease of immuno-expression.

In conclusion, diabetes not only induced impairment of the epidermal integrity and deterioration in the epidermis via loss of gap-junctions which is the most prominent cellular junctional complex, but also caused a dramatically negative impact on the dermal collagen content and integrity of the basement membrane. In this study, diabetic animal model, epidermis and dermis were evaluated in detail, thus offering newer insights into future cellular therapy initiatives.

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Conflict of Interest Authors have no conflict of interest (financial or otherwise).

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ORIGINAL ARTICLE

Familial aggregation of T2DM among Arab diabetic population

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Int J Diab Dev Ctries. 2012 ; 32 : 90-92

Abstract The aim of the study was to estimate the prevalence of familial history of type 2 diabetes among Arab diabetic patients, compare the maternal and paternal transmission of type 2 DM in patients and evaluate its influence on the clinical characteristics of this disease. This was a cross sectional study. The survey was carried out in urban and semi-urban primary health care [PHC] centers. Of the 2,400 registered with diagnosed diabetes, 1,980 agreed and gave their consent to take part in this study, thus giving a response rate of 82.5 %. DM was defined according to the WHO expert group. Of the study population, 72.9 % reported family history of DM. Family history of DM was significantly higher in females (54.2 %; p00.04) and in the age group below 30 years (24 %; p<0.001). The prevalence of diabetes was higher among patients with diabetic mother (25.4 % vs 22.1 %) and maternal aunts/uncles (31.2 % vs 22.2 %) compared to patients with diabetic father and paternal aunts/uncles. Family history of DM was higher in patients of consanguineous parents (77.4 %) than those of nonconsanguineous parents (70.4 %). The present study has found a significant maternal effect in transmission of T2 DM. Family history is associated with the increased incidence of diabetes.

Keywords Diabetes mellitus · Family history · Parental transmission · Genetic disorders

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Introduction

The global prevalence of Type 2 diabetes mellitus (T2DM) is rapidly increasing and currently stands as the fourth or fifth leading cause of death in most developed countries [1]. It has been widely reported that the occurrence of T2DM is triggered by a genetic susceptibility and familial aggregation in several populations [2]. It was estimated that risk for diagnosed T2DM increases approximately two to four fold when one or both parents are affected [3]. Family history reflects both inherited genetic susceptibilities and shared environments which include cultural factors [4]. A recent study has documented a high prevalence of diabetes mellitus and its complications in the population of Qatar [5]. Hence, this study aimed to determine the familial aggregation of T2DM among Arab diabetic patients in Qatar.

Subjects and methods

This is a cross-sectional study conducted among the patients registered in diabetic clinics of primary health care centres during the period January 2010 to January 2011. A total of 2,400 diabetic patients were approached and 1,980 agreed to participate and gave verbal consent to take part in this study, thus giving a response rate of 82.5 %. The study was approved by the Hamad Medical Corporation. A structured questionnaire was developed covering the socio-demographic data, family history of DM, laboratory investigations and complications for data collection. Family physicians and research nurses recorded results of all investigations from their medical files. Physical examination and measurements were performed by a trained nurse.

Pearson Chi-square test was performed for statistical analysis between categorical variables. P-values <0.05 were considered statistically significant.

Results

The prevalence of DM in father, mother, brother and sister was 22.1 %, 25.4 %, 14.2 % and 9.3 % respectively. 72.9 % of the study sample had family history of diabetes. In second degree relatives like uncles and aunts, a positive family history of T2DM was more common among maternal aunts/uncles (31.2 %) than in paternal aunts/uncles (22.1 %; p<0.001). Similarly, a larger proportion of diabetic patients had a mother with T2DM (25.4 %) in comparison to those who had a father with T2DM (22.1 %; p00.015).

No significant difference was found in the metabolic characteristics of diabetic patients according to the family history of DM, except for the systolic (p00.033) and diastolic blood pressure (p00.025).

Discussion

This is the first study providing insight in the familial aggregation of T2DM among Arab population residing in Qatar. The study sample revealed that 72.9 % of the subjects with DM had a positive family history of diabetes among at least one of their parents, siblings, uncles, aunts and grandparents. In Tunisians [6] 70 % of the diabetic patients had a positive family history of diabetes which is nearly identical to our study. A lower rate was observed in a French study [2], where 66 % of the diabetic patients had at least one relative with diabetes among their first and second degree relatives. In the study sample of 1,980 diabetic patients, 71 % of them reported at least one first degree family member which is similar to the study results of Crispim et al. (76.6 %) [7]. These results are in agreement with a study by Hariri et al. [8] that a family history of diabetes in a first-degree relative doubles a person's risk of developing diabetes.

It was observed that on the maternal side 83.6 % of the diabetic patients have affected mother (25.4 %) and at least one relative (58.2 %), whereas a lower rate was observed on the paternal side with 63.7 % of the diabetic patients with affected father (22.1 %) and one family member (45.2 %). This suggests a maternal transmission of T2DM in the Arab population which is in line with other studies from different populations [2, 7]. A positive family history of T2DM was more common among maternal aunts/uncles (31.2 %) than in paternal aunts/uncles (22.2 %), showing that this maternal effect likely extends to the previous generation in 2nd degree relatives as reported in another study [7]. These study results indicating an excess of maternal transmission of T2DM are quite serious in the light of a recent Greek study which found that diabetic subjects with mother with diabetes were significantly younger (P00.003), had lower age at diabetes diagnosis (P<0.001) [9]. Moreover, the absence of a difference in metabolic characteristics among diabetic patients with and without a family history of T2DM is confirmative with a previous study [9].

In conclusion, this study showed an excess maternal transmission of T2DM in a sample of Arab diabetic population residing in Qatar. The data support the dominant maternal role in the development of diabetes mellitus in their offspring.

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ORIGINAL ARTICLE

Blood glucose concentrations after cardiac surgery: the impact of preoperative quality of life

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Abstract We hypothesized that poor quality of life may lead to the development of post-operative hyperglycemia, as it is more likely to be associated with stress-induced impairment of glucose control. A correlation between the components of quality of life, and blood glucose changes after Coronary Artery Bypass Surgery (CABG) was examined. In a cross-sectional study, 268 consecutive patients undergoing CABG at Tehran Heart Center were recruited. Postoperative blood glucose were measured every 2 to 4 h for 24 h following surgery. Quality of life was assessed using the SF-36 questionnaire, and the physical and mental component summary scores were used to analyze data. An adverse relationship was found between mental summary score and the mean of plasma glucose concentrations during 24 h after surgery (β0−0.152, SE00.076, P00.046). No significant association was found between physical summary score and mean of plasma glucose concentration during this time. Postoperative blood glucose changes may be independently associated with patients' mental status preoperative, as reflected by mental component of quality of life.

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Keywords Glucose · Mental stress · Surgery · Quality of life

Introduction

Quality of life has been recognized as an important component of health status which reflects physical and mental health. Several studies have shown that people with complications related to impaired blood glucose have a decreased level of quality of life compared to those without these complications, and that the degree of reduction is associated with the number and severity of the complications [1–3]. It has been also hypothesized that poor quality of life may result in development of type 2 diabetes, as it is likely to be related to less healthy lifestyle [1].

In an observational study, Gandhi et al. suggested that intra-operative hyperglycemia in cardiac surgical patients is an independent predictor of a composite end-point of morbidity and mortality [4]. Recently, the same authors reported the results of a randomized, controlled trial that showed intensive intra-operative insulin therapy to keep blood glucose level normal during surgery which improved outcomes in patients with or without diabetes [5]. These two studies were in line with previous studies [6–8]. Other investigators also demonstrated that inadequate blood glucose control could be associated with in-hospital mortality, postoperative myocardial infarction as well as pulmonary and renal complications after cardiac surgery [9].

So, we know from the literature that poor quality of life in long-term may influence blood glucose regulating processes possibly because of the lack of healthy lifestyle. The question is whether quality of life measured by a standardized self-rating questionnaire in short-term may affect serum

glucose after surgery. Therefore, the purpose of this study was to determine a possible relationship between the components of quality of life and postoperative blood glucose concentration in patients who underwent Coronary Artery Bypass Surgery (CABG).

Materials and methods

This study enrolled 268 consecutive patients who underwent isolated CABG in Tehran Heart Center during a 6 month period. Among them, 113 patients were diabetics and 155 patients were non-diabetics. Diabetes mellitus was diagnosed on the basis of symptoms of diabetes plus at least one of the following: plasma glucose concentration ≥11.1 mmol/L (200 mg/dL), fasting plasma glucose ≥7.0 mmol/L (126 mg/dL), and 2-h plasma glucose ≥11.1 mmol/L [10]. Demographic and clinical characteristics, preoperative risk factors, laboratory tests, and cardiac status variables were analyzed as described previously [11].

Postoperative plasma glucose concentrations (every 2 to 4 h for 24 h following surgery) were also assessed by means of a glucose hexokinase method (Pars Azmoon kits accredited by Bioactiva Diagnostica, Germany). The Health-Related Quality of Life (HRQL) was assessed by a standardized self-rating Short Form 36 Health Questionnaire (SF-36) [12]. On admission, a data manager proposed the SF-36 questionnaire to the patients in the surgical ward. The SF-36 is a 36-item tool that covers eight dimensions of health including: physical functioning, role emotional, role

Table 1 Demographic characteristics, risk factors and laboratory data of diabetics and non-diabetics

Items	Diabetics (N0 113)	Non-diabetics (N0 155)
Gender (Male)	41.6	15.5
Age (year)	60.3±8.4	59.2±9.4
Body mass index (kg/m^2)	28.6±4.9	26.2±3.8
Duration of diabetes (year)	8.8±7.6	–
Family history of CAD	52.2	41.3
History of cigarette smoking	30.1	43.2
History of alcohol use	10.6	12.3
History of opium addiction	8.8	18.1
History of hyperlipidemia	72.6	64.5
History of hypertension	56.6	45.2
History of cerebrovascular disease	8.0	1.3
History of peripheral vascular disease	30.1	14.2
Recent myocardial infarction	47.8	51.6
Ejection fraction (%)	60.0±9.4	48.2±9.9
Functional class:		
I	31.0	35.5
II	47.8	52.9
III	21.2	11.6
Number of involved coronary vessels		
One	1.8	5.2
Two	17.7	25.8
Three	80.5	69.0
Euroscore	2.9±4.9	2.3±2.1
Laboratory data:		
Serum albumin	4.7±0.3	4.7±0.3
Last serum creatinine	1.3±0.2	1.3±0.2
Serum triglyceride	164.2±65.9	172.4±90.9
Serum cholesterol	154.7±43.6	158.4±40.1
Serum high density lipoprotein	40.8±9.2	40.7±8.8
Serum low density lipoprotein	82.6±36.8	84.3±35.9
Serum blood glucose (during 24 hours before) surgery	172.3±68.7	132.5±55.9
Serum blood glucose (during 24 hours after) surgery	187.7±24.5	160.9±22.8
Serum HbA_1C	7.1±1.9	6.2±2.0

CAD coronary artery disease

Data are presented as mean ± SD or percentages

Table 2 The statistical measures of the various dimensions of the SF-36 questionnaire

Subscale	Diabetics	Non-diabetics
Physical functioning	60.4±24.0	69.0±23.8
Role physical	30.8±37.6	38.9±40.5
Bodily pain	64.4±33.6	77.6±28.6
General health	68.6±18.0	70.4±16.4
Vitality	66.7±22.4	71.3±21.7
Social functioning	76.5±26.5	76.4±24.2
Role emotional	55.1±40.6	67.2±39.3
Mental health	65.5±20.0	68.9±21.1
Physical summary score	58.0±20.3	65.3±19.2
Mental summary score	66.5±19.7	70.9±19.4

Data are presented as mean ± SD

physical, bodily pain, social functioning, mental health, vitality, and general health. We considered two main components measuring mental health (as mental summary score) and physical functioning (as physical summary score). Mental health refers to the degree of nervousness or calmness, happiness or sadness and physical functioning refers to the ability to perform activities without limitation [12].

Statistical analysis Results were reported as the mean± standard deviation (SD) for quantitative variables and percentages for categorical variables. The Student'st-test and one-way analysis of variance (ANOVA) were used for group comparison. The predictors exhibiting a statistically significant relationship with the mean plasma glucose concentrations during 24 h after surgery in the univariable analyses were taken for a multivariable linear regression analysis to investigate their independence. P values of 0.05 or less were considered statistically significant. All statistical analyses were performed using SPSS version 13 (SPSS Inc., Chicago, IL, USA) and SAS version 9.1 for windows (SAS Institute Inc., Cary, NC, USA).

Results

Demographic characteristics as well as clinical and laboratory data are summarized in Table 1. The most common risk factors for coronary artery disease in both diabetics and non-diabetics were hyperlipidemia and hypertension. Almost half of the patients had functional class II, and in the majority of studied patients, three coronary arteries were involved.

In patients with diabetes, mean of plasma glucose concentration was significantly increased during 24 h after surgery in comparison with preoperative values (P00.024). This increase was also found in total group (P00.014), however, this change was not observed in non-diabetics (P00.340).

Among the various sub-scales of the SF-36, role physical and social functioning sub-scales reached the lowest and highest values, respectively in diabetic patients (Table 2). Similarly, in non-diabetics the lowest and highest values for the SF-36 related to role physical and bodily pain subscales, respectively.

Linear multivariable regression analysis showed a significant adverse relationship between mental summary score and mean of plasma glucose concentration during 24 h after surgery (β0−0.152, SE00.076, P00.046), whereas, no significant association was found between physical summary score and mean plasma glucose concentration during this period (Table 3).

Discussion

The results of the present study indicated that the mental domain of quality of life was independently associated with the postoperative blood glucose levels in our CABG patients but the physical domain did not show such relationship. To

Table 3 Multivariable analysis of the determinants of the plasma glucose concentrations during 24 h after surgery adjusted for confounders

Item	Univariable P-value	Multivariable P-value	Beta	Standard error
Gender (Male)	0.004	0.769	1.196	4.060
Age	0.056	0.116	0.262	0.166
Diabetes mellitus	<0.001	<0.001	−24.489	3.144
Body mass index	0.008	0.703	0.144	0.376
Cigarette smoking	0.065	0.616	1.771	3.532
Opium addiction	0.070	0.621	2.285	4.611
Peripheral vascular disease	0.032	0.534	−2.314	3.717
Inotropic use	0.007	0.006	−8.098	2.914
Physical summary score	0.009	0.231	−0.093	0.077
Mental summary score	0.008	0.046	−0.152	0.076

our knowledge, there are no such studies in the literature to examine the relationship between the components of quality of life and blood glucose changes following surgery. However, the association between postoperative blood glucose changes with the mental component of quality of life may probably be explained by the patients' preoperative mental situation and operation related stress.

According to a study by D'Arrigo [13], emotional stress causes an increase in blood glucose that seems to be due to a stress response. Activation of beta-adrenoceptors is involved in this response. Mental stress can also stimulate glucose uptake and energy expenditure [14]. In addition, an increase in cortisol levels may cause elevated glucose concentrations [15]; however, the effect of acute psychological stress on glucose concentrations may critically depend on whether stress is applied in the fasting or fed state [16]. Although it has been found that during mental stress, blood glucose concentration can be changed, serum insulin may be unchanged and also this change can be unrelated to age, sex, body mass, and even hemodynamic status [17].

It seems that patient's lifestyle can also affect the relationship between mental stress and blood glucose impairment. Patients under high level of stress, may have unhealthy nutritional status, irregular physical activity, and altered medications. All of these factors along with the physiological failed glucose regulation could dramatically increase blood glucose upto harmful levels. Furthermore, it is believed that not only stress may lead to deterioration of glycemic control due to its effects on the neuroendocrine system; it may also impact more indirectly on changes in health behaviors, such as regimen adherence, smoking, or drinking practices [18, 19]. Therefore, it has been suggested that in addition to preoperative psychological stress, other aspects of lifestyle such as preoperative nutritional status, habits and irregular medication intake may impair postoperative glucose concentrations.

In conclusion, we observed that there is an independent association between preoperative patient's perceptions of mental health state as measured by standardized self-rating SF-36 health questionnaire with the glucose level postoperatively. Further large studies are needed to confirm such relationship and to suggest protocols to improve quality of life of high risk patients as much as possible. This may be reflected by better perioperative glycemic control which results in decreased morbidity and mortality after CABG surgery.

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ORIGINAL ARTICLE

Sociodemographic, clinical and lifestyle factors associated with psychiatric illness among individuals with diabetes

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Abstract The aims of this study were to assess the prevalence of psychiatric illness among patients with diabetes and to elucidate the association of sociodemographic, clinical and lifestyle factors with the presence of psychiatric illness.A random sample of 262 patients with diabetes (age 50.48±12.02 years, duration of diabetes 5.85±5.36 years, 55 % females, 89 % type 2 diabetes) was accrued for the study after taking informed consent and was assessed for clinically relevant anxiety and depression using Aga Khan University Anxiety and Depression Scale (AKUADS). Multivariate logistic regression analysis was used for determining the association of various factors with psychiatric illness.A high prevalence of clinically significant levels of anxiety and depression of 56.49 % (95 % CI=50.50–62.48 %) as defined by a score of ≥ 19 on AKUADS was found in the study population. Factors significantly associated with a higher likelihood of having scores indicative of presence of anxiety and depression included younger age, female sex, being unmarried, being from urban locality, low income, unemployment, poor glycemic control, being a non-smoker and being physically inactive. In conclusion, Age, marital status, locality, income, employment status, glycemic control, smoking and physical activity are associated with an increased likelihood of meeting criteria for psychiatric illness highlighting the need of routine screening of patients with diabetes.

Keywords Depression · Anxiety · Prevalence · Diabetes complications

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Introduction

Diabetes is major public health problem in Pakistan. The prevalence of diabetes in Pakistan is high with the country currently ranked 7th in the list of countries with highest number of estimated cases of diabetes in 2010. It is estimated that 7.1 million Pakistanis have the disease in 2010 which is projected to increase to 13.9 million in 2030 with the country estimated to become top fourth in terms of the highest number of people with diabetes [1].

Studies have shown that there is an association between diabetes and depression [2–10]. The lifetime prevalence of comorbid depression in patients with diabetes has been shown to be 28.5 % [2]. A meta analysis has shown that the presence of diabetes doubles the risk of comorbid depression [2]. Depression affects the progression of diabetes and has been associated with hyperglycemia [11, 12], poor self management[12], failure to attain clinical goals[13], poor glycemic control [14], macrovascular complications[14], microvascular complications including neuropathy [15] and retinopathy[11, 15], increase in diabetes symptoms burden[16], decrease in quality of life[3], high economic burden[17], perceived functional limitation of diabetes[18], and increase in mortality[5]. Anxiety is also a common comorbidity in people with diabetes [19]. It has been reported to be associated with poor glycemic control [20]. However, interventions to manage psychiatric illness in patients with diabetes are effective and improve functioning and quality of life of patients [2, 21]. This

highlights the need for their early recognition and prompt treatment.

There are few studies which have examined the prevalence of comorbid anxiety and depression in people with diabetes. It is important to identify comorbid anxiety and depression because they increase medical utilization and has greater chronicity, slower recovery, increased recurrence and greater disability [22]. There is lack of simultaneous reporting of important sociodemographic, clinical and lifestyle variables in many studies. Most the association of a limited number of variables or only a single type of variables with anxiety and depression. There is a need to explore the effect of these variables on psychiatric morbidity to help identify high risk patients. Some studies have used homogenous populations limiting the generalisibility [3, 8, 9, 11, 12, 18, 23, 24]. Furthermore, there is little data from Pakistan on the prevalence of psychiatric illness in people with diabetes. All of the previous studies done on the subject are from Karachi which no study from Punjab province which has the highest population of the country and has more number of patients with diabetes than any other province [23, 24]. In addition, these studies have reported association of only some of the factors which have been shown to significantly affect the prevalence of psychological distress in studies from other countries. Identifying the prevalence of anxiety and depression and understanding the factors associated with it are important because the public health impact.

We describe the results of a cross-sectional study involving individuals with diabetes attending outpatient clinic of Diabetes Research Centre, Multan. We aimed to determine the preva- lence of anxiety and depressive disorders as defined by a self- reported questionnaire. We also investigated the association of certain sociodemographic, clinical and lifestyle factors with the prevalence of anxiety and depression in the study population.

Patients and methods

This was a cross-sectional analytical study from the outpatient clinic of Diabetes Research Centre, Nishtar Medical College Hospital, Multan. The participants were randomly accrued from the diabetes centre for follow up. Every fifth patient from the database was selected and considered for participation in the study. Those who were more than 18 years old and consented to participate were enrolled. Patients were excluded if they were diagnosed at current visit, reported with any emergency medical condition, and had any language or hearing problem. Patients were also excluded if they had cognitive impairment, family history of psychotic disorder, incomplete medical record or had no recent record of glucose test available. Recruitment details of the study participants are outlined in Fig. 1. The study was approved by the Ethics Review Committee of Nishtar Medical College Hospital, Multan. Informed consent was taken from all the participants before recruitment. The study

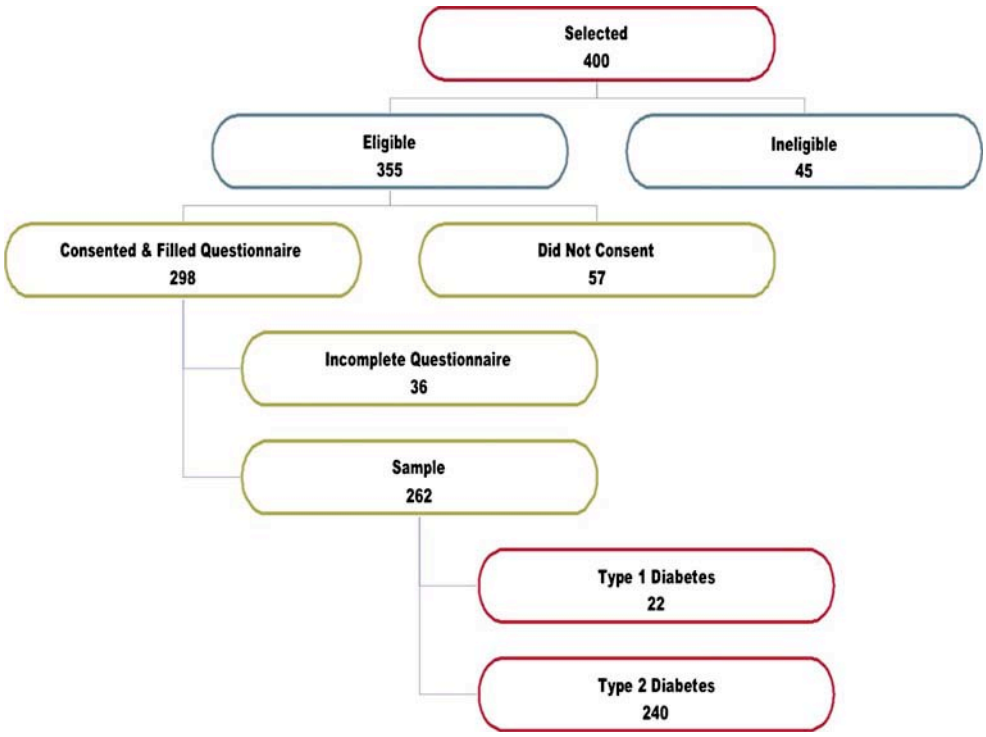

Fig. 1 Recruitment of study participants

was carried out in accordance with the principles laid out in the Declaration of Helsinki.

A structured self reported questionnaire was administered to the patients after consent. The questionnaires were administered by the principal investigator to the participants who were unable to read Urdu. It contained demographic variables including age, gender, marital status, locality, education, income and occupation. Questions related to clinical profile of patients included type of diabetes, age at onset of diabetes and glycemic control measured by a recent fasting or random plasma glucose value. Poor glycemic control was taken as fasting blood sugar value of ≥ 6.5 mmol/l or random blood sugar level ≥ 10 mmol/l. Lifestyle factors which were assessed for a possible association with the prevalence of anxiety and depression included smoking and physical activity. Smoking history was taken and individuals who were current or ex smokers were classified as smokers. Physical activity was defined as exercise of more than 30 minutes on 5 or more days per week. Aga Khan University Anxiety and Depression Scale (AKUADS) was used to screen patients for the presence of psychiatric distress over the past two weeks [25]. It is a self report screening instrument developed from the complaints of anxious and depressed patients from urban areas of Karachi. The questionnaire has 25 items, 13 psychological and 12 somatic, The items are related to both anxiety and depression. For each question (e.g., have you been sleeping less? Have you cried?), there are four possible responses ranging from never (0) to always (3). The participants answer the question according to their feelings over the preceding 2 weeks. The scores from all the 25 items are used to obtain the final summative score with a range of 0 to 75. A score of 19 or more is indicative of presence of clinically significant levels of anxiety and depressive sympomatology. At a cut-off score of 19, it has a sensitivity of 81 %, a specificity of 74 %, and positive predictive value of 63 and a negative predictive value of 88 [25]. The scale is reliable for use as a screening instrument as it has both psychological and somatic items and desirable attribute of a valid screening questionnaire making it generalisable [25].

Statistical analysis was done with Statistical Package for Social Sciences version 16. Descriptive statistics were reported as means and proportions. Multivariate Logistic regression analysis was used to determine the odds of having anxiety and depression using anxiety and depression as the dependent variable after entering AKUADS scores as categorical variable. In the constructed model, 11 variables were examined for the presence of association with psychiatric illness among the study sample. The variables included seven sociodemographic factors (age, gender, marital status, locality, educational status, occupation and total family income; two diabetes related clinical factors (type of diabetes and glycemic control) and two lifestyle factors (smoking and physical activity). Odds ratio and respective 95 % Confidence Intervals were computed for all the 11 variables. A P value of <0.05 was considered statistically significant.

Results

298 patients participated in the study (Response rate 83.94 %). Out of a total of 298 forms filled and returned, 262 were found to be completely filled and were included in final analysis. Characteristics of the study sample are given in Table 1.

Using a cutoff score of 19 on AKUADS, 148 patients (56.49 %, 95 % CI=50.50–62.48 %) had scores indicative of the presence of anxiety and depression. Analysis of the factors associated with the prevalence of anxiety and depressive affect in the study participants are described in Table 2. Sociodemographic characteristics associated with a significantly higher likelihood of having psychological distress were younger age, female gender, being unmarried, belonging to rural; area, being unemployed and having a low total family income. Clinical and lifestyle factors associated with a significantly higher likelihood of having psychiatric illness included having poor glycemic control, being physically inactive and a non-smoker.

Discussion

This study shows that psychosocial morbidity is high, which is related to the prevalence of psychiatric illness in patients with diabetes in this region is high. Second, there are various sociodemographic, clinical and lifestyle factors affecting the odds of having anxiety and depression some of which have not been adequately studies.

The prevalence of anxiety and depression in our study was 56.49 % which is high as compared to earlier studies most of previously done studies reporting prevalence rates varying from 11 % to 31 % [2–4, 6, 7]. There are various reasons for this difference. First, most of the studies are from developed countries which have low prevalence of psychiatric morbidity as compared to developing countries. However, studies from developing countries have shown comparable results with prevalence rates of 28 % in China [26], 43.4 % in Iran [27] and 100 % in Iraq [28]. The results are also in agreement with recent studies from Karachi which reported prevalence rates of 57.9 % for anxiety and 43.5–50.0 % for presence of depressive symptomatology [23, 24]. Second, community based epidemiological studies

Table 1 Characteristics of the study sample[a]

Variables		Frequency	%
Age	<40 Years	62	23.66 %
	40–60 Years	158	60.31 %
	>60 Years	42	16.03 %
Gender	Male	158	60.3 %
	Female	104	39.70 %
Marital status	Married	214	81.68 %
	Unmarried	48	18.32 %
Locality	Urban	218	83.2 %
	Rural	44	16.8 %
Education	Illiterate	76	29.0 %
	Primary	50	19.08 %
	Secondary	74	28.2 %
	HSSC	36	13.74 %
	Bachelor	16	6.11 %
	Masters	10	3.82 %
Income	<Rs.15,000	160	61.07 %
	Rs.15,000-Rs.30,000	76	29.01 %
	>Rs.30,000	26	9.92 %
Occupation	Professional	18	6.9 %
	Skilled worker	30	11.5 %
	Administrative Job	20	7.6 %
	Sales	32	12.2 %
	Farming	16	6.11 %
	Unskilled/ Elementary	32	12.2 %
	Unemployed	114	43.5 %
Duration	(mean ± SD)	79.98 months	68.83 months
Diabetes Type	Type 1	22	8.4 %
	Type 2	240	91.6 %
Glycemic Control	Good Control	144	54.96 %
	Poor Control	118	45.04 %
Physical Activity	Active	42	16.03 %
	Inactive	220	83.97 %
Smoking	Smoker	74	28.24 %
	Non-Smoker	188	71.76 %

[a] n0 262. Data are frequency and percentage except for duration where it is mean ± SD

of psychiatric disorders conducted in general population in Pakistan has shown that prevalence of psychiatric disorders is much higher as compared to western countries. A systematic review in 2004 reported mean overall prevalence of 34 % in general population [29]. Recent studies have reported prevalence rates of 35.7 % in Karachi, 43.9 % in Quetta, 53.4 % in Lahore and 41.04 % in Multan [30, 31]. These population based studies show that even though the prevalence of anxiety and depression in our study sample is high, it is in accordance in earlier studies in being significantly higher in patients with diabetes as compared to general population [2]. Third, income which has been shown to be a significant factor affecting depression in various studies [6, 26, 27, 32] is lower in developing countries which may have affected the result. A study carried out in low income adults in USA showed prevalence of 36 % in patients with diabetes which is higher as compared to other studies [2–4, 6, 7]. Fourth, most of the studies included only patients with type 2 in analysis while in our study individuals with type 1 diabetes were also included in whom prevalence of depression has been reported to be higher (32 % and 47.6 %) as compared to patients with type 2 diabetes [8].

To our knowledge, this is the first study from Pakistan that has examined the prevalence of psychiatric illness among individuals with diabetes and the factors affecting it. In our study, analysis of sociodemographic, clinical and lifestyle factors revealed that age, gender, locality, education, marital status, glycemic control, smoking and physical activity significantly altered the likelihood of having psychiatric illness.

An association between psychiatric illness and younger age was seen in our study. In agreement with some of the earlier studies which also showed the effects of age on psychiatric morbidity [4, 7, 23, 24, 26, 27, 32]. Women were more likely to have anxiety and depression as compared to men in our study. Similarly, to this effect of gender has been highlighted by a number of studies conducted earlier studies [2, 4, 6, 7, 26, 32]. Lower level of education was found to be associated with psychiatric illness in this sample, a finding that is also consistent with other studies [4, 6, 7, 27, 32, 33]. In agreement with other studies, unmarried and single individuals had higher levels of psychiatric morbidity, a finding which may be attributable to social support [4, 32]. Patients with higher scores on AKUADS were more likely to have lower income and be unemployed, showing the effect of financial stress on psychological health of patients. These findings agree with results of several previously conducted studies [6, 26, 27, 32, 33]. Individuals with poor glycemic were five times more likely to have anxiety and depressive symptoms as compared to those having good glycemic control. Effect of glycemic control on psychological helath has also been documented by earlier studies [4, 14, 24]

Other factors associated with anxiety and depression in our sample includes physical activity, smoking and locality of respondents. Only one study documented the association of physical activity and smoking with depression [7] while none of the studies have reported the effect of locality of an individual on the presence of psychiatric morbidity. Individuals who were physically active had less likelihood of anxiety and depression as compared to those who were

Table 2 Multivariate logistic regression model for variables associated with anxiety and depression*

Variables		Anxiety & Depression		AOR[a]	95%CI	P value
		Present	Absent			
Age	<40 Years	42	20	1	–	0.03
	40–60 Years	90	68	0.63	0.26–1.51	
	>60 Years	16	26	0.29	0.09–0.93	
Gender	Male	72	86	1	–	0.002
	Female	76	28	3.24	1.52–6.90	
Marital status	Married	112	102	1	–	0.04
	Unmarried	36	12	2.73	1.0–7.43	
Locality	Urban	14	30	1	–	0.01
	Rural	134	84	3.42	1.29–9.1	
Education	Illiterate	46	30	1	–	0.70
	Primary	34	16	1.39	0.48–4.02	
	Matric	38	36	0.69	0.28–1.73	
	HSSC	18	18	0.65	0.21–2.02	
	Bachelor	8	68	0.65	0.14–3.0	
	Masters	4	6	0.43	0.06–2.89	
Occupation	Professional	2	16	1	–	0.002
	Skilled worker	18	12	12	1.18–122.28	
	Administrative Job	4	16	2	0.15–26.74	
	Sales	20	12	13.33	1.32–134.63	
	Farming	4	12	2.67	0.19–36.74	
	Unskilled/Elementary	22	10	17.6	1.71–181.28	
	Unemployed	78	36	17.33	2.01–149.21	
Income	<Rs.15,000	104	54	1	–	0.04
	Rs.15,000-Rs.30,000	30	48	0.52	0.24–1.13	
	>Rs.30,000	14	12	0.71	0,22–2.31	
Diabetes Type	Type 1	10	12	1	–	0.44
	Type 2	138	102	1.62	0.47–5.61	
Glycemic Control	Good Control	42	76	1	–	0.0001
	Poor Control	106	38	5.05	2.39–10.66	
Physical Activity	Active	26	48	1	–	0.002
	Inactive	122	66	3.41	1.54–7.57	
Smoking	Smoker	14	28	1	–	0.04
	Non-Smoker	134	86	3.12	1.16–8.36	

[a] AOR obtained from multivari- ate logistic regression analysis with anxiety and depression as a dependent variable

physically inactive. This finding is in accordance with that of another study [7]. Possible reasons for this might be decrease in insulin resistance improving control of diabetes and decrease in body mass index which has been positively correlated with presence of psychiatric illness [4]. In our study, smokers which made up 28.24 % of the sample were found to had lower levels of psychiatric morbidity as compared to non smokers which is in contrast to findings documented by Engum et al. [7] who observed that smoking increases the likelihood of having depression. It is possible that this finding might have been caused by mood alleviating effect of smoking on patients who were assessed for psychiatric illness by self reported questionnaire. Locality of the individuals also had an impact on their likelihood of having psychiatric illness in our study with people living in rural areas at greater risk of having anxiety and depression than urban dwellers. It can be explained by low socioeconomic status and lack of easy access to quality healthcare for individuals of rural areas.

Our study has several strengths. This is the first study assessing the prevalence of psychiatric illness among patients with diabetes from Punjab province and the first from the country assessing the association of a number of various factors with presence of psychological distress. The study used validated reliable indigenously developed screening instrument reducing the potential of error. Also,

the study analyses the prevalence of comorbid anxiety and depression which frequently coexist together and increase healthcare services utilization [22]. Furthermore, study reports association of various important sociodemographic, clinical and lifestyle factors which have not been reported previously. This will help in identifying at-risk subgroups for screening and treatment of patients.

It will also be pertinent to discuss certain limitations of the study. First, the study used a self reported questionnaire to measure anxiety and depressive symptoms rather than diagnostic interview by a psychologist which may lead to reporting of higher levels of psychiatric morbidity. However, it is unlikely to result in a major bias as research has shown that there is good agreement between self reported and in-person interviews [34]. Second limitation is the cross-sectional design of the study limiting causality and direction of association interpretation. Also, the study involved people from a specific geographical area. Another limitation of the study is use of instrument having somatic items which may have led to increase presence of symptomatology, however, since there are only a few somatic symptoms related to diabetes, it is not likely to have resulted in major bias. Furthermore, the patients in this cultural context present with more somatic rather than psychological symptoms [29].

Despite these limitations, the study is valuable as it adds to the limited data on psychiatric illness in patients with diabetes in the developing countries. It is important as this is the population with the highest prevalence of diabetes and poor access to healthcare services [1]. It provides important data needed for early recognition and prompt management of depression which can be an additional barrier to achievement of effective control of diabetes [13]. There is a need to undertake coordinated efforts to increase awareness about the problem as psychological care for patients with diabetes is largely unavailable at most centers even in developed countries [35].

Further studies are needed to replicate these findings in other geographical areas employing larger sample sizes. There is also a need of conducting longitudinal studies to determine the mechanism and direction of causation and to get an accurate picture of the relationship between diabetes and psychiatric illness.

In summary, the high prevalence of psychiatric illness in individuals with diabetes is a significant finding as it has negative impact on disease outcome and quality of life. There is a need of effective identification and management of psychiatric illness in patients with diabetes which will lead to increase in treatment regimen adherence, improved functional capacity, and lower health care costs [17, 18]. Furthermore, prompt treatment of psychiatric illness in individuals with diabetes which is on the rise in developing countries will decrease economic burden of disease by decreasing complications of diabetes [11, 13–15]. There should be routine screening of patients with diabetes for psychiatric illness specifically targeting those with risk factors. It is further recommended that there should be adequate training of staff to enable them to recognize anxiety and depression and refer patients for appropriate care.

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Duality of Interest The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Circulating retinol-binding protein 4 concentrations in patients with coronary artery disease and patients with type 2 diabetes mellitus

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Abstract Circulating Retinol-Binding Protein 4 (RBP4) has recently been identified as a marker of insulin resistance. We tested this hypothesis in patients with coronary artery disease (CAD), patients with type 2 diabetes mellitus (T2DM), and in non-diabetic control subjects. We studied plasma RBP4 levels and RBP4-to-transthyrethin (TTR) ratio, estimating the excess circulating RBP4 in proportion to TTR, in 45 individuals divided into three groups (15 CAD, 15 T2DM, and 15 controls). Plasma RBP4 levels were significantly lower in patients with T2DM than in non-diabetic control subjects (P00.05). The RBP4/TTR ratio was not statistically different between the groups. There was no difference in plasma RBP4 levels and RBP4/TTR ratio between non-diabetic CAD patients and control subjects or those with and without metabolic syndrome. No significant associations were found between RBP4 and RBP4/TTR ratio, as dependent parameters, with markers of the metabolic syndrome and lipid metabolism. RBP4 does not seem to be a valuable marker for identification of the metabolic syndrome or insulin resistance in patients with T2DM or CAD.

Keywords Atherosclerosis · Diabetes mellitus · Metabolic syndrome · Retinol-binding protein

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Introduction

Adipose tissues function as an endocrine organ, secreting several bioactive molecules collectively known as adipokines [1]. Retinol-binding protein 4 (RBP4) is a newly discovered adipokine with potential implications in the development of obesity-associated diseases, including insulin resistance, type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD) [2]. RBP4 is mainly synthesized in the liver and secreted bound to retinol, along with transthyretin (TTR), a homotetrameric thyroid hormone transport protein, forming a 1:1 molar complex in the human plasma [3].

Serum RBP4 has been recently reported to be associated with insulin resistance in subjects with obesity, Impaired

Glucose Tolerance (IGT), or T2DM and lean non-diabetic individuals with a strong family history of diabetes [4]. However, whilst the role of RBP4 in human glucose metabolism is considered controversial [5-8], its close association with proatherogenic lipid profile makes it a potential novel marker for identifying those at increased risk of cardiovascular events and premature death [8, 9]. The purpose of the present study was to investigate circulating RBP4 concentrations in patients with CAD, patients with T2DM, and non-diabetic control subjects, correlating RBP4 levels with components of the metabolic syndrome and circulating lipoproteins.

Patients and methods

Participants

A total of 45 subjects were included in this study. All participants underwent elective coronary angiography for the evaluation of stable CAD based on clinical indication. Three cohorts were studied: (1) 15 patients with angiographically confirmed CAD without T2DM; (2) 15 patients with T2DM without CAD by coronary angiography; and (3) 15 non-diabetic control subjects without CAD by coronary angiography. CAD was diagnosed angiographically and defined as ≥50 % diameter stenosis in at least one of the coronary arteries or their branches. T2DM was defined according to the criteria of the American Diabetes Association [10]. Previously diagnosed DM, receiving oral hypoglycemic drugs and/or insulin, and fasting plasma glucose levels of ≥126 mg/dL were considered exclusion criteria for the CAD and control groups. Patients with active infections, autoimmune diseases, malignancies, and recent myocardial infarction were excluded. Written informed consent was obtained from all participants. This study was approved by the Ethics Committee of Tehran University of Medical Sciences and Health Services.

Data collection included demographics, cardiovascular risk factors including diabetes, hypertension, hypercholesterolemia, family history of CAD, smoking, medication in use, measures of weight, height, waist circumference at its narrowest point, and Body Mass Index (BMI) calculated as weight in kilograms divided by height square in meters. Metabolic syndrome was diagnosed according to National Cholesterol Education Program Adult Treatment Panel III criteria [11]. Participants were also divided in two groups according to BMI (BMI>30 or ≤30 kg/m^2).

Biochemical assays

Fasting blood samples were collected for the measurement of plasma glucose, total cholesterol, Low-Density Lipoprotein (LDL) cholesterol, High-Density Lipoprotein (HDL) cholesterol, triglyceride, and C-Reactive Protein (CRP). All samples were frozen at −80°C until assayed. Laboratory measurements were performed using automated enzymatic commercial kits (Pars Azmoon, Tehran, Iran).

RBP4 and TTR assays

Plasma RBP4 and TTR levels were determined in duplicate by sandwich Enzyme-Linked Immunosorbent Assay (ELISA).

Statistical analyses

The statistical analyses were performed using SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). RBP4 and TTR form a 1:1 molar complex in plasma [3]. In order to estimate the excess RBP4 in proportion to TTR, the molar concentrations of RBP4 were divided to the molar concentrations of TTR. Continuous variables were expressed as mean±standard deviation (SD). Comparison between two cohorts of patients was performed by unpaired Student's t-test or Mann–Whitney U test. Three-way analysis of variance (ANOVA) was used to compare RBP4, TTR, and RBP4/TTR ratio according to age and sex among the groups. Categorical variables were expressed as percentages and compared by Pearson's Chi-square test and Fisher's exact test. In univariate analysis models, the associations of plasma RBP4 levels and RBP4/TTR ratio, as dependent parameters, were investigated with markers of the metabolic syndrome and lipid metabolism, as independent variables. Age and sex were also included in all models. Results were reported as Regression Coefficients (RC) with 95 % Confidence interval (CI). A p value of less than 0.05 was considered to be statistically significant.

Results

The baseline characteristics of participants are summarized in Table 1. Compared with control subjects, non-diabetic CAD patients were older and had a lower mean HDL cholesterol level. The prevalence of hypertension was higher in non-diabetic CAD patients than in non-diabetic controls. Abdominal obesity, hypercholesterolemia, and hypertension were more prevalent in patients with T2DM than in non-diabetic controls. The metabolic syndrome was diagnosed in approximately half of all participants (N023). Subjects diagnosed with metabolic syndrome had a higher proportion of diabetes, hypertension, hypertriglyceridemia, abdominal obesity, and angiographically confirmed CAD. CRP was significantly higher in subjects with than in those

Table 1 Clinical characteristics of non-diabetic CAD and diabetic patients and controls

Characteristics	CAD (N0 15)	T2DM (N0 15)	Control (N0 15)	P value
Age (yrs)	55.20±10.97*	56.26±7.55	42.66±5.32*	<0.001
Male gender [N (%)]	15 (100)	9 (60)**	13 (86.67)**	0.014
Hypertension[a] [N (%)]	12 (80)*	8 (54)**	0 (0)*,**	0.001
Hypertriglyceridemia[b] [N (%)]	9 (60)	7 (47)	4 (27)	ns
Hypercholesterolemia[c] [N (%)]	4 (27)	8 (53)**	1 (7)**	0.018
Abdominal obesity[d] [N (%)]	8 (53)	13 (87)**	5 (33)**	0.012
Metabolic syndrome [N (%)]	11 (73)*	12 (80)**	0 (0)*,**	<0.001
Metabolic syndrome score	3.20±1.01*	3.40±0.98**	1.06±0.79*,**	<0.001
Smoking [N (%)]	3 (20)	1 (7)	2 (13)	ns
Waist circumference (cm)	82.76±11.21	85.38±9.39	85.62±19.37	ns
Body mass index (kg/m^2)	29.75±4.24	32.15±3.36	30.46±7.22	ns
Triglyceride (mg/dL)	220.95±123.58	173.34±77.38	141.30±68.97	ns
Total cholesterol (mg/dL)	174.01±37.96	190.85±51.96	213.59±51.33	ns
LDL cholesterol (mg/dL)	91.96±26.46	106.02±36.00	120.07±36.6	ns
HDL cholesterol (mg/dL)	45.15±9.09*	46.50±11.89	55.38±11.65*	0.029
CRP (mg/dL)	4.28±4.13	3.25±4.39	1.52±1.77	ns
RBP4 (μmol/L)	1.30±0.29	1.09±0.23**	1.24±0.15**	0.025
TTR (μmol/L)	0.56±0.15*	0.44±0.1	0.45±0.11*	0.018
RBP4/TTR ratio	2.45±0.65	2.57±0.62	2.93±0.83	ns

[a] Diagnosed hypertension or receiving antihypertensive medications

[b] Elevated triglycerides or receiving lipid lowering medications

[c] Elevated total cholesterol (or low HDL cholesterol) or receiving lipid lowering medications

[d] Elevated waist circumference, i.e. waist circumference ≥88 cm in women and ≥102 cm in men. LDL Low-Density Lipoprotein; HDL High- Density Lipoprotein; CRP C-Reactive Protein; RBP4 Retinol-Binding Protein 4; TTR Transthyretin

*P value<0.05 non-diabetic CAD patients vs. controls

**P value<0.05 diabetic patients vs. controls

***P value<0.05 non-diabetic CAD vs. diabetic patients

ns not significant

without metabolic syndrome (4.34±4.66 vs. 1.64±1.58 mg/dL, p00.014).

Plasma RBP4 and TTR

Univariate linear regression analysis showed a significant correlation between plasma RBP4 levels as the dependent parameter and plasma TTR levels (P<0.0001, r^2: 0.291, RC: 0.539, CI: 0.292–0.718) (Fig. 1). Similar results were obtained after age and sex adjustment.

Plasma RBP4

Plasma RBP4 levels were lower in individuals with T2DM compared with non-diabetic control subjects (1.09±0.23 vs.1.24±0.15 μmol/L, p00.05). There was no difference between non-diabetic CAD patients and control subjects (P00.418) or those with and without metabolic syndrome

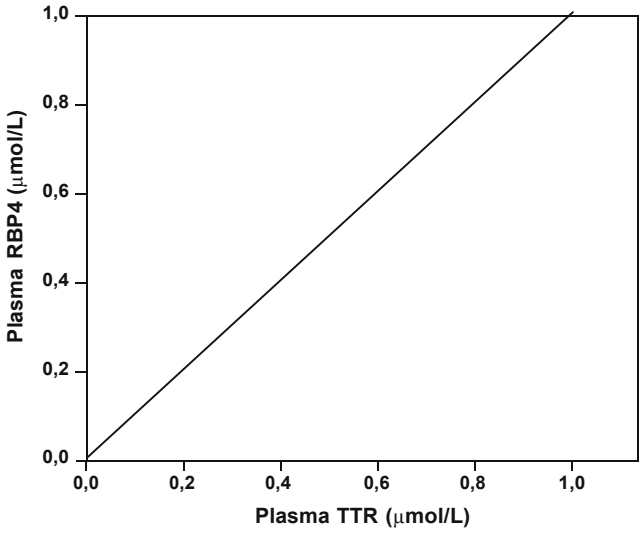

Fig. 1 Significant correlation between plasma levels of RBP4 and TTR (P<0.0001, r^2: 0.291, RC: 0.539, CI: 0.292–0.718)

Table 2 Univariate linear regression analyses for RBP4 and RBP4/TTR ratio in patients with T2DM or controls

Covariates	RBP4 (μmol/L)			RBP4/TTR ratio		
	RC	CI	P	RC	CI	P
Age	−0.324	−0.612 to 0.041	ns	−0.275	−0.578 to 0.094	ns
Sex	0.136	−0.235 to 0.473	ns	−0.234	−0.548 to 0.137	ns
Waist circumference	0.047	−0.318 to 0.4	ns	0.108	−0.262 to 0.45	ns
BMI	0.243	−0.128 to 0.554	ns	0.129	−0.242 to 0.467	ns
Hypertension[a]	−0.137	−0.473 to 0.234	ns	−0.163	−0.494 to 0.209	ns
Triglyceride	0.019	−0.343 to 0.376	ns	0.302	−0.065 to 0.597	ns
Total cholesterol	0.192	−0.18 to 0.516	ns	0.168	−0.204 to 0.498	ns
LDL cholesterol	0.178	−0.194 to 0.505	ns	0.166	−0.206 to 0.496	ns
HDL cholesterol	0.178	−0.194 to 0.505	ns	−0.134	−0.471 to 0.237	ns
CRP	−0.191	−0.515 to 0.181	ns	0.110	−0.26 to 0.452	ns

[a]Diagnosed hypertension or receiving antihypertensive medications. BMI Body Mass Index; HDL High-Density Lipoprotein; LDL Low-Density Lipoprotein; CRP C-Reactive Protein. Results are Regression Coefficient (RC) with 95 % Confidence Interval (CI)

ns not significant

(P 0 0.054). There was no difference in plasma RBP4 according to obesity (P 0 0.261) or sex (P 0 0.969).

Plasma TTR

Plasma TTR levels were not significantly different between patients with T2DM and non-diabetic control subjects (P 0 0.90) or those with and without metabolic syndrome (P 0 0.537). Plasma TTR levels were significantly higher in non-diabetic CAD patients compared with control subjects (0.56±0.15 vs. 0.45±0.11 μmol/L, P 0 0.027). There was no difference in plasma TTR according to obesity (P 0 0.063) or sex (P 0 0.709).

RBP4/TTR ratio

RBP4/TTR ratio was not statistically different between T2DM patients and controls (P 0 0.182), non-diabetic CAD patients and controls (P 0 0.085), or those with and without metabolic syndrome (P 0 0.433)

Associations of RBP4 and RBP4/TTR ratio with markers of the metabolic syndrome and lipid metabolism

In patients with T2DM, univariate regression analyses revealed no significant associations between RBP4 and RBP4/TTR ratio as dependent parameters with markers of the metabolic syndrome and lipid metabolism, including waist circumference, BMI, history of hypertension, triglyceride, total cholesterol, LDL, and HDL (Table 2). There were also no associations found in non-diabetic CAD patients (Table 3).

Discussion

RBP4 was recently reported to be expressed by adipose tissue and associated with insulin resistance and components of the metabolic syndrome in patients with T2DM, IGT, or obesity [4, 5]. No associations between RBP4 and markers of insulin sensitivity have also been reported in

Table 3 Univariate linear regression analyses for RBP4 and RBP4/TTR ratio in patients with CAD or controls

Covariates	RBP4 (μmol/L)			RBP4/TTR ratio		
	RC	CI	P	RC	CI	P
Age	−0.070	−0.419 to 0.297	ns	−0.195	−0.518 to 0.177	ns
Sex	0.208	−0.164 to 0.528	ns	0.032	−0.332 to 0.387	ns
Waist circumference	0.056	−0.31 to 0.408	ns	0.223	−0.149 to 0.539	ns
BMI	0.229	−0.143 to 0.544	ns	0.326	−0.038 to 0.614	ns
Hypertension[a]	−0.029	−0.385 to 0.334	ns	−0.180	−0.507 to 0.192	ns
Triglyceride	0.297	−0.07 to 0.593	ns	−0.130	−0.468 to 0.241	ns
Total cholesterol	−0.010	−0.368 to 0.351	ns	−0.039	0.393 to 0.325	ns
LDL cholesterol	−0.092	−0.437 to 0.277	ns	0.010	−0.351 to 0.368	ns
HDL cholesterol	0.062	−0.305 to 0.413	ns	−0.118	−0.458 to 0.253	ns
CRP	−0.247	−0.557 to 0.124	ns	0.142	−0.23 to 0.477	ns

[a]Diagnosed hypertension or receiving antihypertensive medications. BMI Body Mass Index; HDL High-Density Lipoprotein; LDL Low-Density Lipoprotein; CRP C-Reactive protein. Results are Regression Coefficient (RC) with 95 % Confidence Interval (CI)

ns not significant

patients with T2DM [8] or non-diabetic, insulin-resistant individuals [12, 13]. These findings are in accordance with recent published data showing that circulating RBP4 concentrations were similar in lean, overweight, and obese postmenopausal women and that there was no relationship between the HOMA index of insulin resistance and circulating RBP4 concentrations [14]. Moreover lower RBP4 levels have also been reported in patients with T2DM [15] or IGT [16]. Erikstrup et al. showed that plasma RBP4 and retinol levels were lower, whereas RBP4/retinol ratio was elevated in patients with T2DM compared to controls [15]. It has been postulated that, since the RBP4/retinol ratio estimates the excess of circulating RBP4 in proportion to retinol, free rather than total RBP4 concentration is related to insulin resistance [15]. RBP4 and TTR are also thought to exist as a 1:1 molar complex [3]. Akbay et al. reported comparable levels of RBP4 in type 2 diabetic patients and controls, whereas, the molar ratio of RBP4 to TTR was found to be higher in diabetic patients than that of the control group [17]. We found lower plasma RBP4 levels in patients with T2DM compared to non-diabetic control subjects; however, RBP4/TTR ratio was not significantly different between the groups. Plasma retinol level was not determined in the present study, nevertheless, there might be differences in the ratio between the groups.

It has been demonstrated recently that plasma RBP4 increases with decreasing renal function in type 2 diabetes [17-19]. Since we did not assess renal function in our cohort of patients, it should be considered as a potential confounding factor which might account for the above discrepancies.

These discrepancies among human studies have been attributed to differences in study populations (age, sex composition, levels of fasting insulin, use of glucose or lipid lowering agents, adjusting for cardiovascular risk factors, components of the metabolic syndrome and kidney function), divergent measures of insulin sensitivity (clamp studies vs. HOMA-IR), as well as methodological differences in measuring serum RBP4 levels (ELISA vs. Western blotting) [4, 8, 14, 15]. Therefore, the lack of associations of RBP4 with glucose metabolism and insulin sensitivity needs to be further investigated in a large, well-defined cohort of patients with more sophisticated measures of insulin sensitivity.

Increased RBP4 levels were significantly associated with prior cerebrovascular disease, but not with prior myocardial infarction, in large community-based samples of elderly individuals [9]. It has been postulated that circulating RBP4, as a marker of metabolic complications, might serve as a useful marker in identifying individuals at increased risk for CVD [9]. Additionally, the risk of incident CAD was associated with increasing quartiles of RBP4 concentrations in a nested case–control study; however, RBP4 mostly correlated with triglyceride levels and failed to provide added value beyond traditional cardiovascular risk factors [20]. The presence of clinical atherosclerosis, including coronary, cerebrovascular or peripheral vascular disease, but not sub-clinical atherosclerosis, was associated with higher plasma RBP4 concentrations [18]. On the contrary, serum RBP4 levels have been reported to be significantly lower in patients with CAD than in non-diabetic control subjects [8]. Mean intima media thickness has been reported to correlate with RBP4, retinol, RBP/TTR ratio, and retinol/RBP4 ratio [21]. However, we found no difference in plasma RBP4 levels and RBP4/TTR ratio between non-diabetic CAD patients and controls.

Based on current data, the contributing roles of RBP4 in insulin resistance and metabolic syndrome has been debated, whilst its associations with proatherogenic lipid profile, including increased triglyceride, LDL cholesterol, and very low-density lipoprotein cholesterol and decreased HDL cholesterol levels, as well as key enzymes of lipoprotein metabolism may imply a plausible contribution to the pathogenesis of CVD [2, 4, 5, 8, 9, 15, 22, 23]. However, we failed to demonstrate such an association which may be partly due to recruitment of individuals who were on lipid lowering medications and lack of adjustment for potential confounders. This statement needs to be clarified in experimental and prospective studies.

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EDITORIAL

Clinical scoring in gestational diabetes screening

V. Seshiah

Int J Diab Dev Ctries. 2012; 32: 63-64

Women with a history of GDM are at increased risk of future diabetes, predominantly type 2 diabetes, as are their children [1]. Additionally other compelling reasons for addressing GDM include the elevated risk of adverse pregnancy outcomes, including increased maternal and perinatal morbidity, obstructed labor, infections, spontaneous abortion, congenital abnormalities and macrosomia. Women with GDM have an increased lifetime risk of developing diabetes, which is more than 3 times that of control population at 16 years after index pregnancy [2]. By 17 years of age one-third of children born to GDM mothers have had evidence of IGT or Type 2 DM [3]. Furthermore, women with a history of GDM are also at increased risk of future cardiovascular disease and thus, screening for GDM has become imperative.

Screening test for GDM should identify subjects at risk either using clinical risk factors or by biochemical testing. Traditional risk factors have not included ethnic origin, and are relatively insensitive (less than 70 %) when applied to multiethnic antenatal clinics, as women from ethnic minorities are at substantial greater risk of GDM than Caucasian women [4].

ADA expert committee recommended a strategy for potential "exclusion from blood glucose testing" on the basis of below average risk for GDM rather than testing only on the basis of category of high risk like obesity, strong family history of type 2 DM, previous history of GDM or glucose intolerance outside of pregnancy and glucosuria and history of poor obstetrics outcome [5]. But selective screening based on risk factor scored poorly in predicting GDM [6]. If selective screening is employed it is likely that 27 % of the GDM women will go undetected [6]. GDM diagnosis is overlooked in about one-third of the women where selective rather than universal screening is performed [7]. Further selective screening recommendation by American Diabetes Association (ADA) may be applicable for women belonging to the ethnic group with low prevalence of GDM. Risk factor screening does not take into account the inevitable difficulties in implementation including the potential for substantial under diagnosis of GDM [8].

Compared to risk factor based selective screening, universal screening for diabetes detects more cases and improves maternal and offspring prognosis [9]. Thus, universal screening appears to be the most reliable and desirable method for the detection of GDM [6]. In this aspect, diagnosis of GDM by WHO criteria is cost effective. GDM is diagnosed if 2 h Plasma Glucose (PG)≥140 mg/dl (7.8 mmol/L), with 75 g OGTT similar to that of Impaired Glucose Tolerance outside Pregnancy [10]. WHO criteria of 2 h PG more than 140 mg/dl identifying large number of cases may have greater potential for prevention [11]. But the WHO criteria require women to be in the fasting state. Hence, a procedure that does not require any restriction would be ideal for universal screening. It is important to have a test that detects glucose tolerance without the women necessarily undergoing a test in the fasting state as pregnant women seldom visit an antenatal clinic in the fasting state. The test results should be informative from the blood collected from pregnant women irrespective of their last meal timing. Collecting the blood sample for the measurement of glucose concentration without regard to the time of the day or the interval since the last food intake (random sampling) has also been advocated as a simpler and adequate method for blood sugar screening [12]. A single sample drawn 2 h

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after 75 g glucose load approaches the standard glucose challenge test (GCT) in simplicity [12]. Yet another study suggests a similar procedure in that, a pregnant woman after undergoing preliminary clinical examination in the prenatal clinic is given a 75 g oral glucose load, without regard to the time of the last meal and venous blood sample is collected at 2 h for estimating PG by the GOD-POD method. GDM is diagnosed, if 2 h PG is > 140 mg/dL [13]. Performing this test procedure in the non-fasting state is rational, as glucose concentrations are affected little by the time since the last meal in a normal glucose tolerant woman, whereas it will, in a woman with GDM [14]. After a meal, a normal glucose tolerant woman would be able to maintain euglycemia despite glucose challenge due to brisk and adequate insulin response, whereas in case of a woman with GDM who has impaired insulin secretion [15] glycemic level increases with a meal and the glycemic excursion exaggerates with glucose challenge further. Therefore, this procedure assumes clinical relevance as WHO criteria based on glucose concentration 2 h after 75 g glucose load was able to correctly identify subjects with GDM. This single step procedure serves as both screening and diagnostic test for GDM, and is simple, economical and feasible [13]. The 2 h PG> 140 mg/dl has got clinical significance as both short term and long term morbidity in the offspring of GDM mothers occurs above this glycemic level [16].

International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommendations suggest that GDM can be diagnosed if FPG>92 mg/dl. Thus, estimation of FPG is also an option but has limitations in South Asians and Asian Indian population. This is due to the ethnicity of Asian Indians who have high Insulin Resistance (IR) and as a consequence, their postprandial plasma glucose is higher compared to Caucasians [17]. Asian and South Asian ethnicity are both independently associated with increased IR in late pregnancy [18]. Das et al. documented an increased IR during pregnancy in Asian Indian Women and the IR escalates further in GDM [19]. All these studies indicate that FPG is inadequate to diagnose GDM.

Clinical risk factors were poor predictors of GDM. A possible reason could be that the South Asians are generally at higher risk for GDM and thus more prone to developing it than Caucasians [20]. Important highlight is the need for universal screening in high risk population. Risk factor based screening would miss 10–40 % of women with GDM [21, 22].

Cost analysis of universal screening when compared with risk factor screening showed only negligible difference [6]. For Universal screening, estimating the plasma glucose is the most reliable and desired method. In this regard, the cost effective and evidence based single step procedure serves as a both definitive screening and diagnostic test procedure [13].

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Letter to the editor

Mathew K. Jose, Viswanathan Mohan

Int J Diab Dev Ctries. 2012 ; 32 : 111-112

Dear sir,

Reading the original article by Irving et al, "Mosaic pancreas or type 3 diabetes"[1] and the invited editorial by Mohan, "Type 2 diabetes can be multigenerational"[2], has evoked a mixed sense of intrigue and controversy.

The intrigue is that why Irving et al did not consider mitochondrial diabetes as a possibility among their patients. For maternally inherited diabetes due to mitochondrial disorder the usual clue is sensory neural deafness.

The controversy is why, with all due respect, Mohan is dismissive of Mosaic pancreas or even type 3 DM. Other specific forms of diabetes are now classified into group three or as type 3 diabetes by ADA & IDF. Where do we put those lean, ketosis resistant and poorly controlled "type 2 DM" patients without fibro-calcific pancreatic pathology or auto-immune markers (clinical presumption to be non-immune) needing at times large doses of insulin? Why not as mosaic pancreas or T3DM ? True, it can only be meaningful if a pharmaceutical R & D can define the patho-physiology and find a new drug!

Yours sincerely,
Mathew Jose

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Reply by V. Mohan

I read with interest the letter by Mathew Jose regarding the article by R.R. Irving et al[1] and my editorial[2] and wish to thank him for the same. It is clear that there is a genetic susceptibility to type 2 diabetes and it is quite strong. How- ever, it is true that the present genetic studies in type 2 diabetes have not unraveled all the genes. This does not mean that there is a 'low susceptibility gene penetration'. Particularly in Indi- ans there seems to be a fairly strong genetic susceptibility and the lower the age at onset of type 2 diabetes suggests a stronger role of genes. I agree with Dr. Jose's views regarding the screening for monogenic diabetes. Dr. Jose also raises a good point about screening for mitochondrial diabetes and I
entirely agree that the clinical clue to this type of diabetes is sensory-neural deafness. I also agree that whenever we consider monogenic diabetes, Mitochondrially Inherited Diabetes (MID) should also be kept in mind.

Regarding Jose's final point about why I did not encourage the term mosaic pancreas or type 3 diabetes, to be honest, I think we have enough confusion in the classification of diabetes. Every single clinical variation in presentation of a forum of diabetes cannot be called a different type of diabetes. The ADA and the IDF classifications do not list other forms of diabetes, as type 3 diabetes but they are listed under 'other specific types of diabetes' where we have a big list of types of diabetes including diabetes secondary to exocrine pancreas, endocrine forms of diabetes. Fibrocalculous Pancreatic Diabetes is now classified under diabetes secondary to chronic pancreatitis. The earlier term Malnutrition Related Diabetes which included lean ketosis resistant diabetes has now been deleted by all classifications due to lack of specificity and diagnostic criteria. Just because somebody is ketosis-resistant we cannot club this individual as having a separate type of diabetes. There are many

patients with type 1 diabetes who are not prone to ketosis. Ketosis occurs only when the beta cells have completely failed i.e. more than 90 % of the beta cells are destroyed. In those who have some residual beta cell function they may require insulin but they are resistant to ketosis. To my mind, these are formè frustes of type 1 diabetes and do not merit being called by any other name. In India, it was mostly in the state of Orissa that this type of diabetes was described but recent studies from that very state suggest that they are not seeing these cases any more.

I hope I have clarified all the issues. I entirely agree with Dr. Jose's last remark that such classifications can only be meaningful either if there is a definite marker for the type of diabetes or if a specific therapy is available. Otherwise it doesn't make sense to keep coming up with new terms for diabetes which will only lead to confusion. I had specifically stated this in my editorial where I said that any classification of diabetes should be based on etiopathogenesis, genetic markers etc. Once again, I wish to thank Jose for his astute comments.

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REVIEW ARTICLE

Thymoquinone: a promising antidiabetic agent

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Abstract The antidiabetic property of Nigella sativa seeds oil are attributable to the presence of Thymoquinone (TQ) which is considered as a major phytochemical component of the seeds volatile oil. The aim of this review is to highlight the potential of thymoquinone as an antidiabetic agent and the latest reported research investigations regarding the molecular mechanism of its hypoglycemic effect. For researchers involved in the field of antidiabetic therapy, clinical testing of the efficacy of TQ in human diabetic patients could provide plenty of opportunities for further research.

Keywords Thymoquinone · Diabetes · Hypoglycemic effect · Molecular pharmacology

Introduction

Diabetes Mellitus (DM) is a complex metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia due to disruptions of carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion (β-cell dysfunction), insulin action (insulin resistance) or both [1]. DM has worldwide high prevalence, morbidity and mortality and is regarded as a non-curable but controllable disease [2]. DM is considered to be a major risk factor for cardiovascular disease [3]. Research has shown that diabetic patients have a 2–4 fold higher predisposition to vascular, renal, ophthalmic and neurological complications which impair the quality of life and present a high burden to both individuals and society in terms of morbidity and economic costs [4]. Prevention and control of DM is a major challenge and require a change of lifestyle from avoiding sedentary habits towards more physical activity and less calorie intake. Most people find it difficult to implement this lifestyle change and instead look for alternatives [4]. One such alternative is herbal medicine which is probably an attractive and affordable choice when used alongside with insulin in controlling hyperglycemia. Nigella sativa (also known as black cumin) is one of the most recommended plants for DM treatment due to the known hypoglycemic effect of the oil found in its seeds [5].

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Review and discussion

Nigella sativa, a dicotyledon of the Ranunculaceae family, is a herb with a rich historical and religious background [6]. Nigella sativa grows in the Mediterranean Region and in Western Asian countries including India, Pakistan and Afghanistan. It is a bushy, self-branching plant with white or pale to dark blue flowers. Of all the plant organs, only the seeds attracted interest primarily because of the pharmacological and therapeutic benefits of the oil extracted from the seeds (Fig. 1a) [6]. Most of the reported studies dealt with the volatile oil of the seeds and its major phytochemical components to evaluate its benefits against several in vivo and in vitro disease models. There are many scientific reviews in the literature summarising the pharmacological and therapeutic benefits, including the antidiabetic property, of Nigella sativa seeds oil [5–14]. The authors of these reviews agreed that the antidiabetic property of Nigella sativa seeds is due to the presence of Thymoquinone (TQ) which is a major phytochemical component of the seeds volatile oil. The TQ content in the seeds volatile oil is

Fig. 1 Nigalla sativa and TQ: a the flowering part of Nigella sativa plant and its seeds. b the chemical structure of TQ; 2-isopropyl-5-methyl-1,4-benzo-quinone ($C_{10}H_{12}O_2$); Mwt 164.2

around 27.8 %–57 % [15]. Chemically, TQ belongs to the 2,5-di-substituted benzoquinone class of compounds having methyl and isopropyl groups at C-2 and C-5 positions, respectively (Fig. 1b).

Hawsawi et al. [16] studied the hypoglycemic effect of powdered Nigella sativa seeds when mixed with diet and pure TQ administered intraperitoneally at doses of 0.5–6 mg/kg in normal rats. The results showed that the powdered Nigella sativa seeds and pure TQ both exhibited the hypoglycemic effect of lowering blood glucose concentration within 14 days of treatment. The authors also concluded that pure TQ would induce a much better hypoglycemic effect than powdered Nigella sativa seeds, but failed to explain the possible mechanism of action. There are a few research groups which took the initiative to investigate the mechanism of action of the antidiabetic effect of TQ using streptozotocin induced animal models [17–23]. Fararh et al. [19, 20] showed that daily gastric administration of 50 mg/kg TQ for a period of 30 days in diabetic rats and hamsters reduced both fasting glucose and glycated hemoglobin levels in blood. They elaborated that TQ decreased blood glucose concentration through two possible mechanisms: 1) it decreases the hepatic gluconeogenesis by suppressing the synthesis of gluconeogenic enzymes and 2) it activates glucose utilization in the cytosol of extrapancreatic tissues by enhancing insulin secretion from β-cells of the pancreas. In agreement with Fararh et al. findings, Pari and Sankaranarayanan [21] have shown that the daily gastric administration of 80 mg/kg TQ for 45 days in diabetic rats produced a consistent, dose dependent and significant decrease in blood glucose concentration. Their proposed mechanism of the hypoglycemic effect is a TQ decreased activity of the gluconeogenic enzymes: glucose-6-phosphatase and fructose-1,6-bisphosphatase, in treated rats. In another related study, El-Mahmoudy et al. [17, 18] revealed that giving dia- betic rats 3 mg/kg of TQ intraperitoneally for three days decreased serum and pancreatic nitrites which are the source of nitric oxide. It has been established that nitric oxide is involved in the destruction of β-cells of the pancreas during the development of type I DM. Therefore, the hypoglycemic effect of TQ is due to its protective effect on β-cells of the pancreas through the down regulation of the immunological inflammatory activity towards β-cells mediated by nitric oxide [17, 18]. In addition, Sankaranarayanan and Pari [22] and Abdelmeguid et al. [23] reported that the significant reduction in blood glucose concentration of diabetic rats after TQ treatment is mainly due to the antioxidant property of TQ. Increasing evidence suggests that oxidative stress plays a central role in the onset of DM and its complications [24]. Persistent hyperglycemia causes increased production of Reactive Oxygen Species (ROS) and free radicals as well as a simultaneous decline of antioxidant defence mechanisms which lead to oxidative stress and subsequent cellular damage [25]. Several mechanisms seem to be involved in the generation of the oxidative stress in experimental animal models, as well as in patients with Type 1 and Type 2 diabetes, including glucose auto-oxidation, protein glycation and the formation of advanced glycation end-products [25, 26]. Furthermore, the lack of an effective antioxidant system in the β-cells of the pancreas renders them more susceptible to oxidative damage than other cell types [27]. Therefore, the antioxidant property of TQ protects β-cells from the oxidative stress and preserves their integrity. This ultimately leads to enhanced insulin production and secretion which explains the reduction in blood glucose concentration in the treated rats [22, 23].

From the above discussion, different mechanisms of action were reported for the antidiabetic effect of TQ which can be summarized in two major points: 1) TQ restores the activity of enzymes involved in glucose metabolism such as glucose-6-phosphatase and fructose-1,6-bisphosphatase of gluconeogenesis. 2) TQ has a protective effect on β-cells of the pancreas against the damaging effect of oxidative stress and nitric oxide, leading to enhanced insulin production and secretion. This promotes glucose uptake and utilization by the extrapancreatic tissues.

Prospects for future research

Clinical evaluation of TQ

The antidiabetic property of Nigella sativa seeds oil has been tested on humans. It has been safely given to humans in some clinical trials and shown to be effective in reducing blood glucose concentration if given in appropriate doses [7,

28, 29]. Although TQ shows a promising antidiabetic effect, besides being a relatively safe compound particularly when given orally to experimental animals [30], there is no clinical evaluation for its antidiabetic effectiveness in humans known until now. It is worth mentioning that there is one clinical study which was conducted to evaluate the effectiveness of TQ treatment in adult patients with solid tumors or haematological malignancies [31]. The study concluded that oral administration of TQ with a dose up to 2,600 mg/day was tolerated by patients with no significant systemic toxicities [31].

New TQ analogues

There is a lot of research reporting the antiproliferative effect of TQ against many human cancer cell lines [32–36]. Hence, the molecular pharmacology of TQ regarding its anticancer property is well established [12, 32, 36]. This provided an opportunity to Banerjee et al. [37] to develop new TQ analogues that showed potent anticancer effects against pancreatic cancer. Likewise, further understanding of the molecular pharmacology of TQ regarding its antidiabetic property would open new research avenues for the synthesis of analogues which could have effective antidiabetic activity.

This report gives the reader a glimpse of the collective research work on TQ and is an attempt to bring attention to the promising antidiabetic effect of TQ. It is an invitation for researchers involved in the field of antidiabetic therapy to clinically test the efficacy of TQ in human diabetic patients. Furthermore, it is hoped that this report will promote further research to develop new agents with potential antidiabetic effect based on both a thorough understanding of the antidiabetic activity of TQ and its structural characteristics.

Conflict of interest There is no conflict of interest.

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