CURRENT STATUS AND EMERGING HEALTH PROBLEMS ASSOCIATED WITH DIABETES, IN ASIA AND IN DEVELOPING COUNTRIES

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CURRENT STATUS AND EMERGING HEALTH PROBLEMS ASSOCIATED WITH DIABETES, IN ASIA AND IN DEVELOPING COUNTRIES

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ORIGINAL RESEARCH
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Insulin Sensitivity and Pancreatic β-Cell Function in Ecuadorian Women With Turner Syndrome

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Objective: To assess insulin sensitivity and pancreatic β-cell function in an adult population of Ecuadorian individuals with Turner syndrome (TS).

Design and Methods: This was a cross-sectional correlational study conducted in TS subjects (>20 years old; $n = 38$). A standard 2-h oral glucose tolerance test was performed in both women with TS and the reference group. Glucose, lipids, insulin, and C-peptide concentrations were measured. Homeostasis Model Assessment (HOMA) of Insulin Resistance, Quantitative Insulin Sensitivity Check Index, McAuley, Matsuda, and Belfiore indices were calculated to evaluate the degree of insulin resistance (IR). The pancreatic β-cell function was assessed using HOMA-β, basal C-Peptide Index (CPI), and CPII at 120′.

Results: A higher prevalence of impaired glucose tolerance was found in TS subjects compared with the reference group. Although significant differences were found for glucose concentrations at 60′ and 120′ (but not at 0′), only the baseline insulin concentrations differed significantly between the two groups. The values of the IR indices were statistically different between study and reference groups. A significant number of TS subjects diagnosed with IR were differently classified according to the index applied. The concentrations of C-peptide at 0′ and 120′ of TS subjects were similar to those of the control group. In contrast, the CPI and CPII values in the study group were significantly lower than those in the control group.

Conclusion: It is impossible to select the best surrogate method for the assessment of IR in women with TS. The CPI and CPII values could be preferable to other indices to assess the pancreatic β-cell function in TS subjects. Our findings suggest that IR and pancreatic β-cell dysfunction could be independent events in women with TS, and both conditions seem to be caused by the disease *per se*. Our results imply that early screening and intervention for TS would be therapeutic for TS women.

Keywords: glucose metabolism, impaired glucose tolerance, insulin resistance indices, obesity, overweight, pancreatic β-cell function, Turner syndrome

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INTRODUCTION

Turner syndrome (TS) is the result of a missing or structurally abnormal second sex chromosome associated with several clinical features of variable expressivity that may include dysmorphic stigmata, short stature, gonadal failure, sexual infantilism, and renal, cardiac, skeletal, and other endocrine and metabolic abnormalities. Endothelial dysfunction, impaired glucose tolerance (IGT), insulin resistance (IR), dyslipidemia, and arterial hypertension may lead to the development of type 2 diabetes mellitus (T2DM), metabolic syndrome, and cardiovascular disease (stroke and myocardial infarction) (1). The life expectancy in TS is reduced by at least 10 years due principally to a 3-fold increase in the risk of mortality from cardiovascular disease (2, 3). An altered insulin secretion appears to be a key factor of such comorbidities in TS. However, of the few studies that address insulin secretion in TS, some demonstrate hyperinsulinemia, and others suggest a decreased insulin secretion (4). Also, ∼40% of adult patients with TS are affected with components of metabolic syndrome, especially dyslipidemia (5–7) and IR (8, 9), which are worsened by the coexistence of overweight/obesity (10). Nevertheless, there are conflicting results of pancreatic β-cell function and IR in women with TS (4).

The oral glucose tolerance test (OGTT) was recommended by the International Turner Syndrome Consensus Group (2017) for screening glucose homeostasis disorders in women with TS if hemoglobin A_{1c} (HbA_{1c}) is elevated because they are at high risk of developing IGT (50%) due to a combination of impaired insulin secretion and IR (11). However, the best method of assessment of IR in TS subjects has not been defined. Euglycemic–hyperinsulinemic clamp technique (12) is well documented as the gold standard for assessment of IR, but it is too laborious, time-consuming, expensive, and technically complicated. The OGTT has been reported to be superior to other tests (hemoglobin A_{1c} levels, fasting or postprandial blood glucose concentrations) to detect early abnormalities of glucose metabolism in these patients (4, 13). Therefore, in this study, the objectives were to assess insulin sensitivity and pancreatic β-cell function in an adult population of Ecuadorian individuals with TS. Also, we compared the indices of IR derived from fasting concentrations values [insulin plus glucose and insulin plus triglycerides (TAGs)] such as Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), Quantitative Insulin Sensitivity Check Index (QUICKI), and the McAuley index. Likewise, we wanted to contrast the aforementioned indices of IR with indices of IR derived from glucose and insulin measurements during the 75 g OGTT, such as Matsuda and Belfiore indices. Similarly, to evaluate the pancreatic β-cell function in this group of subjects with TS, we have used the HOMA-β and C-peptide indices.

MATERIALS AND METHODS

Design Study and Study Subjects

This was a cross-sectional correlational study carried out in the School of Biology, Central University of Ecuador, Quito, Ecuador. The study protocol was reviewed and approved by the Ethics Committee for Research in Human Subjects of the Central University of Ecuador. Unrelated adult Ecuadorian TS patients (>20 years old; $n = 38$) were recruited through the Ecuadorian Foundation in Support of Turner Syndrome. They were contacted using letters, e-mails, and telephone calls and subsequently underwent a comprehensive health examination between January and November 2017. None of the subjects had received treatment with growth hormone or anabolic steroids. All except two patients had received conventional sex hormone replacement therapy (HRT). The age at which HRT was started was 17.9 ± 5 years. Of the 38 patients who constituted the study group, 21 (55%) received HRT for more than 5 years. The remaining 18 subjects were either poorly or irregularly treated (<2 years; range = 6 months to 7.3 years). Hormone replacement therapy was discontinued 4 weeks before their assessment. To ensure a comparable reference group, we prospectively recruited all TS subjects first, and then a match among reference group subjects was sought. This recruitment strategy yields 38 healthy women (20–49 years) volunteered matched by body mass index (BMI) and age. These volunteers were required to be free of significant medical illnesses and not to be taking any medication known to affect body weight or metabolic processes. All these women had regular menstrual cycles and had not been treated with or were presently receiving HRT or glucocorticoid therapy.

An appointment was given for each subject during which clinical evaluation and anthropometric measurements were performed and recorded by a single experienced physician (F. A.-N.) according to a standard protocol described elsewhere (10). Each participant was tested individually. Information regarding medical, personal, family, and dietary was obtained in the form of a questionnaire. The data collection was conducted in the morning after an overnight fast of at least 12 h. Systolic and diastolic blood pressures (SBP and DBP, respectively) were measured in each subject using an automated sphygmomanometer monitor (Omron Healthcare Inc., Bannockburn, IL, USA) on the left arm with an appropriate cuff size and in the sitting position, following 10 min of rest. Two readings at 5-min interval were obtained and averaged to determine SBP and DBP for each individual. If the two readings differed by more than 10 mm Hg, additional readings were obtained, and the last additional two readings were averaged.

Blood Collection and Oral Glucose Tolerance Test

After a 10-h overnight fast, a total of 10 mL fasting venous blood samples for measuring serum biochemical and lipid profiles were obtained from each subject before the flavored glucose load. Serum and plasma were immediately separated. Then, a standard 2-h OGTT was performed in both TS and reference groups with 75 g glucose load at 08:00 h. Blood samples were obtained from an antecubital vein with three-point at the predetermined intervals (0, 60, and 120 min).

Analytical Methods

The plasma glucose and serum lipids [total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and TAGs] were immediately measured using commercially available kits with the enzymatic reference method on an autoanalyzer spectophotometer (Humalyzer 3000; HUMAN, Wiesbaden,

Germany). Low-density lipoprotein cholesterol (LDL-c) was derived from the lipids measured using the Friedewald equation. The rest samples were immediately put on at room temperature, centrifuged (2,500 revolutions/min) at 4°C for 30 min to separate sera, and stored at −80°C for measurement of insulin and C-peptide. Insulin and C-peptide levels were measured by electrochemiluminescence analysis using a commercially available kit (Cobas 6000 Chemistry Analyzer; Roche Diagnostics, Indianapolis, IN, USA). Body fat assessment by bioelectrical impedance analysis was estimated using a segmental two-frequency bioimpedance analyzer (Inbody 120; Biospace Industry, Seoul, South Korea) by a trained investigator (F.A.N.) according to a standard protocol described elsewhere (10).

Definitions

The diagnosis of TS was established by lymphocyte chromosomal analysis in combination with the presence of typical clinical features. The nutritional status classification was performed using BMI, according to the cutoffs indicated by the World Health Organization (14). Thus, all participants were classified as overweight/obese ($\geq$25 kg/m^2) or lean ($\geq$18.5 $<$ 25kg/m^2). Essential arterial hypertension was defined by SBP $\geq$130 mm Hg or DBP $\geq$85 mm Hg or taking antihypertensive medication with no identifiable cause. Metabolic syndrome was diagnosed using the International Diabetes Federation criteria, which requires the presence of (1) central obesity (defined as waist circumference $\geq$80 cm) plus any two of the following four factors: (2) fasting plasma glucose 5.6 mmol/L (100 mg/dL); (3) HDL-c level $<$1.29 mmol/L ($<$50 mg/dL); (4) TAGs $\geq$1.7 mmol/L ($>$150 mg/dL) or specific treatment for this; and (5) blood pressure $\geq$130/85 mm Hg or treatment of previously diagnosed hypertension (15). Subjects were defined as having impaired fasting glucose (IFG) if fasting plasma glucose concentration was from 5.6 mmol/L (100 mg/dL) to 6.9 mmol/L (125 mg/dL) and/or IGT if 2-h post–glucose plasma glucose level was $\geq$7.8–11.0 mmol/L (140–199 mg/dL, respectively) according to the diagnostic criteria from American Diabetes Association. Subjects were divided into two groups with (a) normal glucose tolerance (NGT) (IFG and IGT negative) and (b) with IFG and/or IGT positive.

To evaluate the degree of IR, several indexes derived from either fasting or OGTT-stimulated concentrations of glucose, insulin, and TAGs were used:

(1) Homeostatic Model Assessment of Insulin Resistance Index (HOMA-IR) was calculated using the equation:

$$\frac{\text{fasting insulin concentration } (\mu\text{IU/mL}) \times \text{fasting glucose concentration (mmol/L)}}{22.5}$$

(2) Quantitative Insulin Sensitivity Check Index was determined according to the following formula:

$$\frac{1}{\log \text{fasting insulin concentration} + \log \text{fasting glucose concentration}}$$

(3) McAuley index was measured according to the formula:

$$\exp\left[2.63 - 0.28log(\text{fasting insulin concentration } (\mu\text{IU/mL})) - 0.31log(\text{fasting triglyceride concentration (mmol/L)})\right]$$

(4) Matsuda index was calculated according to the formula:

$$\frac{10,000}{\sqrt{[\text{fasting glucose (mg/dL)} \times \text{fasting insulin } (\mu\text{U/L}) \times [\text{MPG} \times \text{MSI during OGTT}]}}$$

where MPG (mg/dL) is mean plasma glucose OGTT, and MSI (μIU/mL) is mean serum insulin during OGTT.

(5) Belfiore index was calculated from changes in plasma glucose and serum insulin using the equation:

$$\frac{2}{(1/(\text{AUC insulin} \times \text{AUC glucose})) + 1}$$

where AUC is the values of the area under curve insulin (μIU/mL) and glucose (mmol/L) concentrations in the course of OGTT, respectively.

The pancreatic β-cell function was evaluated using the formulas of Hovorka et al. with some modifications.

(1) The HOMA-β index, which was calculated using the following equation:

$$\frac{20 \times \text{fasting insulin (mU/mL)}}{(\text{fasting plasma glucose (mmol/L)} - 3.5)}$$

(2) The C-Peptide Index (CPI), which represents the ability of fasting glucose to stimulate pancreatic β-cell secretion (fasting pancreatic β-cell responsiveness), was determined according to the following formula:

$$\frac{100 \times \text{fasting C} - \text{peptide (ug/L)}}{\text{fasting glucose concentration (mg/dL)}}$$

(3) The CPII index, which represents the ability of postprandial glucose to step up pancreatic β-cell secretion, was calculated by

$$\frac{100 \times [120' \text{ C} - \text{peptide(ug/L) during OGTT} - \text{fasting C} - \textit{peptide} \text{ (ug/L)}]}{120' \text{ glucose concentration (mg/dL)during OGTT} - \text{fasting glucose concentration (mg/dL)}}$$

According to different indices, the following thresholds defined IR state among study and reference group subjects: HOMA-IR $\geq$ 2.5, QUICKI $\leq$0.339, McAuley index $\leq$ 5.8, Matsuda index $\leq$ 4.6, and Belfiore index $\geq$1.23 (16–18).

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad InStat 7.00 (GraphPad Software, Inc., La Jolla, CA, USA). The general features of the participants are described as number of cases and mean

and standard deviations unless otherwise mentioned. Results as determined by 95% confidence intervals (95% CIs) and $P < 0.05$ were considered to be statistically significant. The prevalence of metabolic syndrome, overweight/obesity, IGT, and IR were indicated as percentages. To compare the proportions of categorical variables, the two-proportion Z test was used. After checking the normality of the quantitative variables with the Kolmogorov–Smirnoff or the Shapiro–Wilk tests ($P < 0.05$), a Student t-test was performed to compare means between groups for continuous variables. Conversely, when the variables did not fit into the criteria of normality, they were expressed as median (range), and a Mann–Whitney U test was carried out. Likewise, the association between quantitative variables was examined using Pearson or Spearman correlation analysis depending on the distribution assumed by the data. The degree of agreement or concordance between the IR indices derived from fasting concentration values and glucose and insulin levels during OGTT was reported by using the Cohen κ coefficient with 95% CIs, with levels of agreement interpreted according to Landis and Koch (19), $\kappa < 0.40$, 0.41–0.60, 0.61–0.80, and 0.81–1.00 to considered fair, moderate, substantial, and almost perfect agreement, respectively. Bivariable linear regression analysis was performed to identify independent influencing factors for pancreatic β-cell function in TS subjects by a marginal statistical significance $P < 0.2$. These independent variables (age; waist circumference; fat mass; glucose and insulin concentrations at 0′, 60′, and 120′; and HOMA-IR) were considered candidate variables and exported to a multiple linear regression model with HOMA-β as the dependent variable to assess the magnitude of their individual effect on the pancreatic β-cell function and for controlling the possible effect of confounders. In the final multivariable model, $P < 0.05$ for two-sided tests was set as the level of significance.

RESULTS

Clinical, Anthropometric, and Biochemical Features of the Study Group and Reference Groups

The anthropometric and metabolic variables of TS and reference groups are described in **Table 1**. Although no significant difference was found for BMI between both groups, weight and waist circumference, TAGs, TC, LDL-c, fat mass, percentage of fat mass, and the free fat mass–to–fat mass ratio in the study group were significantly greater than those in the reference group.

The prevalence of overweight/obesity was similar between both groups (two-proportion Z test $P > 0.05$, 95% CI = −0.18 to 0.3). Although the prevalence of IR (according to HOMA-IR) was similar between both groups (two-proportion Z test $P > 0.05$, 95% CI = 0.34–2.92), a higher prevalence of IGT was found in TS subjects compared with the reference group (two-proportion Z test $P < 0.05$, 95% CI = 0.428–0.105). Significant differences for fasting glucose ($P < 0.022$) and insulin ($P < 0.004$), and HOMA-IR ($P < 0.005$), Matsuda ($P < 0.004$), and QUICKI ($P < 0.001$) indexes were found between TS women when they were categorized according to their nutritional status (<25 or ≥25 kg/m^2). The concentrations of C-peptide at 0′ and 120′ of TS subjects were similar to those of the control group (**Table 1**). In contrast, the CPI values in the study group were significantly lower than those in the control group [median = 0.89 (range = 0.67–1.56) vs. 1.53 (0.89–1.98), $P = 0.01$]. Similarly, the values of CPII in the study group were significantly lesser than those in the reference group [median = 1.57 (range = 0.67–1.56) vs. 5.23 (0.89–1.98), $P = 0.0001$, 95% CI = 1.093–20.7].

During OGTT, the maximum glucose and insulin concentration peak was found at 60′ for both the study group and the reference group. Although the study group maintained higher glucose and insulin values than the reference group over time (0′, 60′, and 120′), there were only significant differences for glucose concentrations at 60′ and 120′ (P

TABLE 1 | Clinical and metabolic features of TS subjects and reference group.

	TS Subjects	Reference Group	*P*-value
n	38	38	
Age (years)	29.23 ± 8.09	29.81±4.7	0.936
Height (cm)	139 ± 0.04	148 ± 0.01	<0.0001
Weight (kg)	51.01 ± 14.14	54.08 ± 9.08	0.0406
BMI (kg/m^2)	26.17 ± 7.41	24.72 ± 3.89	0.813
Triglycerides (mg/dL)	158.9 ± 67.75	117.87 ± 58.06	0.0071
Total cholesterol (mg/dL)	245.90 ± 38.13	180.99 ± 23.39	<0.0001
LDL-c (mg/dL)	203.69 ± 60.43	101.71 ± 19.64	<0.0001*
HDL-c (mg/dL)	60.68 ± 17.73	55.70 ± 13.19	0.4508
Glucose 0′ OGTT (mg/dL)	81.68 ± 10.28	79.36 ± 6.93	0.3088*
Glucose 60′ OGTT (mg/dL)	132.65 ± 39.91	90.71 ± 1.06	<0.0001*
Glucose 120′ OGTT (mg/dL)	118.50 ± 33.46	85.26 ± 15.37	<0.0001*
Insulin 0′ OGTT (μU/mL)	12.09 ± 8.62	9.18 ± 6.42	0.008
Insulin 60′ OGTT (μU/mL)	90.06 59.85	74.29 ± 54.09	0.1393
Insulin 120′ OGTT (μU/mL)	85.57 ± 56.70	78.24 ± 75.03	0.3994
C-peptide 0′ (ng/mL)	2.58 ± 1.34	1.99 ± 0.97	0.0528
C-peptide 60′ (ng/mL)	8.66 ± 3.12	7.17 ± 2.31	0.0369
C-peptide 120′ (ng/mL)	9.20 ± 3.39	7.16 ± 2.9	0.0963
HOMA-IR	2.57 ± 2.09	2.18 ± 1.22	0.8476
HOMA-β	265.18 ± 83.74	332.38 ± 42.40	0.7227
Matsuda index	5.57 ± 3.72	6.14 ± 3.39	0.3671
QUICKI index, % (*n*)	0.35 ± 0.04	0.34 ± 0.03	0.7695*
Metabolic syndrome	42.1 (16) (0.2–0.5§)	13.33 (4) (0.05–0.3§)	<0.05***
Overweight/obesity	42.11 (16) (0.28–0.61§)	50 (15) (0.33–0.67§)	>0.05**
Insulin resistance	33.33 (10) (0.2–0.5§)	33.33 (10) (0.2–0.5§)	>0.05**
Impaired glucose regulation	26.6 (8) (0.1–0.44§)	0 (0.0–0.113§)	<0.05**

**Student t-test. **Two-proportion Z test. § 95% confidence intervals. Insulin Resistance is defined by HOMA-IR = ≥ 2.5. BMI, body mass index; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; OGTT: oral glucose tolerance test, TC, total cholesterol.*

< 0.001). Also, TS subjects with overweight/obesity ($\geq$25 kg/m^2) showed higher glucose values at 60′ and 120′ than those found for lean TS individuals ($P < 0.0001$) or the two reference subgroups ($P < 0.0001$). When the four subgroups (subcategorized according to BMI) were compared, baseline glucose concentrations did not differ significantly between the four subgroups. However, significant differences were found in the plasma glucose concentration at 60′ and 120′ between the different subgroups analyzed ($P < 0.0001$). In contrast, although baseline serum insulin concentrations between the two subgroups of individuals with TS differed significantly, insulin levels at 60′ and 120′ between the four subgroups were similar. As expected, both in the study and reference groups, there was a significant correlation between the general adiposity variables (weight and BMI) and IR indices. However, HOMA-β weakly correlated (weight) or not correlated (BMI) with these anthropometric adiposity variables ($r = 0.38$; $P < 0.037$; $r = 0.34$; $P < 0.063$, respectively) in TS subjects.

Correlation Between Different Insulin Resistance Indices in the TS Group

The values of 60′ or 120′ glucose concentrations during the OGTT were correlated positively with HOMA-IR ($r = 0.35$, $P < 0.032$, and $r = 0.397$, $P < 0.021$, respectively) and negatively with the Matsuda index ($r = -0.512$, $P = 0.004$, and $r = 0.478$, $P < 0.008$, respectively) in TS subjects. Spearman rank correlations between IR indices derived from fasting values of glucose and insulin and/or TAGs and OGTT in TS subjects are presented in **Table 2**. Among the former, a high degree of correlation between HOMA vs. QUICKI, ($r = -0.994$, $P < 0.0001$) and HOMA-IR vs. McAuley ($r = -0.835$) was detected. In addition, a moderate correlation between OGTT-derived IR indices (Belfiore vs. Matsuda index, $r = -0.748$) was also observed. By contrast, the correlation between IR indices based on fasting values and OGTT was highly variable, ranging from high correlation between HOMA-IR and Matsuda index ($r = -0.953$, $P < 0.0001$) and QUICKI and Matsuda index ($r = -0.959$, $P < 0.0001$), through moderate correlation between McAuley and QUICKI ($r = 0.784$), HOMA-IR, and Belfiore (0.745) or Belfiori vs. QUICKI ($r = -0.692$).

The prevalence of IR was highly variable in the study group according to HOMA-IR (42.1%), QUICKI (44.7%), Belfiore (51.2%), or Matsuda (55.3%) indices. A broad range of concordance was found between fasting or OGTT-stimulated indices. Thus, if IR was defined according to HOMA-IR (cutoff = $\geq$2.5), 97.4% (16/17, $\kappa = 0.946$) of the subjects in the study group had also IR as reported by QUICKI (cutoff = $\geq$0.34), considered as an almost perfect agreement. Besides, if IR was considered according to the Matsuda index (cutoff = $\geq$4.6), 88.9% (16/18, $\kappa = 0.918$) of TS individuals was also IR as stated in the Belfiore index (cutoff = 1.23). Conversely, a lower but substantial concordance was found when the IR indices based on fasting glucose and insulin concentrations were contrasted with those based on the OGTT concentrations. For example, 76.19% (16/21) of TS subjects with IR according to the HOMA-IR were not considered IR if the Matsuda index was applied ($\kappa = 0.741$).

Insulin Sensitivity vs. Pancreatic β-Cell Function in TS Subjects

In TS subjects, HOMA-IR values showed a wide range of IR from 0.3 to 7.2. Similarly, HOMA-β values ranged from 33.7 to 525.6% in TS subjects, indicating a broad range of insulin secretion. A significant ($P < 0.018$) but only moderate correlation ($r = 0.43$) between HOMA-IR and HOMA-β was found in the study group. Thus, elevated IR with an increasing (compensatory) insulin secretion was demonstrated. However, in the TS subject group, this correlation was lower ($r = 0.429$) than that observed in the reference group ($r = 0.487$, $P < 0.0006$).

Because the pancreatic β-cell function may be influenced by a variety of factors, a multiple linear regression analysis was performed with HOMA-β as the dependent variable and age, height, weight, BMI, fat mass, the HOMA-IR index, and glucose and insulin concentrations at 0′, 60′, and 120′ as independent variables. Because of a strong correlation between HOMA-IR and QUICKI indices ($r = -0.994$) in TS subjects, only HOMA-IR was added to this model as an IR index. The model explained a part of the variance of the HOMA-β with adjusted R^2 values of 0.687. Approximately only 20% of the total variation in HOMA-β can be explained by the HOMA-IR index ($R^2 = 0.192$, $P < 0.02$). Also, on stepwise multivariable regression, HOMA-IR and glucose concentration at 0′, 60′, and 120′ were independent factors for HOMA-β in TS subjects, but after adjusting the model for fasting glucose concentration, the influence of HOMA-IR on HOMA-β decreased [standardized regression coefficient (β) = 0.237, $P < 0.0487$].

DISCUSSION

Adult TS subjects are susceptible to IGT and T2DM (8, 20–22). The prevalence of IGT in TS patients is ∼10 to 34% (4), which is higher than that in the healthy population. In a study of 103 TS subjects, the prevalence of IGT and T2DM was reported in 7.48 and 1.9%, respectively (5). In our study, IGT was found in 18 and 38% of lean and overweight/obese TS individuals, respectively, with a global prevalence of 27%. No individual in the reference group had glucose metabolism abnormalities. Subjects with T2DM were excluded from our study. Thus, a higher prevalence of IGT was observed in the present study compared to those previously reported in the literature. This may be due to the different age ranges, ethnic origin, and a higher BMI reported in our study. We included only TS subjects $\geq$20 years of age in our study. In studies in TS subjects with a wide age range, the prevalence of IGT is lower. Our reference group was age- and BMI-matched with the study group. Although overweight/obesity is a well-known risk factor for IRG, BMI did not explain the higher glucose levels during OGTT found in our study group compared with the reference group.

Our results suggest that the HOMA-IR and Matsuda indices may represent a good alternative combination to the euglycemic–hyperinsulinemic clamp in the assessment of IR in TS. Because of a good correlation between these two indices ($r = -0.748$), it is reasonable to think that this combination is the best instrument to evaluate IR in TS. However, some caution should

TABLE 2 | Correlation between insulin resistance indices in subjects with Turner syndrome.

	HOMA-IR	QUICKI	McAuley index	Matsuda index	Belfiore index	HOMA-β
HOMA-IR	—	−0.9937	−0.803	−0.9531	−0.811	0.4342
QUICKI	<0.0001	—	0.792	0.9599	0.692	−0.4165
McAuley index	<0.0001	<0.0001	—	0.750	0.626	−0.148
Matsuda index	<0.0001	<0.0001	<0.0001	—	0.917	−0.4136
Belfiore index	<0.0001	<0.0001	<0.0001	<0.0001	—	−0.403
HOMA-β	0.0165	0.022	0.434	0.023	0.027	—

Spearman correlation coefficients are above the diagonal; P-values for each pairwise correlation are below the diagonal.

be considered. The McAuley index had a strong linear correlation with both HOMA-IR and QUICKI, suggesting that it can be used together with other indices to assess IR in TS subjects in a clinical context. Also, the Belfiore index and HOMA-IR had similar correlations with the Matsuda index (r= −0.744 and 0.748, respectively). In addition, the presence of a good correlation does not necessarily mean that HOMA-IR and Matsuda indices have the best performance predictive in diagnosing IR in our TS patients because there were some borderline values in a relatively small group of TS individuals in our study. Additionally, the correlation coefficients of the Matsuda index with QUICKI (r = 0.9599 vs. −0.954) and the McAuley index (r = 0.795 vs. 0.758) are stronger than those observed with HOMA-IR, which were also calculated from fasting concentrations. Finally, varying correlation and agreement were found between IR indices based on fasting concentrations and those calculated on OGTT values. Consequently, the assessment of IR in TS subjects produced significantly different results according to the method applied (fasting vs. OGTT concentrations). Furthermore, our findings revealed that a significant number of our TS patients were differently classified according to the indices applied. Thus, it is impossible to select the best surrogate method for the assessment of IR in women with TS. Therefore, the utilization of any surrogate IR indices in the clinical scenario of TS must be viewed with extreme caution.

Several studies have examined the sensitivity or secretion of insulin in TS. Insulin resistance is clearly found in many subjects with TS (8, 9, 23). However, different results have been reported, depending on whether insulin sensitivity was assessed from the fasting concentration or in the postabsorptive state. On the other hand, whereas some studies have demonstrated a decreased insulin secretion (9, 24), other studies have reported an increased insulin secretion in TS compared with normal subjects, probably as compensation for IR (25, 26). Thus, although several studies have examined insulin sensitivity or pancreatic β-cell function in TS, thus far, the results are equivocal. A possible confounding factor is that these studies fail to adjust insulin secretion to the prevailing degree of IR. Although compensatory hyperinsulinemia may be detected, it may not be appropriately elevated to match the degree of IR. Also, insulin secretion and insulin sensitivity have been shown to have a hyperbolic association (27). Therefore, it is necessary to adjust the insulin secretion for IR to accurate interpretation of the pancreatic β-cell function. In contrast to several studies examining the sensitivity or secretion of insulin in TS, to the best of our knowledge, this is the first study that explores the relationship between insulin sensitivity (HOMA-IR) and pancreatic β-cell function (HOMA-β) in TS subjects. We tested the association between HOMA-IR and HOMA-β rather than comparing mean values. Because the pancreatic β-cell function, measured by HOMA-β, may be influenced by a variety of factors, we performed a multiple linear regression analysis with the variables showing correlation (age, waist circumference, fat mass, fasting glucose, and insulin concentrations at 0′, 60′, and 120′, HOMA-IR). Overall, the model explained a large part of the variance of the pancreatic β-cell function with adjusted R^2 values of approximately 0.7. However, the influence of BMI was not significant in the model. Also, the model remained explaining much of the variance of the pancreatic β-cell function when it was adjusted by HOMA-IR.

As the BMI does not seem to be an independent factor of the pancreatic β-cell function in our study, we did find an increased but insufficient insulin secretion both in lean and overweight/obese TS subjects in our study. These findings reinforce the idea that despite having a compensatory response to glucose overload during OGTT, insulin secretion is not enough to maintain adequate glucose levels at 60 and 120 min. Thus, a higher IR with a lower insulin secretion seems to indicate a declining pancreatic β-cell function in our TS subjects. We cannot prove whether these findings are a reflection of an evaluation carried out at an early stage of the disorder, because the study group was represented by women with 29.23 ± 8.09 years. However, our findings suggest that a decrease in insulin secretory response observed in our TS subjects is an underlying mechanism that can lead to T2DM in a short time.

In the clinical setting, HOMA-β and insulinogenic indices are usually used to measure the pancreatic β-cell function (28, 29). However, the insulin reserve cannot be accurately estimated on the insulin levels because of its pulsatile release pattern and short half-life. The pancreatic β-cell area has been reported to correlate with the C-peptide–to–glucose ratio calculated from the concentrations of 75 g OGTT but not with HOMA-β (30). C-peptide is produced in equimolar amounts to endogenous insulin, but unlike insulin, this is subject to neither hepatic nor significant peripheral degradation as is mainly removed by the kidneys. Thus, C-peptide can be used to assess the pancreatic β-cell function. The increment of C-peptide immunoreactivity level in the glucagon test is probably the most accurate test to evaluate insulin reserves as has the advantage of being a

more reproducible stimulus and having much faster action on pancreatic β cells (31). However, the OGTT has the advantage of being a better physiological stimulus, avoiding the side effects of glucagon. Besides, compared with the fasting C-peptide level, the C-peptide level at 120′ during OGTT is more capable of representing the maximal pancreatic β-cell secretory capacity (32). In our study, the metabolic variables representing the ability of fasting glucose to stimulate β-cell secretion, such as fasting C-peptide, postprandial insulin, and C-peptide, were similar between the study and reference groups. However, the CPI and CPII indices indicating the ability of postprandial glucose to step up pancreatic β-cell secretion differed significantly between the two groups. This seems to indicate that the higher levels of basal insulin in our TS subjects represent a diminution of peripheral insulin sensitivity rather than a conserved function of the pancreatic β-cells.

The observed variability in correlation coefficients between insulin sensitivity indices calculated from the fasting concentration and those based on OGTT in women with TS led us to ask whether the relationship between HOMA-IR and HOMA-β (a good measure of pancreatic β-cell function) was different between TS and reference subjects. A Spearman correlation showed a significant but moderate association between HOMA-IR and HOMA-β both TS and reference subjects (0.429 vs. 0.487, respectively). The lower effect of HOMA-IR on HOMA-β in our TS subjects indicates that a given increase in HOMA-IR leads to a lesser raising in HOMA-β in TS subjects than in the reference group. Although this difference is minimal, it should also be considered that a significant difference between plasma concentrations of glucose at 60′ and 120′ was found between both groups and that the serum concentrations of insulin during OGTT were similar between the study and reference groups. Thus, all these findings suggest that the pancreatic β-cell function is inadequate. Therefore, our TS individuals seem to have an increased risk of the pancreatic β-cell exhaustion and the development of T2DM. Type 2 diabetes mellitus develops when the pancreatic β cells become "exhausted" and cannot secrete adequate amounts of insulin to preserve normoglycemia in an increasing IR environment. All these altered mechanisms seem to be present in our TS subjects.

Circulating insulin levels at 60′ and 120′ were similar when our TS subjects were compared according to their glucose metabolism status. However, significant differences were found in the plasma glucose concentration at 60′ and 120′ between our TS subjects with or without IGT. This contrasts with the findings in glucose concentration during OGTT in our TS subjects when they were discriminated by BMI. In this scenario, only a significant difference in basal glucose concentration was found between both groups. In addition, TS subjects with IGT showed basal circulating insulin levels similar to those found in NGT-TS subjects. Furthermore, no significant difference was found in serum levels of insulin at 60′, and 120′ during OGTT between lean TS women and overweight/obese TS subjects. Thus, in our TS subjects, an NGT or lean status did not mean that insulin secretion was normal. This probably reflects early IR, and plasma glucose concentration is maintained to a certain degree. Not only do all these findings reflect a lower insulin sensitivity in our TS individuals, but also increased insulin levels are not enough to maintain normal glucose homeostasis. Thus, IR and β-cell dysfunction could be independent events in women with TS, and both conditions seem to be caused by the disease itself. This may be the result of deletions of some genes related to insulin signal transduction and pancreatic β-cell function located on the X chromosome. Bakalov et al. (21) observed that the long arm of the X chromosome (iXq) is associated with a higher incidence of T2DM as compared to the 45,X group. A proposed explanation for this increased risk could be that haploinsufficiency for unknown Xp gene(s) constitutes a "first hit" that causes the basic deficit in pancreatic β-cell function seen in 45,X patients. Excess dosage of Xq genes in isochromosome Xq may provide a "second hit" that exacerbates the deficit, perhaps by altering other genes involved in pancreatic β-cell development and function or survival, and/or by stimulating low-grade chronic autoimmunity that injures but does not obliterate the pancreatic β-cells. However, so far, such mechanisms have not been demonstrated, and potentially involved genes have not been identified. An alternative hypothesis is that both conditions (IR plus pancreatic β-cell dysfunction and TS) are comorbidities caused by a common factor. The cell cycle delay hypothesis states that a retarded cell cycle due to aneuploidy will reduce the number of cell cleavages per time units (33). Therefore, there will not be enough time for pancreatic β cells to develop fully and to grow to their optimal function. Also, a retarded cell cycle might origin a disturbed intracellular metabolism in general as a result of unbalanced gene content. No association was found between IR or pancreatic β-cell dysfunction and karyotype in our study.

Several limitations of our study should be considered. Sufficiently large sample size will be needed to reach reliable conclusions. Also, the cross-sectional nature of our study prevents us from delineating pathogenic interpretations of our findings. A longitudinal evaluation of the capacity of IR indexes to predict β-cell decompensation in adult TS individuals is warranted. Although statistically significant differences were determined between the TS subjects and the reference group and between NGT-TS and IGT-TS, caution should be considered. We applied the Kruskal–Wallis test with *post hoc* correction to multiple comparisons to reduce the chances of obtaining a type I error. Also, the TS group with IGT was small, which prevented us from detecting relevant features of this group. Besides, we were unable to establish cutoff points by percentiles for defining IR to each index. We cannot apply percentile distribution for IR indices derived from other populations (e.g., Caucasian) as this may be very different because our data have been obtained from an entirely Latin American population, and a broad variability in the concordance analysis of selected IR indices was found in our study. As well, we cannot generalize the conclusions to other ethnic populations. Lastly, although euglycemic–hyperinsulinemic clamp and the intravenous glucose tolerance test with minimal modeling techniques are more accurate than OGTT, this latter is much more physiological than the intravenous tests, mainly because glucose sensors disseminated by the gastrointestinal tract take part in insulin secretion (34). In addition, studies

have shown that OGTT is superior to other tests for the diagnosis of early abnormalities of glucose metabolism in these patients (4, 11, 13).

In summary, a broad variety of correlation coefficients between methods based on fasting data of glucose and insulin vs. methods of OGTT-derived values was found in our study. Consequently, adult TS subjects diagnosed with IR by one method might not have equally IR if diagnosed by another method. Therefore, it is impossible to select the best surrogate method for the assessment of IR in women with TS. Based on our findings of OGTT, CPI and CPII values could be preferable to other indices to assess the pancreatic β-cell function in TS patients in a clinical setting. Lastly, an impaired β-cell function was observed in our cohort of TS subjects both in lean and overweight women with TS, as estimated by adjusting for IR. Our findings suggest that the compromised pancreatic β-cell function in TS subjects might differ from that seen in obese women without TS. Thus, an important question raises whether TS *per se* could account for the development of IGT or T2DM. Because this defect was not associated with BMI in our patients, it may be caused by intrinsic factors of TS. Our findings imply that early screening and intervention for TS would be therapeutic for TS women.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Subcomité de Ética de Investigación en Seres Humanos de la Universidad Central del Ecuador. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FÁ-N: study concepts and design, data analysis and interpretation, statistical analysis, obtaining funding, critical revision of the manuscript for important intellectual content, and manuscript preparation. DB: data analysis and interpretation, statistical analysis, and critical revision of the manuscript for important intellectual content. MR-O: biochemical studies and data analysis. JG: statistical analysis. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Mortensen KH, Andersen NH, Gravholt CH. Cardiovascular phenotype in turner syndrome-integrating cardiology, genetics, and endocrinology. *Endocr Rev.* (2012) 33:677–714. doi: 10.1210/er.2011-1059
2. Schoemaker MJ, Swerdlow AJ, Higgins CD, Wright AF, Jacobs PA, United Kingdom Clinical Cytogenetics Group. Mortality in women with turner syndrome in Great Britain : A National Cohort Study. *J Clin Endocrinol Metab.* (2008) 93:4735–42. doi: 10.1210/jc.2008-1049
3. Stochholm K1, Juul S, Juel K, Naeraa RW, Gravholt CH. Prevalence, incidence, diagnostic delay, and mortality in Turner syndrome. *J Clin Endocrinol Metab.* (2006) 91:3897–902. doi: 10.1210/jc.2006-0558
4. Sun L, Wang Y, Zhou T, Zhao X, Wang Y, Wang G, et al. Glucose metabolism in turner syndrome. *Front Endocrinol.* (2019) 10:49. doi: 10.3389/fendo.2019.00049
5. Lebenthal Y, Levy S, Sofrin-Drucker E, Nagelberg N, Weintrob N, Shalitin S, et al. The natural history of metabolic comorbidities in Turner syndrome from childhood to early adulthood: comparison between 45,X monosomy and other karyotypes. *Front Endocrinol.* (2018) 9:27. doi: 10.3389/fendo.2018.00027
6. Pirgon Ö, Atabek ME, Oran B, Güçlü R. Atherogenic lipid profile and systolic blood pressure are associated with carotid artery intima-media thickness in children with Turner Syndrome. *J Clin Res Pediatr Endocrinol.* (2008) 1:62–71. doi: 10.4008/jcrpe.v1i2.9
7. Mavinkurve M, O'Gorman CS. Cardiometabolic and vascular risks in young and adolescent girls with Turner syndrome. *BBA Clin.* (2015) 3:304–9. doi: 10.1016/j.bbacli.2015.04.005
8. Salgin B, Amin R, Yuen K, Williams RM, Murgatroyd P, Dunger DB. Insulin resistance is an intrinsic defect independent of fat mass in women with Turner's syndrome. *Horm Res.* (2006) 65:69–75. doi: 10.1159/000090907
9. Bakalov VK, Cooley MM, Quon MJ, Luo ML, Yanovski JA, Nelson LM, et al. Impaired insulin secretion in the Turner metabolic syndrome. *J Clin Endocrinol Metab.* (2004) 89:3516–20. doi: 10.1210/jc.2004-0122
10. Álvarez-Nava F, Racines M, Witt J, Guarderas J, Estévez M, Lanes R. Anthropometric variables as cardiovascular risk predictors in a cohort of adult subjects with Turner syndrome. *Diabetes Metab Syndr Obes.* (2019) 12:1795–809. doi: 10.2147/DMSO.S214787
11. Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, et al. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur J Endocrinol.* (2017) 3:G1–70. doi: 10.1530/EJE-17-0430
12. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care.* (1999) 22:1462–70. doi: 10.2337/diacare.22.9.1462
13. Choi IK, Kim DH, Kim HS. The abnormalities of carbohydrate metabolism in Turner syndrome: analysis of risk factors associated with impaired glucose tolerance. *Eur J Pediatr.* (2005) 164:442–7. doi: 10.1007/s00431-005-1643-x
14. World Health Organization. Obesity: preventing and managing the global epidemic. *World Health Organ Tech Rep Ser.* (2000) 894i–xii:1–253.
15. Alberti KGMMG, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A consensus statement from the international diabetes federation. *Diabet Med.* (2006) 23:469–80. doi: 10.1111/j.1464-5491.2006.01858.x
16. Petersen KF1, Dufour S, Feng J, Befroy D, Dziura J, Dalla Man C, et al. Increased prevalence of insulin resistance and nonalcoholic fatty liver disease in Asian-Indian men. *Proc Natl Acad Sci USA.* (2006) 103:18273–7. doi: 10.1073/pnas.0608537103
17. Henríquez S, Jara N, Bunout D, Hirsch S, de la Maza MP, Leiva L, et al. Variability of formulas to assess insulin sensitivity and their association with the Matsuda index. *Nutr Hosp.* (2013) 28:1594–8. doi: 10.3305/nh.2013.28.5.6512

18. Lam KD1, Bacchetti P, Abbasi F, Ayala CE, Loeb SM, Shah V, et al. Comparison of surrogate and direct measurement of insulin resistance in chronic hepatitis C virus infection: impact of obesity and ethnicity. *Hepatology.* (2010) 52:38–46. doi: 10.1002/hep.23670
19. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics.* (1977) 33:159–74. doi: 10.2307/2529310
20. Forbes AP, Engel E. The high incidence of diabetes mellitus in 41 patients with gonadal dysgenesis, and their close relatives. *Metabolism.* (1963) 12:428–39.
21. Bakalov VK, Cheng C, Zhou J, Bondy CA. X-chromosome gene dosage and the risk of diabetes in Turner syndrome. *J Clin Endocrinol Metab.* (2009) 94:3289–96. doi: 10.1210/jc.2009-0384
22. Ibarra-Gasparini D, Altieri P, Scarano E, Perri A, Morselli-Labate AM, Pagotto U, et al. New insights on diabetes in Turner syndrome: results from an observational study in adulthood. *Endocrine.* (2018) 59:651–60. doi: 10.1007/s12020-017-1336-z
23. Mazzanti L, Bergamaschi R, Castiglioni L, Zappulla F, Pirazzoli P, Cicognani A. Turner syndrome, insulin sensitivity and growth hormone treatment. *Horm Res.* (2005) 64 (Suppl. 3):51–7. doi: 10.1159/000089318
24. Hjerrild BE, Holst JJ, Juhl CB, Christiansen JS, Schmitz O, Gravholt CH. Delayed β-cell response and glucose intolerance in young women with Turner syndrome. *B BMC Endocr Disord.* (2011) 11:6. doi: 10.1186/1472-6823-11-6
25. O'Gorman CS, Syme C, Lang J, Bradley TJ, Wells GD, Hamilton JK. An evaluation of early cardiometabolic risk factors in children and adolescents with Turner syndrome. *Clin Endocrinol.* (2013) 78:907–13. doi: 10.1111/cen.12079
26. Gravholt CH1, Naeraa RW, Nyholm B, Gerdes LU, Christiansen E, Schmitz O, et al. Glucose metabolism, lipid metabolism, and cardiovascular risk factors in adult Turner's syndrome. *Diabetes Care.* (1998) 21:1062–70. doi: 10.2337/diacare.21.7.1062
27. Bergman RN, Ader M, Huecking K, Van Citters G. Accurate assessment of B-cell function: the hyperbolic correction. *Diabetes.* (2002) 51 (Suppl. 1):S212–20. doi: 10.2337/diabetes.51.2007.S212
28. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* (1985) 28:412–9. doi: 10.1007/BF00280883
29. Pratley RE, Weyer C. The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. *Diabetologia.* (2001) 44:929–45. doi: 10.1007/s001250100580
30. Meier JJ, Menge BA, Breuer TG, Müller CA, Tannapfel A, Uhl W, et al. Functional assessment of pancreatic beta-cell area in humans. *Diabetes.* (2009) 58:1595–603. doi: 10.2337/db08-1611
31. Scheen AJ, Castillo MJ, Lefèbvre PJ. Assessment of residual insulin secretion in diabetic patients using the intravenous glucagon stimulatory test: methodological aspects and clinical applications. *Diabetes Metabol.* (1996) 22:397–406.
32. Saisho Y. Postprandial C-peptide to glucose ratio as a marker of β cell function: implication for the management of type 2 diabetes. *Inter J Mol Sci.* (2016) 17:E744. doi: 10.3390/ijms17050744
33. Barrenäs ML, Landin-Wilhelmsen K, Hanson C. Ear and hearing in relation to genotype and growth in Turner syndrome. *Heart Res.* (2000) 144:21–8. doi: 10.1016/S0378-5955(00)00040-X
34. Holst J. The physiology of glucagon-like peptide 1. *Physiol Rev.* (2007) 87:1409–39. doi: 10.1152/physrev.00034.2006

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Establishment of Clinical Prediction Model Based on the Study of Risk Factors of Stroke in Patients With Type 2 Diabetes Mellitus

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Purpose: Stroke has sparked global concern as it seriously threatens people's life, bringing about dramatic health burdens on patients, especially for type 2 diabetes mellitus (T2DM) patients. Therefore, a risk scoring model is urgently valuable for T2DM patients to predict the risk of stroke incidence and for positive health intervention.

Methods: We randomly divided 4,335 T2DM patients into two groups, training set (n = 3,252) and validation set (n = 1,083), at the ratio of 3:1. Characteristic variables were then selected based on the data of training set through least absolute shrinkage and selection operator regression. Three models were established to verify predictive ability. Foundation model was composed of basic information and physical indicators. Biochemical model consisted of biochemical indexes. Integrated model combined the above two models. Data of three models were then put into logistic regression analysis to form nomogram prediction models. Tools including *C* index, calibration plot, and curve analysis were implemented to test discrimination, calibration, and clinical use. To select the best predicting model, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were put into effect.

Results: Eleven risk factors were determined, including age, duration of T2DM, estimated glomerular filtration rate, systolic blood pressure, diastolic blood pressure, low-density lipoprotein, high-density lipoprotein, triglyceride, body mass index, uric acid, and glycosylated hemoglobin A_{1c}, all with significant *P*-values through logistic regression analysis. In the training set, areas under the curve of three models were 0.810, 0.819, and 0.884, whereas in the validation set, they were 0.836, 0.832, and 0.909. Through calibration plot, the S:P values in the training set were 0.836, 0.754, and 0.621 and were 0.918, 0.682, and 0.666 separately in the validation set. In terms of the decision curve analysis, the risk thresholds were, respectively, 8–73%, 8–98%, and 8%∼ in the training set and 8–70%, 8–90%, and 8–95% in the validation set. With the aid of NRI and IDI, integrated model is proved to be the best model in training set and validation set. Besides, internal validation was conducted on all the subjects in this study, and the *C* index was 0.890 (0.873–0.907).

Conclusion: This study established a model predicting risk of stroke for T2DM patients through a community-based survey.

Keywords: stroke, type 2 diabetes mellitus, risk factor, prediction model, external validation, dynamic nomogram

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INTRODUCTION

Type 2 diabetes mellitus (T2DM), accounting for ∼90% total diabetes cases, is one of the most threatening non-communicable chronic diseases. Data from the latest IDF Diabetes Atlas showed that the number of adults aged 20–79 years in the world suffering from diabetes was ∼463 million in 2019. Diabetes mellitus (DM) is a great growing public health burden in China as the prevalence estimated at 11.6%, whereas that of prediabetes was ∼50.1% (1).

Stroke, as one of the macrovascular complications related to DM, results in extracranial carotid artery disease and intracranial large and small vessel diseases and includes clinical characteristics ranging from asymptomatic carotid artery occlusion or cerebral small vessel disease to transient ischemic attack and hemorrhagic and ischemic stroke (2). Stroke has been acknowledged in the form of a major issue in public health contributing to morbidity and mortality worldwide. According to the *Atlas of Heart Disease and Stroke* released by the World Health Organization, stroke is the third cause of death (ranks after myocardial infarction and cancer) in the world, and every year ∼17 million people die of cardiovascular diseases (CVDs) particularly attributed to heart attacks and strokes.

As one of the related complications of DM, stroke is the condition different from DM but with many common aspects (3). Nearly all types of stroke are known to be influenced by DM, including large artery stroke, lacunar stroke, intracerebral hemorrhage, and embolic stroke (4). Considerable prospective studies have indicated that, in comparison with non-diabetic population, patients with diabetes are at a higher risk of stroke among the western population (5–8). A Chinese hospital study based on 2,532 hospitalized patients with a first stroke showed that diabetes had a remarkable frequency of strokes than non-diabetics (9). In contrast to non-diabetics, the risk of stroke of people with DM is 2.5–3.6 times higher (4). Through a prospective observational study including 210 acute stroke patients, patients with DM were proved to shoulder the huger burden with poorer outcome brought by acute stroke compared with non-diabetic patients (3). According to statistics, 80% of DM patients eventually died of macrovascular complications (10). Accordingly, risk factors of stroke for T2DM patients urgently need to be determined.

Related studies of T2DM patients with stroke have provided evidence for us to identify corresponding risk factors. Through studies on diabetes and stroke, Wang et al. (11), Li et al. (12), and Bos et al. (13) stated that gender, estimated glomerular filtration rate (eGFR), duration of T2DM (course), postprandial blood glucose (PBG), fasting blood glucose (FBG), glycosylated hemoglobin A_{1c} (HbA_{1c}), systolic blood pressure (SBP), diastolic blood pressure (DBP), age, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG), body mass index (BMI), and uric acid (UA) are risk factors for stroke among T2DM patients. Based on the previous studies and community survey in this study, we involved basic information indicators including gender, age, course, BMI, SBP, DBP, and family history of DM; disease record information including hypertension, hyperlipemia, and microvascular disease; lifestyle factors containing smoking and alcohol; and biochemical indicators including FBG, PBG, HbA_{1c}, TC, TG, HDL-C, LDL-C, blood urea nitrogen (BUN), UA, eGFR, and the ratio of urinary microalbumin to uric creatinine (ACR) in this study.

At present, many studies on T2DM or stroke describe risk factors of stroke and T2DM patients, respectively. The study combined the two diseases and aimed to find out risk factors of stroke for T2DM patients.

This study aimed to build a simple, convenient, and efficient prediction model because of the main risk factors affecting stroke for T2DM patients. In this study, three nomogram plots were demonstrated, and the most predictive, accurate, and effective one was found through net reclassification improvement (NRI) and integrated discrimination improvement (IDI). At the same time, we also developed an online application for predicting T2DM patients with stroke based on the nomogram plot. The work can be used for clinically evaluating T2DM patients to assess the risk of stroke incidence for them.

MATERIALS AND METHODS

Patients

We worked with Shanghai University of Traditional Chinese Medicine–affiliated community health center hospitals for this study. From September 2014 to September 2019, we conducted baseline and follow-up study on all the patients in the seven communities including Community of Huamu, Community of Jinyang, Community of Sanlin, Community of Siping, Community of Yinhang, Community of Daqiao, and Community of Jiangpu in Shanghai and finally included 4,335 subjects in this study. Subjects were determined based on their medical history information. Patients with T2DM with a history of stroke were valid to be involved in this study. Questionnaire survey, physical examination, and biochemical examination contained values of each influencing factor in this study, which were crucial for forming results. In order not to affect the model establishment and results, accordingly, for data screening, we checked the missing values at the beginning. Patients with any lack of needed information would be excluded. After obtaining all the data and comparing the various data values in the population, subjects would be eliminated with any abnormal value of influencing factors. With exclusion of invalid questionnaires and those without complete information from all the collected questionnaires, we eventually involved 4,335 subjects in the study. Before enrolling the subjects in this study, we received written informed consent from all of them.

Abbreviations: T2DM, Type 2 diabetes mellitus; DM, Diabetes mellitus; eGFR, Estimated glomerular filtration rate; PBG, Postprandial blood glucose; FBG, Fasting blood glucose; HbA_{1c}, Glycosylated hemoglobin A_{1c}; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; TC, Total cholesterol; TG, Triglyceride; BMI, Body mass index; UA, Uric acid; BUN, Blood urea nitrogen; ACR, Ratio of urinary microalbumin to uric creatinine; NRI, Net reclassification improvement; IDI, Integrated discrimination improvement; LASSO, Least absolute shrinkage and selection operator; OR, Odds ratio; CI, Confidence interval; ROC, Receiver operating characteristic; DCA, Decision curve analysis; SE, Standard error.

Procedure

We performed survey, including questionnaire surveys, physical examination, and biochemical test, and investigated all T2DM patients in seven communities with support from affiliated community health centers and central hospitals. All the researchers and investigators involved in the survey were well-trained and qualified to ensure standardization and scientific rigor in the procedure. A structured questionnaire survey was composed of social demographic characteristics, lifestyle factors, DM status, disease history, and drug history (lipid-lowering, blood pressure-lowering, aspirin, and insulin). Besides, to determine the subjects precisely, we checked the electronic medical records of all the participants for filtering. Patients with T2DM were determined as the initial population. The diagnosis of T2DM was in accordance with the criteria defined by the World Health Organization in 1999 (14). Patients with stroke were then determined through rigorous screening of medical records to ensure validity for this study and were finally included.

All the physical indicators were measured with standard electronic devices. Systolic blood pressure and DBP were measured in standard sitting with OMRON blood pressure monitors. According to the Guidelines for the Prevention and Treatment of T2DM in China, BMI was calculated with weight (in kilograms) divided by square height (in meters squared). Biochemical indexes included FBG, PBG, HbA_{1c}, TC, TG, HDL-C, LDL-C, BUN, UA, and ACR. Estimated glomerular filtration rate was computed according to serum creatinine, age, and gender according to Modification of Diet in Renal Disease Trial. To test blood indicators, all the participants need to keep fasting for at least 10 h and took the examination at 7 in the morning. Two hours after the meal, urine was collected from participants for glycosuria measurement. All the blood samples were required to be taken for the operation of *in situ* centrifugation within 30 min after collection and stored in refrigerators at −80°C for further study. All the samples were at once sent to hematology department of Ruijin Hospital Affiliated to Shanghai Jiaotong University and community health centers and central hospitals affiliated to Shanghai University of Traditional Chinese Medicine for testing after the scientific operation. Urine-related biochemical indicators were analyzed by uritest-500b (URIT, China).

Statistical Analysis

Through the community survey, we collected 4,335 T2DM patients, including 2,504 female patients and 1,831 male patients. With the aid of R software (version 3.6.2; https://www.R-project.org), we randomly divided patients into two groups, training set (n = 3,252) and validation set (n = 1,083) for external validation at a theoretical ratio of 3:1 (15). In the first step, we used data of the training set and took the least absolute shrinkage and selection operator (LASSO) regression method to analyze the data. Least absolute shrinkage and selection operator are a method applied for data dimensional reduction. Besides, the LASSO regression model takes double-standard error by constructing a penalty function. Concerning the characteristics of this method, we screened suitable and effective risk factors for T2DM patients with stroke in the LASSO regression analysis and selected 11 non-zero characteristic factors. We then obtained three models: foundation model, biochemical model, and integrated model, respectively, including basic physical indicators, biochemical indicators, and both indicators, and separately put into the multivariate logistic regression analysis. Variables selected through logistic regression analysis were considered of odds ratio (OR) and P-value with 95% confidence interval (CI), and the statistical significance levels were all two-sided. Based on the logistic regression results, we selected risk factors with the P-value of and <0.05 and constructed a nomogram prediction model. In this study, all the variables were selected. For the validation of the three models, we, respectively, calculated C index, receiver operating characteristic (ROC) curve, and dynamic component analysis (DCA) measurements based on the data from training set and validation set (16).

We used NRI and IDI to choose the best predictive model. NRI and IDI are two mutually complementary validation method to compare the accuracy and predictive ability of two prediction models, evaluating the effectiveness of index change compared with the old one. The difference between NRI and IDI is that the NRI only considers the improvement setting a certain cutoff point while the IDI inspects the overall improvement of the model. When NRI >0.1, the prediction model is improved, and if IDI >0.1, it indicates that this is an improvement and that the new model is better than the old model. The difference between NRI and IDI is that the NRI only considers the improvement when setting a certain cutoff point, while the IDI inspects the overall improvement of the model.

After selecting the best model, we applied the variables of the model to all the subjects in this study for internal validation to ensure the predictive ability of the model.

RESULTS

This study involved 4,335 T2DM patients, including 1,831 (42.24%) male participants and 2,504 (57.76%) female participants from seven communities in Shanghai. Among all the included T2DM patients, there were 379 patients (8.74%) with stroke and 3,956 patients (91.26%) without stroke. The average age of the participants was 64.54 ± 6.79 years. The prevalence of stroke among all participants was 8.74% (379 participants). The mean LDL-C and HDL-C levels in patients with stroke were 1.75 ± 0.48 and 1.47 ± 0.36 mmol/L and were 1.48 ± 0.46 and 1.73 ± 0.38 mmol/L separately in those without stroke. The median TG level was 2.00 (1.61, 2.59) mmol/L in patients with stroke and 1.24 (0.81, 1.91) mmol/L in those without stroke. The median HbA_{1c} and FBG levels of T2DM patients with stroke were separately 7.30% (6.60%, 8.30%) and 7.60 (6.35, 9.10) mmol/L, whereas those of patients without stroke were 6.57% (5.87%, 7.57%) and 7.03 (5.83, 8.63) mmol/L. In this study, the mean SBP and DBP levels were 148.38 ± 19.34 mmHg and 81.90 ± 10.76 mmHg in patients with stroke, whereas the median SBP and mean DBP levels were 132.00 (119.00, 145.00) mmHg and 77.18 ± 10.52 mmHg in patients without stroke. Among 4,335 T2DM patients, 2,880 (66.44%) people used antihypertensive drugs, 692 (15.96%) people used

TABLE 1 | Characteristics of the participants in different groups.

	Total (n = 4,335)	Stroke (n = 379)	No stroke (n = 3,956)	Training set (n = 3,252)	Validation set (n = 1,083)	P
Gender						0.648
Male	1,831 (42.24%)	176 (46.44%)	1,655 (41.84%)	1,380 (42.44%)	451 (41.64%)	
Female	2,504 (57.76%)	203 (53.56%)	2,301 (58.16%)	1,872 (57.56%)	632 (58.36%)	
Diagnosed stroke	379 (8.74%)			284 (8.73%)	95 (8.77%)	0.969
Age (years)	64.54 ± 6.79	66.97 ± 6.21	64.31 ± 6.79	64.46 ± 6.73	64.80 ± 6.95	0.146
Course (years)	9.00 (4.00, 14.00)	11.00 (6.00, 16.00)	8.00 (4.00, 14.00)	9.00 (4.00, 14.00)	9.00 (4.00, 14.00)	0.925
BMI (kg/m^2)	24.23 ± 3.47	26.94 ± 3.36	23.97 ± 3.37	24.20 ± 3.44	24.32 ± 3.54	0.342
Hypertension						0.845
No	1,656 (38.20%)	85 (22.43%)	1,571 (39.71%)	1,245 (38.28%)	411 (37.95%)	
Yes	2,679 (61.80%)	294 (77.57%)	2,385 (60.29%)	2,007 (61.72%)	672 (62.05%)	
Hyperlipemia						0.912
No	2,716 (62.65%)	194 (51.19%)	2,522 (63.75%)	2,039 (62.70%)	677 (62.51%)	
Yes	1,619 (37.35%)	185 (48.81%)	1,434 (36.25%)	1,213 (37.30%)	406 (37.49%)	
Microvascular disease						0.191
No	2,155 (49.71%)	163 (43.01%)	1,992 (50.35%)	1,598 (49.14%)	557 (51.43%)	
Yes	2,180 (50.29%)	216 (56.99%)	1,964 (49.65%)	1,654 (50.86%)	526 (48.57%)	
Family history of DM						0.278
No	2,565 (59.17%)	225 (59.37%)	2,340 (59.15%)	1,909 (58.70%)	656 (60.57%)	
Yes	1,770 (40.83%)	154 (40.63%)	1,616 (40.85%)	1,343 (41.30%)	427 (39.43%)	
Smoking						0.184
No	3,544 (81.75%)	317 (83.64%)	3,227 (81.57%)	2,644 (81.30%)	900 (83.10%)	
Yes	791 (18.25%)	62 (16.36%)	729 (18.43%)	608 (18.70%)	183 (16.90%)	
Alcohol						0.094
No	3,288 (75.85%)	283 (74.67%)	3,005 (75.96%)	2,487 (76.48%)	801 (73.96%)	
Yes	1,047 (24.15%)	96 (25.33%)	951 (24.04%)	765 (23.52%)	282 (26.04%)	
SBP (mmHg)	133.00 (120.00, 147.00)	148.38 ± 19.34	132.00 (119.00, 145.00)	133 (120.00, 148.00)	133.00 (120.00, 146.00)	0.110
DBP (mmHg)	77.59 ± 10.62	81.90 ± 10.76	77.18 ± 10.52	77.73 ± 10.66	77.18 ± 10.51	0.139
FBG (mmol/L)	7.03 (5.73, 8.63)	7.60 (6.35, 9.10)	6.93 (5.73, 8.63)	7.03 (5.83, 8.63)	7.00 (5.70, 8.73)	0.871
PBG (mmol/L)	11.28 ± 4.81	12.45 ± 4.27	11.17 ± 4.84	7.70 (10.90, 14.40)	11.13 ± 4.92	0.246
HbA_{1c} (%)	6.57 (5.87, 7.67)	7.30 (6.60, 8.30)	6.57 (5.87, 7.57)	6.57 (5.87, 6.67)	6.57 (5.87, 7.67)	0.987
TC (mmol/L)	4.51 ± 1.09	4.86 ± 1.12	4.48 ± 1.08	4.50 ± 1.09	4.53 ± 1.06	0.463
TG (mmol/L)	1.32 (0.85, 2.00)	2.00 (1.61, 2.59)	1.24 (0.81, 1.91)	1.31 (0.85, 2.00)	1.34 (0.88, 1.99)	0.909
LDL-C (mmol/L)	1.51 ± 0.47	1.75 ± 0.48	1.48 ± 0.46	1.50 ± 0.47	1.52 ± 0.46	0.432
HDL-C (mmol/L)	1.71 ± 0.39	1.47 ± 0.36	1.73 ± 0.38	1.71 ± 0.39	1.72 ± 0.39	0.290
BUN (mmol/L)	5.81 (4.21, 6.25)	5.63 (4.72, 6.65)	5.14 (4.18, 6.20)	5.19 (4.24, 6.27)	5.14 (4.14, 6.18)	0.171
UA (μmol/L)	298.31 ± 79.89	343.43 ± 81.33	293.99 ± 78.39	298.66 ± 80.33	297.25 ± 78.52	0.614
eGFR (mL/min)	53.35 (33.05, 77.67)	72.37 (52.90, 97.59)	51.58 (31.49, 74.82)	53.31 (33.39, 77.74)	53.63 (32.44, 76.97)	0.794
ACR (mg/g)	23.33 (10.64, 59.08)	46.01 (30.95, 96.33)	20.36 (9.74, 54.60)	23.58 (10.64, 58.10)	22.71 (10.77, 60.75)	0.845

Data are presented as n (%), mean ± SD, or median (IQR).

lipid-lowering drugs, 1,162 (26.81%) people used aspirin, and 772 (17.81%) people used insulin. Among the 3,956 T2DM patients without stroke, 2,702 (68.30%) patients used antihypertensive drugs, 562 (14.21%) patients used lipid-lowering drugs, 1,021 (25.81%) patients used aspirin, and 674 (17.04%) patients used insulin. Among the 379 stroke patients, 178 (46.97%) used antihypertensive drugs, 130 (34.30%) used lipid-lowering drugs, 141 (37.20%) used aspirin, and 98 (25.86%) used insulin.

For external verification, we divided two groups, training set (n = 3,252) and validation set (n = 1,083), at a ratio of 3:1. The training set composed of 1,380 (42.44%) male patients and 1,872 (57.56%) female patients, with average age of 64.46 ± 6.73 years. There were 284 patients (8.73%) complicated with stroke. In the validation set, 451 (41.64%) male patients and 632 (58.36%) female patients were included. The average age was 64.80 ± 6.95 years. Ninety-five patients (8.77%) were complicated with stroke. The detailed demographic and clinical characteristics are given in **Table 1**.

Through the analysis of literature search results and questionnaire results, 23 potential risk factors from physical examination indicators and biochemical examination indicators were included in the LASSO regression analysis (**Figures 1A,B**).

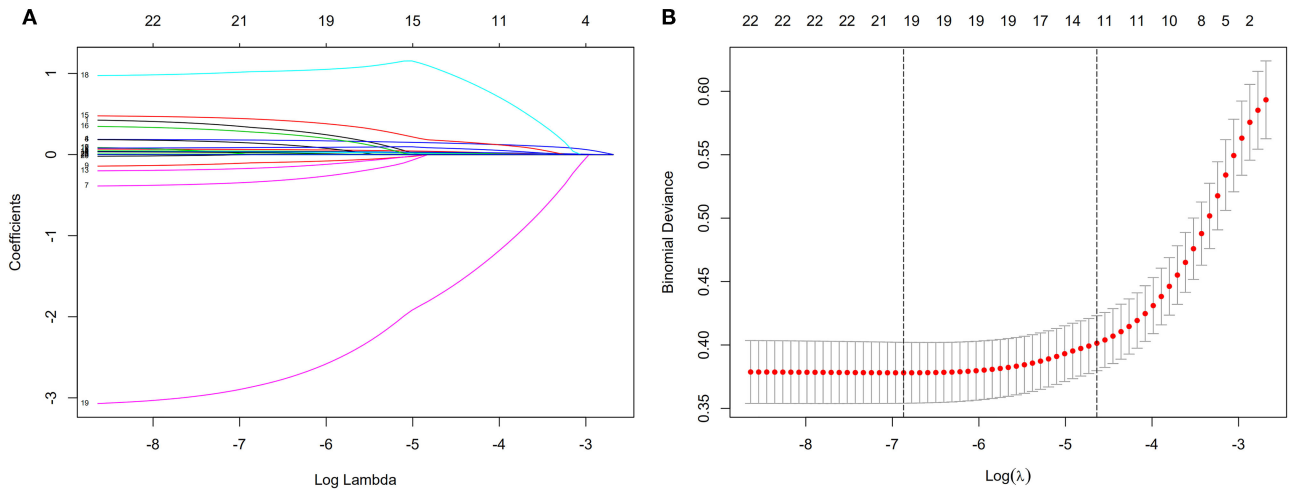

FIGURE 1 | Demographic and clinical feature selection using the LASSO binary logistic regression model. **(A)** The selection of the best parameter (lambda) in the LASSO model uses 5-fold cross-validation with the lowest standard. The relationship curve between partial likelihood deviation (binomial deviation) and log(lambda) was plotted. Dotted vertical lines were drawn at the optimal values by using the minimum criteria and the 1 SE of the minimum criteria (the 1 – SE criteria). **(B)** LASSO coefficient profiles of the 11 features. A coefficient profile plot was produced against the log(lambda) sequence. Vertical line was drawn at the value selected using 5-fold cross-validation, where optimal lambda resulted in five features with non-zero coefficients. LASSO, least absolute shrinkage and selection operator; SE, standard error.

TABLE 2 | Coefficients and lambda.min value of the LASSO regression.

Factors	Coefficients	Lambda.min
Age (years)	0.036	0.010
Course (years)	0.022	
BMI (kg/m^2)	0.142	
SBP (mmHg)	0.027	
DBP (mmHg)	0.013	
HbA_{1c} (%)	0.170	
TG (mmol/L)	0.079	
LDL-C (mmol/L)	1.023	
HDL-C (mmol/L)	−1.689	
UA (μmol/L)	0.003	
eGFR (mL/min)	0.003	

We selected 11 non-zero characteristic variables in the LASSO regression results, including AGE, course, BMI, SBP, DBP, HbA_{1c}, TG, LDL-C, HDL-C, UA, and eGFR (**Table 2**).

For external validation, three models were constructed. Foundation model, composed of basic information indicators and physical indicators, included AGE, course, SBP, DBP, and BMI (**Figure 2A**). Biochemical model consisted of biochemical indexes, including HbA_{1c}, TG, LDL-C, HDL-C, UA, and eGFR (**Figure 2B**). Integrated model contained all the variables of the above two models (**Figure 2C**). To give a plain and clarified illustration of integrated model, an example of a T2DM patient demonstrated in **Figure 2D**. If the subject is at the age of 68 years, duration of 2 years, SBP of 151 mmHg, DBP of 83 mmHg, BMI of 22.84 kg/m^2, HbA_{1c} of 6.5%, TG of 2.42 mmol/L, HDL-C of 1.04 mmol/L, LDL-C of 1.77 mmol/L, UA of 362 μmol/L, and eGFR of 85.89 mL/min, the probability of stroke is estimated to be 31.7%.

Through logistic regression analysis, *p*-values of all risk characteristic factors were proved to be significant in the three models, respectively (**Tables 3–5**). The *C* index of foundation model, biochemical model, and integrated model were 0.810 (0.783–0.837), 0.819 (0.792–0.845), and 0.884 (0.863–0.905) (**Table 6**). Area under the curve (AUC) values of ROC for foundation model, biochemical model, and integrated model in training set (**Figure 3G**) were 0.810 (**Figure 3A**), 0.819 (**Figure 3C**), and 0.884 (**Figure 3E**) (**Table 7**), whereas in validation set (**Figure 3H**) correspondingly were 0.836 (**Figure 3B**), 0.832 (**Figure 3D**), and 0.909 (**Figure 3F**) (**Table 7**). Calibration plot indicated that S:P of foundation model, biochemical model, and integrated model in training set is 0.836 (**Figure 4A**), 0.754 (**Figure 4C**), and 0.621 (**Figure 4E**), whereas in validation set is, respectively, 0.918 (**Figure 4B**), 0.682 (**Figure 4D**), and 0.666 (**Figure 4F**). The DCA decision curve demonstrated that the threshold probability of foundation model, biochemical model, and integrated model in training set is 8–73%, 8–98% and ∼8% (**Figure 5A**), whereas in validation set is 8–70, 8–90, and 8–95% (**Figure 5B**).

Through calculating the NRI, the cutoff in the training set was 0.088 (0.804, 0.785) (**Figure 3E**). Integrated model demonstrated to be 0.131 better than foundation model (**Figure 6A**) and 0.113 better than biochemical model (**Figure 6C**) (**Table 8**). In the validation set, the cutoff was 0.087 (0.811, 0.853) (**Figure 3F**). Integrated model was 0.133 better than foundation model (**Figure 6B**) and 0.118 better than biochemical model (**Figure 6D**) (**Table 8**). Through calculating the IDI in training set, integrated model was 0.148 (0.124, 0.172) better than foundation model and 0.139 (0.115, 0.164)

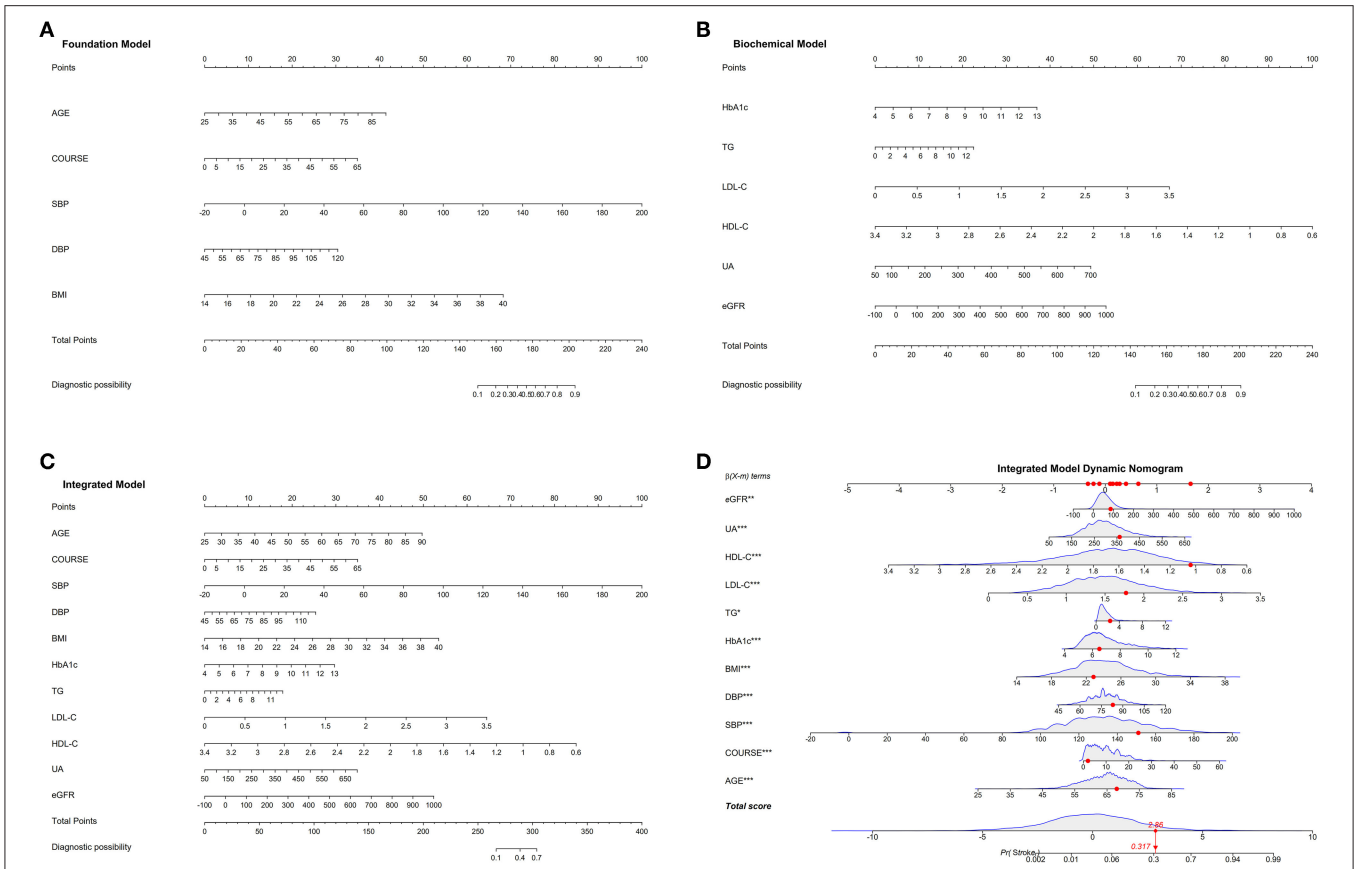

FIGURE 2 | Developed nomograms of three T2DM combined stroke models. **(A)** Foundation model: the medication stroke nomogram for T2DM patients was developed in the cohort, with age, course, SBP, DBP, and BMI incorporated. **(B)** Biochemical model: the medication stroke nomogram for T2DM patients was developed in the cohort, with HbA_{1c}, TG, LDL-C, HDL-C, UA, and eGFR incorporated. **(C)** Integrated model: the medication stroke nomogram for T2DM patients was developed in the cohort, with age, course, BMI, SBP, DBP, HbA_{1c}, TG, LDL-C, HDL-C, UA, and eGFR incorporated. **(D)** An example of nomogram based on integrated model.

TABLE 3 | Foundation model established by logistic regression analysis based on the training set.

	β Coefficient	Wald test	*P*	OR (95% CI)
Age (years)	0.052	4,696	<0.001	1.054 (1.031–1.078)
Course (years)	0.044	5.035	<0.001	1.045 (1.027–1.063)
SBP (mmHg)	0.037	10.798	<0.001	1.038 (1.031–1.045)
DBP (mmHg)	0.033	5.158	<0.001	1.034 (1.021–1.047)
BMI (kg/m^2)	0.216	11.137	<0.001	1.241 (1.195–1.290)

TABLE 4 | Biochemical Model established by logistic regression analysis based on the training set.

	β Coefficient	Wald test	*P*	OR (95% CI)
HbA_{1c} (%)	0.312	6.931	<0.001	1.366 (1.251–1.492)
TG (mmol/L)	0.132	2.817	0.005	1.141 (1.039–1.248)
LDL-C (mmol/L)	1.459	9.431	<0.001	4.301 (3.184–5.842)
HDL-C (mmol/L)	−2.711	−10.558	<0.001	0.066 (0.040–0.109)
UA (μmol/L)	0.006	6.556	<0.001	1.006 (1.004–1.008)
eGFR (mL/min)	0.004	2.835	0.005	1.004 (1.001–1.006)

better than biochemical model (**Table 8**). In the validation set, integrated model was 0.157 (0.115, 0.200) better than foundation model and 0.166 (0.120, 0.213) better than biochemical model (**Table 8**). Therefore, based on the above results, we can conclude that compared with foundation model and biochemical model; integrated model is improved, indicating that integrated model meets the clinical predictive modeling standards.

After obtaining integrated model, we verified on all the subjects included in this study with all the characteristic variables of integrated model and the variables proved to have a fairly good ability of predicting risk of stroke among T2DM patients. The result has been showed in **Table 9**.

Based on the results, the integrated model was confirmed to have moderate predictive ability. To better aid prevention and treatment of T2DM patients with stroke clinically and in the community, we developed an online application that could

predict quickly and directly. The URL of the application is https://doctorhu.shinyapps.io/T2DM_Stroke_DynNomapp/.

DISCUSSION

Prevalence of Stroke, Differences in Clinical Characteristics, and Medication Conditions of T2DM Patients

The prevalence of stroke in T2DM patients was 8.74%, and those in training set and validation set were, respectively, 8.73 and 8.77% in the study, which were consistent with some other previous studies. In a national observational cohort study in Sweden, in 26,380 T2DM patients, 6.5% were diagnosed with a stroke with the stroke incidence rate of 10.12 events 1,000 person-years (17). A study including multivariate analysis conducted in Spain found 41.2% T2DM patients with atherothrombotic stroke and 35.1% with lacunar infarction (18). Shen et al. (19) performed a retrospective cohort study composed of 27,113 blacks and 40,431 whites with T2DM and found that 8,496 (12.57%) participants developed stroke during a mean follow-up period of 3 years. A Chinese study was conducted on 9,374 T2DM patients in total to establish a risk score system; among all the participants, 11.48% developed ischemic stroke with a mean follow-up of 8 years (12). Xuebing et al. (20) performed a study in Beijing, China, on 4,639 T2DM patients, and among all the subjects, the prevalence of stroke was 5.5%.

The biochemical indicator characteristics of the general population in this study, T2DM patients with stroke, were generally higher in the levels of clinical indicators, including LDL-C, TG, HbA_{1c}, FBG, SBP, and DBP than those of patients without stroke, and HDL-C level was lower among patients with stroke, which were consistent with other studies. A study on Chinese T2DM patients indicated that LDL-C and TG were higher in patients with CVD, and HDL-C was lower than those without CVD (21). A study exploring risk factors of ischemic stroke on 2,769 DM patients found that the mean HbA_{1c} and FBG levels were significantly higher in patients with stroke when compared with patients without stroke (22). A Taiwanese study of 16,994 T2DM patients demonstrated that compared with those without stroke, patients with stroke were higher in the prevalence of hypertension with a rate of 74.5% (23).

During this study, more than two-thirds of patients took antihypertensive drugs, and nearly a third of patients use aspirin. A case-control study conducted in 32 countries/regions indicated that the occurrence of stroke is related to hypertension (24). A systematic review also showed that lowering blood pressure can significantly reduce various baseline blood pressure levels and vascular risk of complications (25). In our study, there were 2,702 T2DM patients without stroke taking antihypertensive drugs, accounting for 68.30% of all patients without stroke, which showed that taking antihypertensive drugs has significance on controlling blood pressure and then reducing the stroke incidence. According to a review comprehensively including randomized controlled trials of aspirin therapy, it is estimated that aspirin would reduce the risk of myocardial infarction and stroke by ∼10% in DM patients, indicating that low-dose aspirin therapy (75–162 mg) would be reasonable for DM patients in the primary prevention for stroke (26).

TABLE 5 | Integrated Model established by logistic regression analysis based on the training set.

	β Coefficient	Wald test	*P*	OR (95% CI)
Age (years)	0.063	5.144	<0.001	1.065 (1.040–1.091)
Course (years)	0.044	4.204	<0.001	1.045 (1.024–1.067)
SBP (mmHg)	0.037	9.380	<0.001	1.038 (1.030–1.046)
DBP (mmHg)	0.028	3.835	<0.001	1.028 (1.014–1.043)
BMI (kg/m^2)	0.168	7.500	<0.001	1.183 (1.133–1.237)
HbA_{1c} (%)	0.270	5.038	<0.001	1.310 (1.179–1.455)
TG (mmol/L)	0.112	2.146	0.032	1.119 (1.007–1.237)
LDL-C (mmol/L)	1.508	8.766	<0.001	4.518 (3.24–6.354)
HDL-C (mmol/L)	−2.483	−9.024	<0.001	0.083 (0.048–0,142)
UA (μmol/L)	0.004	4.455	<0.001	1.004 (1.002–1.006)
eGFR (mL/min)	0.004	2.777	0.005	1.004 (1.001–1.007)

Risk Factors for T2DM Patients With Stroke

We utilized the nomogram in the study. A nomogram is a superior visual tool with the user-friendly display, precise calculation, and easy to understand and effective prognoses (27), which is expert in developing a graphic continuous scoring system based on incorporated related factors and calculating precisely the risk probability of adverse results according to individual characteristics (28). In terms of all the bright points, the nomogram was applied for predicting the risk of stroke incidence among T2DM patients and clinical evaluation and displayed decent predictive power through internal and external validation.

Eleven risk characteristic variables considered as factors affecting stroke incidence among T2DM patients in this study, including age, course, SBP, DBP, HDL-C, LDL-C, BMI, TG,

TABLE 6 | *C* index in the array on the training set.

	C index (95% CI)	Dxy	aDxy	Variance	*Z*	*P*	*n*
Foundation model	0.810 (0.783–0.837)	0.619	0.619	0.028	22.43	0	3,252
Biochemical model	0.819 (0.792–0.845)	0.639	0.639	0.027	23.88	0	3,252
Integrated model	0.884 (0.863–0.905)	0.768	0.768	0.021	36.38	0	3,252

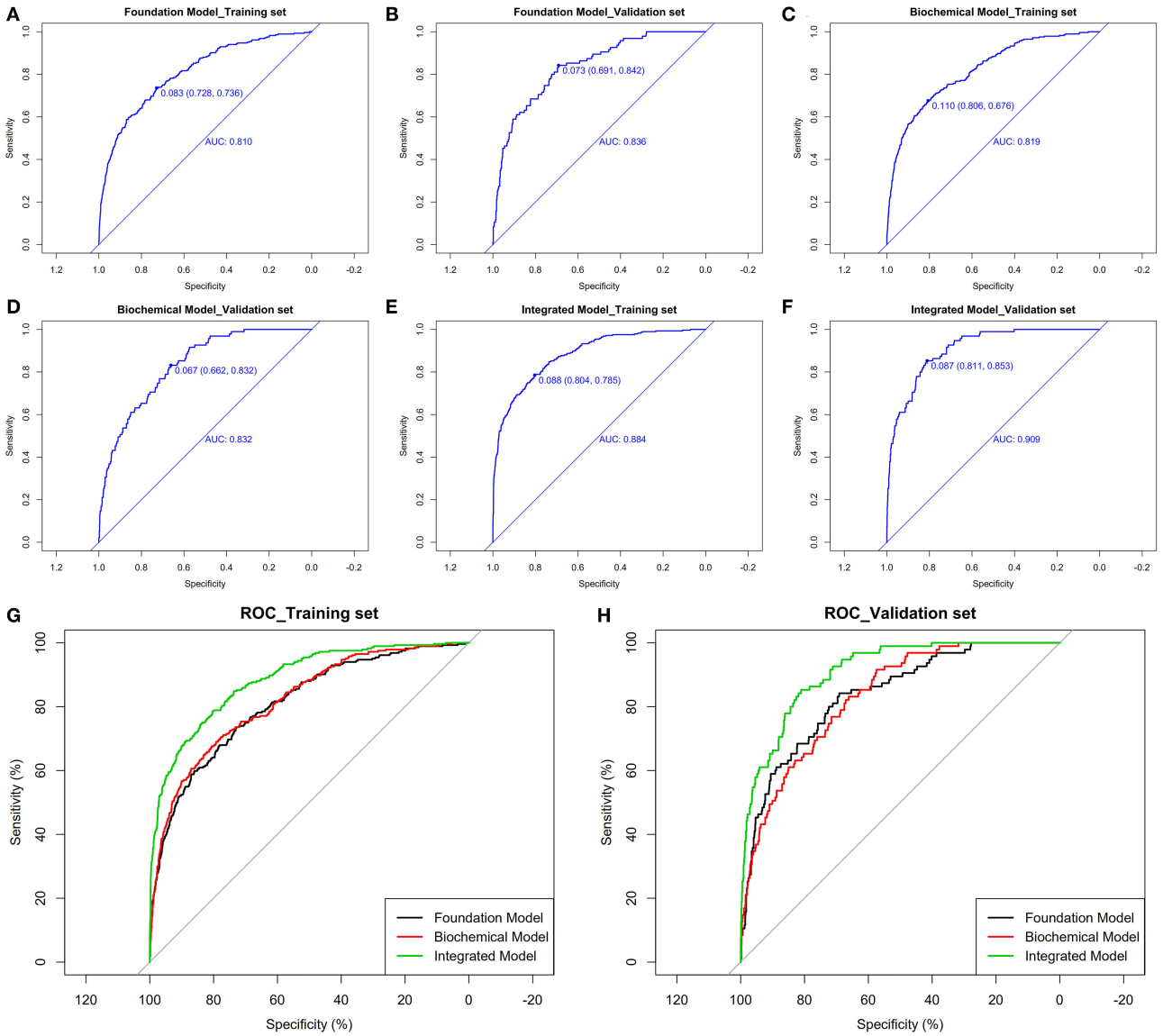

FIGURE 3 | The pooled AUC of the ROC curve in training set and validation set. Foundation model **(A,B)**, biochemical model **(C,D)**, integrated model **(E,F)**: The *y* axis measures the net benefit. The dotted line represents the stroke incidence risk nomogram for T2DM patients. The thin solid line represents the assumption that all patients are diagnosed as stroke. The thick solid line represents the assumption that no patients are diagnosed as stroke. **(G,H)**: Integration of above decision curve analysis for the stroke incidence risk nomogram based on three models in training set and validation set.

TABLE 7 | Comparison of ROC between different models using training set and validation set.

	Training set (n = 3,252)					Validation set (n = 1,083)				
	ROC A	**ROC B**	**ROC C**	**A–C**	**B–C**	**ROC A**	**ROC B**	**ROC C**	**A–C**	**B–C**
AUC	80.950	81.927	88.380			83.642	83.196	90.942		
Z				−7.394	−6.684				−4.646	−5.048
P				<0.001	<0.001				<0.001	<0.001

eGFR, UA, HbA_{1c}, were selected through LASSO and logistic regression analysis based on training set. Among three different models we established, integrated model incorporating all the 11 variables showed the best predictive ability through NRI and IDI validation, which displayed the necessity of each of the 11 risk factors in predicting the risk of stroke among T2DM patients.

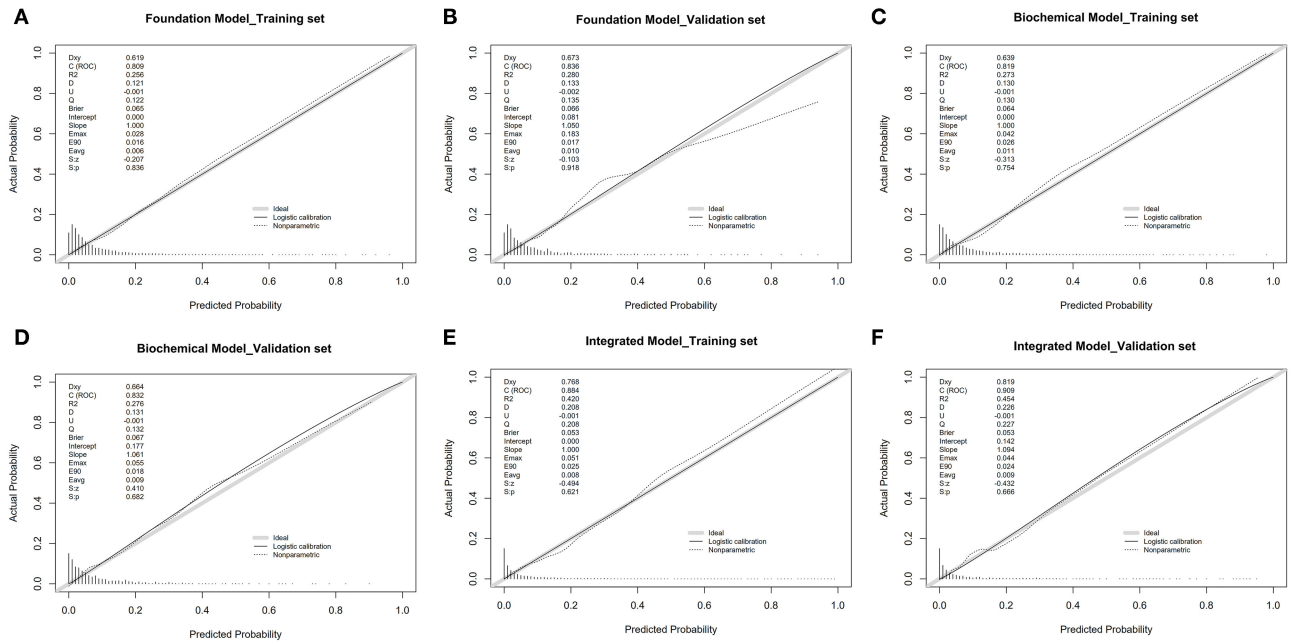

FIGURE 4 | Calibration curves of the stroke incidence risk nomogram prediction in the array in training set and validation set. Foundation model **(A,B)**, biochemical model **(C,D)**, integrated model **(E,F)**: The *x* axis represents the predicted T2DM patients with stroke incidence risk. The *y* axis represents the actual diagnosed T2DM patients with stroke. The diagonal dotted line represents a perfect prediction by an ideal model. The solid line represents the performance of the nomogram, of which a closer fit to the diagonal dotted line represents a better prediction.

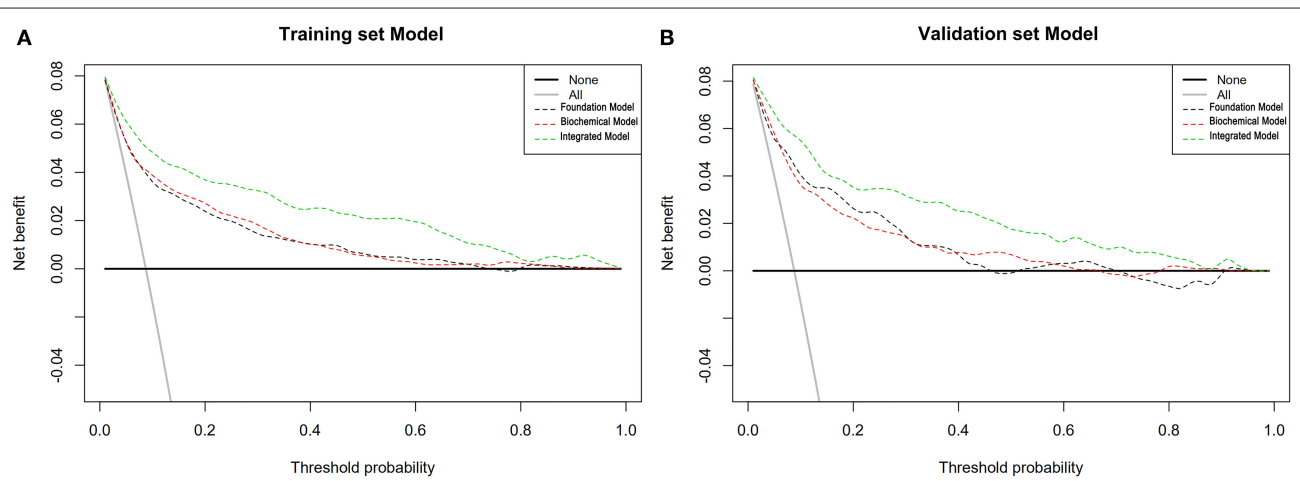

FIGURE 5 | Decision curve analysis for the T2DM patients with stroke incidence risk nomogram based on three models in training set and validation set. **(A)** Training set and **(B)** validation set: The *y* axis means the true positive rate of the risk prediction of T2DM patients with stroke. The *x* axis means the false positive rate of the risk prediction of T2DM patients with stroke. The black line represents the performance of the nomogram of foundation model. The red line represents the performance of the nomogram of biochemical model. The green line represents the performance of the nomogram of integrated model.

A risk study on T2DM patients with stroke obtained 14 risk factors, among which four risk factors, including age, disease course, blood pressure, and HbA_{1c} level, were consistent with this study (12).

According to the results, this study suggested that age and the course of diabetes in T2DM patients are important and immutable predictive risk factors for T2DM patients with stroke. Old age means the decline of the function of various tissues and organs of the body, pointing out that the risk of T2DM patients with stroke is affected by age (1). A study of 3,776 T2DM subjects identified age as an important risk factor (29). As the age of T2DM patients with stroke continues to increase, with the decline of physical function and the prolongation of the duration of diabetes, blood glucose fluctuations are obvious, exacerbating

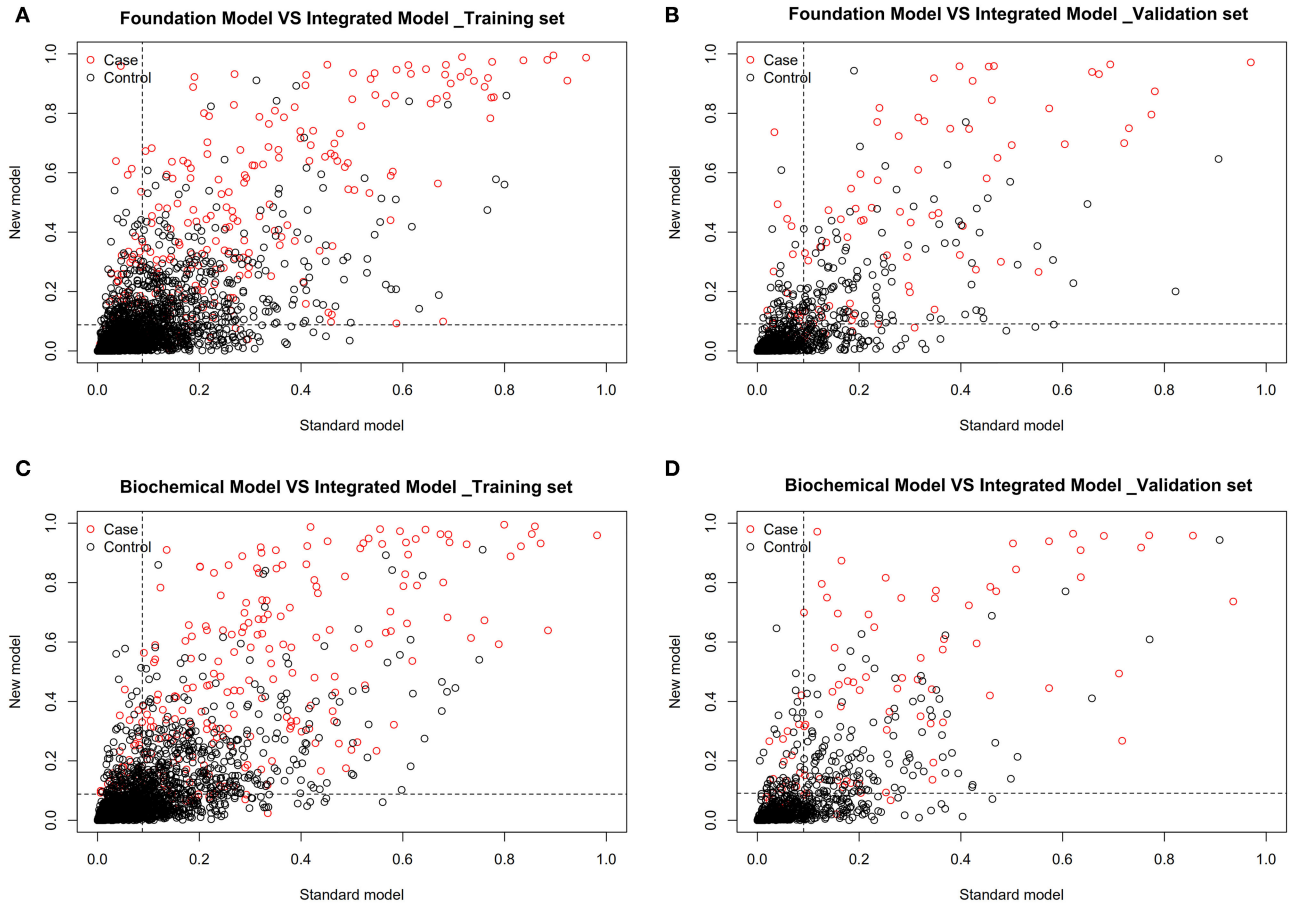

FIGURE 6 | Model comparison based on NRI in training set [cutoff is 0.088 (0.804, 0.785)] and validation set [cutoff is 0.087 (0.811, 0.853)]. Foundation model vs. integrated model **(A,B)**: Integrated model is 0.131 better than foundation model in training set. Integrated model is 0.133 better than foundation model in validation set. Biochemical model vs. integrated model **(C,D)**: Integrated model is 0.113 better than biochemical model in training set. Integrated model is 0.118 better than biochemical model in validation set.

vascular endothelial damage and inflammatory stimuli, thereby accelerating the formation of stroke (30). Khalid Al-Rubeaan et al. (22) performed a study on ischemic stroke and its risk factors in a diabetic cohort in countries facing diabetes prevalence and showed the prevalence of ischemic stroke was 4.42% and was higher in the older age group with longer diabetes duration.

The result of this study illustrated that there was a positive correlation between BMI, TG, and stroke prevalence in T2DM patients. High BMI and TG indicate that patients are obese, having a higher possibility of blood lipid status. According to the American Heart Association, American Stroke Association, and many other global guidelines, maintaining a healthy weight is recommended as an important intervention for stroke outbreaks. The BMI, as an important measure of physical health, plays an important role in preventing the onset of disease in the brain of diabetic patients. For a cohort including patients with first-ever stroke, higher BMI was confirmed as an independent indicator for long-term survival according to a randomized controlled trial–based study on the effect of interventions targeting risk factors prevention (31). The study has shown that BMI has an impact on stroke risk in diabetic patients (11). A study of Chinese patients with T2DM showed that TG is a risk factor for stroke in T2DM patients and that female's elevated TG levels are more likely to be the risk factor to cause strokes than those of males (32). During the literature search, it was found that the results of some studies on BMI risk factors pointed out that the BMI of patients with type 2 diabetes was negatively related to the risk of stroke (33), which was consistent with the same results we obtained according to the available data.

The result of our study showed that there is a significantly positive association between the prevalence of stroke and blood pressure in patients with DM. The study has indicated that high blood pressure is the factor leading to increased stroke risk (34). A meta-analysis of randomized controlled trials comparing the effects including BP lowering on cardiovascular outcomes of DM patients concluded that BP-lowering treatment would significantly reduce cardiovascular risk in DM patients (35). According to the *Journal of the American Heart Association*, different from the cutoff point (BP $\geq$140/90 mmHg) for the diagnosis of hypertension in non-diabetic population, the

TABLE 8 | Comparison of the prediction ability between different models through NRI and IDI.

	Training set (n = 3,252)		Validation set (n = 1,083)	
	Foundation model ~ Integrated model	Biochemical model ~ Integrated model	Foundation model ~ Integrated model	Biochemical model ~ Integrated model
NRI	0.131	0.113	0.133	0.118
P	0	0	0.004	<0.001
2.5% CI	0.078	0.065	0.080	0.068
97.5% CI	0.185	0.162	0.185	0.163
IDI	0.148	0.139	0.157	0.166
P	0	0	0	0
2.5% CI	0.124	0.115	0.115	0.120
97.5% CI	0.172	0.164	0.200	0.213

TABLE 9 | *C* index in the array in 4,335 T2DM patients.

C index (95% CI)	Dxy	aDxy	Variance	*Z*	*P*	*n*
0.890 (0.873–0.907)	0.781	0.781	0.017	45.48	0	4,335

diagnostic criteria of hypertension in diabetic patients are SBP ≥130 mmHg and/or DBP ≥80 mmHg (BP ≥130/80 mmHg) (36). Patients with T2DM often have comorbidities such as hypertension, obesity, and depression (37). Systolic blood pressure is one of the main diagnostic indicators of hypertension. Hypertension is the basis of arteriosclerosis, which can cause endothelial hyperplasia, sclerosis, vascular stenosis, and even occlusion. It is for this reason that strokes eventually occur. A study on high blood pressure showed that SBP and DBP are related to the occurrence of stroke (38). A review summarizing evidence mainly based on randomized controlled trials for the effect of BP management on the primary and secondary prevention of stroke determined that adequate BP lowering is of great significance and is expected to bring benefits for stroke prevention (39). Therefore, it is necessary to control SBP and DBP among T2DM patients.

HbA_{1c} is a parameter of sugar, indicating the 2- or 3-month mean level of blood glucose control and has a close link with the risk of diabetic complications (40). According to the result, glycemic control is essential as a preventable measure of stroke incidence for its influence on T2DM patients. A prospective cohort study conducted on 563 qualified T2DM patients showed that HbA_{1c} could affect the development of microvascular complications (41). A study in Pakistan that worked on the difference of HbA_{1c} values among diabetics and non-diabetics with stroke demonstrated that HbA_{1c} level was higher in the diabetic group (42). Through a Swedish study of 406,271 T2DM patients in total, T2DM patients were proved to have a higher risk of stroke and death with a lack of proper glycemic control, measured by the HbA_{1c} index (17). A systematic review including meta-analysis indicated that a rising HbA_{1c} level would be associated with the elevated risk of first-ever stroke, with average hazard ratios (95% CI) among DM cohorts of 1.17 (1.09, 1.25) as HbA_{1c} increased 1% (43). According to a study in Thailand based on T2DM patients with and without ischemic stroke, the risk of ischemic stroke would be raised 7.9–10.9 times with HbA_{1c} of 8–8.9% and higher (44).

Both LDL-C and HDL-C were considered as risk factors affecting stroke incidence of T2DM patients based on the result. A population-based retrospective cohort study on 144,271 Chinese T2DM patients found control of LDL-C was considerably related with 42% reduction of CVDs and should be given priority for treatment in primary care (45). Based on extensive clinical trials, a meta-analysis showed that the incidence rate of stroke among T2DM patients decreased by 21% with LDL-C level decreasing by 1 mmol/L (38.7 mg/dL) (46). High-density lipoprotein cholesterol is known for its antithrombotic influencing platelets, endothelial cells, and the blood coagulation–fibrinolysis system (47) and as a prevention factor of atherosclerosis. A meta-analysis on data of 61 studies indicated a strong association between HDL-C cholesterol and high risk of CVD and death (48). Through a retrospective cohort study and a mean follow-up of 3 years, including 67,544 T2DM patients, a significant adverse connection was found between HDL-C cholesterol among T2DM patients and the risk of total, ischemic, and hemorrhagic stroke (19). High-density lipoprotein cholesterol was an influencing factor involved in a Chinese retrospective cohort study aiming at establishing a predictive model of ischemic stroke among T2DM patients (12).

Uric acid is considered as a risk factor affecting the stroke incidence according to the result. Previous studies have shown that T2DM patients with stroke are usually considered to have a high level of serum UA. Through meta-analysis, a Chinese work proved that T2DM patients were vulnerable to cerebral infarction with a high level of serum UA, along with a finding that the UA level among T2DM patients with cerebral infarction was 29% higher than those without the symptom (49). A study on 1,017 non–insulin-independent DM patients with a 7-year follow-up for each patient demonstrated that a high UA level was considerably related to fatal and non-fatal stroke, thus proving the significant association between UA and stroke among T2DM patients (50). A study exploring links between serum UA level and cardiovascular complications in T2DM patients found that the hazard ratio (95% CI) of stroke was 1.19 (1.08, 1.31)

with correspondence to every 59 μmol/L increase in UA level, indicating the serum UA level was related to the risk of stroke incidence among T2DM patients (51).

Estimated glomerular filtration rate is the indicator of renal function. In this study, eGFR was proved to be a risk factor of stroke in T2DM patients. Based on the discussions above, in a Roman study, eGFR was found to have a strong negative correlation to UA, thus indicating the association between eGFR and risk of stroke among DM patients in terms of the act of UA on stroke incidence (52). A study implemented in Poland found in a multivariate analysis that eGFR was considered as a risk factor of both diabetic and non-diabetic patients with ischemic stroke (53). A cross-sectional study conducted in Thailand based on 30,423 T2DM patients showed the association between decreased eGFR and increased risk of ischemic stroke, especially for those of eGFR $<$60 mL/min per 1.73 m^2 (54).

Limitations

However, our study still has a few limitations objectively. First, the number of subjects in our study is insufficient. In this study, all of the subjects were T2DM patients in seven communities in Shanghai, whereas still many patients were unable to participate in this study because of their serious condition. The prediction of risk factors for type 2 diabetes with stroke in other regions of China still requires more data to improve the prediction model. Second, there are relatively few indicators included in our study. Some indicators of lifestyle and socioeconomic factors should also be included in the study, such as smoking, drinking habits, education, income, and medication status (hypertensive drugs and lipid-lowering drugs). Also, we worked on the cross-sectional data without conducting subsequent related investigations. If the patient's indicators are followed up, the accuracy of this prediction model will be improved to a certain extent.

At the same time, current studies on the risk of stroke in T2DM patients in China mainly obtained data of hospitalized patients. There are insufficient epidemiological surveys conducted on T2DM patients in the community. At the beginning of this study, foundation model, biochemical model, and integrated model incorporating different risk factors were established at the step of external verification, which can be used to assess the risk of stroke in T2DM patients. Based on NRI and IDI, model C was finally identified as the best prediction model. That is to say, age, course, BMI, SBP, DBP, HbA_{1c}, TG, HDL-C, LDL-C, UA, and eGFR are valuable predictors of risk. When applying the nomogram to T2DM patient evaluation, doctors must carry out health education from the perspective of medicine and skills guidance to help patients develop a healthier lifestyle.

CONCLUSION

Based on a survey collecting basic information, physical data, and biochemical indicators of T2DM patients in seven communities in Shanghai, and processing-related data, this study established three predictive models of stroke risk for T2DM patients through risk factor analysis. To effectively apply the prediction model to T2DM patients and meet the needs of community management and clinical practice, tools including ROC, NRI, IDI, and internal verification were implemented in this study to determine the integrated model as the optimal and most accurate model among the three models.

DATA AVAILABILITY STATEMENT

Considering the privacy of patients, if readers have similar research and want to obtain data related to the article, they can contact the corresponding author, the corresponding research data can be obtained with permission.

ETHICS STATEMENT

Shanghai Medical Ethics Society Committee waived the requirement for ethical approval for this study, which won support from Shanghai Municipal Health Commission before it began. The study was in accordance with the China Guideline for Type 2 Diabetes. All the subjects were carefully informed about the protocol and provided written informed consent before their inclusion in the study. This study protected the subject's anonymity. There is no identifiable information in this manuscript. Researchers kept all the questionnaires and signed informed consent forms.

AUTHOR CONTRIBUTIONS

RS was mainly responsible for data acquisition, including questionnaire design, recruitment and training of volunteers for questionnaire survey, communication with community health center affiliated to Shanghai University of traditional Chinese medicine. FH was responsible for the overall framework design of the paper, including experimental ideas, writing methods, data processing, building models, writing code by RStudio, completed community questionnaire recovery, and biochemical index test entry. TZ and HS were mainly responsible for the literature review, data interpretation, and manuscript compilation. All authors revised the manuscript and approved the current version submitted.

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REFERENCES

1. Akin S, Boluk C. Prevalence of comorbidities in patients with type-2 diabetes mellitus. *Prim Care Diabetes.* (in press) doi: 10.1016/j.pcd.2019.12.006
2. Dal Canto E, Ceriello A, Ryden L, Ferrini M, Hansen TB, Schnell O, et al. Diabetes as a cardiovascular risk factor: An overview of global trends of macro and micro vascular complications. *Euro J Prev Cardiol.* (2019) 26:25–32. doi: 10.1177/2047487319878371
3. Altemimi MT, Hashim AR. Acute stroke in diabetes mellitus: a prospective observational study evaluating the course and short-term outcome in Basrah, Southern Iraq. *Cureus.* (2019) 11:e6017. doi: 10.7759/cureus.6017
4. Gujjar AR. Diabetes and stroke: more than just accelerated atherosclerosis? *Sultan Qaboos Univ Med J.* (2018) 18:e261–e3. doi: 10.18295/squmj.2018.18.03.001
5. Almdal T, Scharling H, Jensen JS, Vestergaard H. The independent effect of type 2 diabetes mellitus on ischemic heart disease, stroke, and death - A population-based study of 13000 men and women with 20 years of follow-up. *Arch Intern Med.* (2004) 164:1422–6. doi: 10.1001/archinte.164.13.1422
6. Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS. Stroke in patients with diabetes - the copenhagen-stroke-study. *Stroke.* (1994) 25:1977–84. doi: 10.1161/01.STR.25.10.1977
7. Burchfiel CM, Curb JD, Rodriguez BL, Abbott RD, Chiu D, Yano K. Glucose intolerance and 22-year stroke incidence. the honolulu heart program. *Stroke.* (1994) 25:951–7. doi: 10.1161/01.STR.25.5.951
8. Janghorbani M, Hu FB, Willett WC, Li TY, Manson JE, Logroscino G, et al. Prospective study of type 1 and type 2 diabetes and risk of stroke subtypes: the Nurses' Health study. *Diabetes Care.* (2007) 30:1730–5. doi: 10.2337/dc06-2363
9. Zhang XD, Chen YR, Ge L, Ge ZM, Zhang YH. Features of stroke in Chinese diabetes patients: a hospital-based study. *J Int Med Res.* (2007) 35:540–6. doi: 10.1177/147323000703500414
10. Bhattacharyya OK, Shah BR, Booth GL. Management of cardiovascular disease in patients with diabetes: the 2008. Canadian Diabetes Association guidelines. *Canad Med Assoc J.* (2008) 179:920–6. doi: 10.1503/cmaj.080554
11. Wang S, Chen J, Wang Y, Yang Y, Zhang D, Liu C, et al. Cigarette smoking is negatively associated with the prevalence of Type 2 diabetes in middle-aged men with normal weight but positively associated with stroke in men. *J Diab Res.* (2019) 2019:1853018. doi: 10.1155/2019/1853018
12. Li T-C, Wang H-C, Li C-I, Liu C-S, Lin W-Y, Lin C-H, et al. Establishment and validation of a prediction model for ischemic stroke risks in patients with type 2 diabetes. *Diabetes Res Clin Pract.* (2018) 138:220–8. doi: 10.1016/j.diabres.2018.01.034
13. 13. Bos MJ, Koudstaal PJ, Hofman A, Witteman JCM, Breteler MMB. Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. *Stroke.* (2006) 37:1503–7. doi: 10.1161/01.STR.0000221716.55088.d4
14. Gavin JR, Alberti K, Davidson MB, DeFronzo RA, Drash A, Gabbe SG, et al. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care.* (1997) 20:1183–97. doi: 10.2337/diacare.20.7.1183
15. Li W, Xie B, Qiu S, Huang X, Chen J, Wang X, et al. Non-lab and semi-lab algorithms for screening undiagnosed diabetes: a cross-sectional study. *Ebiomedicine.* (2018) 35:307–16. doi: 10.1016/j.ebiom.2018.08.009
16. Lyu J, Li Z, Wei H, Liu D, Chi X, Gong D-W, et al. A potent risk model for predicting new-onset acute coronary syndrome in patients with type 2 diabetes mellitus in Northwest China. *Acta Diabetol.* (2020) 57:705–13. doi: 10.1007/s00592-020-01484-x
17. Zabala A, Darsalia V, Holzmann MJ, Franzen S, Svensson A-M, Eliasson B, et al. Risk of first stroke in people with type 2 diabetes and its relation to glycaemic control: a nationwide observational study. *Diabetes Obes Metabol.* (2020) 22:182–90. doi: 10.1111/dom.13885
18. Arboix A, Rivas A, Garcia-Eroles L, de Marcos L, Massons J, Oliveres M. Cerebral infarction in diabetes: clinical pattern, stroke subtypes, and predictors of in-hospital mortality. *BMC Neurol.* (2005) 5:9. doi: 10.1186/1471-2377-5-9
19. Shen Y, Shi L, Nauman E, Katzmarzyk PT, Price-Haywood EG, Bazzano AN, et al. Inverse association between HDL (high-density lipoprotein) cholesterol and stroke risk among patients with Type 2 Diabetes Mellitus. *Stroke.* (2019) 50:291–7. doi: 10.1161/STROKEAHA.118.023682
20. Zhang XB, Mu YM, Yan WH, Ba JM, Li HM. Prevalence of stroke and metabolic disorders in the middle-aged and elderly Chinese with type 2 diabetes. *Chin Med J.* (2014) 127:3537–42.
21. Ren Y, Jin N, Hong T, Mu Y, Guo L, Ji Q, et al. Interactive effect of serum uric acid and total bilirubin for cardiovascular disease in Chinese patients with type 2 diabetes. *Sci Rep.* (2016) 6:36437. doi: 10.1038/srep36437
22. Al-Rubeaan K, Al-Hussain F, Youssef AM, Subhani SN, Al-Sharqawi AH, Ibrahim HM. Ischemic stroke and its risk factors in a registry-based large cross-sectional diabetic cohort in a country facing a diabetes epidemic. *J Diabetes Res.* (2016) 2016:4132589. doi: 10.1155/2016/4132589
23. Tseng CH, Chong CK, Sheu JJ, Wu TH, Med CP. Prevalence and risk factors for stroke in Type 2 diabetic patients in Taiwan: a cross-sectional survey of a national sample by telephone interview. *Diabetic Med.* (2005) 22:477–82. doi: 10.1111/j.1464-5491.2005.01452.x
24. O'Donnell MJ, Chin SL, Rangarajan S, Xavier D, Liu L, Zhang H, et al. Global and regional effects of potentially modifiable risk factors associated with acute stroke in 32 countries (INTERSTROKE): a case-control study. *Lancet.* (2016) 388:761–75. doi: 10.1016/S0140-6736(16)30506-2
25. Ettehad D, Emdin CA, Kiran A, Anderson SG, Callender T, Rahimi K, et al. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. *Euro Heart J.* (2016) 37:639. doi: 10.1016/S0140-6736(15)01225-8
26. Pignone M, Williams CD. Aspirin for primary prevention of cardiovascular disease in diabetes mellitus. *Nat Rev Endocrinol.* (2010) 6:619–28. doi: 10.1038/nrendo.2010.169
27. Wei L, Champman S, Li X, Li X, Li S, Chen R, et al. Beliefs about medicines and non-adherence in patients with stroke, diabetes mellitus and rheumatoid arthritis: a cross-sectional study in China. *BMJ Open.* (2017) 7:e017293. doi: 10.1136/bmjopen-2017-017293
28. Sun C, Li X, Song B, Chen X, Nyame L, Liu Y, et al. A NADE nomogram to predict the probability of 6-month unfavorable outcome in Chinese patients with ischemic stroke. *BMC Neurol.* (2019) 19:274. doi: 10.1186/s12883-019-1464-6
29. Davis TME, Millns H, Stratton IM, Holman RR, Turner RC, Grp UKPDS. Risk factors for stroke in type 2 diabetes mellitus - United Kingdom prospective diabetes study (UKPDS) 29. *Arch Intern Med.* (1999) 159:1097–103. doi: 10.1001/archinte.159.10.1097
30. Banerjee C, Moon YP, Paik MC, Rundek T, Mora-McLaughlin C, Vieira JR, et al. Duration of diabetes and risk of ischemic stroke: the Northern Manhattan study. *Stroke.* (2012) 43:1212–7. doi: 10.1161/STROKEAHA.111.641381
31. Hagberg G, Fure B, Sandset EC, Thommessen B, Ihle-Hansen H, Oksengard AR, et al. Long-term effects on survival after a 1-year multifactorial vascular risk factor intervention after stroke or TIA: secondary analysis of a randomized controlled trial, a 7-year follow-up study. *Vasc Health Risk Manag.* (2019) 15:11–18. doi: 10.2147/VHRM.S191873
32. Cui R, Qi Z, Zhou L, Li Z, Li Q, Zhang J. Evaluation of serum lipid profile, body mass index, and waistline in Chinese patients with type 2 diabetes mellitus. *Clin Interv Aging.* (2016) 11:445–52. doi: 10.2147/CIA.S104803
33. Li W, Katzmarzyk PT, Horswell R, Zhang Y, Zhao W, Wang Y, et al. Body mass index and stroke risk among patients with type 2 diabetes mellitus. *Stroke.* (2015) 46:164. doi: 10.1161/STROKEAHA.114.006718
34. Hu G, Sarti C, Jousilahti P, Peltonen M, Qiao Q, Antikainen R, et al. The impact of history of hypertension and type 2 diabetes at baseline on the incidence of stroke and stroke mortality. *Stroke.* (2005) 36:2538–43. doi: 10.1161/01.STR.0000190894.30964.75
35. Thomopoulos C, Parati G, Zanchetti A. Effects of blood-pressure-lowering treatment on outcome incidence in hypertension: 10 – should blood pressure management differ in hypertensive patients with and without diabetes mellitus? Overview and meta-analyses of randomized trials. *J Hypertens.* (2017) 35:922–44. doi: 10.1097/HJH.0000000000001276
36. White WB, Jalil F, Cushman WC, Bakris GL, Bergenstal R, Heller SR, et al. Average clinician-measured blood pressures and cardiovascular outcomes in patients with Type 2 diabetes mellitus and ischemic heart

disease in the EXAMINE trial. *J Am Heart Assoc.* (2018) 7:e009114. doi: 10.1161/JAHA.118.009114
37. Campbell RK. Type 2 diabetes: Where we are today: An overview of disease burden, current treatments, treatment strategies. *J Am Pharm Assoc.* (2009) 49:S3–9. doi: 10.1331/JAPhA.2009.09077
38. Willmot M, Leonardi-Bee J, Bath PMW. High blood pressure in acute stroke and subsequent outcome - a systematic review. *Hypertension.* (2004) 43:18–24. doi: 10.1161/01.HYP.0000105052.65787.35
39. Hong K-S. Blood pressure management for stroke prevention and in acute stroke. *J Stroke*. (2017) 19:152–65. doi: 10.5853/jos.2017.00164
40. Nalysnyk L, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in patients with diabetes mellitus: evidence from a systematic review of the literature. *Diabetes Obes Metab.* (2010) 12:288–98. doi: 10.1111/j.1463-1326.2009.01160.x
41. Yozgatli K, Lefrandt JD, Noordzij MJ, Oomen PHN, Brouwer T, Jager J, et al. Accumulation of advanced glycation end products is associated with macrovascular events and glycaemic control with microvascular complications in Type 2 diabetes mellitus. *Diab Med.* (2018) 35:1242–8. doi: 10.1111/dme.13651
42. Nomani AZ, Nabi S, Ahmed S, Iqbal M, Rajput HM, Rao S. High HbA1c is associated with higher risk of ischaemic stroke in Pakistani population without diabetes. *Stroke Vasc Neurol.* (2016) 1:133–9. doi: 10.1136/svn-2016-000018
43. Mitsios JP, Ekinci EI, Mitsios GP, Churilov L, Thijs V. Relationship between glycated hemoglobin and stroke risk: a systematic review and meta-analysis. *J Am Heart Assoc.* (2018) 7:53. doi: 10.1161/JAHA.117.007858
44. Chaveepojnkamjorn W, Boonrasri W, Viwatwongkasem C, Siri S, Kriengkaisakda W. Relationship between hemoglobin A1c and ischemic stroke among patients with type-2 diabetes. *Southeast Asian J Trop Med Public Health.* (2019) 50:935–41. doi: 10.1007/s13410-020-00806-7
45. Wan EYF, Fung CSC, Yu EYT, Chin WY, Fong DYT, Chan AKC, et al. Effect of multifactorial treatment targets and relative importance of hemoglobin A1c, blood pressure, and low-density lipoproteincholesterol on cardiovascular diseases in chinese primary care patients with Type 2 diabetes mellitus: a population-based retrospective cohort study. *J Am Heart Assoc.* (2017) 6:13. doi: 10.1161/JAHA.117.006400
46. 日本老年医学会雑誌 細高齢者の脳卒中リスク管理. (2017) 54:524–30. doi: 10.3143/geriatrics.54.524
47. Annema W, von Eckardstein A, Kovanen PT. HDL and atherothrombotic vascular disease. *Handb Exp Pharmacol.* (2015) 224:369–403. doi: 10.1007/978-3-319-09665-0_11
48. Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, et al. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55000 vascular deaths. *Lancet.* (2008). 372:292. doi: 10.1016/S0140-6736(07)61778-4
49. Du L, Ma JH, Zhang XN. Higher serum uric acid may contribute to cerebral infarction in patients with type 2 diabetes mellitus: a meta-analysis. *J Mol Neurosci.* (2017) 61:25–31. doi: 10.1007/s12031-016-0848-y
50. Lehto S, Niskanen L, Ronnemaa T, Laakso M. Serum uric acid is a strong predictor of stroke in patients with non–insulin-dependent diabetes mellitus. *Stroke.* (1998) 29:635–9. doi: 10.1161/01.STR.29.3.635
51. Shao YX, Shao H, Sawhney MS, Shi LZ. Serum uric acid as a risk factor of all-cause mortality and cardiovascular events among type 2 diabetes population: Meta-analysis of correlational evidence. *J Diab Complic.* (2019) 33:7. doi: 10.1016/j.jdiacomp.2019.07.006
52. Gaita L, Timar R, Lupascu N, Roman D, Albai A, Potre O, et al. The impact of hyperuricemia on cardiometabolic risk factors in patients with diabetes mellitus: a cross-sectional study. *Diabetes Metab Syndr Obesity-Targets Ther.* (2019) 12:2003–10. doi: 10.2147/DMSO.S222570
53. Snarska KK, Bachorzewska-Gajewska H, Kapica-Topczewska K, Drozdowski W, Chorazy M, Kulakowska A, et al. Hyperglycemia and diabetes have different impacts on outcome of ischemic and hemorrhagic stroke. *Arch Med Sci.* (2017) 13:100–8. doi: 10.5114/aoms.2016.61009
54. Kaewput W, Thongprayoon C, Rangsin R, Mao MA, Satirapoj B, Cheungpasitporn W. The association between renal function and neurological diseases in type 2 diabetes: a multicenter nationwide cross-sectional study. *Hosp Pract.* (1995) 47:46–52. doi: 10.1080/21548331.2019.1549916

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Metformin Use and Leukemia Risk in Patients With Type 2 Diabetes Mellitus

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Background: The effect of metformin on leukemia risk remains unknown.

Methods: The Taiwan's National Health Insurance database was used to enroll 610,089 newly diagnosed type 2 diabetes patients on at least 2 anti-diabetic prescriptions during 1999–2009. We followed-up these patients until 31 December 2011, in order to determine the incidence of leukemia. We used Cox regression model (incorporated with the inverse probability of treatment-weighting using propensity scores) to estimate hazard ratios in both intention-to-treat and per-protocol analyses.

Results: We enrolled 414,783 metformin initiators and 195,306 non-metformin initiators. Among them, 598 and 372 patients developed new-onset leukemia after a median follow-up period of 5.08 years and 6.79 years, respectively. The respective incidence rates were 26.52 and 28.40 per 100,000 person-years. The hazard ratio for metformin initiators versus non-metformin initiators was 0.943 (95% confidence interval 0.828–1.074) in the intention-to-treat analysis and 0.852 (95% confidence interval 0.705–1.031) in the per-protocol analysis. Sensitivity analyses after excluding patients using the exclusion criteria (a follow-up duration < 24 and < 36 months, respectively, patients with incretin-based therapies during follow-up, and patients enrolled during 2 different periods of 1999–2003 and 2004–2009) consistently showed a neutral effect. However, metformin initiators had a significantly higher risk of leukemia in the per-protocol analyses when censoring patients at a time without regular follow-up.

Conclusion: Metformin use has an overall neutral effect on leukemia but we cannot exclude a significantly higher risk in patients who persistently use the drug.

Keywords: diabetes mellitus, metformin, leukemia, National Health Insurance, Taiwan

INTRODUCTION

According to a study on the global burden of cancer in 2015, the estimated number of new cases of leukemia was 606,000 and 353,000 deaths were related to leukemia, ranking it as eighth for all cancer incidences and ninth for all cancer deaths, respectively (1). Risk factors include some genetic syndromes, ionizing radiation, some environmental or occupational exposures and medications (2, 3). Diabetes patients may also suffer from a significantly higher risk of leukemia. In 2010, a Swedish study reported a significantly higher risk of leukemia in patients with type 2 diabetes mellitus (T2DM) following hospitalization (4). The estimated standardized incidence ratio compared to the general Swedish population was 1.95 (95% confidence interval: 1.72–2.17) (4). In a meta-analysis of 11 studies, the estimated odds ratio of leukemia for patients with T2DM was 1.22 (95% confidence interval: 1.03–1.44, P = 0.02) (5).

Some *in vitro* studies suggest that metformin may induce cell cycle arrest and apoptosis in leukemic cells (6, 7), and leukemic cell growth is activated by the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway (8, 9). Therefore, being recognized for its activating effect on the liver kinase B1/adenosine monophosphate kinase (LKB1/AMPK) resulting in the inhibition of mTOR pathway, metformin theoretically inhibits leukemic cell growth (10, 11). However, the involvement of other pathways may also provide a protective effect of metformin on leukemia because a recent *in vitro* study suggested that metformin may suppress the growth of leukemia cells through the downregulation of AXL receptor tyrosine kinase (12).

Some investigators envisage metformin as a new adjuvant therapeutic agent for the treatment of leukemia (8, 13). However, there is lack of evidence from human studies and several clinical trials are ongoing in order to demonstrate the therapeutic effects of metformin on leukemia in patients with or without diabetes (14). On the other hand, there is paucity of data on whether metformin is preventive for the occurrence of new-onset leukemia or not. The present study investigated the effect of metformin on the risk of leukemia in T2DM patients.

MATERIALS AND METHODS

The National Health Insurance (NHI) implemented since March 1995 in Taiwan covers > 99% of the population and has contracts with 93% of medical institutions and all in-hospitals nationwide. The reimbursement database of the NHI and the methods applied in the present study were described in details in previously published papers (15, 16). This database keeps all records of diseases diagnosed, medications prescribed and procedures performed. It serves as an important base for academic research after approval by an ethics review board of the National Health Research Institutes. The approval number of the present study is 99274.

The NHI database coded the different diagnoses using the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) during the study period. Diabetes was coded 250.XX and leukemia, 204–208.

Metformin initiators [metformin (+)] and non-metformin initiators [metformin (–)] were defined according to the prescriptions of anti-diabetic drugs after diagnosis during the initial 12-month period (17). Metformin (+) were patients who had been prescribed metformin during this initial 12-month period and metformin (–) were those without any metformin prescription during this period.

Figure 1 shows the procedures used to create metformin (+) and metformin (–). At first, we identified 778,300 newly diagnosed T2DM patients followed-up from 1999 to 2009 and on at least 2

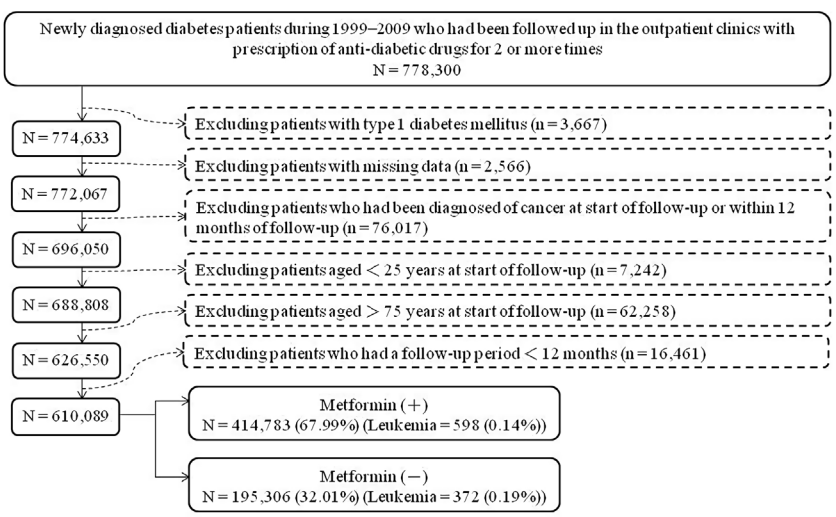

FIGURE 1 | Flowchart showing the procedures followed in creating a cohort of metformin initiators [Metformin (+)] and non-metformin initiators [Metformin (–)] from the reimbursement database of Taiwan's National Health Insurance.

anti-diabetic prescriptions in the outpatient clinics. The following patients were then excluded: patients with type 1 diabetes mellitus (n = 3,667), patients with missing data (n = 2,566) and patients with a diagnosis of any cancer prior to the start of follow-up or within 12 months of follow-up (n = 76,017). We also excluded patients aged < 25 years at the start of follow-up (n = 7,242), patients aged > 75 years at the start of follow-up (n = 62,258), and patients with a follow-up duration < 12 months (n = 16,461). As a result, we included 610,089 patients for analyses. Among them, 414,783 were metformin (+), and 195,306 were metformin (–).

Table 1 shows the baseline characteristics in metformin (+) and metformin (–). These included demographic data [age, time elapsed since diabetes diagnosis (the time between diabetes diagnosis and the time of the first prescription of anti-diabetic drugs), sex, occupation and living region], major comorbidities (hypertension, dyslipidemia, and obesity), diabetes-related complications (nephropathy, eye disease, stroke, ischemic heart disease, and peripheral arterial disease), diagnoses that may be associated with cancer risk (chronic obstructive pulmonary disease, tobacco abuse, alcohol-related diagnoses, gallstone,

TABLE 1 | Baseline characteristics in non-metformin initiators and metformin initiators.

Variable	Metformin (–) (n = 195,306)		Metformin (+) (n = 414,783)		Standardized difference
	n	%	n	%	
Demographic data					
Age* (years)	55.61	10.92	53.85	11.07	-16.41
Time elapsed since diabetes diagnosis* (years)	1.61	1.38	1.83	1.54	17.77
Sex (men)	108,070	55.33	228,303	55.04	-0.13
Occupation**					
I	76,442	39.14	173,894	41.92	
II	42,149	21.58	94,215	22.71	3.23
III	44,014	22.54	75,968	18.32	-11.17
IV	32,701	16.74	70,706	17.05	0.77
Living region					
Taipei	60,319	30.88	143,892	34.69	
Northern	21,872	11.20	53,148	12.81	5.19
Central	34,916	17.88	74,326	17.92	0.75
Southern	34,639	17.74	62,944	15.18	-7.52
Kao-Ping and Eastern	43,560	22.30	80,473	19.40	-7.86
Major comorbidities					
Hypertension	115,512	59.14	250,686	60.44	3.95
Dyslipidemia	91,557	46.88	236,370	56.99	21.82
Obesity	3,837	1.96	18,177	4.38	13.28
Diabetes-related complications					
Nephropathy	26,286	13.46	51,212	12.35	-2.54
Eye disease	8,072	4.13	29,763	7.18	13.14
Stroke	31,085	15.92	63,101	15.21	-1.32
Ischemic heart disease	53,609	27.45	112,417	27.10	0.17
Peripheral arterial disease	19,779	10.13	45,226	10.90	3.42
Diagnoses that may be associated with cancer risk					
Chronic obstructive pulmonary disease	61,853	31.67	138,049	33.28	4.11
Tobacco abuse	2,112	1.08	7,487	1.81	6.19
Alcohol-related diagnoses	9,028	4.62	20,151	4.86	1.60
Gallstone	14,771	7.56	30,391	7.33	-0.89
History of Helicobacter pylori infection	28,737	14.71	65,870	15.88	3.89
Epstein-Barr virus-related diagnoses	923	0.47	2,118	0.51	0.67
Hepatitis B virus infection	1,934	0.99	6,698	1.61	5.70
Hepatitis C virus infection	4,799	2.46	10,726	2.59	1.21
Diseases of the musculoskeletal system and connective tissue	153,214	78.45	342,498	82.57	12.06
Human immunodeficiency virus disease	99	0.05	211	0.05	0.12
Medications that are commonly used in diabetes patients or may affect cancer risk					
Angiotensin converting enzyme inhibitor/angiotensin receptor blocker	88,148	45.13	195,569	47.15	5.03
Calcium channel blocker	87,182	44.64	173,559	41.84	-5.24
Statin	50,026	25.61	137,451	33.14	17.37
Fibrate	43,828	22.44	101,518	24.47	5.44
Aspirin	68,807	35.23	154,083	37.15	4.84

**Age and time elapsed since diabetes diagnosis are expressed as mean and standard deviation.*

***Refer to Materials and Methods for the classification of occupation.*

history of Helicobacter pylori infection, Epstein-Barr virus-related diagnoses, hepatitis B virus infection, hepatitis C virus infection, diseases of the musculoskeletal system and connective tissue, and human immunodeficiency virus disease) and medications that are commonly used in diabetes patients that affect cancer risk (angiotensin converting enzyme inhibitor/angiotensin receptor blocker, calcium channel blocker, statin, fibrate, and aspirin). We classified the residence and occupation elsewhere in details (18). We coded diseases of musculoskeletal system and connective tissue as ICD-9-CM 710–739 and human immunodeficiency virus disease as 042. The ICD-9-CM codes for other diagnoses can be found in previously published papers (15, 16, 19).

We calculated the standardized difference for each covariate as proposed by Austin and Stuart and a value > 10% was used as an indication for potential confounding in the analyses (20).

We conducted both intention-to-treat and per-protocol analyses to emulate a target trial that compares the risk of leukemia associated to the use of metformin relative to non-metformin anti-diabetic drugs. For intention-to-treat analyses, the numerator of the incidence of leukemia was the number of newly diagnosed cases during the follow-up, and the denominator was the person-years of follow-up. We commenced follow-up at the end of the initial 12-month period for the assessment of metformin (+) and metformin (–), and ended at the diagnosis of leukemia, death or the date of the last medical record by 31 December 2011 (whichever occurred first, with no exclusion according to switching to or adding other anti-diabetic drugs thereafter).

In the per-protocol analyses, we excluded patients who were not adherent to the assigned treatment within the initial 12-month period of exposure. We then followed-up the remainder for the incidence of leukemia. Follow-up started at the end of the 12-month period and ended at the first of the following events by 31 December 2011: leukemia diagnosis, death, the last reimbursement record, or non-adherence to the assigned treatment.

We used Cox regression incorporated with the inverse probability of treatment-weighting using propensity scores (PS) to estimate hazard ratios and their 95% confidence intervals for metformin (+) versus metformin (–). This method was recommended by Austin to reduce the potential confounding effect in the different distribution in characteristics (21). We created PS using the logistic regression model from the start of follow-up plus all baseline characteristics in **Table 1**. The inclusion of the start of follow-up partly accounted for some unknown risk factors: such as the introduction of novel therapeutic agents or changes in treatment guidelines during the long inclusion period.

Furthermore, we examined the consistency of findings using sensitivity analyses. Firstly, we excluded the interpretation of leukemia cases followed up for < 24 and < 36 months, respectively, in the analyses (as an effect of treatment assignment) (17). Secondly, we excluded patients treated with incretin-based therapies during follow-up to avoid their potential confounding because these therapies were introduced in Taiwan only during the follow-up period. Thirdly, we conducted analyses separately with included participants during two time intervals (1999–2003 and 2004–2009), to avoid further potential effects of some unknown risk factors such as the introduction of novel therapeutic agents and changes in treatment guidelines. Finally, we censored patients at 4 months and 6 months, respectively, after the last prescription. These would have excluded patients who had not received regular refills of anti-diabetic drugs because in Taiwan, the NHI Bureau allows a prescription of not more than 3 months each time. Because metformin use can cause anemia (22–27), associated with a higher risk of leukemia (28–30), prompting the conduction of additional sensitivity analyses after excluding patients who had ever been diagnosed of anemia (ICD-9-CM 280–285).

We performed data analyses using SAS statistical software (version 9.3, SAS Institute, Cary, NC), meanwhile we considered $P < 0.05$ as statistically significant.

RESULTS

Table 1 displays the baseline characteristics of metformin (–) and metformin (+). We observed standardized difference values > 10% for age, time elapsed since diabetes diagnosis, occupation, dyslipidemia, obesity, eye disease, diseases of the musculoskeletal system, and connective tissue and statin.

The median follow-up duration for metformin (–) was 6.79 years and was 5.08 years for metformin (+) in the intention-to-treat analyses. They were 2.38 and 4.58 years, respectively, in the per-protocol analyses.

Table 2 illustrated the incidence rates of leukemia and the hazard ratios with their 95% confidence intervals comparing

TABLE 2 | Incidence rates of leukemia and hazard ratios comparing metformin initiators versus non-metformin initiators.

Model	Incident case number of leukemia	Cases followed	Person-year	Incidence rate (per 100,000 person-years)	Hazard ratio	95% Confidence interval	*P* value
Intention-to-treat							
Metformin (–)	372	195,306	1,309,950.45	28.40	1.000		
Metformin (+)	598	414,783	2,254,885.22	26.52	0.943	(0.828–1.074)	0.3793
Per-protocol							
Metformin (–)	151	195,306	620,459.93	24.34	1.000		
Metformin (+)	379	362,455	1,823,252.50	20.79	0.852	(0.705–1.031)	0.1000

*Cox regression was constructed with the inverse probability of treatment-weighting using propensity scores derived from variables in **Table 1** plus the date of start of follow-up.*

TABLE 3 | Sensitivity analyses.

Model	Incident case number of leukemia	Cases followed	Person-year	Incidence rate (per 100,000 person-years)	Hazard ratio	95% Confidence interval	*P* value
I. Excluding patients followed up for < 24 months							
Intention-to-treat							
Metformin (–)	283	177,902	1,288,885.66	21.96	1.000		
Metformin (+)	397	346,503	2,168,791.69	18.31	0.891	(0.765–1.038)	0.1394
Per-protocol							
Metformin (–)	82	177,902	602,830.64	13.60	1.000		
Metformin (+)	239	310,991	1,764,390.14	13.55	0.808	(0.628–1.039)	0.0970
II. Excluding patients followed up for < 36 months							
Intention-to-treat							
Metformin (–)	230	164,514	1,255,216.89	18.32	1.000		
Metformin (+)	297	298,978	2,050,558.13	14.48	0.862	(0.725–1.024)	0.0920
Per-protocol							
Metformin (–)	56	164,514	578,594.22	9.68	1.000		
Metformin (+)	172	271,053	1,677,117.72	10.26	0.760	(0.562–1.029)	0.0760
III. Excluding patients who had been treated with incretin-based therapies during follow-up							
Intention-to-treat							
Metformin (–)	358	166,985	1,087,074.03	32.93	1.000		
Metformin (+)	573	343,004	1,794,891.99	31.92	0.979	(0.857–1.118)	0.7526
Per-protocol							
Metformin (–)	149	166,985	549,293.74	27.13	1.000		
Metformin (+)	366	292,660	1,398,347.23	26.17	0.961	(0.794–1.164)	0.6876
IV. Patients enrolled during 1999–2003							
Intention-to-treat							
Metformin (–)	275	110,596	920,620.00	29.87	1.000		
Metformin (+)	376	175,567	1,384,679.02	27.15	0.910	(0.779–1.063)	0.2328
Per-protocol							
Metformin (–)	102	110,596	392,730.35	25.97	1.000		
Metformin (+)	247	158,915	1,144,021.56	21.59	0.832	(0.657–1.053)	0.1263
V. Patients enrolled during 2004–2009							
Intention-to-treat							
Metformin (–)	97	84,710	389,330.46	24.91	1.000		
Metformin (+)	222	239,216	870,206.21	25.51	1.044	(0.819–1.330)	0.7299
Per-protocol							
Metformin (–)	49	84,710	227,729.58	21.52	1.000		
Metformin (+)	132	203,540	679,230.94	19.43	0.912	(0.656–1.269)	0.5852
VI. Censoring patients from the time 4 months have elapsed since the last prescription							
Intention-to-treat							
Metformin (–)	335	195,306	1,172,870.89	28.56	1.000		
Metformin (+)	491	414,783	1,950924.96	25.17	0.895	(0.779–1.029)	0.1203
Per-protocol							
Metformin (–)	135	195,306	1,172,870.89	11.51	1.000		
Metformin (+)	303	362,455	1,748,533.00	17.33	1.452	(1.185–1.780)	0.0003
VII. Censoring patients from the time 6 months have elapsed since the last prescription							
Intention-to-treat							
Metformin (–)	345	195,306	1,192,566.74	28.93	1.000		
Metformin (+)	510	414,783	1,999,911.72	25.50	0.894	(0.779–1.026)	0.1099
Per-protocol							
Metformin (–)	140	195,306	1,192,566.74	11.74	1.000		
Metformin (+)	319	362,455	1,796,167.77	17.76	1.456	(1.193–1.778)	0.0002
VIII. Excluding patients with a diagnosis of anemia							
Intention-to-treat							
Metformin (–)	342	191,348	1,283,062.87	26.65	1.000		
Metformin (+)	555	408,109	2,214,811.83	25.06	0.949	(0.829–1.087)	0.4499
Per-protocol							
Metformin (–)	137	191,348	606,481.33	22.59	1.000		
Metformin (+)	354	356,832	1,793,992.76	19.73	0.874	(0.717–1.067)	0.1865

Cox regression was constructed with the inverse probability of treatment-weighting using propensity scores derived from variables in ***Table 1*** *plus the date of start of follow-up.*

metformin (+) versus metformin (–) in the intention-to-treat and the per-protocol analyses. Both analyses suggested a null association between metformin use and leukemia risk. The hazard ratio in the intention-to-treat analysis was 0.943 (95% confidence interval: 0.828–1.074) and was 0.852 (95% confidence interval: 0.705–1.031) in the per-protocol analysis.

Sensitivity analyses in **Table 3** supported the finding of a null association in the main analyses displayed in **Table 2** (seen above). However, when censoring patients at the time of not receiving regular refills, metformin (+) had a significantly higher risk of leukemia in the per-protocol analyses (Models VI and VII). After excluding patients with a diagnosis of anemia (Model VIII), the results in the analyses did not deviate much from the main analyses shown in **Table 2**.

DISCUSSION

This is the first human study investigating the risk of leukemia after metformin use in T2DM patients. Unlike many previous studies that showed beneficial effects of metformin on the prevention of solid cancers (14) and some recent *in vitro* studies suggesting an inhibitory effect of metformin on the growth of leukemic cells (11, 12), the findings of the present study did not support a beneficial effect of metformin on leukemia in human beings (**Tables 2** and **3**). In the main analyses, the risk of leukemia was neutral while comparing metformin (+) versus metformin (–) in either the intention-to-treat or the per-protocol analysis (**Table 2**). However, from the sensitivity analyses in the per-protocol models that censored patients at the time of without regular refills, we observed a significantly increased risk among metformin (+) (Models VI and VII, **Table 3**). These sensitivity analyses (by including only patients with regular refills and adhering to metformin treatment within the desired follow-up person-years), implied a possible devastating effect of metformin on leukemia.

Deficiency in vitamin B12 (22, 23, 26, 27), folic acid (24), and/or iron (25) is a known potential long-term side effect of metformin treatment and deficiency in these micronutrients has been known to increase the risk of leukemia (28–30). Though not yet clarified, one of the possible explanations for a neutral or even devastating effect of metformin on leukemia is that the beneficial effects observed in *in vitro* studies (11, 12) could be obliterated by the deficiency in these micronutrients after long-term use of metformin.

Metformin is well known for its activation of AMPK and one of the mechanisms of preventing cancer is through its activation of AMPK resulting in the inhibition of mTOR (10, 11). A recent *in vitro* study suggested another potential mechanism through the downregulation of the AXL receptor tyrosine kinase (12). However, another recent *in vitro* and *in vivo* study suggested that only phenformin but not metformin could delay the development of T cell acute lymphoblastic leukemia/lymphoma (31). It is worth mentioning that some recent studies suggested that AMPK activators can also exert an opposite effect of promoting tumor cell survival through alternative pathways [involving redox regulation to maintain nicotinamide adenine dinucleotide phosphate (NADPH) and inhibit cell death] (32). Therefore, it is possible that metformin may exert anti-leukemic effects on one hand, but counteracted by its pro-survival pathways and its side effect of deficiency in micronutrients. The possible differentiation between solid tumors and leukemia in the activation of signaling pathways by AMPK on either the inhibition of mTOR or the activation of NADPH, could explain the different effects of metformin on the prevention of solid tumors and leukemia seen in previous observational studies. A recent study in a cancer center in New York compared the overall and disease-free survival rates in 924 diabetes patients on metformin with newly diagnosed solid tumors or acute myeloid leukemia. This analysis revealed a lack of metformin benefit in leukemia but a significant benefit for patients with solid tumors (33). The "two-faces" of AMPK on cancer has been an issue under vigorous discussion by some investigators recently (34–36). However, these speculations require further investigation for confirmation.

The present study has several clinical implications. Firstly, *in vitro* studies usually investigate a specific pathway and ignore the complex actions of and interactions with other biological pathways. Therefore, we cannot readily interpret the findings of metformin benefits on leukemic cells derived from *in vitro* studies (6, 7, 11, 12). Secondly, an overall neutral effect of metformin on leukemia risk (**Table 2**) and the potentially higher risk observed in patients in persistent use (per-protocol analyses, Models VI and VII, **Table 3**), calls for a cautious attention to the ongoing preclinical trials investigating the use of metformin as a therapeutic agent for leukemia (14). Thirdly, the possibility of differential responses to AMPK activators (such as metformin) between solid tumors and leukemia opens a new interesting venue for future research. Finally, though not proven, the hypothetical role of deficiency in vitamin B12, folic acid, or iron in the increased risk of leukemia speculated from the results (Models VI and VII, **Table 3**), calls for an attention to closely monitor levels of these micronutrients among metformin users. The timing for supplementation of these micronutrients among metformin users is an issue of clinical importance that requires vigorous research.

This study has been conducted with special attention to the potential methodological limitations commonly seen in pharmacoepidemiological studies such as selection bias, prevalent user bias, immortal time bias, and confounding by indication, as discussed previously (37).

Limitations of the study include the lack of measurement data on some potential risk factors such as hemoglobin level, serum concentrations of vitamin B12, folic acid, and iron, anthropometric factors, lifestyle, smoking, alcohol drinking, nutritional status, dietary patterns, family history, genetic markers, ionizing radiation, and environmental/occupational exposures. There are different categories of leukemia but we could not evaluate the effect of metformin on each specific category due to the lack of data. Whether the findings derived from the diabetes patients can be applied to non-diabetes ones, await additional confirmation.

In summary, this is the first observational study evaluating the effect of metformin on the risk of leukemia in humans. The

findings suggest an overall neutral effect, but we cannot exclude a significantly higher risk in patients who adhered persistently to the treatment for up to five years. We recommend further studies in other populations and in non-diabetes participants in order to consolidate the findings of this study.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available because public availability of the dataset is restricted by local regulations to protect privacy. Requests to access the datasets should be directed to C-HT, ccktsh@ms6.hinet.net.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the National Health Research Institutes. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REFERENCES

1. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2015: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol* (2017) 3:524–48. doi: 10.1001/jamaoncol.2016.5688
2. Advani SH, Doval DC, Gopal R, Nair CN, Kutty PM. Therapy-related leukemia. A report of five patients and a review of the literature. *Oncology* (1983) 40:268–72. doi: 10.1159/000225741
3. Schüz J, Erdmann F. Environmental Exposure and Risk of Childhood Leukemia: An Overview. *Arch Med Res* (2016) 47:607–14. doi: 10.1016/j.arcmed.2016.11.017
4. Hemminki K, Li X, Sundquist J, Sundquist K. Risk of cancer following hospitalization for type 2 diabetes. *Oncologist* (2010) 15:548–55. doi: 10.1634/theoncologist.2009-0300
5. Castillo JJ, Mull N, Reagan JL, Nemr S, Mitri J. Increased incidence of non-Hodgkin lymphoma, leukemia, and myeloma in patients with diabetes mellitus type 2: a meta-analysis of observational studies. *Blood* (2012) 119:4845–50. doi: 10.1182/blood-2011-06-362830
6. Rodríguez-Lirio A, Pérez-Yarza G, Fernández-Suárez MR, Alonso-Tejerina E, Boyano MD, Asumendi A. Metformin Induces Cell Cycle Arrest and Apoptosis in Drug-Resistant Leukemia Cells. *Leuk Res Treat* (2015) 2015:516460. doi: 10.1155/2015/516460
7. Bruno S, Ledda B, Tenca C, Ravera S, Orengo AM, Mazzarello AN, et al. Metformin inhibits cell cycle progression of B-cell chronic lymphocytic leukemia cells. *Oncotarget* (2015) 6:22624–40. doi: 10.18632/oncotarget.4168
8. Rosilio C, Ben-Sahra I, Bost F, Peyron JF. Metformin: a metabolic disruptor and anti-diabetic drug to target human leukemia. *Cancer Lett* (2014) 346:188–96. doi: 10.1016/j.canlet.2014.01.006
9. Tabe Y, Tafuri A, Sekihara K, Yang H, Konopleva M. Inhibition of mTOR kinase as a therapeutic target for acute myeloid leukemia. *Expert Opin Ther Targets* (2017) 21:705–14. doi: 10.1080/14728222.2017.1333600
10. Green AS, Chapuis N, Maciel TT, Willems L, Lambert M, Arnoult C, et al. The LKB1/AMPK signaling pathway has tumor suppressor activity in acute myeloid leukemia through the repression of mTOR-dependent oncogenic mRNA translation. *Blood* (2010) 116:4262–73. doi: 10.1182/blood-2010-02-269837
11. Yuan F, Cheng C, Xiao F, Liu H, Cao S, Zhou G. Inhibition of mTORC1/P70S6K pathway by Metformin synergistically sensitizes Acute Myeloid Leukemia to Ara-C. *Life Sci* (2020) 243:117276. doi: 10.1016/j.lfs.2020.117276
12. Saito T, Itoh M, Tohda S. Metformin suppresses the growth of leukemia cells partly through downregulation of AXL receptor tyrosine kinase. *Leuk Res* (2020) 94:106383. doi: 10.1016/j.leukres.2020.106383
13. Biondani G, Peyron JF. Metformin, an anti-diabetic drug to target leukemia. *Front Endocrinol (Lausanne)* (2018) 9:446. doi: 10.3389/fendo.2018.00446
14. Cunha Júnior AD, Pericole FV, Carvalheira JBC. Metformin and blood cancers. *Clinics (Sao Paulo)* (2018) 73(suppl 1):e412s. doi: 10.6061/clinics/2018/e412s
15. Tseng CH. Metformin is associated with a lower risk of colorectal cancer in Taiwanese patients with type 2 diabetes: a retrospective cohort analysis. *Diabetes Metab* (2017) 43:438–45. doi: 10.1016/j.diabet.2017.03.004
16. Tseng CH. Metformin and lung cancer risk in patients with type 2 diabetes mellitus. *Oncotarget* (2017) 8:41132–42. doi: 10.18632/oncotarget.17066
17. Tsilidis KK, Capothanassi D, Allen NE, Rizos EC, Lopez DS, van Veldhoven K, et al. Metformin does not affect cancer risk: a cohort study in the U.K. clinical practice research datalink analyzed like an intention-to-treat trial. *Diabetes Care* (2014) 37:2522–32. doi: 10.2337/dc14-0584
18. Tseng CH. Diabetes, metformin use, and colon cancer: A population-based cohort study in Taiwan. *Eur J Endocrinol* (2012) 167:409–16. doi: 10.1530/EJE-12-0369
19. Tseng CH. Diabetes, insulin use and Helicobacter pylori eradication: a retrospective cohort study. *BMC Gastroenterol* (2012) 12:46. doi: 10.1186/1471-230X-12-46
20. Austin PC, Stuart EA. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. *Stat Med* (2015) 34:3661–79. doi: 10.1002/sim.6607
21. Austin PC. The performance of different propensity score methods for estimating marginal hazard ratios. *Stat Med* (2013) 32:2837–49. doi: 10.1002/sim.5705
22. Chapman LE, Darling AL, Brown JE. Association between metformin and vitamin B(12) deficiency in patients with type 2 diabetes: A systematic review and meta-analysis. *Diabetes Metab* (2016) 42:316–27. doi: 10.1016/j.diabet.2016.03.008
23. Yang W, Cai X, Wu H, Ji L. Associations between metformin use and vitamin B(12) levels, anemia, and neuropathy in patients with diabetes: a meta-analysis. *J Diabetes* (2019) 11:729–43. doi: 10.1111/1753-0407.12900

24. Maniar K, Moideen A, Mittal A, Patil A, Chakrabarti A, Banerjee D. A story of metformin-butyrate synergism to control various pathological conditions as a consequence of gut microbiome modification: Genesis of a wonder drug? *Pharmacol Res* (2017) 117:103–28. doi: 10.1016/j.phrs.2016.12.003
25. Stynen B, Abd-Rabbo D, Kowarzyk J, Miller-Fleming L, Aulakh SK, Garneau P, et al. Changes of cell biochemical states are revealed in protein homomeric complex dynamics. *Cell* (2018) 175:1418–29. doi: 10.1016/j.cell.2018.09.050
26. Iftikhar R, Kamran SM, Qadir A, Iqbal Z, bin Usman H. Prevalence of vitamin B12 deficiency in patients of type 2 diabetes mellitus on metformin: a case control study from Pakistan. *Pan Afr Med J* (2013) 16:67. doi: 10.11604/pamj.2013.16.67.2800
27. Ko SH, Ko SH, Ahn YB, Song KH, Han KD, Park YM, et al. Association of vitamin B12 deficiency and metformin use in patients with type 2 diabetes. *J Korean Med Sci* (2014) 29:965–72. doi: 10.3346/jkms.2014.29.7.965
28. Murphy G, Dawsey SM, Engels EA, Ricker W, Parsons R, Etemadi A, et al. Cancer risk after pernicious anemia in the US elderly population. *Clin Gastroenterol Hepatol* (2015) 13:2282–9.e1-4. doi: 10.1016/j.cgh.2015.05.040
29. Cantarella CD, Ragusa D, Giammanco M, Tosi S. Folate deficiency as predisposing factor for childhood leukaemia: a review of the literature. *Genes Nutr* (2017) 12:14. doi: 10.1186/s12263-017-0560-8
30. Hung N, Shen CC, Hu YW, Hu LY, Yeh CM, Teng CJ, et al. Risk of cancer in patients with iron deficiency anemia: a nationwide population-based study. *PloS One* (2015) 10:e0119647. doi: 10.1371/journal.pone.0119647
31. Vara-Ciruelos D, Dandapani M, Russell FM, Grzes KM, Atrih A, Foretz M, et al. Phenformin, but not metformin, delays development of T cell acute lymphoblastic leukemia/lymphoma via cell-autonomous AMPK activation. *Cell Rep* (2019) 27:690–698.e4. doi: 10.1016/j.celrep.2019.03.067
32. Jeon SM, Chandel NS, Hay N. AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. *Nature* (2012) 485:661–5. doi: 10.1038/nature11066
33. Ceacareanu AC, Nimako GK, Wintrob ZAP. Missing the benefit of metformin in acute myeloid leukemia: a problem of contrast? *J Res Pharm Pract* (2017) 6:145–50. doi: 10.4103/jrpp.JRPP_17_37
34. Bonini MG, Gantner BN. The multifaceted activities of AMPK in tumor progression–why the "one size fits all" definition does not fit at all? *IUBMB Life* (2013) 65:889–96. doi: 10.1002/iub.1213
35. Hardie DG. Molecular Pathways: Is AMPK a friend or a foe in cancer? *Clin Cancer Res* (2015) 21:3836–40. doi: 10.1158/1078-0432.CCR-14-3300
36. Faubert B, Vincent EE, Poffenberger MC, Jones RG. The AMP-activated protein kinase (AMPK) and cancer: many faces of a metabolic regulator. *Cancer Lett* (2015) 356:165–70. doi: 10.1016/j.canlet.2014.01.018
37. Tseng CH. Metformin reduces risk of benign nodular goiter in patients with type 2 diabetes mellitus. *Eur J Endocrinol* (2019) 180:367–74. doi: 10.1530/EJE-19-0133

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Additional Benefit of Chinese Medicine Formulae Including *Dioscoreae rhizome (Shanyao)* for Diabetes Mellitus: Current State of Evidence

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Background: Chinese medicine has been used to treat diabetes symptoms for thousands of years. *Dioscoreae rhizome* or *Shanyao* is a Chinese medicinal herb that is routinely used in the treatment of diabetes mellitus (DM).

Objective: The purpose of this study is to evaluate the evidence of the added benefits and safety of herbal formulae containing *Shanyao* in clinical studies and the possible mechanisms of *Shanyao* in the prevention and treatment of DM in experimental studies.

Methods: We searched nine databases for randomized controlled trials (RCTs) that included *Shanyao* in the formulae in the treatment of type 2 DM. Furthermore, experimental studies on the prevention and treatment of DM by *Shanyao* in English- and Chinese-language databases were identified.

Results: Fifty-three moderate quality RCTs with herbal formulae containing *Shanyao* were identified. Results from meta-analysis indicated that *Shanyao* alone or formulae containing *Shanyao* in addition to conventional treatments could benefit people with type 2 DM in lowering blood glucose, blood lipids and reducing insulin resistance. Moreover, adverse events were significantly lower in the CHM plus conventional group than those in the conventional group. *Shanyao* may exert the benefit through various mechanisms including inhibition of α-glucosidase and DPP-IV activity, increase of endogenous GLP-1 and immune regulating activities.

Conclusion: Evidence from this review suggested that there appeared to be added clinical benefits associated with the use of *Shanyao* for DM, whether as a food supplement or as a CHM combined with hypoglycemic agents with a good safety profile.

Keywords: *Dioscoreae rhizome*, *Shan yao*, Chinese medicine, diabetes, systematic review, efficacy, safety, mechanism

INTRODUCTION

Diabetes mellitus (DM) prevalence is growing at an alarming rate worldwide. According to the International Diabetes Federation, about 463 million adults worldwide suffer from DM (1). Among them, China is the country with the largest number of people suffering from DM.

Approximately 95% of the diabetic population is T2DM, followed by type 1 diabetes mellitus, gestational diabetes mellitus, and specific types of diabetes due to other causes (2). Patients with type 1 diabetes mellitus and gestational diabetes mellitus are mainly treated with insulin supplementation and replacement therapy. Specific etiology and pathogenesis of other types of DM vary, and the treatment plan is not unified. Therefore, this study focuses on patients with T2DM.

Epidemiological figures showed that the prevalence of type 2 diabetes mellitus (T2DM) in adults aged 18 and over in China is 10.9% and that of pre-diabetes is 35.7% (3). T2DM is a chronic progressive disease. Da Qing research, a registry cohort study with long term follow-up in China showed that 67.7% of pre-diabetic people will develop DM after six years without early intervention, but the risk of T2DM in the combined diet and exercise group can be reduced by 42% (4). UK Prospective Diabetes Study suggested that strict glycemic control can reduce the risk of diabetic complications (5). Early active management can be more beneficial for diabetic people, which coincides with the idea of "cure disease before disease onset" in traditional Chinese medicine (TCM).

Treating diabetes by conventional medicine includes oral and injectable hypoglycemic agents. Diabetic patients often need a combination of several drugs. Common concerns of doctors and diabetic patients include hypoglycemia, gastrointestinal discomfort, inconvenience of missing medicines and injectable medicines. The Chinese Diabetes Society (CDS) guidelines of 2017 presented information of TCM for diabetes and recommended the use of TCM (6). In China, it has been observed that doctors in the field of diabetes are also looking for diverse treatments including TCM (6).

In TCM, DM belongs to the category of disease called "*xiao ke* 消渴". Disease characteristics include thirst, excessive drinking, polyuria, and weight loss. The pathogenesis of DM in TCM is *qi* and *yin* deficiency and excessive dryness and heat. Commonly used formulae by TCM practitioners include *Liu wei di huang wan* 六味地黄丸, *Shen qi wan*肾气丸, and *Ba wei wan*八味丸 (7). *Shanyao* (*Dioscoreae rhizome*) is the root of *Dioscorea opposita* Thunb. (8); it is one of the main herbs in the above formulae. *Shanyao* has been widely used in the treatment of *xiao ke*消渴or diabetes since the ancient times. It is a popular herb and can also be consumed as a type of food. Active ingredients of *Shanyao* include polysaccharides, flavonoids, allantoin, choline, and dioscin (9–14).

This study will review evidence of *Shanyao* for DM from clinical research and experiment research results from Chinese- and English-language databases and present the evidence on added benefits and safety of herbal formula containing *Shanyao* in clinical studies. Possible mechanisms of *Shanyao* in the prevention and treatment of DM in experimental studies are also investigated.

METHODS

Systematic Review of Clinical Trials

Search Strategy

We searched English- and Chinese-language databases and followed the methods outlined in the Cochrane Handbook of Systematic Reviews (15). English-language databases included PubMed, ExcerptaMedica Database (Embase), Cumulative Index of Nursing and Allied Health Literature (CINAHL), Cochrane Central Register of Controlled Trials (CENTRAL), including the Cochrane Library, and Allied and Complementary Medicine Database (AMED); Chinese-language databases included China SinoMed, China National Knowledge Infrastructure (CNKI), Chongqing VIP (CQVIP), and Wanfang. Databases were searched from inception to March 2019. No restrictions were applied.

Search terms were grouped into three blocks: 1) intervention (formulae including *Shanyao*, *dioscoreae rhizome*); 2) clinical condition (including type 2 diabetes mellitus); and 3) trial design (including clinical trial, randomized controlled trial).

We also searched reference lists of previous systematic reviews and included studies. Clinical trial registries were also searched including the Australian New Zealand Clinical Trial Registry (ANZCTR), Chinese Clinical Trial Registry (ChiCTR), European Union Clinical Trials Register (EU-CTR) and USA National Institutes of Health register (ClinicalTrials.gov). When required, we contacted trial investigators by email or telephone to obtain data. If we didn't receive a response after four weeks, we marked the unknown information 'not available'.

Protocol of the review was registered with the PROSPERO international prospective register of systematic reviews (CRD 42019145668).

Study Inclusion Criteria

Study design

Randomized controlled trials (RCTs) were eligible.

Participants

Adults were diagnosed with T2DM using the following guidelines: 1999 World Health Organization (WHO) (16), Chinese Diabetes Society (17, 18), American Diabetes Association (2), or a description of diagnostic criteria including:

- Fasting blood glucose (FBG, defined as no caloric intake for at least 8 h) ≥126 mg/dl (7.0 mmol/L),
- Or 2-h plasma glucose (PG) ≥200 mg/dl (11.1 mmol/L) during an oral glucose tolerance test (OGTT). The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
- Or A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program certified and standardized to the Diabetes Control and Complications Trial assay.

- Or in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mmol/L). In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

Type of Interventions

CHM, other CM therapies (for example, Chinese medicine dietary therapy), and integrative medicine such as CHM plus hypoglycemic drugs; all the interventions should include *Shanyao*.

Type of Controls

Conventional therapies recommended in guidelines, including pharmacotherapy, diet therapy and lifestyle interventions.

Outcomes

The primary outcome measures were:

- Blood glucose tests (fasting blood glucose, post-prandial blood glucose, hemoglobin a_{1c});
- Adverse events (AEs);

The secondary outcomes were:

- Blood lipid metabolism indicators (triglyceride, cholesterol, low-density lipoprotein, high-density lipoprotein);
- β-cell function indicators: fasting serum insulin (Fins) the unit of FINS is pmol/L or μu/ml will be included (pmol/L = μu/ml × 6.965); HOMA-IR(IR) = FBG × FINS/22.5; HOMA-IS (IS) = 1/(Fins × FPG);
- Body Mass Index (BMI).

Study Exclusion Criteria

- Quasi-randomized controlled trials;
- Prediabetic state;
- Type 1 diabetes;
- Gestational diabetes;
- Other specific types of diabetes included in the American Diabetes Association and Chinese Diabetes Society:
    - Genetic defects of beta-cells
    - Genetic defects in insulin action
    - Diseases of the exocrine pancreas
    - Endocrinopathies
    - Drug or chemical induced diabetes
    - Infections
    - Uncommon forms of immune-mediated diabetes
    - Other genetic syndromes sometimes associated with diabetes
- Diabetic complications and comorbidities;
- Integrative medicine studies that used different therapies in the intervention group and control group;
- If the control group uses a form of Chinese medicine.

Data Extraction and Management

Search results were synthesized by removing duplicates, followed by screening of titles and abstracts by LS and YD. Full texts were obtained and screened by two reviewers (LS and YD). Eligible studies satisfying the inclusion criteria were extracted using EpiData software (EpiData Association, Odense, Denmark). LS and YD extracted the data from the included studies independently and double-checked the data to obtain information on authors, publication year, title, journal, participants' characteristics, sample size, methodological details, intervention details, treatment duration, outcome measures, and AEs.

Assessment of Risk of Bias in Included Studies

Risk of bias was assessed using the Cochrane Collaboration's procedures (15). RevMan software (Version 5.2.4, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012) was used for risk of bias analysis. Items of bias assessed included sequence generation, allocation concealment, blinding of participants, blinding of personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting and other bias including baseline imbalance and funding. Egger's test was used to assess publication bias. Publication bias was assessed when the subgroup included more than 10 studies.

Risk of bias assessment was conducted by two independent reviewers (LS and YD) and disagreement was resolved by discussion or consultation with a third person (TZ).

Data Analysis

Continuous outcomes were presented as mean difference (MD) with 95% confidence interval (CI) between two groups, whereas dichotomous data were presented as relative risk (RR) with 95% CI. Stata software (13.0) was used for data analysis. Considering heterogeneities among trials, all meta-analyses were performed with random effects model. Heterogeneities between studies was estimated by I^2. An I^2 score greater than 50% was considered to indicate substantial heterogeneity.

Predefined comparisons in the meta-analysis were as follows: (1) CHM plus hypoglycemic agents *versus* hypoglycemic agents, (2) CHM plus lifestyle intervention *versus* lifestyle intervention, (3) CHM diet therapy plus lifestyle intervention *versus* lifestyle intervention.

Subgroup analysis were performed where possible, including studies with low risk for sequence generation, FBG level at baseline(6–≤8 mmol/L, 8–10 mmol/L, ≥10 mmol/L), patient age groups (18–40 years, 41–64 years, >65 years), BMI (normal < 24 kg/m^2, overweight ≥ 24–28 kg/m^2, obese ≥ 28 kg/m^2), disease duration (<5 years, ≥5–10years, ≥10 years), treatment duration (≤3 months, 3–6 months, and ≥6 months), comparator drugs class and CM syndrome differentiation (19–23).

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was used to assess the quality of evidence.

Pharmacological Research Evidence of *Shanyao* for DM

The constituent compounds were identified by searching herbal monographs, high quality reviews of CHM, pharmacopoeia of the People's Republic of China (24), and PubMed. To identify preclinical publications a literature search of PubMed and China

National Knowledge Infrastructure was undertaken. The search strategy included the terms for *Shanyao* and its constituent compounds and T2DM. Relevant data were extracted, and a summary of the findings are reported here.

RESULTS

Modern Literature Results

Description of Included Studies

Search Results

Our search identified 44,958 articles in the included databases. Fifty-three (53) RCTs involving 4,905 participants were included in the systematic review (25–77). The screening process is shown in **Figure 1**.

Characteristics of the Included Studies

All studies were randomized, parallel-group, controlled trials conducted in China between 2002 and 2018. One study published was in English (44) and the rest in Chinese language. All studies included participants diagnosed in accordance with the 1999 WHO, Chinese Diabetes Society or American Diabetes Association diagnostic criteria for T2DM. In total, 4,905 participants were included in these RCTs; participants' age ranged from 45 to 74 years. Duration of T2DM ranged from 1 week to 20 years. Treatment duration ranged from 2 to 24 weeks. Only one study had a follow-up for 30 weeks (65). Characteristics of included studies are summarized in **Table 1**.

Fifty-three (53) RCTs assessing CHM as food or integrative medicine for T2DM were identified from the search. 51 studies assessed the combination of CHM with conventional medication (integrative medicine) (27–77). One study compared CHM to lifestyle intervention (26); one study compared Chinese medicine diet therapy to lifestyle intervention (25).

Ninety-eight herbs were used in the formulae, and the most commonly used herb used with *Shanyao* 山药 were*huangqi* 黄芪 (42 studies), *shengdihuang* 生地黄 (30 studies), *gegen* 葛根 (28 studies), *tianhuafen* 天花粉 (28 studies), *danshen* 丹参 (26 studies), *fuling* 茯苓 (23 studies), *maimendong* 麦门冬 (22 studies), *shanzhuyu* 山茱萸 (21 studies), and *huanglian* 黄连 (16 studies).

Comparators included pharmacologic therapy and lifestyle intervention. Pharmacologic therapy used in the included RCTs includes biguanides, sulfonylureas, thiazolidinediones, a-glucosidase inhibitors, DPP-4 inhibitors, and insulins. Lifestyle management of T2DM includes diabetes self-management education and support, medical nutrition therapy, physical activity, and psychosocial care.

CM syndrome differentiation was described in thirty-four studies (26, 28, 29, 31–41, 43, 46, 48–50, 52, 53, 55, 57, 59–62, 64, 67, 69, 71, 74, 75, 77). The most common syndromes described in the studies include *qi* and *yin* deficiency 气阴两虚, *yin* deficiency and excessive heat 阴虚热盛, damp heat retention 湿热阻滞, spleen deficiency 脾虚, phlegm-dampness, and blood stasis 痰湿血瘀.

Risk of Bias in the Included Studies

The results of the risk of bias judgements are presented in **Figure 2** and **Figure 3**. All included 53 studies were randomized; 15 trials described the process of random sequence generation (25, 26, 31, 32, 34, 37, 38, 43, 44, 46, 62, 64, 65, 69, 73); two trials implemented allocation concealment (25, 37). One study was described as "single-blind" trial with no further details (37). Considering all included outcome measures were objective outcomes, blinding of assessors has low risk of influencing the outcome measures and was judged as low risk of bias in all included studies. Twelve trials did not report on all outcome measures described in the *Methods* section (37, 40, 44, 53, 56–58, 60, 62, 63, 75, 77), three trials reported on AEs which were not

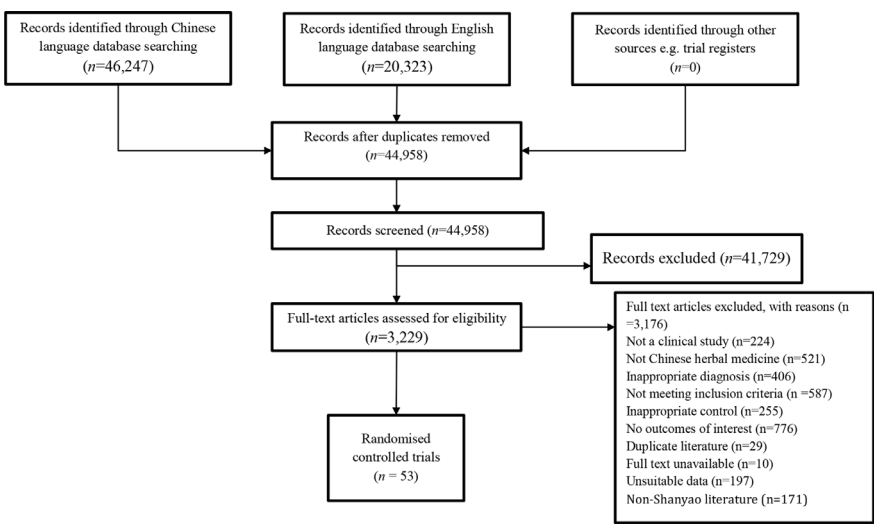

FIGURE 1 | Flow chart of study selection process.

TABLE 1 | Basic characteristics of the included studies in modern literature.

NO.	Study	Sample size	Sample size	Mean age(y)	Mean age(y)	Treatment	Control	Duration (w)	FBG	2hPG	HbA_{1c}	TG	TC	LDL	HDL	BMI	FINS	IR	IS	AEs
		(I)	(C)	(I)	(C)															
1	Bai (42)	40	30	48.27	49.23	SMF&Bigu&Sulf	Bigu/Sulf	4	√	√	×	√	√	×	×	×	×	×	×	×
2	Cao et al. (77)	68	68	48	47	SMF&Bigu	Bigu	8	√	√	√	√	√	×	×	×	×	×	×	√
3	Cao and Zou (76)	54	54	56.7	54.5	Baihurenshen Formula&Sulf	Sulf	8	√	√	×	×	×	×	×	×	√	√	√	√
4	Chen (30)	24	26	60.33	61.35	a-Glucosidase or Insulins	a-Glucosidase/ Insulins	6	√	√	√	√	√	√	√	×	×	×	×	√
5	Cui and Lou (75)	40	40	56.75	52.57	SMF&Bigu&Sulf	Bigu&Sulf	8	√	√	√	×	×	×	×	×	×	×	×	×
6	Fan et al. (74)	40	40	42–74	40–73	Shenqijiangtang Granule&Bigu	Bigu	12	√	√	√	×	×	×	×	×	×	×	×	×
7	Gao et al. (73)	90	90	58.9	57.6	Shenqijiangtang Capsule&Bigu	Bigu	12	√	√	√	√	√	√	√	×	×	×	×	×
8	Hou (72)	30	30	46.12	46.78	seld-made Formula&Bigu&a-Glucosidase	Bigu&a-Glucosidase	8	√	√	√	√	√	√	√	√	√	√	×	√
9	Li (25)	45	45	69.76	69.44	Chinese medicine diet therapy	No treatment	12	√	√	×	√	√	√	√	×	×	×	×	×
10	Li (32)	30	30	NS	NS	SMF&Bigu&a-Glucosidase	Bigu&a-Glucosidase	4	√	√	√	×	×	×	×	×	×	×	×	√
11	Li et al. (71)	102	100	57.20	58.46	Jiangtang Capsule&Sulf	Sulf	4	√	√	×	√	√	√	√	×	×	×	×	×
12	Lin (40)	20	20	56.2	54.8	Zhibodihuang Formula&Bigu	Bigu	4	√	√	√	×	×	×	×	×	×	×	×	×
13	Liu et al. (70)	29	29	46.5	46.9	SMF&Bigu	Bigu	12	√	×	√	√	√	√	√	×	×	×	×	×
14	Liu et al. (69)	30	30	NS	NS	SMF&DPP4	DPP4	12	√	√	×	×	×	×	×	×	√	×	×	×
15	Liu (29)	30	30	57.3	56.7	SMF&Bigu&Sulf	Bigu&Sulf	12	√	√	√	√	√	√	√	×	×	×	×	√
16	Lou and Zhao (68)	52	50	53.5	54	SMF&Bigu	Bigu	8	√	√	√	×	×	×	×	×	×	×	×	×
17	Lv et al (35)	60	60	54.61	55.77	Yuye Formula&insulin	insulin	4	√	√	×	√	√	√	√	×	×	×	×	×
18	Lv et al. (67)	45	30	58.9	58.8	SMF&a-Glucosidase	a-Glucosidase	4	√	√	√	×	×	×	×	×	×	×	×	×
19	Peng et al. (66)	25	25	57	58	SMF&Bigu&a-Glucosidase	Bigu&a-Glucosidase	8	√	√	×	√	√	√	√	×	×	×	×	×
20	Peng and Xu (36)	68	68	74.86	75.01	Shenqi JiangTang Pill&a-Glucosidase	a-Glucosidase	7	√	√	×	×	×	×	×	×	×	×	×	×
21	Shang et al. (65)	30	30	48.36	47.97	SMF&CSII	CSII	8	×	×	×	×	×	×	×	×	√	√	×	×
22	Tang and Li (64)	69	69	51.6	50.4	Liuweidihuang Pill&Bigu	Bigu	12	√	√	√	√	√	√	√	×	√	√	×	√
23	Wang and Wang (27)	29	27	59.1	59.2	SMF&Bigu&Sulf	Bigu&Sulf	8	√	√	√	√	√	√	√	×	×	×	√	×
24	Wang (37)	30	30	68.2	67.3	SMF&CSII	CSII	2	√	×	√	√	√	√	√	×	×	×	×	√
25	Wang (43)	30	30	50.3	53.2	Gankujiangtang Formula&Bigu	Bigu	12	√	√	√	√	√	√	√	×	√	√	×	√
26	Wang (33)	30	30	50	53.7	Zhibodihuang Formula&Sulf	Sulf	4	√	√	×	×	×	×	×	×	√	√	√	×
27	Wu (28)	20	18	51.85	54.78	SMF&Bigu	Bigu	8	√	√	√	√	√	×	×	√	×	×	√	√
28	Xie and Lu (63)	135	133	35-64	36–60	Dihuangjiangtang pill&Bigu	Bigu	12	×	×	×	√	√	√	√	×	×	×	×	×
29	Xu et al. (62)	20	20	54	55	Shenlinbaizhu Formula&insulin	insulin	12	×	×	×	×	×	×	×	√	×	×	×	×
30	Yang (39)	30	30	NS	NS	Qianwenwu Formula&Bigu	Bigu	9	√	√	√	√	√	√	√	×	×	×	×	×
31	Yu et al. (60)	40	40	56.2	55.4	Taipingtangke Pill&Bigu	Bigu	12	√	√	√	×	×	×	×	×	×	×	×	×
32	Yu et al. (61)	30	30	32-64	34–67	SMF&Bigu	Bigu	8	√	√	×	×	×	×	×	×	×	×	×	×
33	Zhang et al. (59)	30	30	46.4	47.3	SMF&Bigu	Bigu	4	√	√	√	×	×	×	×	×	×	×	×	×
34	Zhang et al. (52)	32	30	54.4	56.5	SMF&Bigu	Bigu	8	√	√	√	×	×	×	×	×	×	×	×	√
35	Zhang et al. (57)	36	20	42–59	44–57	SMF&Bigu	Bigu	4	√	√	√	×	×	×	×	×	×	×	×	×
36	Zhang et al. (58)	100	50	52.6	50.5	SMF&Bigu&Sulf	Bigu&Sulf	8	√	√	×	√	√	√	√	×	√	×	×	√
37	Zhang and Pan (56)	120	60	46.5	46.4	Shendishengjin Capsule&Sulf	Sulf	8	√	√	×	√	√	√	√	×	×	×	×	√
38	Zhang (41)	30	30	NS	NS	SMF&Bigu&a-Glucosidase	Bigu&a-Glucosidase	8	√	√	√	×	×	×	×	×	×	×	×	√
39	Zhang (38)	32	31	54	56	SMF&Bigu&a-Glucosidase	Bigu&a-Glucosidase	12	√	√	√	×	×	×	×	×	×	×	×	×
40	Zhang (26)	34	32	51	49	self-made Formula	No treatment	4	√	√	×	×	×	×	×	×	×	×	×	√
41	Zhang (34)	48	44	52.3	54.2	Jiaweishenqidihuang Formula&Bigu	Bigu	12	√	×	√	√	√	√	√	√	√	√	×	×

(Continued)

TABLE 1 | Continued

NO.	Study	Sample size		Mean age(y)		Treatment	Control	Duration (w)	FBG	2hPG	HbA_{1c}	TG	TC	LDL	HDL	BMI	FINS	IR	IS	AEs
		(I)	(C)	(I)	(C)															
42	Zhang et al. (55)	30	30	45.6	46.9	SMF&Sulf	Sulf	8	√	√	√	×	×	×	×	×	√	×	×	√
43	Zhang et al. (54)	93	79	NS	NS	Xiaotangping Capsule&Bigu	Bigu	12	√	√	√	√	√	√	√	×	×	×	√	×
44	Zhang (31)	20	20	56.9	57.1	SMF&insulin	insulin	12	√	√	√	√	√	√	√	×	×	×	×	×
45	Zhang et al. (44)	109	110	56.9	57.1	Shen-Qi formula & insulin	insulin	12	√	√	√	√	√	√	√	×	×	×	×	√
46	Zhang et al. (53)	48	50	55.2	53.1	SMF&insulin	insulin	ns	√	×	√	×	×	×	×	×	×	×	×	√
47	Zhao and Song (51)	70	68	56.5	53.5	SMF&Sulf&Bigu	Sulf/Bigu	24	√	√	√	×	×	×	×	×	√	×	×	√
48	Zhou et al. (50)	50	50	40–74	41–72	SMF&Sulf	Sulf	12	√	√	×	×	×	×	×	×	×	×	×	×
49	Zhou and Dong (49)	30	30	59.2	58.6	Yiqiyangyin Capsule&Sulf	Sulf	4	√	√	√	×	×	×	×	×	×	×	×	×
50	Zhou and Shen (48)	20	20	60	59.8	SMF&Routine hypoglycemic agents	Routine hypoglycemic agents	12	√	√	√	×	×	×	×	×	×	×	×	×
51	Zhou et al. (47)	124	124	NS	NS	Erban Formula&Bigu	Bigu	12	√	√	√	×	×	×	×	×	×	×	×	×
52	Zhu and Li (45)	30	30	74.2	76.8	Jiangtangqing Granule&insulin secretagogues&a-Glucosidase	insulin secretagogues&a-Glucosidase	12	√	×	√	√	√	√	√	×	√	×	×	√
53	Zhu et al. (46)	35	35	51.7	53	SMF&Bigu&Sulf	Bigu&Sulf	12	√	√	×	×	×	×	×	×	×	×	×	√

C, control; I, intervention; CHM, Chinese herbal medicine; F, female; M, male; w, week/weeks; y, year/years; Sulf, Sulfonylureas; Bigu, Biguanides; CSII, continuous subcutaneous insulin infusion; TZDs, Thiazolidinediones; DPP-4, Dipeptidyl Peptidse-4; SMF, self-made Formula.

included in the methods section (38, 50, 64). Publication bias was not detected (Egger's test, t = −0.71, P = 0.48). The overall methodological quality of the included studies was moderate.

Effects of Intervention

CHM Plus Hypoglycaemic Agents Versus Hypoglycaemic Agents

Fasting Blood Glucose. Forty-eight RCTs including 4,375 participants assessed the effects of CHM plus hypoglycemic agents *versus* hypoglycemic agents alone; these studies used the same hypoglycemic agents in both groups (27–61, 64, 66–77). Treatment duration ranged from 2 to 24 weeks. Different classes of hypoglycemic agents were used across studies including biguanides, sulfonylureas, α-Glucosidase inhibitors, DPP-4 inhibitors and insulins. Specific hypoglycemic agents include metformin, gliclazide, glibenclamide, gliquidone, glimepiride, acarbose, sitagliptin, and insulin.

The integrative use of CHM plus hypoglycemic agents was superior to hypoglycemic agents alone at reducing FBG levels at the end of treatment [MD −0.93 (−1.10, −0.76); I^2 = 84.2%], although heterogeneity was high. Meta-analysis of studies assessed as low risk of bias for sequence generation produced a similar result to the overall results with reduced heterogeneity [11 RCTs, 1,050 participants, MD −0.68 (−0.89, −0.47); I^2 = 67.9%] (31, 32, 34, 37, 38, 43, 44, 46, 64, 69, 73).

Subgroup analyses based on comparator drug class, patient age groups, baseline levels of FBG, CM syndrome differentiation, treatment duration, disease duration, and baseline levels of BMI all showed significant differences between groups (**Table 2**).

2-h Postprandial Blood Glucose. Forty-three RCTs including 4,004 participants assessed the effects of CHM plus hypoglycemic agents compared to hypoglycemic agents alone (27–33, 35, 36, 38–44, 46–52, 54–61, 64, 66–69, 71–77). All studies used the same hypoglycemic agent in both groups. Hypoglycemic agents included biguanides, sulfonylureas, α-glucosidase inhibitors, DPP-4 inhibitors, and insulins. Specific agents include metformin, gliclazide, glibenclamide, glimepiride, gliquidone, glipizide, pioglitazone, acarbose, voglibose, sitagliptin, and insulin. Treatment duration ranged from 4 to 24 weeks.

Meta-analysis results showed that as integrative medicine, CHM plus hypoglycemic agents was superior to hypoglycemic agents alone at reducing 2hPG levels at the end of treatment [MD −1.46 (−1.73, −1.20), I^2 = 86.2%]. Heterogeneity remained high after sensitivity analysis with studies with low risk of bias for sequence generation [nine studies, n = 895, MD −1.64 (−2.27, −1.02); I^2 = 91.8%] (31, 32, 38, 43, 44, 46, 64, 69, 73) (**Table 2**).

Grouping of studies using metformin produced the biggest pool with 16 RCTs and 1,572 participants. The effect on 2hPG is similar to the overall result with reduced heterogeneity [MD −1.46 (−1.73, −1.20); I^2 = 44.3%] (28, 39, 40, 43, 47, 52, 54, 57, 59–61, 64, 68, 73, 74, 77). Combinations of different drug classes also showed a significant difference between groups, but heterogeneity remained high. Additional subgroup analyses based on patient age groups, baseline levels of FBG, CM

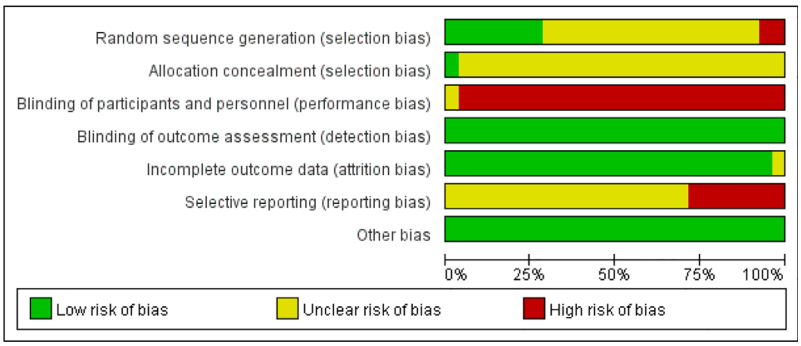

FIGURE 2 | Risk of bias of included studies.

syndrome differentiation, treatment duration, disease duration, and baseline levels of BMI all showed significant differences between groups (**Table 2**).

Hemoglobin A1c. Thirty-five RCTs including 3,009 participants assessed the effects of CHM plus hypoglycemic agents *versus* hypoglycemic agents alone (27–32, 34, 37–45, 47–49, 51–55, 57, 59, 60, 64, 67, 68, 70, 72–77). All studies used the same hypoglycemic agents in both groups, including biguanides, sulfonylureas, α-glucosidase inhibitors, and insulins. Specific agents include metformin, gliclazide, glipizide, gliquidone, voglibose, acarbose, and insulin. Treatment duration ranged from 2 to 24 weeks.

CHM plus hypoglycemic agents was superior to hypoglycemic agents alone [MD −0.84 (−1.05, −0.64), I^2 = 88.2%]. Heterogeneity remained high in subgroup analysis except in two subgroups where sulfonylureas or biguanides plus sulfonylureas were combined with CHM (**Table 2**). In two RCTs with 120 participants, the result indicated that the value of HbA_{1c} was reduced in people receiving CHM plus sulfonylureas compared to sulfonylureas alone [MD −0.65 (−1.07, −0.24), I^2 = 0.0%] (49, 55). CHM plus biguanides and sulfonylureas were used as comparator in three RCTs, including 196 participants; the result showed that in the integrative medicine group, HbA_{1c} significantly lower compared to pharmacotherapy group alone [MD −0.35 (−0.68, −0.02), I^2 = 0.0%] (27, 29, 75).

Blood Lipid Metabolism Indicators. Total Cholesterol. Twenty-four RCTs including 2,582 participants assessed the effects of CHM plus hypoglycemic agents *versus* hypoglycemic agents alone (27–31, 34, 35, 37, 39, 42–45, 54, 56, 58, 63, 64, 66, 70–73, 77). All studies used the same hypoglycemic agents in both groups; specific agents include metformin, glibenclamide, glipizide, glimepiride, acarbose, and insulin. Treatment duration ranged from 2 to 12 weeks.

Meta-analyses showed that CHM in addition to hypoglycemic agents was superior to hypoglycemic agents alone at reducing TG levels in T2DM patients [MD −0.40 (−0.51, −0.29), I^2 = 79.2%]. Meta-analysis of studies assessed as low risk for sequence generation produced a similar result that was more homogeneous [seven RCTs, 792 participants, MD −0.36 (−0.50, −0.22); I^2 = 50.5%] (31, 34, 37, 43, 44, 64, 73).

Subgroup analyses by patient age groups, FBG level at baseline, disease duration and BMI levels at baseline showed similar effects on TG (**Table 2**). Combined use of CHM with biguanides, insulin, biguanides plus sulfonylureas and biguanides plus a-Glucosidase also produced significant difference between groups; results are like the overall analysis (**Table 2**).The combination of CHM to a sulfonylureas did not produce a better result than these agents alone (56, 71) (**Table 2**). In studies that provided details on CM syndrome differentiation, adding CHM to hypoglycemic agents showed more benefit in reducing TG levels in patients with *qi* and *yin* deficiency (29, 31, 34, 35, 37, 39, 71, 77), *yin* deficiency with excessive heat (43, 64), but not those with spleen deficiency (28) (**Table 2**).

Triglyceride. Twenty-four RCTs including 2,582 participants assessed the effects of CHM plus hypoglycemic agents *versus* hypoglycemic agents alone (27–31, 34, 35, 37, 39, 42–45, 54, 56, 58, 63, 64, 66, 70–73, 77). All studies used the same hypoglycemic agents in both groups; specific agents include metformin, glibenclamide, glipizide, glimepiride, acarbose, and insulin. Treatment duration ranged from 2 to 12 weeks.

Meta-analyses showed that CHM in addition to hypoglycemic agents was superior to hypoglycemic agents alone at reducing TC levels in T2DM patients [MD −0.40 (−0.58, −0.22), I^2 = 95.2%]. Meta-analysis of studies assessed as low risk of bias for sequence generation showed no difference between groups [seven RCTs, 792 participants, MD −0.34 (−0.86, 0.19); I^2 = 98.3%] (31, 34, 37, 43, 44, 64, 73).

Subgroup analysis showed that the combination of CHM with sulfonylureas (56, 71), insulin (31, 35, 44), or biguanides plus sulfonylureas (27, 29, 58) was not superior at reducing TC levels (**Table 2**). Studies with patient's age group of 41–61years (20 RCTs, n = 2,230) (27–31, 34, 35, 42–44, 56, 58, 63, 64, 66, 70–73, 77) showed a significant difference between the two groups [MD −0.39 (−0.59, −0.18); I^2 = 96.0%] but not in the two studies with patients who are older [n = 120, MD −0.46 (−0.99, 0.08); I^2 = 57.8%] (37, 45). Subgroup analyses by FBG level at baseline showed similar effects on TC. Analysis on studies that provided CM syndrome information showed that integrative medicine was superior to hypoglycemic agents alone in patients with *qi* and *yin* deficiency (29, 31, 34, 35, 37, 39, 71, 77), but not those with *yin*

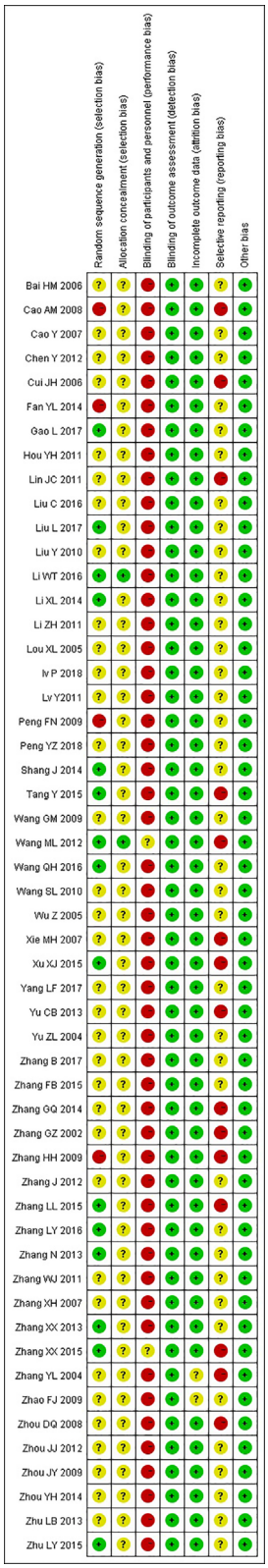

FIGURE 3 | Risk of bias summy of included studies.

deficiency and excessive heat (43, 64) or spleen deficiency (28) (**Table 2**). Studies with disease duration was 5–10 years [six RCTs, n = 612, MD −0.63 (−0.77, −0.50); I^2 = 76.8%] (29, 54, 66, 70, 71) showed a significant difference between the two groups but not in the study with disease duration of less than 5 years (27, 28, 43, 56, 63, 64, 72) or equal or more than 10 years (31, 37, 44, 45, 73, 77) disease duration subgroup (**Table 2**). Additional subgroup analyses by BMI level at baseline showed no difference between groups (**Table 2**).

Low Density Lipoprotein and High-Density Lipoprotein. Twenty-one RCTs including 2,338 participants assessed the effects of CHM plus hypoglycemic agents *versus* hypoglycemic agents alone (27, 29–31, 34, 35, 37, 39, 43–45, 54, 56, 58, 63, 64, 66, 70–73). Different classes of hypoglycemic agents were used, and treatment duration ranged from 2 to 12weeks.

Meta-analysis of 21 studies showed that CHM plus hypoglycemic agents was superior to hypoglycemic agents alone at reducing LDL levels at the end of treatment [MD −0.45 (−0.65, −0.27); I^2 = 97.4%]; however, heterogeneity was high. Seven studies were assessed as low risk of bias for sequence generation, subgroup analysis showed similar results to the overall studies (31, 34, 37, 43, 44, 64, 73). Subgroup analysis by drug class showed that CHM added to sulfonylureas (56, 71) or insulin (31, 35, 44) was no superior at reducing LDL levels compared to sulfonylureas or insulin alone (**Table 2**). Subgroup analyses by FBG level at baseline, disease duration or BMI level at baseline showed similar effects on LDL. Studies with age group of 41–64 years showed significant between group results (27, 29–31, 34, 35, 43, 44, 56, 58, 63, 64, 66, 70–73). In studies that provided details on CM syndrome differentiation, subgroup analysis showed that CHM is better at reducing LDL levels in patients with *qi* and *yin* deficiency (29, 31, 34, 35, 37, 39, 71), but not in those with *yin* deficiency and excessive heat (43, 64) (**Table 2**).

Meta-analysis result of 21 studies showed that CHM plus hypoglycemic agents was superior to hypoglycemic agents alone at improving HDL levels [MD 0.15 (0.08, 0.22); I^2 = 93.9%]; however, heterogeneity was high. Results from studies with a low risk of bias for sequence generation produced a similar result (31, 34, 37, 43, 44, 64, 73) (**Table 2**). Subgroup analysis by drug class showed that CHM in combination with biguanides (34, 39, 43, 54, 63, 64, 70, 73), or insulin (31, 35, 44) can improve HDL levels better than using the agents alone (**Table 2**). Studies with patient age group of 41–64 years showed significant between group results (27, 29–31, 34, 35, 43, 44, 56, 58, 63, 64, 66, 70–73) but not in two studies with older age groups (37, 47). Subgroup analysis using FBG level at baseline showed significant differences between groups with high heterogeneity (**Table 2**). In T2DM patients with *qi* and *yin* deficiency (29, 31, 34, 35, 37, 39, 71), integrative medicine was better at improving HDL levels than hypoglycemic agents alone (**Table 2**). Studies with disease duration was 5–10 years (five RCTs, n = 542) (29, 54, 66, 70, 71) showed a significant difference between the two groups but not in studies with a disease duration of less than 5 years (27, 43, 56, 63, 64, 72) or more than 10 years (31, 37, 44, 45, 73) (**Table 2**). Additional subgroup analyses by BMI level at baseline showed no difference between groups (**Table 2**).

TABLE 2 | Summary of meta-analysis results and sub-group analysis.

Treatment *Vs*. Comparison	Outcomes(unit)	Group	Subgroup	No. of Studies	MD[95% CI]	I^2
CHM plus Hypoglycaemic agents *versus* Hypoglycaemic agents	FBG (mmol/L)	All studies	All studies	48(4,375)	−0.93[−1.10, −0.76]*	84.2%
		Risk of bias SG	Low risk of bias SG	11(1,050)	−0.68[−0.89, −0.47]*	67.9%
		comparator Drug class	Biguanides	18(1,722)	−0.91 [−1.11, −0.70]*	63.8%
			Sulfonylureas	7(770)	−0.92 [−1.58, −0.26]*	91.9%
			Insulin	4(477)	−0.96[−1.57, −0.35]*	85.0%
			Biguanides + Sulfonylureas	5(416)	−1.18[−1.46, −0.89]*	0.0%
			Biguanides+ a-Glucosidase	5(293)	−0.97[−1.69, −0.24]*	96.4%
		Age of patients	41–64 years	36(3,219)	−0.79[−1.02, −0.57]*	86.7%
			>65 years	4(504)	−1.28[−1.51, −1.06]*	0.0%
		FBG level at baseline	8–10 mmol/L	27(2,413)	−0.77[−0.95, −0.59]*	74.9%
			≥10 mmol/L	21(1,962)	−1.15[−1.48, −0.82]*	89.2%
		CM syndrome differentiation	*qi* and *yin* deficiency	23(1,886)	−0.97[−1.23, −0.72]*	81.6%
			yin deficiency and excessive heat	6(430)	−0.82[−1.05, −0.59]*	23.0%
			Spleen deficiency	3(158)	−0.57[−1.10, −0.04]*	87.6%
		treatment duration	≤3 months	46(4,139)	−0.91[−1.08, −0.73]*	83.9%
			≥6 months	1(138)	−1.00[−1.34, −0.66]*	NA
		Disease duration	<	14(1,093)	−0.80[−1.09, −0.51]*	77.2%
			≥5–10 years	10(924)	−1.07[−1.55, −0.59]*	90.7%
			≥10 years	10(1,001)	−0.88[−1.14, −0.62]*	72.5%
		BMI level at baseline	≥24–28 kg/m^2	3(193)	−0.53[−0.87, −0.19]*	0.0%
	2hPG (mmol/L)	All studies	All studies	43(4,004)	−1.46[−1.73, −1.20]*	86.2%
		Risk of bias SG	Low risk of bias SG	9(895)	−1.64[−2.27, −1.02]*	91.8%
		comparator Drug class	Biguanides	16(1,572)	−1.36[−1.62, −1.11]*	44.3%
			Sulfonylureas	7(770)	−1.60[−2.48, −0.72]*	89.6%
			Insulin	3(379)	−1.71[−3.37, −0.05]*	91.2%
			Biguanides + Sulfonylureas	5(416)	−1.12[−2.22, −0.02]*	86.4%
			Biguanides + a-Glucosidase	5(298)	−1.58[−2.41, −0.74]*	94.7%
		Age of patients	41–64 years	33(2,968)	−1.45[−1.78, −1.12]*	86.7%
			>65 years	2(384)	−1.65[−2.92, −0.39]*	94.1%
		FBG level at baseline	8–10 mmol/L	24(2 200)	−1.38[−1.74, −1.03]*	85.6%
			≥10 mmol/L	19(1,804)	−1.56[−1.98, −1.15]*	87.3%
		CM syndrome differentiation	*qi* and *yin* deficiency	20(1,633)	−1.25[−1.67, −0.84]*	85.4%
			yin deficiency and excessive heat	6(430)	−1.51[−2.16, −0.87]*	71.4%
			Spleen deficiency	3(158)	−0.96[−1.29, −0.63]*	44.3%
		treatment duration	≤3 months	42(3,866)	−1.46[−1.73, −1.18]*	86.3%
			≥6 months	1(138)	−1.73[−2.07, −1.39]*	NA
		Disease duration	<5 years	14(1,093)	−1.57[−2.00, −1.15]*	75.9%
			≥5–10 years	9(866)	−1.35[−2.11, −0.59]*	92.7%
			≥10 years	7(783)	−1.38[−2.16, −0.61]*	92.4%
		BMI level at baseline	≥24–28 kg/m^2	2(98)	−1.35[−2.25, −0.46]*	46.6%
	HbA_{1c} (%)	All studies	All studies	35(3,009)	−0.84[−1.05, −0.64]*	88.2%
		Risk of bias SG	Low risk of bias SG	9(920)	−0.74[−1.16, −0.32]*	93.9%
		comparator Drug class	Biguanides	17(1,665)	−0.85[−1.04, −0.66]*	75.1%
			Sulfonylureas	2(120)	−.65[−1.07, −0.24]*	0.0%
			Insulin	3(357)	−1.43[−2.69, −0.18]*	94.8%
			Biguanides + Sulfonylureas	3(196)	−0.35[−0.68, −0.02]*	0.0%
			Biguanides + a-Glucosidase	4(248)	−0.85[−1.68, −0.02]*	96.7%
		Age of patients	41–64 years	27(2,209)	−0.77[−0.99, −0.55]*	85.1%
			>65 years	3(368)	−0.95[−1.76, −0.14]*	77.2%
		FBG level at baseline	8–10 mmol/L	22(1,935)	−0.73[−0.97, −0.50]*	87.5%
			≥10 mmol/L	13(1,074)	−1.04[−1.41, −0.67]*	86.9%
		CM syndrome differentiation	*qi* and *yin* deficiency	17(1,208)	−0.67[−0.91, −0.44]*	72.8%
			yin deficiency and excessive heat	4(300)	−0.82[−1.21, −0.43]*	69.3%
			Spleen deficiency	3(158)	−1.07[−2.21, 0.07]	97.3%
		treatment duration	≤3 months	33(2,773)	−0.82[−1.04, −0.61]*	88.5%
			≥6 months	1(138)	−0.60[−0.99, −0.21]*	NA
		Disease duration	<5 years	11(717)	−0.72[−0.97, −0.47]*	64.6%
			≥5–10 years	6(532)	−0.91[−1.34, −0.48]*	79.8%
			≥10 years	9(893)	−0.90[−1.39, −0.42]*	94.0%
		BMI level at baseline	≥24–28 kg/m^2	3(193)	−0.30[−0.62, 0.02]	39.9%
	TG (mmol/L)	All studies	All studies	24(2,582)	−0.40[−0.51, −0.29]*	79.2%
		Risk of bias SG	Low risk of bias SG	7(792)	−0.36[−0.50, −0.22]*	50.5%
		comparator Drug class	Biguanides	10(1,205)	−0.39[−0.53, −0.24]*	65.5%

(Continued)

TABLE 2 | Continued

Treatment *Vs.* Comparison	Outcomes(unit)	Group	Subgroup	No. of Studies	MD[95% CI]	I^2
			Sulfonylureas	2(382)	−0.06[−0.12, 0.00]	0.0%
			Insulin	3(379)	−0.46[−0.74, −0.18]*	56.5%
			Biguanides + Sulfonylureas	3(266)	−0.37[−0.58, −0.17]*	40.0%
			Biguanides + a-Glucosidase	2(110)	−0.37[−0.52, −0.21]*	0.0%
		Age of patients	41–64 years	20(2,230)	−0.37[−0.48, −0.26]*	73.3%
			>65 years	2(120)	−0.59[−0.81, −0.37]*	0.0%
		FBG level at baseline	8–10 mmol/L	13(1,342)	−0.36[−0.45, −0.27]*	32.1%
			≥10 mmol/L	10(972)	−0.43[−0.64, −0.22]*	88.1%
		CM syndrome differentiation	*qi* and *yin* deficiency	8(773)	−0.34[−0.52, −0.15]*	76.1%
			yin deficiency and excessive heat	2(198)	−0.19[−0.33, −0.05]*	0.0%
			Spleen deficiency	1(38)	−0.85[−1.73, 0.03]	NA
		Disease duration	<5 years	7(800)	−0.31[−0.45, −0.18]*	48.3%
			≥5–10years	6(612)	−0.49[−0.81, −0.17]*	92.7%
			≥10 years	6(695)	−0.49[−0.62, −0.37]*	6.8%
		BMI level at baseline	≥24–28 kg/m^2	3(193)	−0.57[−0.87, −0.28]*	44.1%
	TC	All studies	All studies	24(2,582)	−0.40[−0.58, −0.22]*	95.2%
	(mmol/L)	Risk of bias SG	Low risk of bias SG	7(792)	−0.34[−0.86, 0.19]	98.3%
		comparator Drug class	Biguanides	10(1,205)	−0.36[−0.70, −0.02]*	97.6%
			Sulfonylureas	2(382)	−0.28[−0.82, 0.25]	89.1%
			Insulin	3(379)	−0.13[−0.60, 0.34]	77.1%
			Biguanides + Sulfonylureas	3(266)	−0.69[−1.59, 0.21]	73.9%
			Biguanides + a-Glucosidase	2(110)	−0.44[−0.74, −0.15]*	0.0%
		Age of patients	41–64 years	20(2,230)	−0.39[−0.59, −0.18]*	96.0%
			>65 years	2(120)	−0.46[−0.99, 0.08]	57.8%
		FBG level at baseline	8–10 mmol/L	13(1,342)	−0.39[−0.70, −0.08]*	97.0%
			≥10 mmol/L	10(972)	−0.50[−0.66, −0.34] *	66.5%
		CM syndrome differentiation	*qi* and *yin* deficiency	8(773)	−0.40 [−0.67, −0.12] *	95.9%
			yin deficiency and excessive heat	2(198)	−0.77[−1.92, 0.38]	98.7%
			Spleen deficiency	1(38)	0.12[−0.38, 0.62]	NA
		Disease duration	<5 years	7(800)	−0.39[−0.94, 0.16]	96.0%
			≥5–10 years	6(612)	−0.63[−0.77, −0.50]*	76.8%
			≥10 years	6(695)	−0.16[−0.43, 0.11]	71.3%
		BMI level at baseline	≥24–28 kg/m^2	3(193)	−0.02[−0.15, 0.10]	10.6%
	LDL	All	All studies	21(2,338)	−0.45[−0.65, −0.27]*	97.4%
	(mmol/L)	Risk of bias SG	Low risk of bias SG	7(792)	−0.68[−1.10, −0.26]*	97.9%
		comparator Drug class	Biguanides	8(1,031)	−0.51[−0.88, −0.14]*	98.5%
			Sulfonylureas	2(382)	−0.06[−0.16,0.04]	0.0%
			Insulin	3(379)	−0.57[−1.18, 0.05]	97.6%
			Biguanides + Sulfonylureas	3(266)	−0.45[−0.60, −0.30]*	0.0%
			Biguanides + a-Glucosidase	2(110)	−0.39[−0.66, −0.12]*	78.1%
		Age of patients	41–64 years	17(1,986)	−0.45[−0.69, −0.21]*	97.7%
			>65 years	2(120)	−0.73[−1.82, 0.35]	78.9%
		FBG level at baseline	8–10 mmol/L	12(1,206)	−0.53[−0.83, −0.22]*	97.7%
			≥10 mmol/L	8(864)	−0.23[−0.33, −0.13]*	68.4%
		CM syndrome differentiation	*qi* and *yin* deficiency	7(637)	−0.29[−0.44, −0.15]*	75.1%
			yin deficiency and excessive heat	2(198)	−0.79[−2.03, 0.46]	99.4%
		Disease duration	<5 years	6(762)	−0.59[−1.08, −0.09]*	98.8%
			≥5–10 years	5(542)	−0.31[−0.46, −0.16]*	80.7%
			≥10 years	5(559)	−0.64[−1.11, −0.17]*	95.5%
		BMI level at baseline	≥24–28 kg/m^2	2(155)	−0.27[−0.35, −0.20]*	0.0%
	HDL	All	All studies	21(2,338)	0.15[0.08, 0.22]*	93.9%
	(mmol/L)	Risk of bias SG	Low risk of bias SG	7(792)	0.18[0.07, 0.29]*	93.3%
		comparator Drug class	Biguanides	8(1,031)	0.24[0.14, 0.33]*	89.6%
			Sulfonylureas	2(382)	0.18[−0.21, 0.58]	96.8%
			Insulin	3(379)	0.20[0.05, 0.34]*	93.6%
			Biguanides + Sulfonylureas	3(266)	0.02[−0.17, 0.21]	81.0%
			Biguanides + a-Glucosidase	2(110)	−0.04[−0.12, 0.04]	21.1%
		Age of patients	41–64 years	17(1 986)	0.16[0.08, 0.25]*	94.6%
			>65 years	2(120)	0.01[-0.05,0.07]	0.0%
		FBG level at baseline	8–10 mmol/L	12(1,206)	0.13[0.04, 0.22]*	93.5%
			≥10 mmol/L	8(864)	0.12[0.02, 0.22]*	89.8%
		CM syndrome differentiation	*qi* and *yin* deficiency	7(637)	0.17[0.09, 0.25]*	75.5%
			yin deficiency and excessive heat	2(198)	0.16[−0.04, 0.35]	82.7%

(Continued)

TABLE 2 | Continued

Treatment *Vs.* Comparison	Outcomes(unit)	Group	Subgroup	No. of Studies	MD[95% CI]	I^2
		Disease duration	<5 years	6(762)	0.13[–0.04, 0.31]	94.3%
			≥5–10 years	5(542)	0.17[0.07, 0.27]*	84.9%
			≥10 years	5(559)	0.16[–0.00, 0.33]	95.7%
		BMI level at baseline	≥24–28 kg/m^2	2(155)	0.03[–0.18, 0.23]	90.8%
	FINS	All studies	All studies	12(1,049)	–1.03 [–2.35, 0.29]	86.9%
	(mU/Lor μIU/ml)	Risk of bias SG	Low risk of bias SG	5(413)	–1.60[–4.42, 1.21]	92.3%
		comparator Drug class	Biguanides	3(293)	–2.89[–6.96, 1.18]	95.7%
			Sulfonylureas	3(228)	–2.03[–3.21, –0.84]*	0.0%
			Biguanides + Sulfonylureas	1(150)	0.60[–1.16, 2.36]	NA
			Biguanides + a-Glucosidase	1(60)	–2.10[–2.79, –1.41]	NA
		Age of patients	41–64 years	10(929)	–1.46[–2.85, –0.07]*	88.2%
			>65 years	1(60)	1.32[-2.11,4.75]	NA
		FBG level at baseline	8–10 mmol/L	8(641)	-1.95[-3.45,-0.45]*	87.4%
			≥10 mmol/L	3(348)	1.62[0.09,3.15]	32.2%
		CM syndrome differentiation	*qi* and *yin* deficiency	3(215)	0.17[–2.08, 2.42]	47.4%
			yin deficiency and excessive heat	3(258)	–3.43[–7.37, 0.49]	95.3%
		treatment duration	≤3 months	11(911)	–1.41[–2.68, –0.15]*	84.5%
			≥6 months	1(138)	2.85[0.96, 4.73]	NA
		Disease duration	<5 years	5(378)	–2.96[–4.93, –0.99]	90.7%
			≥10 years	2(168)	–0.80[–4.34, 2.74]	70.1%
		BMI level at baseline	≥24–28 kg/m^2	2(155)	–1.19[–3.21, 0.82]	81.8%
	IR	All studies	All studies	7(581)	–1.11[–1.44, –0.77]*	55.0%
		Risk of bias SG	Low risk of bias SG	4(353)	–1.14[–1.67, –0.60]*	72.6%
		comparator Drug class	Biguanides	3(293)	–0.80[–1.04, –0.56]*	0.0%
			Sulfonylureas	2(168)	–1.30[–1.85, –0.75]*	0.0%
			Biguanides + a-Glucosidase	1(60)	–0.98[–1.52, –0.44]*	NA
		FBG level at baseline	8–10 mmol/L	6(521)	–0.91[–1.12, –0.69]*	5.5%
		CM syndrome differentiation	*qi* and *yin* deficiency	1(95)	–0.90[–1.28, –0.52]	NA
			yin deficiency and excessive heat	3(258)	–0.79[–1.10, –0.48]*	3.5%
		Disease duration	<5 years	4(318)	–0.82[–1.08, –0.57]*	0.0%
			≥10 years	1(108)	–1.49[–2.23,-0.75]*	NA
		BMI level at baseline	≥24–28 kg/m^2	2(155)	–0.93[–1.24, –0.62]*	0.0%
	IS	All studies	All studies	5(434)	0.09[-0.26,0.43]	95.0%
		comparator Drug class	Biguanides	2(210)	–0.14[–0.97, 0.69]	98.7%
			Sulfonylureas	2(168)	0.17[0.05, 0.28]*	0.0%
			Biguanides + Sulfonylureas	1(56)	0.56[0.05, 1.07]*	NA
		Age of patients	41–64 years	4(262)	0.23[0.12, 0.34]*	34.6%
		FBG level at baseline	8–10 mmol/L	3(224)	0.19[0.03, 0.36]*	35.4%
			≥10 mmol/L	2(210)	–0.14[–0.98, 0.69]	98.7%
		CM syndrome differentiation	*yin* deficiency and excessive heat	1(60)	0.21[0.06,0.36]*	NA
			Spleen deficiency	1(38)	0.28[0.18, 0.38]*	NA
		Disease duration	<5 years	3(154)	0.27[0.19, 0.35]*	0.0%
			≥5–10 years	1(172)	–0.57[–0.74, –0.40]	NA
			≥10 years	1(108)	0.09[–0.11, 0.29]	NA
		BMI level at baseline	≥24–28 kg/m^2	1(38)	0.28[0.18,0.38]*	NA
	BMI	All studies	All studies	4(233)	–0.45[–0.99, 0.08]	9.4%
	(kg/m^2)	Risk of bias SG	Low risk of bias SG	2(135)	–0.38[–2.83, 2.07]	68.4%
CHM *vs.* lifestyle intervention	FBG (mmol/L)	All studies	All studies	1(72)	–0.62 [–1.28, 0.04]	NA
	2hPG (mmol/L)	All studies	All studies	1(72)	–2.14[–2.81, –1.47]*	NA
CHM as food *vs.* Lifestyle intervention	FBG (mmol/L)	All studies	All studies	1(90)	–1.08[–1.86, –0.30]*	NA
	2hPG (mmol/L)	All studies	All studies	1(90)	–1.23[–1.96, –0.50]*	NA
	TG (mmol/L)	All studies	All studies	1(90)	–0.30[–0.69, 0.09]	NA
	TC (mmol/L)	All studies	All studies	1(90)	–0.66[–1.10, –0.23]*	NA

**Statistically significant difference between groups.*

CI, confidence interval; CM, Chinese medicine; FBG, fasting blood glucose; 2hPG, 2-hour postprandial blood glucose; HbA1c, hemoglobin A1c; TG, triglyceride; TC, total cholesterol; LDL, low density lipoprotein; HDL, high-density lipoprotein; FINS, fasting insulin; IR, insulin resistance; IS, insulin resistance index; BMI, body mass index; MD, mean difference; SG, sequence generation.

β-Cell Function Indicators. Fasting Insulin. Twelve RCTs including 1,049 participants assessed the effects of CHM plus hypoglycemic agents *versus* hypoglycemic agents alone (33, 34, 43, 45, 51, 55, 58, 64, 65, 69, 72, 76). Different classes of hypoglycemic agents were included and treatment duration ranged from 4 to 24 weeks.

At the end of treatment, CHM plus hypoglycemic agents was not superior to hypoglycemic agents alone at reducing FINS levels [MD −1.03 (−2.34, 0.29), I^2 = 86.9%]. Meta-analysis of results from studies with a low risk of bias for sequence generation showed similar results from the overall result (34, 43, 64, 65, 69). Subgroup analysis by hypoglycemic agent class showed varied results for different class of drugs; there were benefits seen in the addition of CHM to sulfonylureas but not in other hypoglycaemic agents (**Table 2**). Studies with patient age group of 41–64 years showed significant difference between group results (33, 34, 43, 51, 55, 58, 64, 65, 72, 76) but not in older subgroup (45). Subgroup analysis based on FBG level at baseline, the lower reading of 8–10 mmol/L subgroup (33, 34, 43, 55, 64, 69, 72, 76) showed more benefit but not in higher subgroup (45, 51, 58). Studies with a shorter treatment duration of less than 3 months showed benefit in adding CHM to hypoglycaemic agents (33, 34, 43, 45, 55, 58, 64, 65, 69, 72, 76), but not in studies with a longer treatment (51). Additional subgroup analyses by CM syndrome differentiation, disease duration and BMI level at baseline showed no difference between groups (**Table 2**).

Homeostatic model assessment of insulin resistance. Seven RCTs including 581 participants assessed the effects of CHM plus hypoglycemic agents *versus* hypoglycemic agents alone (33, 34, 43, 64, 65, 72, 76). Hypoglycemic agents included biguanides, sulfonylureas, α-glucosidase inhibitors, and insulins. Treatment duration ranged from 4 to 12 weeks.

CHM plus hypoglycemic agents was superior to hypoglycaemic agents alone [MD −1.11 (−1.44, −0.77); I^2 = 55.0%]. Meta-analysis of results from studies with a low risk of bias for sequence generation showed similar results (34, 43, 64, 65); however, heterogeneity was high. Subgroup analyses based on drug class, baseline levels of FBG, disease duration and baseline levels of BMI all showed significant differences between groups (**Table 2**). Subgroup analysis by CM differentiation showed benefit of adding CHM to hypoglycemic group than hypoglycemic agents alone in patients with *yin* deficiency and excessive heat (33, 43, 64) (**Table 2**).

Insulin resistance index. Five RCTs including 434 participants assessed the effects of CHM plus hypoglycemic agents *versus* hypoglycemic agents alone (27, 28, 33, 54, 76). Various hypoglycemic agents were used including metformin, gliclazide, and glimepiride. All studies had a treatment duration of less than or equal to 12 weeks; the shortest treatment duration was 4 weeks.

Meta-analysis showed that CHM plus hypoglycemic agents was not superior to hypoglycemic agents alone [MD 0.09 (−0.26, 0.43), I^2 = 95.0%]. Subgroup analysis by drug class showed benefit in adding CHM to sulfonylureas (33, 76) and the combination of biguanides with sulfonylureas (27). No significant difference was observed when CHM was added to biguanides (28, 54). Subgroup analysis using age of patients of 41–64 years showed benefit (27, 28, 33, 76). Subgroup analysis based on lower FBG level at baseline (8–10 mmol/L) (27, 33, 76) showed more benefit but not in the higher subgroup (28, 54). In studies that provided information on CM differentiation, meta-analyses showed a benefit in adding CHM to hypoglycemic agents in patients with *yin* deficiency and excessive heat (33) or spleen deficiency (28). Studies with disease duration of less than 5 years (three RCTs, n = 154) (27, 28, 33) showed a significant difference between the two groups but not those studies with more than 5 years of disease duration (54, 76). Additional subgroup analyses by BMI level at baseline showed difference between groups (28) (**Table 2**).

Body Mass Index. Four RCTs including 233 participants assessed the effects of CHM plus hypoglycemic agents ***versus*** hypoglycemic agents alone (28, 34, 62, 72). Hypoglycemic agents included biguanides, α-Glucosidase inhibitors and insulins. Specific agents include metformin, acarbose, and insulin. Treatment duration ranged from 8 to 12 weeks.

Meta-analysis results showed that CHM plus hypoglycemic agents was not superior to hypoglycemic agents alone at improving BMI [MD −0.45 (−0.99, 0.08); I^2 = 9.4%). Meta-analysis of studies assessed as low risk for sequence generation produced a similar result [two studies, 135 participants, MD −0.38 (−2.83, 2.07); I^2 = 68.4%] (34, 62), and no difference was found between groups.

CHM Plus Lifestyle Intervention Versus Lifestyle Intervention Alone

One RCT with 72 participants compared CHM (including *Shanyao*) plus lifestyle intervention with lifestyle intervention for 4 weeks (26). The result showed CHM together with lifestyle intervention was superior to lifestyle intervention alone in reducing 2hPG and TC levels at the end of treatment [MD −2.14 (−2.81, −1.47), −0.66 (−1.10, −0.23)]; there was no difference in the FBG and TG levels between the two groups [MD −0.62 (−1.28, 0.04), −0.30 (−0.69, 0.09)].

CHM Diet Therapy Plus Lifestyle Intervention Versus Lifestyle Intervention Alone

One RCT (n = 90) compared CHM as diet therapy (including *Shanyao*) with lifestyle intervention with a treatment duration of 12 weeks (25). The result showed that combination of Chinese diet therapy using *Shanyao* and lifestyle intervention was superior to lifestyle intervention alone at reducing FBG and 2hPG levels at the end of treatment [MD −1.08 (−1.86, −0.30), −1.23 (−1.96, −0.50)].

Adverse Events

Out of the 53 studies, 21 studies reported on AEs. Of these, 10 studies provided specific details about the AEs.

CHM Plus Hypoglycaemic Agents vs. Hypoglycaemic Agents. In twenty RCTs of CHM plus hypoglycemic drugs *versus* hypoglycemic drugs, ten studies reported no AEs (28, 30, 32, 41, 51, 52, 55, 72, 76, 77), ten studies provided specific details about AEs (29, 37, 43–46, 53, 56, 58, 64). In the integrative medicine group, the most common AEs were hypoglycemia (four cases), nausea (three cases), hypertension (two cases), insomnia (one case), epigastric discomfort (two cases), diarrhea (two cases), frequency of urine (three cases), drug-related adverse reactions (one case unknown). There were three

cases of diarrhea reported; however, it was not clear whether the AEs were from the treatment group or the control group (43).

Twenty-four AEs were reported in the hypoglycemic drug group. In the hypoglycemic agents group, hypoglycemia (seven cases) was the most common AE. Other AEs included nausea (three cases), headache (two cases), stomach distention (three cases), dyspepsia (two cases), fatigue (two cases), rash (one case), and four other AEs were not described in detail.

One study that compared CHM plus lifestyle intervention to lifestyle intervention reported no AEs (26).

Assessment Using GRADE

An assessment of the quality of the evidence from RCTs was undertaken using GRADE. Interventions, comparators, and outcomes included were selected based on a consensus process. Comparisons were: CHM plus hypoglycemic agents *versus* hypoglycemic agents, CHM plus lifestyle intervention *versus* lifestyle intervention and CHM diet therapy plus lifestyle intervention *versus* lifestyle intervention.

Evidence of *Shanyao* formulae for T2DM was low to moderate quality (**Table 3**). The results showed that oral

TABLE 3 | GRADE: Quality of the evidence of *Shanyao* formulae for T2DM.

Outcomes	№ of participants (studies)Follow-up	Certainty of the evidence(GRADE)	Anticipated absolute effects	
CHM plus Hypoglycemic agents vs. Hypoglycemic agents			**Risk with [Hypoglycemic drugs]**	**Risk difference with [CHM plus hypoglycemic drugs]**
Fasting blood glucose (FBG) Treatment duration: mean 8.85 weeks	4,375 (48 RCTs)	⨁⨁⨁◯ MODERATE[a]	The mean fasting blood glucose was **7.43** mmol/L	MD **0.93 mmol/L lower** (1.1 lower to 0.76 lower)
2-hour Postprandial blood glucose (2hPG) Treatment duration: mean 8.79 weeks	4,004 (43 RCTs)	⨁⨁⨁◯ MODERATE[a]	The mean postprandial blood glucose was **10.44** mmol/L	MD **1.46 mmol/L lower** (1.73 lower to 1.2 lower)
Glycosylated Hemoglobin A1c (HbA1c) Treatment duration: mean 9.32 weeks	3,009 (35 RCTs)	⨁⨁◯◯ LOW [a,b]	The mean glycosylated Hemoglobin A1c was **7.44%**	MD **0.84% lower** (1.05 lower to 0.64 lower)
Triglyceride (TG) Treatment duration: mean 9.04 weeks	2,582 (24 RCTs)	⨁⨁◯◯ LOW [a,b]	The mean triglyceride was **2.41** mmol/L	MD **0.4 mmol/L lower** (0.51 lower to 0.29 lower)
Cholesterol (TC) Treatment duration: mean 9.04 weeks	2,582 (24 RCTs)	⨁⨁⨁◯ MODERATE[a]	The mean cholesterol was **5.15** mmol/L	MD **0.4 mmol/L lower** (0.58 lower to 0.22 lower)
Fasting insulin (FINS) Follow-up: range 1 to 30 weeks Treatment duration: mean 10.67 weeks	1,049 (12 RCTs)	⨁⨁◯◯ LOW [a,b]	The mean fasting insulin was **15.11** μU/ml	MD **1.03 μU/ml lower** (2.35 lower to 0.29 higher)
CHM plus lifestyle intervention vs. lifestyle intervention			**Risk with [lifestyle intervention]**	**Risk difference with [CHM]**
Fasting blood glucose (changed from baseline)(FBG) Treatment duration 4 weeks	72 (one RCT)	⨁⨁⨁◯ MODERATE[c]	The mean fasting blood glucose (changed from baseline) was –**0.32** mmol/L	MD **0.62 mmol/L lower** (1.28 lower to 0.04 higher)
2-hour Postprandial blood glucose (changed from baseline)(2hPG) Treatment duration 4 weeks	72 (one RCT)	⨁⨁⨁◯ MODERATE[c]	The mean postprandial Blood Glucose (changed from baseline) was –**0.77** mmol/L	MD **2.14 mmol/L lower** (2.81 lower to 1.47 lower)
CHM diet therapy plus lifestyle intervention vs. lifestyle intervention			**Risk with [lifestyle intervention]**	**Risk difference with [CHM]**
Fasting blood glucose (changed from baseline)(FBG) Treatment duration 12 weeks	90 (one RCT)	⨁⨁⨁◯ MODERATE[c]	The mean fasting blood glucose (changed from baseline) was –**0.87** mmol/L	MD **1.08 mmol/L lower** (1.86 lower to 0.30 lower)
2-hour Postprandial blood glucose (changed from baseline)(2hPG) Treatment duration 12 weeks	90 (one RCT)	⨁⨁⨁◯ MODERATE[c]	The mean postprandial Blood Glucose (changed from baseline) was **–1.34** mmol/L	MD **1.23 mmol/L lower** (1.96 lower to 0.50 lower)
Triglyceride(changed from baseline) (TG) Treatment duration 12 weeks	90 (one RCT)	⨁⨁⨁◯ MODERATE[c]	The mean triglyceride(changed from baseline) was **–0.41** mmol/L	MD **0.3 mmol/L lower** (0.69 lower to 0.09 higher)
Cholesterol(changed from baseline) (TC) Treatment duration 12 weeks	90 (one RCT)	⨁⨁⨁◯ MODERATE[c]	The mean cholesterol(changed from baseline) was **–0.45** mmol/L	MD **0.66 mmol/L lower** (1.1 lower to 0.23 lower)

**The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CHM, Chinese herbal medicine; CI, Confidence interval; GRADE, Grading of Recommendations Assessment, Development and Evaluation; MD, Mean difference; RCTs, randomized controlled trials;*

Explanations

[a]High statistical heterogeneity, $p < 0.05$; [b]Funnel plot not symmetrical; [c]Small sample size.

Bold values is used to highlight.

formulae containing *Shanyao* may improve glycolipid metabolism and fasting insulin level.

Experiment Research Evidence of *Shanyao* for Diabetes

The major component groups of *Shanyao* are saponins, phenolic compounds, sterols, and mucilage (8). Identified active ingredients of *Shanyao* include polysaccharides, flavonoids, allantoin, choline, dioscin, and so on (9–14). *Shanyao* has shown immunomodulatory and anti-inflammatory effects (78, 79). Hpyoglycemic effects of *Shanyao* as food and pharmacological effects in relation to T2DM are reviewed below.

Nutritional Study on the Hypoglycemic Effect of *Shanyao*

Shanyao has been studied to explore its effect on blood glucose as a food. A study indicated that meal A (maize flour meal) was composed of 81% carbohydrate, 3% protein, and 11% fat; meal B (cassava flour meal) was composed of 76% carbohydrate, 3% protein, and 15% fat; while meal C (yam flour meal) was composed of 85% carbohydrate, 2% protein, and 8% fat. Analysis of the results demonstrated a better glycemic response with meals A and C compared with meal B; *Shanyao* as food may bring more benefits to blood glucose (80).

Shanyao's glycemic index has also been investigated. Glycemic index was introduced to rank how slowly or quickly carbohydrate containing foods are digested and increase postprandial blood glucose (24). A study has shown that the actual calorie input may be much lower in *Shanyao* than in brown rice and white bread, showing that the glycemic value of *Shanyao* is lower than brown rice (81), which is beneficial for controlling postprandial blood glucose, in turn may benefit diabetic patients.

An experimental research conducted in Brazil showed that *Shanyao* flour alleviated the consequences of the experimental diabetic disease. It showed that *Shanyao* flour could control the rise in blood glucose levels in diabetic rats and significantly greater radiodensity of femoral head when compared to DM group, suggesting protection in oxidative agents and postpone bone damage caused by diabetes (82).

Different *Shanyao* species are present depending on place of production. *Dioscorea alata* is known to have the highest yields among the *Shanyao* species with tubers. *Dioscorea alata* has shown to have higher amylose and total dietary fiber contents, resulting in slower absorption rates and can be particularly useful in diets for diabetics (83).

Pharmacological Effects in Relation to Diabetes of *Shanyao* and Its Compounds

The pharmacological effects and preventative effects for T2DM of *Shanyao* or its compounds have been tested in various animal models.

As we mentioned earlier on, a popular formula used for DM is Liu wei di huang wan made up of six herbs including *Shanyao*. In fructose-rich chow fed rats, after feeding of Liu wei di huang wan at 26 mg/kg for 60 min, reduction in plasma glucose was observed (84). When *Shanyao* was removed from the mixture, plasma glucose was not modified while this action was not modified by the removal of the other five herbs, indicating important hypoglycemic roles of *Shanyao* (84). Further, *Shanyao* produced similar hypoglycemic effects of the Liu wei di huang wan formula, while other herbs in the formula failed to produce the same effects. The authors also investigated the role of *Shanyao* in improving insulin sensitivity and found that oral administration of *Shanyao* at 4.2 mg/kg three times daily into streptozotocin-induced diabetic rats increased the response to exogenous insulin (84).

In vehicle-controlled mice and in alloxan-induced diabetic mice, *Shanyao* decoction concentrate at 300 mg and 600 mg/kg for 10 consecutive days can significantly reduce blood glucose level (85). Further, *Shanyao* has shown preventative effects on induced blood glucose elevation due to different causes including adrenaline, alloxan, and glucose feeding (85). As for hypoglycemic effects, *Shan yao* can reduce total cholesterol and triglyceride levels in diabetic rats, showing lipid lowering effects (86).

Polysaccharides

In alloxan-induced diabetic rats and mice, high dose DOTP-80 water-soluble polysaccharide (400 mg/kg) had strong hypoglycemic activity (87). Moreover, water-soluble polysaccharide could increase the level of antioxidant enzyme (superoxide dismutase) activity in alloxan-induced diabetic mice and stimulated an increase in glucose disposal in diabetic rats (87).

In high fat fed streptozotocin-induced type 2 diabetic rats, *Shanyao* polysaccharide administration significantly reduced fasting plasma glucose levels, increased serum insulin levels, and decreased glucagon levels, showing hypoglycemic effects (88, 89). The hypoglycemic effects of *Shanyao* polysaccharides are comparable to metformin (88, 89).

In vitro testing of *Shanyao* polysaccharide (YP-1) from a variety of Chinese yam revealed that YP-1 could stimulate ConA-induced T lymphocyte proliferation, and its branches are extremely important for the expression of the enhancement of the immunological activity (90). Considering the important role of the immune system in the progression of T2DM, YP-1 could play a part in enhancing immunological activities in T2DM.

Shanyao polysaccharides have shown antioxidant activities. Purified *Shanyao* polysaccharide could scavenge hydroxyl radical and superoxide radical. Additionally, it displayed inhibitory activity against *E. coli*, with a minimal inhibitory concentration of 2.5 mg/ml (91).

Dexamethasone-induced insulin resistance glucose/lipid metabolism diabetic mice model was established to evaluate the hypoglycemic effect of different concentrations of *HuaiShanyao* and different molecular weights of polysaccharide HSY-I, HSY-II, and HSY-III. The results indicated that the Chinese yam polysaccharide mixture had hypoglycemic effect (92).

Saponins and Flavones

The metabolic syndrome is a term for cluster of multiple metabolic risk criteria which is positively correlated with type

2 diabetes mellitus. *Shanyao* dioscorin interventions exhibit improved metabolic syndrome activities in obese rats, and peptic hydrolysates of *Shanyao* dioscorin *in vitro* exhibit DPP IV inhibitory activities (93).

After intragastric administration of dioscoreae flavone in diabetic mice, it was found that blood glucose of diabetic mice was decreased, the amount of drinking water decreased, and the weight of mice recovered (94). Further experiments showed that dioscoreae flavone could inhibit α-glucosidase activity, effectively reduce superoxide dismutase activity and malondialdehyde content in diabetic mice, and have antioxidant effect (94).

Experimental results show that the saponins and flavones of *Shanyao* had a marked inhibition effect on α-amylase. The kinetic analysis indicates that the inhibition type of saponins and flavones on α-amylase was competitive, and the enzyme-substrate apparent dissociation constant (Km′) of acarbose (the control), saponins, and flavones is 118.86, 79.23, 49.51 mg/ml, respectively (95).

Allantoin

Allantoin is known as the active principle richly contained in *Shanyao* (Dioscorea spp.). It is identified as an abundant and active component in *Shanyao* (Dioscorea spp.) (96). Allantoin may improve glucose utilization in the skeletal muscle through β-endorphin dependent- and independent-pathways that decrease plasma glucose in STZ-diabetic rats (97).

Allantoin can increase β-endorphin release through activation of I-2A receptors to lower blood glucose. Also, allantoin can activate I-2B receptors in the skeletal muscle or adipose tissues and brain and others to reduce blood glucose in STZ-diabetic rats. Both actions of allantoin may assist the increase of insulin sensitivity in diabetic rats (98).

Ligands

α-Glucosidase inhibitors are widely used in the treatment of patients with T2DM, which delay the absorption of carbohydrates from the small intestine and result in lowered postprandial blood glucose and insulin levels (99). Two ligands identified as 2,4-dimethoxy-6,7-dihydroxyphenanthrene and batatasin I were extracted from *Shanyao* using α-glucosidase functionalized magnetic nanoparticles as a solid phase extraction absorbent. Their α-glucosidase inhibitory activities were significantly higher than acarbose (100).

DISCUSSION

Summary of Evidence

In this study, we searched clinical studies and experimental studies to present an overall picture of the evidence for *Shanyao* and its formulae for additional benefits to conventional therapies in the management of DM. We also explored the possible mechanisms of actions for *Shanyao*.

This study presents a comprehensive and up-to-date evidence for the treatment of T2DM with *Shanyao* formulae. A systematic review of 53 randomized controlled trials was identified in English- and Chinese-language databases, and meta-analysis was conducted to evaluate the additive benefits and safety of *Shanyao* formulae. Systematic evaluation of modern literatures suggested that in terms of controlling blood glucose, blood lipid and improving insulin resistance, traditional Chinese medicine formulae containing *Shanyao* combined with conventional therapies do show added benefits when compared to hypoglycemic agents and lifestyle intervention alone.

Systematic review of clinical studies indicates that *Shanyao*-containing herbal formulae can improve important outcome measures for T2DM including FBG, 2hPG, HbA_{1c}, TG, TC, and IR in people with T2DM. Further, the adverse events in the herbal formula group are lower than in the control group, suggesting a good safety profile. Taken together, Chinese herbal medicine therapy including *Shanyao* can be of beneficial for patients with T2DM. The heterogeneity in our meta-analysis was high. We performed a subgroup analysis based on pre-set conditions, but the results of subgroup analysis did not fully explain the source of heterogeneity. Considering different herb usages in different clinical trials, this could be a possible source of heterogeneity in the meta-analysis. Therefore, it is necessary to interpret these results with caution.

The occurrence of DM in the current day and age is closely related to lifestyle change. We found that *Shanyao* 山药, *Fuling* 茯苓, *Maimendong*麦门冬, *Shanzhuyu* 山茱萸, *Haungqi* 黄芪, *Shengdihuang* 生地黄, *Gegen* 葛根, *Danshen* 丹参, *Tianhuafen* 天花粉, and *Huanglian* 黄连are used more in modern literatures. The use of Chinese herbs that taste sweet and bitter, has the effect of invigorating *qi* and nourishing *yin*, infiltrating dampness and solidifying astringency, clearing heat, generating body fluid, and detoxifying. In recent years, the prevalence of diabetes in obese and overweight people has doubled in modern China (6). TCM believes that phlegm-dampness is the basic cause of obesity. An ancient practitioner Zhu Danxi clearly put forward in "*Dan Xi Zhi Fa Xin Yao*丹溪治法心要" that "people who are obese have excessive dampness and phlegm in the body". Obesity combined with diabetes is characterized by *yin* deficiency, internal heat, and phlegm dampness. Therefore, modern diabetes treatment focuses on nourishing *yin* and clearing heat, drying dampness and resolving phlegm.

To sum up the experimental evidence, *Shanyao* as a food, is rich in dietary fiber with low glycemic index, which is beneficial to improving postprandial blood glucose of diabetic patients. In diabetic animals, *Shanyao* can reduce blood sugar, regulate blood lipid, resist oxidation and be beneficial to the bone. The main active ingredients of *Shanyao* can regulate blood glucose by improving insulin resistance, inhibiting α-glucosidase activity, delaying the absorption of glucose in intestine, inhibiting DPP-IV activity, increasing the concentration of endogenous GLP-1, antioxidation and regulating immunity.

Limitations of the Current Review

The included clinical studies also present methodological shortfalls. In randomized clinical trials, appropriate randomization and allocation concealment methods can reduce

bias, but only 25.81% of the included studies described the randomization methods, and only 2.58% of the studies described allocation concealment. Inappropriate randomization and allocation concealment may exaggerate the efficacy. Inadequate blinding method can also lead to overestimation of the effect. Chinese herbal medicine has many obstacles to the implementation of blinding in clinical trials due to the herbal preparation and odor; it is easy for participants to identify which group they are in. Active exploration of the preparation of placebo and the implementation of blinding method of traditional Chinese medicine may be a way to solve this problem. In addition, the included studies did not publish study protocols, and the non-standardization of clinical research reports is not helpful to the dissemination and recognition of research results.

Modern literature systematic reviews included the study of traditional Chinese medicine formulae containing *Shanyao*. The included studies all used *Shanyao* as part of a traditional Chinese medicine formula for T2DM. Therefore, the results of modern literature analysis do not fully represent the role of *Shanyao* in the treatment of T2DM in reducing blood sugar, lipid and improving IR.

Implications in Research and Clinical Practice

Future clinical studies should be recommended to design and report data following the items required by the Consolidated Standards of Reporting Trials (CONSORT) (101) and its extensions for herbal medicine and traditional CM (102, 103).

Rigorous methodology is recommended when designing future clinical trials with correct methods of sequence generation and allocation concealment. Protocols should be published and be registered to minimize reporting bias and increase transparency in the reporting the results.

The included studies reported outcome measures directly related to blood glucose metabolism, lipid profiles, and β-cell function. As we know, T2DM is a chronic and progressive disease, and those who are affected may have drastic lifestyle changes. Other clinically important outcomes such as quality of life would provide another aspect of understanding the effect of CM therapies for T2DM.

As we all know, T2DM is a progressive and lifelong disease. The included clinical studies had treatments between 2 and 24 weeks with only few studies reporting on follow-up data. Future studies may consider using longer treatment durations and lengthy follow-up period to reflect clinical practice and provide evidence for the long-term effects of CM treatments using *Shanyao*.

Although we present in the study available experimental evidence for *Shanyao*, the volume of studies is small compared to other commonly used herbs such as ginseng. More experimental study data will improve the understanding of mechanisms of action of hypoglycemic effects of *Shanyao*. Results may provide future directions for the development of new and improved hypoglycemic agents.

Based on clinical studies, herbs that are commonly used with *Shanyao* in the modern context include *huangqi* 黄芪, *shengdihuang* 生地黄, *gegen* 葛根, *tianhuafen* 天花粉, *danshen* 丹参, *fuling* 茯苓, *maimendong* 麦门冬, *shanzhuyu* 山茱萸, and *huanglian* 黄连.

Finally, this study provides comprehensive information about *Shanyao*'s use for T2DM in clinical studies. At the same time, it explores possible mechanisms through experimental studies, providing evidence of hypoglycemic mechanism of *Shanyao* at a cellular and animal model levels.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REFERENCES

1. Federation. ID. *IDF Diabetes Atlas. 9th edn.* Belgium (2019). Available at: https://www.diabetesatlas.org. [cited 2020 March 23rd].
2. Association AD. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2020. *Diabetes Care* (2020) 43:s14–31. doi: 10.2337/dc20-S002
3. Wang L, Gao P, Zhang M, Huang Z, Zhang D, Deng Q, et al. Prevalence and Ethnic Pattern of Diabetes and Prediabetes in China in 2013. *Jama* (2017) 317:2515–23. doi: 10.1001/jama.2017.7596
4. Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, et al. Effects of Diet and Exercise in Preventing NIDDM in People With Impaired Glucose Tolerance: The Da Qing Igt and Diabetes Study. *Diabetes Care* (1997) 20:537–44. doi: 10.2337/diacare.20.4.537
5. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular andmicrovascular complications of type 2 diabetes (UKPDS 35):prospective observational study. *BMJ* (2000) 321:405–12. doi: 10.1136/bmj.321.7258.405
6. Chinese Diabetes Society. version) [Article in Chinese]. *Chin J Diabetes* (2018) 10:4–50. doi: CNKI:SUN:SYNK.0.2018-04-009

7. Shi Y. *Internal medicine of traditional Chinese medicine (Textbook for the 13th five year plan of general higher education, textbook for the plan of national higher medical institutions)*. Beijing, China: Science Press (2017).
8. Bensky D, Clavey S, Stoger E. *Chinese herbal medicine Materia Medica. 3rd ed.* Seattle, US: Eastland Press, Inc (2004).
9. Yang MH, Chin YW, Yoon KD, Kim J. Phenolic compounds with pancreatic lipase inhibitory activity from Korean yam (Dioscorea opposita). *J Enzyme Inhibition Med Chem* (2014) 29:1–6. doi: 10.3109/14756366.2012.742517
10. Yang MH, Yoon KD, Chin YW, Park JH, Kim J. Phenolic compounds with radical scavenging and cyclooxygenase-2 (COX-2) inhibitory activities from Dioscorea opposita. *Bioorg Med Chem* (2009) 17:2689–94. doi: 10.1016/j.bmc.2009.02.057
11. Kong XF, Zhang YZ, Yin YL, Wu GY, Zhou HJ, Tan ZL, et al. Chinese yam polysaccharide enhances growth performance and cellular immune response in weanling rats. *J Sci Food Agricult* (2009) 89:2039–44. doi: 10.1002/jsfa.3688
12. Jeon JR, Lee JS, Lee CH, Kim JY, Kim SD, Nam DH. Effect of Ethanol Extract of Dried Chinese Yam (Dioscorea batatas) Flour Containing Dioscin on Gastrointestinal Function in Rat Model. *Arch Pharm Res* (2006) 29:348–53. doi: 10.1007/bf02968583
13. Fu YC, Ferng LH, Huang PY. Quantitative analysis of allantoin and allantoic acid in yam tuber, mucilage, skin and bulbil of the Dioscorea species. *Food Chem* (2006) 94:541–9. doi: 10.1016/j.foodchem.2004.12.006
14. Fu YC, Chen SH, Huang PY, Li YJ. Application of Bubble Separation for Quantitative Analysis of Choline in Dioscorea (Yam) Tubers. *J Agric Food Chem* (2005) 53:2392–6. doi: 10.1021/jf048501h
15. Green J. *Cochrane Handbook for Systematic Reviews of Interventions(Version 5.1.0)*. UK: The Cochrane Collaboration (2011). March.
16. World Health Organisation. *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications, Part 1: Diagnosis and Classification of Diabetes Mellitus*. Geneva (1999).
17. Chinese Diabetes Society. *Prevention and Treatment of Type 2 Diabetes Mellitus in China (2010 version)*. Beijing China: Beijing University Publisher (2011).
18. Chinese Diabetes Society. Prevention and Treatment of Type 2 Diabetes Mellitus in China (2013 version). *Chin J Diabetes* (2014) 6:447–98. doi: 10.3760/cma.j.issn.1000-6699.2014.10.020
19. Zhou ZY. *Internal medicine of traditional Chinese medicine / national planning textbook for "12th Five Year Plan", "11th Five Year Plan" and "15th five year plan" of Higher Education*. Beijing, China: China Press of Traditional Chinese Medicine (2007).
20. Zhang BY. *Internal medicine of traditional Chinese medicine / teaching materials of medical colleges and Universities*. Shanghai, Chian: Shanghai Science and Technology Press (1985).
21. Zhang BL. *Internal medicine of traditional Chinese medicine / textbook for the 13th five year plan of higher education in the industry of traditional Chinese Medicine*. Beijing, China: China Press of Traditional Chinese Medicine (2017).
22. Fan GJ. *Series on clinical diagnosis and treatment of specialist diseases in traditional Chinese Medicine - clinical diagnosis and treatment of specialist diseases in endocrinology and rheumatism in traditional Chinese medicine. 2nd Edition*. Beijing, China: People's Medical Publishing House (2013).
23. Wu MH. *Internal medicine of traditional Chinese medicine / textbook for the 12th Five Year Plan of higher education in the industry of traditional Chinese Medicine*. Beijing, China: China Press of Traditional Chinese Medicine (2012).
24. Wolever TM. The glycemic index. *World Rev Nutr Diet* (1990) 62:120–85. doi: 10.1007/s11883-007-0064-x
25. Li WT. *The effect of Dioscorea porridge on blood sugar and blood lipid in patients with type 2 diabetes mellitus. Master dissertation*. Fuzhou: Fujian University of Traditional Chinese Medicine (2016).
26. Zhang LY. *Clinical Observation on the Treatment of Damp-heat Syndrome in Type 2 Diabetes Mellitus by Clearing Heat and Removing Dampness. Master dissertation*. Beijing: Beijing University of Chinese Medicine (2016).
27. Wang GM, Wang ZG. Clinical Study on the Treatment of Insulin Resistance in Type 2 Diabetes Mellitus with the Method of Benefiting Kidney and Strengthening Spleen [Article in Chinese]. *Gansu J TCM* (2009) 22:25–6. doi: 10.3969/j.issn.1004-6852.2009.04.016
28. Wu Z. *Study on Jianpi Jiangtang Decoction in the Treatment of Spleen Deficiency and Phlegm Stasis Syndrome of Diabetes Mellitus. Master dissertation*. Nanjing: Nanjing University of Traditional Chinese Medicine (2005).
29. Liu Y. *Huang Qi Tang in type 2 diabetes Qi and Yin Deficiency and Blood Stasis Syndrome Clinical Study. Master dissertation*. Xianyang: ShanXi University Of Chinese Medicine (2010).
30. Chen Y. *The Clinic research of treating Type 2 Diabetes by thetherapy of nourishing spleen and kidneys,heat-clearing and yin –nourishing. Master dissertation*. Nanjing: Nanjing University of Traditional Chinese Medicine (2012).
31. Zhang XX. *Clinical Observation and Mechanism Study on the Effect of Shenqi Compound and Insulin on Cell Function, Oxidative Stress and Blood Level in Patients with Diabetes Mellitus. Master dissertation*. Chengdu: Chengdu University of Chinese Medicine (2013).
32. Li XL. *Clinical studies on insulin from the intestine to explore the mechanism of Spleen Qi Treatment of type 2 diabetes. Master dissertation*. Jinan: Shandong University Of Chinese Medicine (2014).
33. Wang SL. *The early Protective effect on β-cell function in Type 2 diabetes with the therapy of nourishing yin and clearing away heat. Master dissertation*. Guangzhou: Guangzhou University of Chinese Medicine (2010).
34. Zhang N. *Clinical Study on Invigorating Qi and Nourishing Yin Therapy for Insulin Resistance in Initial Type 2 Diabetes Mellitus. Master dissertation*. Taian: Tianshan Medical University (2013).
35. Lv P, Chen L, Bi NN. Clinical Study on Yuye Decoction in Treatment of Type 2 Diabetes with Qi and Yin Deficiency Syndrome [Article in Chinese]. *China J Chin Med* (2018) 33:1244–7. doi: 10.16368/j.issn.1674-8999.2018.07.294
36. Peng YZ, Xu Y. Clinical efficacy of the Shenmai Jiangtang tablet on senile patients with type 2 diabetes mellitus of Qiyin Liangxu [Article in Chinese]. *Clin Res Tradit Chin Med* (2018) 10:67–9. doi: CNKI:SUN:ZYLY.0.2018-06-031
37. Wang ML. *Clinical Observation of tang yi prescription on Improving Symptom of Diabetes mellitus Patients. Master dissertation*. Guangzhou: Guangzhou University of Chinese Medicine (2012). p. 15–24.
38. Zhang LL. *Clinical Observation of Yiqi Yangyin Jiangtang Decoction in the Treatment of Type 2 Diabetes Mellitus. Master dissertation*. Beijing: Beijing University of Chinese Medicine (2015).
39. Yang LF. *Clinical Study of Qianjin Wenwu Decoction Combined with Metformin in the Treatment of Type 2 Diabetes in Taiyin People. Master dissertation*. Yanji: Yanbian University (2017).
40. Lin JC. *Observation on the curative effect of traditional Chinese medicine combined with Baduanjin on type 2 diabetes mellitus. Master dissertation*. Nanjing: Nanjing University of Traditional Chinese Medicine (2011).
41. Zhang J. *Clinical Observation and Gastrointestinal Hormone Study on Diabetic Spleen Deficiency Syndrome Treated by Jianpi Yiqi Method. Master dissertation*. Jinan: Shangdong University of Chinese Medicine (2012).
42. Bai HM. 40 Cases of Type 2 Diabetes Treated with Yiqi Yangyin Huoxue Decoction [Article in Chinese]. *Shanxi Tradit Chin Med* (2006) 27:1237–328. doi: 10.3969/j.issn.1000-7369.2006.10.053
43. Wang HQ. *The Clinical Research of combination of sweet and bitter herbs for the yin dificiency and heat accumulation of Type 2 diabetes millitus. Master dissertation*. Jinan: Shandong University Of Chinese Medicine (2016).
44. Zhang XX, Liu Y, Xiong DQ, Xie CG. Insulin combined with Chinese medicine improves glycemic outcome through multiple pathways in patients with type 2 diabetes mellitus. *J Diabetes Investigation* (2015) 6:708–15. doi: 10.1111/jdi.12352
45. Zhu LB, Li J. Effect of Jiangtangjing Granule on Prethrombotic State in Elderly Patients with Type 2 Diabetes Mellitus [Article in Chinese]. *J Gansu Coll Tradit Chin Med* (2013) 30:31–4. doi: CNKI:SUN:GSZX.0.2013-05-016
46. Zhu LY, Wu ZW, Cheng ZJ. Clinical Curative Effect of self-made Herbal Medicine combined with western medicine on diabetes [Article in Chinese]. *Liaoning J Tradit Chin Med* (2015) 42:561–3. doi: 10.13192/j.issn.1000-1719.2015.03.048
47. Zhou YH, Zhong ZY, Deng LH, Pen NX, Shen QS. Effect of Erban Decoction on the Blood Sugar of Senile patients with type 2 diabetes [Article in Chinese]. *J New Chin Med* (2014) 46:90. doi: 10.13457/j.cnki.jncm.2014.10.037
48. Zhou JY, Shen W. Clinical Observation of Shenqi Ningxiao Decoction in the Treatment of Type 2 Diabetes Mellitus [Article in Chinese]. *J Clin Chin Med* (2009) 21:204–5. doi: 10.16448/j.cjtcm.2009.03.007

49. Zhou JJ, Dong YY. Clinical Study of Yiqi Yangyin Capsule in the Treatment of Diabetes Mellitus (Type 2 Diabetes Mellitus) with Deficiency of Qi and Yin [Article in Chinese]. *Drugs Clin* (2012) 8:252–3.
50. Zhou DQ, Feng P, Wan P. Clinical Observation on 50 Cases of Type 2 Diabetes Treated with Jiangtang Mixture [Article in Chinese]. *Zhejiang J Tradit Chin Med* (2008) 43:431. doi: 10.3969/j.issn.0411-8421.2008.07.047
51. Zhao FJ, Song J. Clinical Study on the Treatment of Type 2 Diabetes Mellitus with the Method of Supplementing Qi and Nourishing Yin [Article in Chinese]. *J Heze Med College* (2009) 21:39–41. doi: 10.3969/j.issn.1008-4118.2009.01.019
52. Zhang FB, Tao Y, Ning J. Observation on the efficacy of no-named Chinese herbal Formula in the adjuvant treatment of type 2 diabetes mellitus [Article in Chinese]. *Chin Tradit Med Sci Technol* (2015) 22:186–7. doi: CNKI:SUN:TJYY.0.2015-02-034
53. Zhang YL, Zhang J, Xie FM. Combined Chinese and Western Medicine for 98 Cases of Secondary Type 2 Diabetes Mellitus Irresponsive Sulfonylurea Drugs [Article in Chinese]. *Shanghai J Tradit Chin Med* (2004) 38:18–9. doi: CNKI:SUN:SHZZ.0.2004-07-007
54. Zhang HX, Chang FY, Zhang BR, Deng GX, Yu YX, Zhang GL. Clinical Observation on 109 Cases of Insulin Resistance in Type 2 Diabetes Mellitus Treated with Xiaotangping Capsule [Article in Chinese]. *J Tradit Chin Med* (2007) 48:812–3. doi: 10.3321/j.issn:1001-1668.2007.09.016
55. Zhang WJ, Yang HJ, Zhang ZJ, Zhang ZX, Wu LL, Chen M. Effect of Qilian Xiaoke Formula on Diabetes Insulin Resistance and Related Inflammatory Factors [Article in Chinese]. *Hunan J Tradit Chin Med* (2011) 27:4–6. doi: 10.3969/j.issn.1003-7705.2011.01.002
56. Zhang HH, Pan BY. Clinical Observation on 120 Cases of Type 2 Diabetes Mellitus with Deficiency of both Qi and Yin and Blood Stasis Treated by Combination of Traditional Chinese Medicine and Western Medicine [Article in Chinese]. *Beijing J Tradit Chin Med* (2009) 28:538–40. doi: 10.3969/j.issn.1002-2619.2009.06.011
57. Zhang GQ, You Q, Lv M, Chen DL. 36 Cases of Type 2 Diabetes Treated with Yiqi Yangyin Huoxue Decoction [Article in Chinese]. *New World Diabetes* (2014) 11:35–6. doi: CNKI:SUN:TNBX.0.2014-22-031
58. Zhang GZ, Sun QF, Guo JF, Huang BT, Li SH, Wang FH, et al. Clinical study on the treatment of type 2 diabetes mellitus with integrated traditional Chinese and Western Medicine [Article in Chinese]. *Modern J Integrated Tradit Chin Western Med* (2002) 11:5–7. doi: 10.3969/j.issn.1008-8849.2002.01.003
59. Zhang B, Nan Z, Xu CM, Liu GP, Guo XT, Yang LY. et al. Clinical Observation on 30 Cases of Type 2 Diabetes Treated by Combination of Traditional Chinese Medicine and Western Medicine [Article in Chinese]. *Hunan J Tradit Chin Med* (2017) 33:13–5. doi: 10.16808/j.cnki.issn1003-7705.2017.08.005
60. Yu CB, Yan DB, Zhang MZ, Chen Y, Wang Y. Clinical Observation of Self-made Taiping Tangke Concentrated Pill in the Treatment of Type 2 Diabetes Mellitus [Article in Chinese]. *Pract Clin Appl Integrated Tradit Chin Western Med* (2013) 13:16–7. doi: 10.3969/j.issn.1671-4040.2013.01.010
61. Yu ZL, Zhou ZK, Quan FL. Clinical Observation on 30 Cases of Type 2 Diabetes Treated by Combination of Traditional Chinese Medicine and Western Medicine [Article in Chinese]. *Modern J Integrated Tradit Chin Western Med* (2004) 13:186. doi: 10.3969/j.issn.1008-8849.2004.02.037
62. Xu XJ, Liu D, Zhang W, Wang J. Study of the effect of add itive Shenling Baizhu san decoction to insulin sensitivity index of patients with type 2 Diabetes Mellitus [Article in Chinese]. *Clin J Tradit Chin Med* (2015) 27:954–6. doi: 10.16448/j.cjtcm.2015.0360
63. Xie MH, Lu CM. Observation on the therapeutic effect of Dihuang Jiangtang Pill on type 2 diabetes mellitus [Article in Chinese]. *Jilin J Tradit Chin Med* (2007) 27:22–3. doi: 10.13463/j.cnki.jlzyy.2007.06.016
64. Tang Y, Li JS. Research on Treating Syndrome of Endogenous Heat Due to Yin Deficiency Type 2 Diabetes Patients with Liuwei Dihuang Bolus Combined Metformin Hydrochloride Tablets [Article in Chinese]. *J Huan Normal Univ (Med Sci)* (2015) 12:28–31. doi: CNKI:SUN:HNYG.0.2015-04-010
65. Shang J, Zheng JQ, Su XL. Effect of integrated traditional Chinese and Western Medicine on insulin resistance and islet beta cell function in type 2 diabetes mellitus [Article in Chinese]. *Guangming J Tradit Chin Med* (2014) 29:1197–200. doi: 10.3969/j.issn.1003-8914.2014.06.038
66. Peng MN, Wang HZ, Zheng CF. Observation of the Effect of Using Ginseng and Astragalus Arbran Decoction with Western Medicine on the Type-2 Diabetes [Article in Chinese]. *Int J Tradit Chin Med* (2009) 31:241. doi: 10.3760/cma.j.issn.1673-4246.2009.03.028
67. Lv Y, Hou JJ, Lu YE. 45 Cases of Type 2 Diabetes Treated by the Method of Supplementing Qi, Nourishing Yin and Promoting Blood Circulation [Article in Chinese]. *Shanxi Tradit Chin Med* (2011) 32:1485–6. doi: 10.3969/j.issn.1000-7369.2011.11.030
68. Luo XL, Zhao C. Clinical Observation on the Treatment of 52 Cases of DM II With ZhiboDihuang Decoction and Metform [Article in Chinese]. *Guiding J TCM* (2005) 11:15–7. doi: 10.3969/j.issn.1672-951X.2005.06.008
69. Liu L, Liu DL, Liu HL, Zhao HX, Qu X, Li ZY, et al. On Treatment of 30 Cases of Type 2 Diabetes with Deficiency of Qi and Yin Deficiency Syndrome by Huoxue Jiangtang Potion [Article in Chinese]. *Modern Tradit Chin Med* (2017) 37:16–20. doi: CNKI:SUN:XDZY.0.2017-05-007
70. Liu C, Ge H, Li M, Li SY, Yuan W, Zhang J. Effect of Yiqi Yangyin Qingre Formula on serum levels of blood lipids, blood gluose and 1,2 5-dihydroxyvitamin D3 in patients with type 2 diabetes mellitus [Article in Chinese]. *Chin J Biochem Drugs* (2016) 36:145–7. doi: CNKI:SUN:SHYW.0.2016-02-050
71. Li ZH, Yang M, Tang D. Shen Li. Clinical Observation of Jiangtang Capsule Combined with Western Medicine in Treating 102 Cases of Type 2 Diabetes with Deficiency of Qi and Yin [Article in Chinese]. *Guiding J TCM Pharmacy* (2011) 17:34–6. doi: 10.3969/j.issn.1672-951X.2011.02.016
72. Hou YH. *Clinical Study of Treating Type 2 Diabetes Mellitus with the Method of Smoothing the Liver and Strengthening The Spleen. Master dissertation*. Jinan: Shandong University of Traditional Chinese Medicine (2011).
73. Gao L, Chen YY, Huo GS, Wan YX. Clinical efficacy of metformin combined with Shenqi Jiangtang Capsule in the treatment of type 2 diabetes mellitus and its influence on blood sugar, lipid and inflammatory factors [Article in Chinese]. *Chin J Clin Rational Drug Use* (2017) 10:1–3. doi: 10.15887/j.cnki.13-1389/r.2017.36.001
74. Fan YL, Huang XJ, Gao JN, Chen HW. Clinical Observation of Shenqi Jiangtang Granule in the Assistant Treatment of Type 2 Diabetes Mellitus [Article in Chinese]. *Chin J Med Equipment* (2014) 11:142. doi: CNKI:SUN:YXZB.0.2014-S1-170
75. Cui JH, Luo HX. Observation on curative effect of 40 cases of type 2 diabetes mellitus treated by integrated traditional Chinese and Western Medicine [Article in Chinese]. *Yunnan J Tradit Chin Med* (2006) 27:4–5. doi: 10.3969/j.issn.1007-2349.2006.04.003
76. Cao Y, Zou BY. Clinical Observation on Treating 54 Cases of Type 2 Diabetes with Modified Baihurensheng Decoction and Western Medicine [Article in Chinese]. *Guiding J TCM* (2007) 13:18–20. doi: 10.3969/j.issn.1672-951X.2007.11.009
77. Cao AM, Zhang GJ, Zheng J, Dan YL. Clinical Observation on 68 Cases of Type 2 Diabetes Treated with Wuhuang Tongmai Decoction [Article in Chinese]. *Beijing J Tradit Chin Med* (2008) 27:957–8. doi: 10.16025/j.1674-1307.2008.12.006
78. Kim S, Shin S, Hyun B, Kong H, Han S, Lee A, et al. Immunomodulatory Effects of Dioscoreae Rhizome Against Inflammation through Suppressed Production of Cytokines Via Inhibition of the NF-kappaB Pathway. *Immune Netw* (2012) 12:181–8. doi: 10.4110/in.2012.12.5.181
79. Lee SC, Tsai CC, Chen JC, Lin CC, Hu ML, Lu S. The evaluation of reno- and hepatoprotective effects of huai-shan-yao (Rhizome Dioscoreae). *Am J Chin Med* (2002) 30:609–16. doi: 10.1142/S0192415X02000624
80. Bakare-Odunola MT, Mustapha A, Abdu Aguye I. Effect of Nigerian meals on the Pharmacokinetics of chlorpropamide in type II diabetic patients. *Eur J Drug Metab Pharmacokinet* (2008) 33:31–5. doi: 10.1007/BF03191016
81. Lin MH, Wu MC, Lu S, Lin J. Glycemic index, glycemic load and insulinemic index of Chinese starchy foods. *World J Gastroenterol* (2010) 16:4973–9. doi: 10.3748/wjg.v16.i39.4973
82. Rego Tde S, Ash Lda S, Pessoa L, Feijo MB, Leite J, dos Santos Ade S, et al. The intake of yam (Dioscorea bulbifera Linn) attenuated the hyperglycemia and the bone fragility in female diabetic rats. *Nutricion Hospitalaria* (2014) 29:370–5. doi: 10.3305/nh.2014.29.2.7046
83. Faustina Dufie WM, Oduro I, Ellis WO, Asiedu R, Maziya-Dixon B. Potential health benefits of water yam (Dioscorea alata). *Food Funct* (2013) 4:1496–501. doi: 10.1039/c3fo60064e

84. Hsu JH, Wu YC, Liu IM, Cheng JT. Dioscorea as the principal herb of Die-Huang-Wan, a widely used herbal mixture in China, for improvement of insulin resistance in fructose-rich chow-fed rats. *J Ethnopharmacol* (2007) 112:577–84. doi: 10.1016/j.jep.2007.05.013
85. Hao ZQ, Hang BQ, Wang Y. Effect of Shan Yao decoction on reducing blood glucose levels in diabetic mice [Article in Chinese]. *J China Pharm Univ* (1991) 22:158–60. doi: CNKI:SUN:ZGYD.0.1991-03-014
86. Hang YY. Pharmacological experiments on lowing the blood sugar and blood lipids level of Rhizoma Dioscoreae [Article in Chinese]. *J Plant Resour Environment* (1994) 3:59–60. doi: CNKI:SUN:ZWZY.0.1994-04-012
87. Fan YJ, He QY, Luo AS, Wang MY, Luo AX. Characterization and antihyperglycemic activity of a polysaccharide from Dioscorea opposita Thunb roots. *Int J Mol Sci* (2015) 16:6391–401. doi: 10.3390/ijms16036391
88. Lv J, Wei PF, Bai F. Effect of Chinese yam polysaccharide on platelet and enzyme activity in type 2 diabetic rats [Article in Chinese]. *Chin J Gerontol* (2017) 37:3186–7. doi: 10.3969/j.issn.1005-9202.2017.13.027
89. Yang HL, Zhang HX, Li LH, Wang Y, Zhang WW, Jiang X. Study of the hypoglycemic mechanism of Chinese yam polysaccharide in type 2 diabetic rats [Article in Chinese]. *J Agricult Univ Hebei* (2010) 33:100–3. doi: 10.3969/j.issn.1000-1573.2010.03.022
90. Zhao GH, Kan JQ, Li ZX, Chen ZD. Structural features and immunological activity of a polysaccharide from Dioscorea opposita Thunb roots. *Carbohydr Polym* (2005) 61:125–31. doi: 10.1016/j.carbpol.2005.04.020
91. Yang WF, Wang Y, Li XP, Yu P. Purification and structural characterization of Chinese yam polysaccharide and its activities. *Carbohydr Polym* (2015) 117:1021–7. doi: 10.1016/j.carbpol.2014.09.082
92. Li Q, Li WZ, Gao QY, Zou YX. Hypoglycemic Effect of Chinese Yam (Dioscorea opposita rhizoma) Polysaccharide in Different Structure and Molecular Weight. *J Food Sci* (2017) 82:2487–94. doi: 10.1111/1750-3841.13919
93. Shih SL, Lin YS, Lin SY, Hou WC. Effects of yam dioscorin interventions on improvements of the metabolic syndrome in high-fat diet-induced obese rats. *Bot Stud* (2015) 56:4. doi: 10.1186/s40529-015-0084-8
94. He F. *Extraction Active components in dioscoreae rhizome and Research on hypoglycemic bioactive. Master dissertation*. Chongqing: Southwestern University (2011).
95. Qu S, Zhang LM, Zhang XB, Li ZJ. Inhibition of α-Amylase Activities by Extracts of Chinese Yam. In: *Proceedings of the 2012 International Conference on Applied Biotechnology (ICAB 2012)* SpringerLink (2014). p. 1499–506. doi: 10.1007/978-3-642-37925-3_159
96. Kazahiko Sagara MO. Kejichi Suto, and Tsuguchika Yoshida. Quantitative Determination of Allantoin in Dioscorea Rhizome and an Oriental Pharmaceutical Preparation, Hachimi-Gan, by High-Performance Liquid Chromatography. *Planta Med* (1989) 55:93. doi: 10.1055/s-2006-961841
97. Niu CS, Chen W, Wu HT, Cheng KC, Wen YJ, Lin KC, et al. Decrease of plasma glucose by allantoin, an active principle of yam (Dioscorea spp.), in streptozotocin-induced diabetic rats. *J Agric Food Chem* (2010) 58:12031–5. doi: 10.1021/jf103234d
98. Lin KC, Yeh LR, Chen LJ, Wen YJ, Cheng KC, Cheng JT. Plasma Glucose-lowering Action of Allantoin is Induced by Activation of Imidazoline I-2 Receptors in Streptozotocin-induced Diabetic Rats. *Horm Metab Res* (2012) 44:14–46. doi: 10.1055/s-0031-1295439
99. van de Laar FA, Lucassen PL, Akkermans RP, van de Lisdonk EH, Rutten GE, van Weel C. α-Glucosidase Inhibitors for Patients With Type 2 Diabetes. *Diabetes Care* (2005) 28:154. doi: 10.2337/diacare.28.7.1840
100. Zhang SS, Wu DD, Li H, Zhu JH, Hu WP, Lu MH, et al. Rapid identification of alpha-glucosidase inhibitors from Dioscorea opposita Thunb peel extract by enzyme functionalized Fe_3O_4 magnetic nanoparticles coupled with HPLC-MS/MS. *Food Funct* (2017) 8:3219–27. doi: 10.1039/C7FO00928C
101. Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Trials* (2010) 11:32. doi: 10.1016/j.ijsu.2011.09.004
102. Bian Z, Liu B, Moher D, Wu T, Li Y, Shang H, et al. Consolidated standards of reporting trials (CONSORT) for traditional Chinese medicine: current situation and future development. *Front Med* (2011) 5:171–7. doi: 10.1007/s11684-011-0132-z
103. Gagnier JJ, Boon H, Rochon P, Moher D, Barnes J, Bombardier C. Reporting randomized, controlled trials of herbal interventions: an elaborated CONSORT statement. *Ann Intern Med* (2006) 144:364–7. doi: 10.7326/0003-4819-144-5-200603070-00013

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Rates and Correlates of Incident Type 2 Diabetes Mellitus Among Persons Living With HIV-1 Infection

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The prevalence of various comorbidities continue to rise in aging persons living with HIV-1 infection (PLWH), and our study here aimed to assess the rates and correlates of incident type 2 diabetes mellitus (T2DM) in PLWH from a retrospective, southeastern U.S. cohort. Based on electronic health records, we examined patient demographics, body mass index (BMI), HIV-1-related outcomes, hepatitis C virus co-infection, common comorbidities (e.g. shingles and asthma), usage of protease inhibitors, and usage of statins as potential correlates for T2DM occurrence. Among 3,975 PLWH with ≥12 months of follow-up between January 1999 and March 2018, the overall rate of incident T2DM was 135 per 10,000 person-years, almost 2-fold higher than the rate reported for the general U.S. population. In multivariable models (354 T2DM patients and 3,617 control subjects), sex, BMI, nadir $CD4^+$ T-cell count, HIV-1 viral load (VL) and duration of statin use were independent correlates of incident T2DM (adjusted P <0.05 for all), with clear consistency in several sensitivity analyses. The strongest associations (adjusted odds ratio/OR >2.0 and P <0.0001) were noted for: i) statin use for ≥6 months (OR = 10.2), ii) BMI ≥30 kg/m^2 (OR = 3.4), and iii) plasma VL ≥200 copies/ml (OR = 2.2). Their collective predictive value was substantial: the C-statistic for area under the receiver operating characteristics curve was 0.87 (95% CI = 0.84-0.91), showing close similarity between two major racial groups (C-statistic = 0.87 for African Americans and 0.91 for European Americans). Overall, these findings not only establish a promising algorithm for predicting incident T2DM in PLWH but also suggest that patients who are obese and use statins should require special consideration for T2DM diagnosis and prevention.

Keywords: HIV-1 infection, obesity, statin, type 2 diabetes mellitus, race

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INTRODUCTION

In persons living with human immunodeficiency virus type 1 (HIV-1) infection (PLWH), adherence to combination antiretroviral therapy (cART) can effectively suppress viral replication and drive plasma viral load (VL) to an undetectable level, followed by partial immune reconstitution and a close-to-normal life expectancy (1). While mortality from AIDS-defining conditions has dramatically declined in the cART era, the burden of chronic, non-AIDS comorbidities continues to

rise in the aging PLWH population (2). For example, the prevalence of diabetes is known to be much higher among PLWH than the general population (3), but longitudinal data on incident type 2 diabetes mellitus (T2DM) in PLWH are lacking.

According to recent estimates from the American Diabetes Association (ADA), about 1.5 million new diabetes cases are diagnosed every year in the United States (U.S.) general population (4), and there is abundant evidence that age, obesity, race, and genetic factors contribute to T2DM (5–7). These factors are also known to influence T2DM in PLWH, but additional factors, including chronic inflammation and long-term cART use, and exposure to various HIV-1 protease inhibitors can alter the risk for and complicate the management of T2DM and other metabolic disorders among PLWH (8–11). Our analyses of retrospective data from a southeastern U.S. clinical cohort were intended to gain new insights into key factors associated with incident T2DM in the contemporary PLWH population.

SUBJECTS AND METHODS

Study Cohort

PLWH attending an outpatient HIV clinic (The 1917 Clinic) at University of Alabama at Birmingham (UAB) (12) were enrolled and continuously followed between January 1999 and March 2018 for routine care and monitoring of coinfections and comorbidities. For this sub-study, diagnosis of T2DM followed the new ADA recommendations based on a combination of any two tests of hemoglobin A1c (≥42 mmol/mol), fasting plasma glucose (≥126 mg/dl), and random plasma glucose test (≥200 mg/dl) (13–15). In most cases, medication records (e.g., prescription of metformin) matched T2DM diagnosis, and a total of 354 incident T2DM cases and 3,621 control subjects were identified based on the following exclusion criteria (**Figure S1 in Supplemental Materials**): i) unknown or ambiguous self-reported races, ii) less than 18 years old at the time of enrollment, iii) receiving organ transplantation (systemic immunosuppression other than HIV-1 infection), iv) less than 12-month active follow-up, v) diagnoses of T2DM before enrollment (prevalent cases, $n = 159$), vi) confirmed type 1 diabetes mellitus, vii) missing two critical, HIV-1-related variables ($CD4^+$ T-cell counts and VL). All subjects provided written informed consent for participation in the study, and research protocols pertinent to this sub-study were approved by an Institutional Review Board at UAB.

Dataset Retrieved for Various Analyses

The following variables were retrieved from a central, electronic database: i) enrollment date, ii) demographics (sex, birth date, race/ethnicity), iii) risk factors for HIV-1 acquisition (sexual orientation and injection drug use), iv) the duration of protease inhibitor use; v) the category and duration of statin use; vi) nadir $CD4^+$ T-cell (CD4) count (cells/μl) in the last 2 years before the incident T2DM diagnosis (for T2DM incident cases) or the last clinical visit (for control subjects), and vii) HIV-1 viremia (VL, RNA copies/ml of plasma) in the last 2 years before the incident T2DM diagnosis (for T2DM cases) or the last clinical visit (for control subjects).

Descriptive Statistics

Data from the overall PLWH cohort and several stratified groups (defined by sex, race, and enrollment periods) were first analyzed for incident rates of T2DM per 10,000 person-years (PY) of follow-up, along with their 95% confidence intervals (CI). Subjects were then stratified by T2DM status (incident cases vs. controls) for direct comparison of baseline characteristics (**Table 1**), which included i) counts and percentages for each categorical variable, ii) mean and standard deviation (SD) of continuous variables, iii) median and interquartile range for CD4 and VL. Collinearity among all variables was assessed by nonparametric (Spearman) pairwise correlation analyses. To focus on robust findings, we adopted a newly proposed *P*-value threshold ($P < 0.005$) for statistical significance (16). This *P*-value cut-off was intended to ensure a corresponding Bayes factor between 14 and 26, which is in favor of rejecting null hypothesis while reducing false positive rate (16).

Multivariable Models to Identify Independent Correlates of Incident T2DM

A linear regression model was fitted to test multicollinearity between independent correlates before fitting them to the final multivariable model. A backward stepwise selection procedure was adopted to identify factors independently associated with T2DM (adjusted *P*-value less than 0.05), and a joint, multivariable model was used to assess the relative impact of each factor on T2DM, with adjusted odds ratios and 95% CIs as the key summary statistics. The clinical utility of these factors in predicting T2DM was assessed by the C-statistic and its 95% CI for area under the receiver operating characteristics curve (ROC) (13). These analytical procedures were performed using the statistical analysis software (SAS), version 9.3 (SAS Institute, Cary, North Carolina, USA).

Secondary Analysis and Sensitivity Analysis

To fully assess the timing of T2DM in our study cohort, several potential correlates (e.g., sex and race) of T2DM were also evaluated in Kaplan-Meier (KM) curves and Cox-proportional hazard models, using enrollment date as the starting point. Moreover, several sensitivity analyses considered the enrollment periods as proxies for the evolving cART regimens, as well as varying guidelines for HIV-1 screening and cART initiation. The new guidelines of statin therapy, as introduced in 2013, also required separate analyses of T2DM cases diagnosed before 2013 and since 2013. Consistent findings from the final (reduced) multivariable model were also validated separately in EA and AA subgroups. Potential fluctuations in rates over the 18-year follow-up period was assessed by comparing rates among four time intervals (4.5 years each) in two major racial groups (AA and EA). The Joinpoint Trend Analysis software was used to detect possible trends, as described elsewhere (17, 18).

TABLE 1 | Overall characteristics of the study cohort stratified by type 2 diabetes mellitus (T2DM).

Characteristics	No T2DM (*n* = 3,621)	Incident T2DM (*n* = 354)	*P*-value[b]	*Q*
Age at enrollment (mean ± SD)	38.8 ± 10.9	44.3 ± 9.4	<0.0001	<0.001
Age group at incident T2DM or last clinical visit			<0.0001	<0.001
18–44 years	1738 (48.0)	111 (31.4)	Ref.	
45–64 years	1712 (47.3)	223 (63.0)	<0.0001	<0.001
≥65 years	171 (4.7)	20 (5.6)	0.002	0.003
Sex			<0.001	<0.001
Men	2821 (21.5)	245 (69.2)	Ref.	
Women	779 (77.9)	108 (30.5)	0.0001	<0.001
Transgenders	21 (0.6)	1 (0.3)	>0.50	>0.50
Race/ethnicity			0.49	>0.50
African American (AA)	2124 (58.7)	202 (57.1)	Ref.	
European American (EA)	1419 (39.2)	147 (41.5)	0.45	0.50
Others	78 (2.2)	5 (1.4)	0.40	0.46
BMI group at incident DM or last clinical visit (Missing 364 subjects)			<0.0001	<0.001
<25 *(kg/m^2)*	1348 (40.6)	58 (19.9)	Ref.	
25–29 *(kg/m^2)*	1091 (32.9)	79 (27.0)	0.003	0.005
≥30 *(kg/m^2)*	880 (26.5)	155 (53.1)	<0.0001	<0.001
Median nadir CD4 count over the last 24 months			0.021	0.027
<200 cells/μl	850 (23.5)	81 (22.9)	0.36	0.43
200-500 cells/μl	1231 (34.0)	145 (41.0)	0.006	0.008
>500 cells/μl	1540 (42.5)	128 (36.1)	ref	
Highest VL over the last 24 months			<0.0001	<0.001
<200	2108 (58.2)	168 (47.5)	Ref.	
200-23,596 (Q3)	614 (17.0)	92 (26.0)	<0.0001	<0.001
>23,596	899 (24.8)	94 (26.5)	0.044	0.055
Protease inhibitor (PI) use			<0.001	<0.001
No PI in the last 2 years	2267 (62.6)	184 (52.0)	Ref.	
PI use for 0–2 years	435 (12.0)	54 (15.2)	0.010	0.014
PI use for ≥2 years	919 (25.4)	116 (32.8)	0.001	0.002
Statin use				<0.001
Never	2753 (76.0)	111 (31.4)	Ref.	
Atorvastatin	379 (10.5)	104 (29.4)	<0.0001	<0.001
Pravastatin	312 (8.6)	82 (23.2)	<0.0001	<0.001
Pitavastatin	2 (0.1)	0 (0.00)	0.69	0.69
Others	175 (4.8)	57 (16.0)	<0.0001	<0.001
Duration of statin use			<0.0001	<0.001
No use	2753 (76.0)	111 (31.3)	Ref.	
Use for <6 months	115 (3.2)	30 (8.5)	<0.0001	<0.001
Use ≥6 months	753 (20.8)	213 (60.2)	<0.0001	<0.001

[a]*For comparisons involve multiple categories within a specific variable, the group with the largest sample size is set as the norm/reference.*

Data and Resource Availability

The retrospective datasets analyzed during this study are available from the corresponding author upon reasonable request.

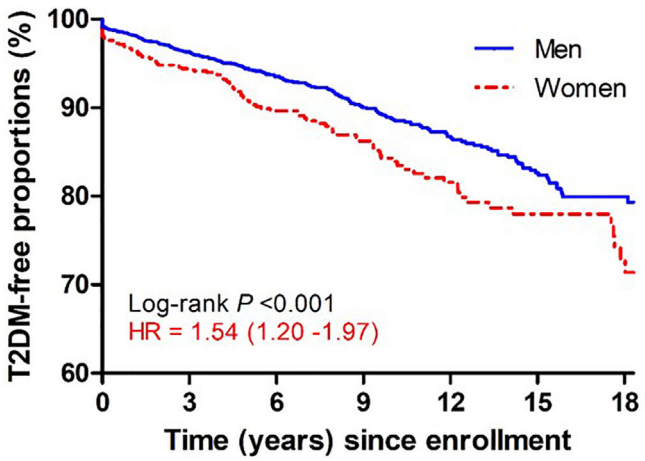
RESULTS

Overall T2DM Rates and Disparity Between Men and Women

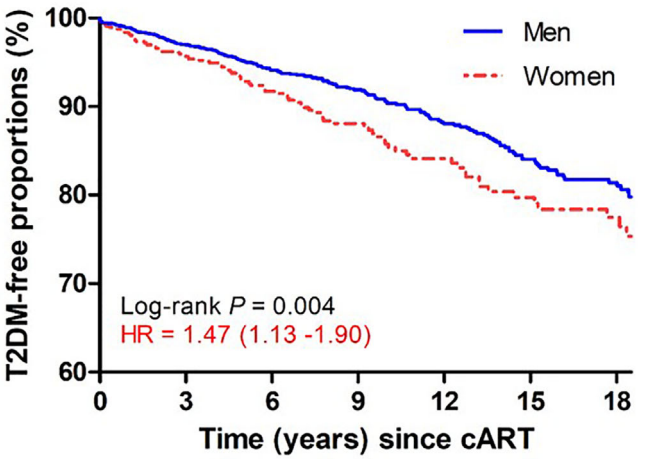
Among 3,975 subjects included in this study (**Figure S1 in Supplemental Materials**), 354 incident T2DM cases were diagnosed after a total of 26,256 PY of follow-up, with an overall rate of 135 incidents per 10,000 PY (95% CI = 121-149 per 10,000 PY), which was almost identical to pooled data from a recent meta-analysis (137 per 10,000 PY, 95% CI = 130-200) (19) but two-fold higher than the general U.S. population (67 per 10,000 PY) (20). When several subgroups were compared, the rates were similar for AA (144 cases per 10,000 PY) and EA (123 cases per 10,000 PY) (P >0.50) but clearly differed between men (122 cases per 10,000 PY) and women (179 cases per 10,000 PY) (P <0.0001). Kaplan-Meier (KM) curves (**Figure 1**) indicated that women progressed to T2DM faster (hazards ratio = 1.54; 95% CI = 1.20-1.97) than men during the study period (P <0.001). Sex-specific disparity was also evident (P = 0.004) when all follow-up time before cART initiation was disregarded (HR = 1.47, 95% CI = 1.13-1.90 for women after cART initiation) (**Figure 1**).

No Trend Over Time

When the 18-year study interval was divided into four equal periods (i.e., 4.5 years each), the rates of T2DM diagnosis were highly comparable for all pairwise comparisons (P >0.50), before and after stratification by two major racial groups (AA and EA) (**Figure S2 in Supplemental Materials**). The Joinpoint regression did not reveal any linear trend for both AA and EA (P > 0.20).



Time (years)	0	3	6	9	12	15	18
Women	887	696	361	232	149	102	50
Men	3066	2240	1264	786	476	297	137



Time (years)	0	3	6	9	12	15	18
Women	887	698	405	258	181	131	84
Men	3066	2358	1422	911	605	423	275

FIGURE 1 | Kaplan-Meier curves showing time from study enrollment or initiation of combination antiretroviral therapy (cART) to diagnosis of type 2 diabetes mellitus (T2DM) in PLWH stratified by sex. Numbers of subjects remaining at various visit intervals (between January 1999 and March 2018) are indicated for each group (excluding 22 transgender subjects). Estimates of hazards ratios (HR) use men as the reference group.

Age at T2DM Onset

The median age of T2DM diagnosis was 49.4 years (IQR: 43.2-55.6) (**Figure S3 in Supplemental Materials**), about 5 years younger than reports (median age = 54 years) for the general U.S. population (21). Subjects between 45 and 65 years old were at the highest risk for incident T2DM (**Table 1**), being consistent with the general U.S. population. The median age of T2DM was similar for AA (48.7 years, IQR = 40.7-65.3) and EA (51.2 years, IQR = 45.0-65.8) (P = 0.31) (**Figure S3 in Supplemental Materials**).

Screening for Factors Associated With T2DM Occurrence

As an initial screening step, univariate analyses (**Table 1**) revealed that T2DM cases and control subjects differed in age at study enrollment, age groups at the end of follow-up, sex, BMI, peak VL over the last 24 months of follow-up, cART regimens containing PI, statin use, and length of statin use (P <0.005 for at least one group in each variable). By contrast, the two groups were quite similar (P >0.05) in terms of their

racial composition, while nadir CD4 count over the last 24 months differed slightly for one of these three subgroups (200–500 cells/μl, $P = 0.008$) (**Table 1**).

Independent Correlates of T2DM Occurrence

In a reduced multivariable model (**Table 2**) applicable to 3,975 subjects (including 354 incident T2DM cases), sex, obesity, nadir CD count, HIV-1 viremia, and statin use were independently associated with the onset of T2DM (adjusted $P < 0.05$ for all). Individual factors with the strongest effect (adjusted OR, aOR >2.0, $P < 0.0001$) were: i) statin use for ≥6 months (aOR = 10.21), ii) BMI ≥30 kg/m^2 (aOR = 3.36), and iii) plasma VL ≥200 copies/ml (aOR = 2.24). The effects of age, sex, and PI-containing cART regimens were attenuated when other factors were accounted for (i.e., through statistical adjustments). In multicollinearity test, there was no evidence to support multicollinearity between the key predictors in the multivariable model.

When all factors independently associated with T2DM were combined, their collective predictive value was substantial: the C-statistic for ROC was 0.87 (95% CI = 0.84-0.91) (**Figure 2**), with close similarity for two major racial groups (C-statistic = 0.87 and 0.91 for AA and EA, respectively). Removing statin use from the model decreased the overall C-statistic to 0.78 (95% CI = 0.73-0.83) (**Figure 2**). In a sensitivity analysis, the C-statistics were similar between T2DM diagnosed before 2013 (when new guidelines of statin therapy were introduced) (ROC = 0.86; 95% CI = 0.83-0.90) and T2DM diagnosed since 2013 (ROC = 0.81; 95% CI = 0.78-0.84) (**Figure 2**).

Partial Collinearity Among Factors Associated With T2DM Occurrence

In pairwise correlation analyses, Spearman $|\rho|$ was <0.30 for all (**Table 3**) except for i) age group and BMI group $\rho = 0.80$, $P < 0.0001$), ii) peak VL and nadir CD4 groups over the final 24 months of follow-up ($\rho = -0.51$, $P < 0.0001$), and iii) statin use and age ($\rho = 0.40$, $P < 0.0001$). These relationships were all well expected.

Discussion

Consistent with data from a recent meta-analysis, which included 44 studies across the world (except for the Eastern Mediterranean area) (19), our findings based on a southeastern U.S. clinical cohort clearly indicate that the rates of incident T2DM have doubled in PLWH as compared with the general U.S. population. These observations appear to be generalizable, as separate analyses of two major racial groups (AA and EA) and four follow-up intervals (calendar dates) produced highly comparable summary statistics for the rates and correlates of T2DM. On the basis of a reduced multivariable model, the final estimates for ROC statistics hovered around 90% for both AA and EA subgroups, well exceeding the commonly used clinical diagnostic cut-off of 80% (22). The final algorithms for predicting incident T2DM in PLWH, which have not been attempted before, now provide promising leads for further refinements and clinical considerations.

Apart from the confirmation of several conventional risk factors (age, obesity and the length of PI-containing cART) associated with T2DM, our analyses here revealed a strong and independent impact of statin use (**Table 2**), regardless of statin categories (**Table 1**). In contrast, earlier studies only suggest a moderate risk (OR <2.0) in adults without HIV-1 infection (23).

TABLE 2 | Independent correlates of incident T2DM, as defined by a reduced multivariable model.

Factors in the final model[a]	n (%)	Adjusted OR[b]	95% CI[b]	*P*-value
Sex				
Men	3,063 (77.1)	Ref.	–	
Women	886 (22.3)	1.47	1.13-1.93	0.005
Transgenders	22 (0.55)	1.23	0.16-9.71	>0.50
BMI at incident DM or last visit (missing 364)				
<25 (kg/m^2)	1,406 (38.9)	Ref.	–	
25–29 (kg/m^2)	1,168 (32.4)	1.46	1.01-2.10	0.043
≥30 (kg/m^2)	1,033 (28.7)	3.36	2.40-4.70	<0.0001
Nadir CD4$^+$ T-cell count[a] in the last 24 months				
<200 cells/μl	931 (23.4)	1.02	0.69-1.49	>0.50
200–500 cells/μl	1,374 (34.6)	1.40	1.06-1.42	0.020
>500 cells/μl	1,666 (42.0)	Ref.	–	
Highest plasma viral load[a] in the last 24 months				
<200	2,273 (57.2)	Ref.	–	
200–23,596 (Q3)	705 (17.8)	2.24	1.64-3.05	<0.0001
>23,596	993 (25.0)	2.21	1.55-3.15	<0.0001
Duration of statin use				
No use	2,864 (72.1)	Ref	–	
Use for <6 months	145 (3.7)	8.92	5.87-13.58	<0.0001
Use ≥6 months	962 (24.3)	10.21	7.71-13.53	<0.0001

[a]*At least one entry in each categorical variable has shown independent association with T2DM (adjusted P < 0.05).*

[b]*The odds ratio (OR) and 95% confidence interval have been adjusted for all factors in the model, as well as age, race, study enrollment dates, exposure to HIV-1 protease inhibitors, and statin groups (atorvastatin, lovastatin, and simvastatin as lipophilic; pravastatin, rosuvastatin, and fluvastatin as hydrophilic). For comparisons involve multiple categories within a specific variable, the group with the largest sample size is set as the norm/reference.*

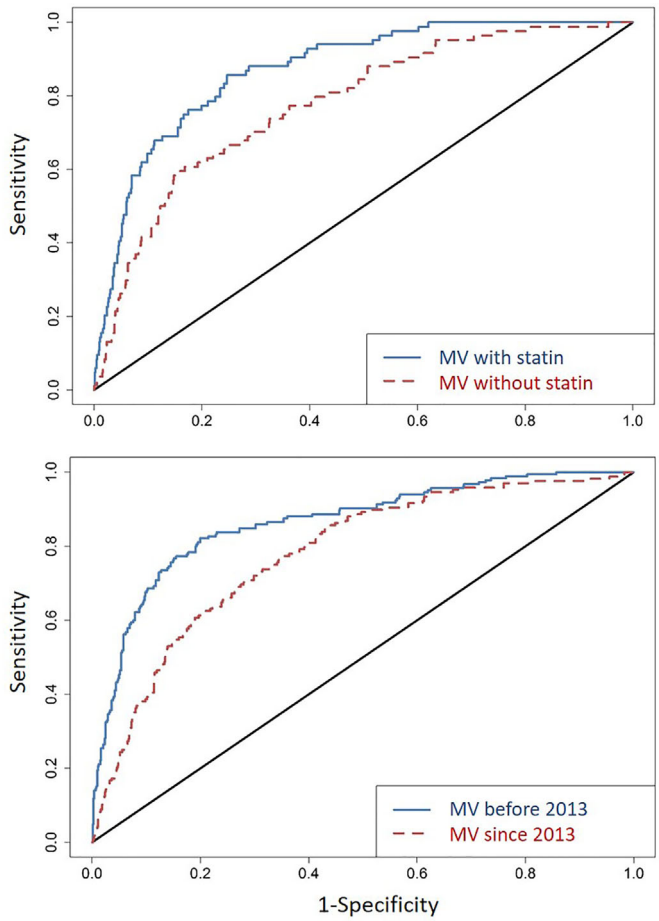


FIGURE 2 | Area under the receiver operating characteristics curves (ROCs) in several rounds of comparative analyses. First (top panel), for all factors identified as independent predictor of incident T2DM in a reduced multivariable (MV) model (**Table 2**), the C-statistic for ROC (line in blue) is 0.87 (95% CI = 0.84-0.91); removing statin use from the model (line in red) leads to an ROC of 0.78 (95% CI = 0.73-0.83). Second (bottom panel), with reference to the new guidelines of statin therapy (introduced in 2013), T2DM diagnosed before 2013 (line in blue) and since 2013 (line in red) have ROC of 0.86 (95% CI = 0.83-0.90) and 0.86 (95% CI = 0.78-0.84), respectively.

TABLE 3 | Partial correlation among factors associated with T2DM[a].

	Sex	Age	BMI	Statin use	CD4 count	HIV-1 VL
Sex	1.00	0.05	0.15	-0.02	0.03	<0.01
Age	0.001	1.00	0.80	0.40	0.07	-0.22
BMI	<0.0001	0.001	1.00	0.16	0.21	-0.21
Statin use	0.294	<0.0001	<0.0001	1.00	0.13	-0.18
CD4 count	0.053	<0.0001	<0.0001	<0.0001	1.00	-0.51
HIV-1 VL	0.862	<0.0001	<0.0001	<0.0001	<0.0001	1.00

[a]*Defined by positive or negative Spearman rho (ρ) and restricted to variables shown in* ***Table 2****. The ρ values (two decimals) for pairwise correlation are shown on and above the diagonal; the corresponding P values (at least three decimals) are listed below the diagonal.*

Statins are cost-effective and widely prescribed drugs for lowering cholesterol level and preventing CVD in the U.S., especially since 2003 (24). The U.S. National Center for Health Statistics reported that more than 26% of American adults aged 40 and above used statin in 2012 (24). In previous reports, the benefits of statins outweigh all known disease risks, including T2DM or prediabetes (25, 26). A recent systematic review suggested that the reduction of LDL-C level through upregulation of LDL receptor expression by statin therapy should not introduce any adverse effects. However, if the LDL-C reduction is not related to LDL receptors, the possible drug-induced adverse effects could counterbalance the clinical benefits

of statin therapy (27), suggesting that drug-drug interactions or effects through an alternative drug target are possible.

In 2013, an ACC/AHA Task Force introduced a comprehensive discussion on the initiation of statin therapy. In addition to LDL-C level and its corresponding age at test, history of diabetes and its diagnosis age, along with life style and family histories of ASCVD are now all taken into consideration before the initiation of statin therapy (28). According to the new guidelines for risk assessment, patients diagnosed with diabetes and LDL-C between 70–189 mg/dl at ages between 40 and 75 years are all recommended for moderate to high-intensity statin therapies (28). However, the 2013 ACC/AHA Blood Cholesterol Guideline did not consider the risk of T2DM after the initiation of statin therapy. Even with 2013 as a new milestone for prescribing statins, our statistical models for T2DM diagnosed before and since 2013 (**Figure 2**) did not reveal striking differences. Both models retained satisfactory fitting (ROC >0.8), even when the available follow-up period was still relatively short (about 5 years) after 2013.

PLWH are known to face high risks for CVD as a result of persistent immune activation, arterial inflammation, endothelial dysfunction, elevated coagulation, and various side effects of cART (29–31). Thus, statins have an increasing clinical application for managing maladaptive inflammatory cascades, and their usage is expected to gain further momentum in the PLWH population. If both lipophilic (atorvastatin, lovastatin, and simvastatin) and hydrophilic (pravastatin, rosuvastatin, and fluvastatin) statins indeed pose a clear risk for T2DM in PLWH, even during a short period of exposure (e.g., <6 months) (**Table 2**), follow-up studies may need to closely monitor the changes in glycated hemoglobin levels (32) and develop a concurrent strategy for minimizing the risk of T2DM. For example, high-dose vitamin D supplementation has shown convincing benefits for insulin sensitivity in pre-diabetic subjects (33, 34), PLWH with vitamin D deficiency and insufficiency may require timely vitamin D supplementation.

Another observation that seems to distinguish PLWH from the general population is the modest disparity in T2DM incidence between male and female PLWH (**Figure 1**). The difference is readily detected in both cross-sectional and longitudinal data, before and after statistical adjustments for other prominent factors (**Table 2**). In the general U.S. population, rates of incident T2DM are comparable for men and women, but differences in rates of pre-diabetes are discernible (20). The underlying biology is still elusive.

When HIV-1-specific variables are considered, CD4 T-cell count and viremia also show modest impact on T2DM onset (**Table 2**). As both can fluctuate over time with and without cART, our focus was on the last 24 months of follow-up when most PLWH were under effective treatment. The occasional viremia (viral blips), defined by plasma VL ≥200 RNA copies/ml, is well expected, and at least one other longitudinal study (a French HIV-1 cohort) has reported that detectable VL is associated with a modest risk for incident T2DM when compared to non-detectable VL (35). In the new era that calls for prompt HIV-1 diagnosis and linkage to care (the 90:90:90 initiative) (36), at least 27% of PLWH will continue to be viremic and thus at high risk for T2DM.

One major limitation of this study is the lack of family history for study participants. T2DM tends to run in families, mostly through shared genetic and environmental factors, as well as similarity in lifestyle (37). Some of these gaps can be closed for PLWH with active follow-up, and the addition of high-throughput genotyping, especially the use of fine-mapping platforms, should facilitate the analyses of "causal variants" previously established in the general population (38). Genomic and epigenomic datasets can also help define the underlying biological pathways for T2DM (39) or enhance predictive algorithms (40). These frontiers will remain as active research areas to further benefit precision medicine.

Overall, adequate sample size and sufficient follow-up in our study cohort have provided a valuable opportunity for illustrating the complexity of T2DM risks pertinent to contemporary PLWH population. Although replication of key findings cannot be done for additional cohorts right away, multiple sensitivity analyses and stratifications by sex, race, and various study intervals have provided promising assurance that the statistical models are robust enough to be clinically relevant. In particular, appropriate interventions for statin users are expected to bear the bulk of immediate impact on the T2DM landscape in PLWH populations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board at University of Alabama at Birmingham. All subjects provided written informed consent for participation in the study.

AUTHOR CONTRIBUTIONS

JT, SS, AB, and NE designed the study. YY and GB managed the datasets. YY, HW, and JT analyzed the data. YY and JT wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.555401/full#supplementary-material

REFERENCES

1. AIDSmap. *Life expectancy now near-normal in people with CD4 counts over 350 a year after starting therapy*. Available at: http://www.aidsmap.com/news/may-2017/life-expectancy-now-near-normal-people-cd4-counts-over-350-year-after-starting (Accessed October 12, 2020).
2. Serrao R, Pinero C, Velez J, Coutinho D, Maltez F, Lino S, et al. Non-AIDS-related comorbidities in people living with HIV-1 aged 50 years and older: The AGING POSITIVE study. *Int J Infect Dis* (2019) 79:94–100. doi: 10.1016/j.ijid.2018.10.011
3. AIDSinfo (DHHS). *Side Effects of HIV Medicines: HIV and Diabetes*. Available at: https://aidsinfo.nih.gov/understanding-hiv-aids/fact-sheets/22/59/hiv-and-diabetes (Accessed October 12, 2020).
4. American Diabetes Association. *Statistics about Diabetes*. Available at: http://www.diabetes.org/diabetes-basics/statistics/ (Accessed October 12, 2020).
5. Hertel JK, Johansson S, Sonestedt E, Jonsson A, Lie RT, Platou CG, et al. *FTO*, type 2 diabetes, and weight gain throughout adult life: a meta-analysis of 41,504 subjects from the Scandinavian HUNT, MDC, and MPP studies. *Diabetes* (2011) 60:1637–44. doi: 10.2337/db10-1340
6. Ma RC, Chan JC. Type 2 diabetes in East Asians: similarities and differences with populations in Europe and the United States. *Ann N Y Acad Sci* (2013) 1281:64–91. doi: 10.1111/nyas.12098
7. Ng MC, Shriner D, Chen BH, Li J, Chen WM, Guo X, et al. Meta-analysis of genome-wide association studies in African Americans provides insights into the genetic architecture of type 2 diabetes. *PLoS Genet* (2014) 10:e1004517. doi: 10.1371/journal.pgen.1004517
8. Reust CE. Common Adverse Effects of Antiretroviral Therapy for HIV Disease. *AFP* (2011) 83:1443–51.
9. Levitt NS, Peer N, Steyn K, Lombard C, Maartens G, Lambert EV, et al. Increased risk of dysglycaemia in South Africans with HIV; especially those on protease inhibitors. *Diabetes Res Clin Pract* (2016) 119:41–7. doi: 10.1016/j.diabres.2016.03.012
10. Maganga E, Smart LR, Kalluvya S, Kataraihya JB, Saleh AM, Obeid L, et al. Glucose Metabolism Disorders, HIV and Antiretroviral Therapy among Tanzanian Adults. *PloS One* (2015) 10:e0134410. doi: 10.1371/journal.pone.0134410
11. Rhee JY, Bahtila TD, Palmer D, Tih PM, Aberg JA, LeRoith D, et al. Prediabetes and diabetes among HIV-infected adults in Cameroon. *Diabetes Metab Res Rev* (2016) 32:544–9. doi: 10.1002/dmrr.2792
12. Mugavero MJ, Lin H-Y, Allison JJ, Giordano TP, Willig JH, Raper JL, et al. Racial disparities in HIV virologic failure: do missed visits matter? *J Acquir Immune Defic Syndr* (2009) 50:100–8. doi: 10.1097/QAI.0b013e31818d5c37
13. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* (1993) 39:561–77. doi: 10.1093/clinchem/39.4.561
14. American Diabetes Association. *Diabetes Overview: Diagnosis*. Available at: http://www.diabetes.org/diabetes-basics/diagnosis/ (Accessed October 12, 2020).
15. American Diabetes Association. *Diagnosing Diabetes and Learning about Prediabetes*. Available at: http://www.diabetes.org/diabetes-basics/diagnosis/ (Accessed October 9, 2020).
16. Benjamin DJ, Berger JO, Johannesson M, Nosek BA, Wagenmakers EJ, Berk R, et al. Redefine statistical significance. *Nat Hum Behav* (2018) 2:6–10. doi: 10.1038/s41562-017-0189-z
17. Ye Y, Burkholder GA, Wiener H, Griffin R, Aslibekyan S, Fry K, et al. Comorbidities associated with HPV infection among people living with HIV-1 in the southeastern US: a retrospective clinical cohort study. *BMC Infect Dis* (2020) 20:144. doi: 10.1186/s12879-020-4822-5
18. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. *Stat Med* (2000) 19:335–51. doi: 10.1002/(SICI)1097-0258(20000215)19:3<335::AID-SIM336>3.0.CO;2-Z
19. Nansseu JR, Bigna JJ, Kaze AD, Noubiap JJ. Incidence and Risk Factors for Prediabetes and Diabetes Mellitus among HIV-infected Adults on Antiretroviral Therapy: A Systematic Review and Meta-analysis. *Epidemiology* (2018) 29:431–41. doi: 10.1097/EDE.0000000000000815
20. National Center for Chronic Disease Prevention and Health Promotion. *National Diabetes Statistics Report*. Available at: http://www.diabetes.org/assets/pdfs/basics/cdc-statistics-report-2017.pdf (Accessed October 12, 2020).
21. Centers for Disease Control and Prevention (CDC). *Mean and median age at diagnosis of diabetes among adult incident cases aged 18–79 years* (1997-2011). United States. Available at: https://www.cdc.gov/diabetes/data/statistics/statistics-report.html (Accessed October 12, 2020).
22. Draelos R. *Measuring Performance: AUC (AUROC)*. Available at: https://glassboxmedicine.com/2019/02/23/measuring-performance-auc-auroc/ (Accessed October 11, 2020).
23. Crandall JP, Mather K, Rajpathak SN, Goldberg RB, Watson K, Foo S, et al. Statin use and risk of developing diabetes: results from the Diabetes Prevention Program. *BMJ Open Diabetes Res Care* (2017) 5:e000438. doi: 10.1136/bmjdrc-2017-000438
24. Gu Q, Paulose-Ram R, Burt VL, Kit BK. Prescription cholesterol-lowering medication use in adults aged 40 and over: United States, 2003-2012. *NCHS Data Brief* (2014) 177:1–8.
25. Maki KC, Ridker PM, Brown WV, Grundy SM, Sattar N. The Diabetes Subpanel of the National Lipid Association Expert P. An assessment by the Statin Diabetes Safety Task Force: 2014 update. *J Clin Lipidol* (2014) 8(3 Suppl):S17–29. doi: 10.1016/j.jacl.2014.02.012
26. Zigmont VA, Shoben AB, Lu B, Kaye GL, Clinton SK, Harris RE, et al. Statin users have an elevated risk of dysglycemia and new-onset-diabetes. *Diabetes/Metab Res Rev* (2019) 35:e3189. doi: 10.1002/dmrr.3189
27. Silverman MG, Ference BA, Im K, Wiviott SD, Giugliano RP, Grundy SM, et al. Association Between Lowering LDL-C and Cardiovascular Risk Reduction Among Different Therapeutic Interventions: A Systematic Review and Meta-analysis. *JAMA* (2016) 316:1289–97. doi: 10.1001/jama.2016.13985
28. Stone NJ, Robinson JG, Lichtenstein AH, Noel Bairey Merz C, Blum CB, Eckel RH, et al. 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults. *Circulation* (2014) 129(25 suppl 2):S1–45. doi: 10.1161/01.cir.0000437738.63853.7a
29. Freiberg MS, Chang C-CH, Kuller LH, Skanderson M, Lowy E , Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. *JAMA Intern Med* (2013) 173(8):614–22. doi: 10.1001/jamainternmed.2013.3728
30. Beltrán LM, Rubio-Navarro A, Amaro-Villalobos JM, Egido J, García-Puig J, Moreno JA. Influence of immune activation and inflammatory response on cardiovascular risk associated with the human immunodeficiency virus. *Vasc Health Risk Manag* (2015) 11:35–48. doi: 10.2147/VHRM.S65885
31. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab* (2007) 92:2506–12. doi: 10.1210/jc.2006-2190
32. Ridker PM, Danielson E, Fonseca FAH, Genest J, Gotto AM Jr., Kastelein JJP, et al. Rosuvastatin to prevent vascular events in men and women with elevated

C-reactive protein. *N Engl J Med* (2008) 359:2195–207. doi: 10.1056/NEJMoa0807646
33. Gagnon C, Daly RM, Carpentier A, Lu ZX, Shore-Lorenti C, Sikaris K, et al. Effects of Combined Calcium and Vitamin D Supplementation on Insulin Secretion, Insulin Sensitivity and β-Cell Function in Multi-Ethnic Vitamin D-Deficient Adults at Risk for Type 2 Diabetes: A Pilot Randomized, Placebo-Controlled Trial. *PloS One* (2014) 9:e109607. doi: 10.1371/journal.pone.0109607
34. Lemieux P, Weisnagel JS, Caron AZ, Julien A-S, Morisset A-S, Carreau A-M, et al. Effects of 6-month vitamin D supplementation on insulin sensitivity and secretion: a randomized, placebo-controlled trial. *Eur J Endocrinol* (2019) 181:287–99. doi: 10.1530/EJE-19-0156
35. Provoost A, Drame M, Cotte L, Cuzin L, Garraffo R, Rey D, et al. Risk of diabetes in HIV-infected patients is associated with cirrhosis but not with chronic HCV coinfection in a French nationwide HIV cohort. *Aliment Pharmacol Ther* (2018) 48:281–9. doi: 10.1111/apt.14812
36. UNAIDS. *90-90-90: Treatment for All*. Available at: https://www.unaids.org/en/resources/909090 (Accessed October 9, 2020).
37. American Diabetes Association. *Learn the Genetics of Diabetes*. Available at: http://www.diabetes.org/are-you-at-risk/lower-your-risk/nonmodifiables.html (Accessed October 9, 2020).
38. Farh KK, Marson A, Zhu J, Kleinewietfeld M, Housley WJ, Beik S, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* (2015) 518:337–43. doi: 10.1038/nature13835
39. Palomaki GE, Melillo S, Marrone M, Douglas MP. Use of genomic panels to determine risk of developing type 2 diabetes in the general population: a targeted evidence-based review. *Genet Med* (2013) 15:600–11. doi: 10.1038/gim.2013.8
40. Hivert MF, Vassy JL, Meigs JB. Susceptibility to type 2 diabetes mellitus–from genes to prevention. *Nat Rev Endocrinol* (2014) 10:198–205. doi: 10.1038/nrendo.2014.11

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Prevalence and Risk Factors of Sensory Symptoms in Diabetes Patients in Taiwan

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Background: Diabetic sensory neuropathy has rarely been studied in the Asian populations. This study investigated the prevalence and risk factors of sensory symptoms (SS) in the Taiwanese diabetes patients.

Methods: A total of 1,400 diabetes patients received a health examination together with a structured questionnaire interview for three categories of abnormal sensation of numbness or tingling pain, electric shock, and skin thickness sensation on seven anatomical sites on upper limbs and six sites on lower limbs. Prevalence of SS was defined using nine different criteria, with the least stringent criterion of "any positive symptom on at least 1 site" and the most stringent criterion of "any positive symptom on at least bilateral and symmetrical 2 sites involving the lower limb." Logistic regression was used to estimate the odds ratios and their 95% confidence interval for SS by the different definitions. Fasting plasma glucose and hemoglobin A_{1c} were entered in separate models to avoid hypercollinearity.

Results: The prevalence of SS was 14.4 and 54.0% when using the most stringent and least stringent criterion, respectively. Women consistently had a significantly higher prevalence than men did. Among the three categories of symptoms, numbness or tingling pain was the most common, and fingers and toes were the most commonly involved anatomical sites. For any symptoms, 37.1% of the patients had any symptoms on the upper limbs and 41.7% had any symptoms on the lower limbs. Female sex, diabetes duration, hemoglobin A_{1c}, and hypertension were associated with SS in all models.

Conclusions: Taiwanese diabetes patients may have a high prevalence of SS if a structured questionnaire is used for screening. Female sex, diabetes duration, hemoglobin A_{1c}, and hypertension are associated with SS.

Keywords: diabetes mellitus, sensory symptoms, epidemiology, risk factors, Taiwan

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INTRODUCTION

Diabetes mellitus is a chronic non-communicable disease characterized by various vascular complications involving the arterial system (macrovascular complications) and the capillaries (microvascular complications). "Diabetes triopathy" has been used to refer to the microvascular complications including retinopathy, nephropathy, and neuropathy (1). Diabetic polyneuropathy (DPN) may involve the sensory, motor, and/or autonomic nervous system. Sensory neuropathy can be divided clinically into painful and non-painful subtypes (2, 3). Painful diabetic peripheral neuropathy (pDPN) may impair the patients' mood, daily function, sleep, and quality of life; and its humanistic and economic burdens are immense (4, 5).

The prevalence of pDPN varies from 6 to 34% in different reports by using different diagnostic criteria in Europe (4). A recent phone/internet survey conducted in the USA showed an ethnic difference in the prevalence of pDPN: 49% in Hispanics, 65% in African-Americans, and 87% in Caucasians ($P < 0.05$) (6).

Epidemiological studies on DPN are rare in Asian populations. In Taipei City of Taiwan, among 217 diabetes patients derived from an epidemiological survey in 1978, 44 patients (20.3%) were diagnosed as having DPN which was defined as a motor nerve conduction velocity below the normal mean minus 2 standard deviations (1). While comparing patients with and without DPN in that study, only diabetes duration, fasting glucose level, insulin use, and blood urea nitrogen (but not creatinine level) differed significantly between the two groups (1). A nationwide hospital-based study conducted in Korea suggested a prevalence of 14.4% for pDPN in patients with type 2 diabetes mellitus (7). This Korean study identified age, female sex, glycemic control, hypertension, and previous cardiovascular events as risk factors of pDPN (7). In Myanmar, a study conducted in 975 diabetes patients who attended the outpatient clinics of four hospitals reported a prevalence rate of 33.7% for DPN and 59.5% for pDPN (8). The investigators found an association between DPN and older age, longer diabetes duration, and smoking (8). In a cross-sectional study that aimed at investigating the prevalence of diabetic retinopathy in 1,008 diabetes patients enrolled from a hospital in Shijiazhuang, Hebei, China, the investigators reported a prevalence of 11% for DPN among the patients and found a close association between DPN and diabetic retinopathy (9). Another recently published cross-sectional study that enrolled patients from 17 primary care clinics across Japan showed a prevalence of 27.7% for DPN among 9,914 patients surveyed (10). Among the patients with DPN, 61.5% had DPN-related sensory symptoms/signs, which were significantly associated with female sex, smoking, and alcohol drinking (10).

Under-diagnosis is very common for pDPN in clinical practice (11). A USA study showed that while 83% of diabetes patients might report symptoms of pDNP at study, only 41% of them had ever been diagnosed as having DPN by healthcare practitioners (11). Another Japanese study showed that although 22.1% of the 298 studied patients might have pDPN, only 36.4% of them were recognized by the physicians (12). A recent multinational survey conducted in five countries in South-East Asia indicated that there might be significant gaps between physicians and patients in the perception of pDPN (13). The investigators advocated physician-patient dialogue to maximize patient outcomes (13).

Because reports on diabetic sensory neuropathy are rare and its true prevalence requires careful and systematic evaluation, the present study aimed at investigating the prevalence and risk factors of sensory symptoms (SS) in a representative cohort of diabetes patients derived from an epidemiological study in Taiwan.

MATERIALS AND METHODS

Study Subjects

The study was approved by an ethics committee of the Department of Health of Taiwan (DOH89-TD-1035) and the subjects participated in the study voluntarily. The participants were informed of the purpose of the study and the funding of the study by the Department of Health of Taiwan. They were able to opt out of the study according to their free will. At the time of questionnaire interview and blood sampling, signed inform consent was not required according to local regulations. More than 96% of the Taiwanese population is covered by a universal and compulsory National Health Insurance at the time of the study. A total of 256,036 diabetes patients using this health insurance were assembled from 1995 to 1998 to investigate a series of epidemiologic issues (14). Baseline data on the onset symptoms and confirmation of diabetes diagnosis were collected by a questionnaire from 93,484 patients (15). At random, 4,164 patients living in the Northern Region of Taiwan from the main cluster of 93,484 patients were selected and invited to participate in a health examination. From March 1998 to September 2002, a total of 1,441 patients participated in the health examination. No significant differences in age or sex were noted among the main national sample and those who participated in the health examination (16, 17).

Questionnaire Interview for Sensory Symptoms

For those who participated in the health examination, a structured questionnaire (**Supplementary File**) was interviewed by a well-trained interviewer for the symptoms of three categories of sensory abnormalities: 1) numbness or tingling pain; 2) electric shock; and 3) skin thickness. Seven sites on each upper limb (i.e., fingertip, other parts of the finger, palm, dorsum of hand, wrist, lower arm, and upper arm) and six sites on each lower limb (toe tip, other parts of the toe, plantar surface of foot, dorsum of foot, lower leg, and thigh) were recorded for the respective symptoms.

Measurements of Covariates

Age, sex, diabetes duration, fasting plasma glucose/hemoglobin A_{1c} (A1C), smoking, obesity, hypertension, dyslipidemia, proteinuria, and use of insulin were treated as covariates.

Diabetes duration was defined as the time period in years between the time of receiving health examination and the time

when diabetes was diagnosed. Patients who smoked one or more cigarettes per day were defined as smokers.

Anthropometric factors including body height, body weight, and waist circumference were measured as described in detail previously (18, 19). Body mass index was calculated as body weight in kg divided by the square of body height in meters. Obesity was defined as a body mass index ≥25 kg/m^2 (20), and/or a waist circumference ≥90 cm for men or ≥80 cm for women.

A mercury sphygmomanometer was used to measure blood pressure on the right arm after 20 min rest in a sitting position. Definition of hypertension was based on one of the following three criteria: 1) being under treatment with antihypertensive drugs; 2) having systolic blood pressure ≥140 mmHg; or 3) having diastolic blood pressure ≥90 mmHg.

Subjects were instructed to avoid any vigorous physical activities one day before attending the health examination, to prevent any undue influence on the urinary excretion of albumin. In the early morning of the date of health examination, urine and blood samples were collected after fasting for a minimum duration of 12 h. First voided mid-stream urine was collected and then venous blood sample was taken. Urinary albumin concentration was measured by a particle-enhanced turbidimetric immunoassay (Biolatex®, Logroño, Spain) (21, 22) and urinary creatinine concentration was measured after dilution (×10) on an automated chemistry analyzer (Cobas Mira S, Roche Diagnostica, Basel, Switzerland) with reagents obtained from Randox Laboratories Ltd. (Antrim, UK). Proteinuria was defined by an albumin-to-creatinine ratio ≥300 μg/mg. Fasting plasma glucose and serum lipid profiles were measured by an automatic biochemistry analyzer (Cobas Mira S, Roche Diagnostica, Basel, Switzerland) with reagents obtained from Randox Laboratories Ltd. (Antrim, UK). A1C was measured by means of boronate affinity chromatography with reagents obtained from the Primus Corporation (Primus CLC385, Kansas City, MO, USA). Dyslipidemia was defined as a triglyceride level ≥1.7 mmol/L and/or low-density lipoprotein cholesterol ≥2.59 mmol/L and/or high-density lipoprotein cholesterol <0.9 mmol/L for men or <1.0 mmol/L for women, and/or those undergoing treatment for lipid disorder.

Statistical Analyses

Analyses were conducted using SAS statistical software, version 9.4 (SAS Institute, Cary, NC, USA). *P*-value <0.05 was considered statistically significant.

The distributions of the three categories of sensory abnormalities (i.e., numbness or tingling pain, electric shock, and skin thickness) and any symptom by sites on upper and lower limbs were first tabulated. SS was defined by nine different criteria with positive symptom: 1) any positive symptom on at least one site; 2) any positive symptom on at least one site involving the lower limb; 3) any positive symptom on at least two sites with at least one involving the lower limb; 4) any positive symptom on at least three sites with at least one involving the lower limb; 5) any positive symptom on at least four sites with at least one involving the lower limb; 6) any positive symptom on at least two sites with at least two involving the lower limb; 7) any positive symptom on at least three sites with at least two involving the lower limb; 8) any positive symptom on at least four sites with at least two involving the lower limb; and 9) any positive symptom on at least bilateral and symmetrical two sites involving the lower limb.

The prevalence of SS according to the different criteria was then calculated for all patients and for men and women, respectively. Chi square test was used to compare the difference of SS prevalence between men and women.

To identify potential risk factors of SS, logistic regression models were created to estimate the odds ratios and their 95% confidence intervals. SS defined by different criteria was treated as a dependent variable, and all covariates including age, sex, diabetes duration, fasting plasma glucose/A1C, smoking, obesity, hypertension, dyslipidemia, proteinuria, and use of insulin were treated as independent variables. Because fasting plasma glucose and A1C were highly correlated, separate models were created for these two covariates.

RESULTS

A total of 1,400 patients received questionnaire interview for the sensory symptoms. Among them, 1,395 patients received health examination and had complete data of the measured covariates.

Table 1 shows the distribution of the three categories of sensory abnormalities by sites on the upper limbs and lower limbs, respectively. The most common complaint was numbness or tingling pain, which involved mainly the fingers or toes. For any symptoms, 37.1% of the patients had any symptoms on the upper limbs and 41.7% had any symptoms on the lower limbs.

Table 2 shows the prevalence of SS by using the nine different definitions. If any positive symptom on any one site was defined as SS, then 54.0% of the patients would have SS. If the most stringent definition was applied (i.e., any positive symptom on at least bilateral and symmetrical two sites involving the lower limb), then 14.4% of the patients would have SS. When comparing the prevalence between men and women, it is evident that a significantly higher prevalence was observed in women disregarding the definitions used.

Tables 3 and **4** show the odds ratios and their 95% confidence intervals in models using the nine different definitions of SS. **Table 3** shows the models created with fasting plasma glucose and **Table 4** shows the models created with A1C. Female sex, diabetes duration, A1C, and hypertension were the four variables that were significantly associated with SS in all models. Fasting plasma glucose was not as good as A1C in the association with SS. Smoking, obesity, and dyslipidemia were not associated with SS in all models. Except for model II in **Table 4**, age was not associated with SS. Other covariates including proteinuria and use of insulin were not consistently associated with SS.

DISCUSSION

This is the first population-based observational study evaluating the sensory abnormalities in Taiwanese diabetes patients. The prevalence of 14.4% by using the most stringent criterion in the

TABLE 1 | Distribution of positive sensory symptoms by sites on upper and lower limbs in 1,400 diabetes patients.

Limb/Site	Numbness or tingling pain				Electric shock				Skin thickness				Any symptoms			
	No		Yes		No		Yes		No		Yes		No		Yes	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Upper limb																
Finger tip	1,012	72.29	388	27.7	1,323	94.50	77	5.5	1,352	96.57	48	3.4	979	69.93	421	30.1
Other parts of finger	1,016	72.57	384	27.4	1,336	95.43	64	4.6	1,353	96.64	47	3.4	987	70.50	413	29.5
Palm	1,177	84.07	223	15.9	1,360	97.14	40	2.9	1,366	97.57	34	2.4	1,146	81.86	254	18.1
Dorsum of hand	1,307	93.36	93	6.6	1,377	98.36	23	1.6	1,388	99.14	12	0.9	1,287	91.93	113	8.1
Wrist	1,342	95.86	58	4.1	1,380	98.57	20	1.4	1,386	99.00	14	1.0	1,320	94.29	80	5.7
Lower arm	1,361	97.21	39	2.8	1,383	98.79	17	1.2	1,389	99.21	11	0.8	1,345	96.07	55	3.9
Upper arm	1,350	96.43	50	3.6	1,378	98.43	22	1.6	1,386	99.00	14	1.0	1,333	95.21	67	4.8
Any site	916	65.43	484	34.6	1,305	93.21	95	6.8	1,331	95.07	69	4.9	881	62.93	519	37.1
Lower limb																
Toe tip	1,049	74.93	351	25.1	1,331	95.07	69	4.9	1,349	96.36	51	3.6	1,011	72.21	389	27.8
Other parts of toe	1,027	73.36	373	26.6	1,334	95.29	66	4.7	1,318	94.14	82	5.9	972	69.43	428	30.6
Plantar surface of foot	1,110	79.29	290	20.7	1,342	95.86	58	4.1	1,303	93.07	97	6.9	1,036	74.00	364	26.0
Dorsum of foot	1,264	90.29	136	9.7	1,361	97.21	39	2.8	1,371	97.93	29	2.1	1,231	87.93	169	12.1
Lower leg	1,260	90.00	140	10.0	1,356	96.86	44	3.1	1,380	98.57	20	1.4	1,227	87.64	173	12.4
Thigh	1,336	95.43	64	4.6	1,371	97.93	29	2.1	1,384	98.86	16	1.1	1,314	93.86	86	6.1
Any site	903	64.50	497	35.5	1,286	91.86	114	8.1	1,255	89.64	145	10.4	816	58.29	584	41.7

present study was lower than the reported 20.3% in our early study conducted in 1978 in Taipei City by using a definition of a slowed motor nerve conduction velocity (1). However, this prevalence rate was the same as that reported in the nationwide hospital-based survey conducted in Korea (7). The identified independent risk factors of female sex and hypertension in the present study (**Tables 3** and **4**) were also observed in the Korean study (7). The present study suggested that diabetes duration was significantly associated with SS after multivariate adjustment (**Tables 3** and **4**). On the contrary, the Korean study showed that age but not diabetes duration was significantly associated with pDPN (7). Glycemic control, especially when indicated by A1C, was significantly associated with SS in the present study (**Tables 3** and **4**), but the Korean study suggested that fasting plasma glucose was better associated with pDPN than A1C (7). The higher risk associated with the use of insulin in our previous study (1) and in the present cross-sectional study in some models (**Tables 3** and **4**) might just indicate a poor glycemic control in patients who required insulin for treatment rather than a true cause-effect relationship.

Sex differences in sensory perception have long been recognized either in animals or humans (23–25). Females are more sensitive to pain than males (23–25). The real mechanisms for such a sex discrepancy in sensory perception remain to be explored, but biological, sociocultural, and psychological factors may play some roles (24, 25). Recent studies suggested a role of estrogen in the regulation of pain processing pathway with the involvement of the adaptive immune system (22, 26, 27). It is not known whether the higher prevalence of SS in women than in men was due to sex difference in the thresholds of sensory perception. A female preponderance of pDPN was also observed in the Korean study (7) and the Japanese study (10). Animal *in vitro* and *in vivo* studies suggested that estradiol may upregulate the nocisensor transient receptor potential (TRP) vanilloid 1 receptor in sensory neurons, resulting in lowered thresholds of sensation (28). Animal studies also supported that female sex may experience greater pain related to inflammation, because of the upregulation of TRP vanilloid 1, TRP ankyrin 1, and TRP melastatin 8 by prolactin; and such effects are more

TABLE 2 | Prevalence rates of sensory symptoms by using different definitions in different sexes.

Definition	All patients		Men		Women		P-value*
	n/N	PR (%)	n/N	PR (%)	n/N	PR (%)	
Any positive symptom on at least 1 site	756/1,400	54.0	339/672	50.5	417/728	57.3	0.0104
Any positive symptom on at least 1 site involving the lower limb	584/1,400	41.7	258/672	38.4	326/728	44.8	0.0155
Any positive symptom on at least 2 sites with at least one involving the lower limb	498/1,400	35.6	221/672	32.9	277/728	38.1	0.0438
Any positive symptom on at least 3 sites with at least one involving the lower limb	422/1,400	30.1	184/672	27.4	238/728	32.7	0.0305
Any positive symptom on at least 4 sites with at least one involving the lower limb	346/1,400	24.7	146/672	21.7	200/728	27.5	0.0128
Any positive symptom on at least 2 sites with at least two involving the lower limb	447/1,400	31.9	193/672	28.7	254/728	34.9	0.0134
Any positive symptom on at least 3 sites with at least two involving the lower limb	390/1,400	27.9	165/672	24.6	225/728	30.9	0.0081
Any positive symptom on at least 4 sites with at least two involving the lower limb	333/1,400	23.8	139/672	20.7	194/728	26.7	0.0088
Any positive symptom on at least bilateral and symmetrical 2 sites involving the lower limb	202/1,400	14.4	82/672	12.2	120/728	16.5	0.0228

n, cases with symptoms; N, cases interviewed; PR, prevalence rate.

**Chi-square test comparing the prevalence in men and women.*

TABLE 3 | Logistic regression evaluating risk factors of sensory symptoms according to different definitions (models with fasting plasma glucose).

Risk factor	Interpretation	n/N	Odds ratio	95% Confidence interval	*P*-value
I. Any positive symptom on at least 1 site					
Age	Every 1-yr increment	753/1,395	1.000	(0.990–1.011)	0.9431
Sex	Men *vs.* women	338/669; 415/726	0.701	(0.528–0.932)	0.0143
Diabetes duration	Every 1-yr increment	753/1,395	1.026	(1.010–1.042)	0.0011
Fasting glucose	Every 1-mg/dl increment	753/1,395	1.002	(0.999–1.003)	0.0694
Smoking	Yes *vs.* no	262/492; 491/903	1.280	(0.956–1.716)	0.0977
Obesity	Yes *vs.* no	535/966; 218/429	1.070	(0.841–1.361)	0.5811
Hypertension	Yes *vs.* no	468/821; 285/574	1.259	(1.001–1.583)	0.0495
Dyslipidemia	Yes *vs.* no	489/886; 264/509	0.824	(0.606–1.121)	0.2185
Proteinuria	Yes *vs.* no	97/148; 656/1,247	1.512	(1.048–2.181)	0.0269
Use of insulin	Yes *vs.* no	126/196; 627/1,199	1.354	(0.970–1.889)	0.0746
II. Any positive symptom on at least 1 site involving the lower limb					
Age	Every 1-yr increment	581/1,395	1.010	(0.999–1.021)	0.0542
Sex	Men *vs.* women	257/669; 324/726	0.687	(0.512–0.922)	0.0123
Diabetes duration	Every 1-yr increment	581/1,395	1.024	(1.008–1.039)	0.0024
Fasting glucose	Every 1-mg/dl increment	581/1,395	1.002	(0.999–1.003)	0.0723
Smoking	Yes *vs.* no	203/492; 378/903	1.319	(0.975–1.785)	0.0726
Obesity	Yes *vs.* no	406/966; 175/429	0.895	(0.700–1.145)	0.3784
Hypertension	Yes *vs.* no	371/821; 210/574	1.290	(1.021–1.631)	0.0330
Dyslipidemia	Yes *vs.* no	381/886; 200/509	0.837	(0.614–1.140)	0.2578
Proteinuria	Yes *vs.* no	83/148; 498/1,247	1.731	(1.214–2.470)	0.0025
Use of insulin	Yes *vs.* no	104/196; 477/1,199	1.478	(1.067–2.047)	0.0187
III. Any positive symptom on at least 2 sites with at least one involving the lower limb					
Age	Every 1-yr increment	495/1,395	1.008	(0.998–1.019)	0.1276
Sex	Men *vs.* women	220/669; 275/726	0.711	(0.525–0.962)	0.0271
Diabetes duration	Every 1-yr increment	495/1,395	1.028	(1.013–1.044)	0.0004
Fasting glucose	Every 1-mg/dl increment	495/1,395	1.002	(0.999–1.004)	0.0512
Smoking	Yes *vs.* no	174/492; 321/903	1.308	(0.958–1.787)	0.0913
Obesity	Yes *vs.* no	340/966; 155/429	0.828	(0.644–1.065)	0.1419
Hypertension	Yes *vs.* no	317/821; 178/574	1.296	(1.018–1.651)	0.0352
Dyslipidemia	Yes *vs.* no	328/886; 167/509	1.002	(0.727–1.382)	0.9893
Proteinuria	Yes *vs.* no	66/148; 429/1,247	1.355	(0.949–1.936)	0.0950
Use of insulin	Yes *vs.* no	89/196; 406/1,199	1.393	(1.004–1.934)	0.0473
IV. Any positive symptom on at least 3 sites with at least one involving the lower limb					
Age	Every 1-yr increment	419/1,395	1.006	(0.995–1.018)	0.2922
Sex	Men *vs.* women	183/669; 236/726	0.699	(0.509–0.961)	0.0273
Diabetes duration	Every 1-yr increment	419/1,395	1.030	(1.014–1.046)	0.0003
Fasting glucose	Every 1-mg/dl increment	419/1,395	1.002	(0.999–1.004)	0.0521
Smoking	Yes *vs.* no	145/492; 274/903	1.278	(0.921–1.773)	0.1417
Obesity	Yes *vs.* no	288/966; 131/429	0.824	(0.634–1.072)	0.1494
Hypertension	Yes *vs.* no	275/821; 144/574	1.403	(1.089–1.809)	0.0088
Dyslipidemia	Yes *vs.* no	284/886; 135/509	1.077	(0.767–1.512)	0.6681
Proteinuria	Yes *vs.* no	57/148; 362/1,247	1.331	(0.924–1.917)	0.1246
Use of insulin	Yes *vs.* no	73/196; 346/1,199	1.229	(0.876–1.725)	0.2327
V. Any positive symptom on at least 4 sites with at least one involving the lower limb					
Age	Every 1-yr increment	344/1,395	1.006	(0.994–1.019)	0.3085
Sex	Men *vs.* women	145/669; 199/726	0.693	(0.493–0.973)	0.0343
Diabetes duration	Every 1-yr increment	344/1,395	1.034	(1.017–1.052)	<0.0001
Fasting glucose	Every 1-mg/dl increment	344/1,395	1.002	(1.000–1.004)	0.0396
Smoking	Yes *vs.* no	114/492; 230/903	1.190	(0.837–1.691)	0.3327
Obesity	Yes *vs.* no	240/966; 104/429	0.870	(0.657–1.152)	0.3318
Hypertension	Yes *vs.* no	227/821; 117/574	1.353	(1.032–1.774)	0.0286
Dyslipidemia	Yes *vs.* no	241/886; 103/509	1.084	(0.753–1.560)	0.6648
Proteinuria	Yes *vs.* no	51/148; 293/1,247	1.480	(1.016–2.156)	0.0412
Use of insulin	Yes *vs.* no	62/196; 282/1,199	1.480	(0.848–1.724)	0.2951
VI. Any positive symptom on at least 2 sites with at least two involving the lower limb					
Age	Every 1-yr increment	444/1,395	1.006	(0.995–1.017)	0.2767
Sex	Men *vs.* women	192/669; 252/726	0.717	(0.525–0.980)	0.0367
Diabetes duration	Every 1-yr increment	444/1,395	1.028	(1.012–1.044)	0.0005
Fasting glucose	Every 1-mg/dl increment	444/1,395	1.003	(1.001–1.005)	0.0031
Smoking	Yes *vs.* no	150/492; 294/903	1.193	(0.865–1.646)	0.2825
Obesity	Yes *vs.* no	310/966; 134/429	0.893	(0.689–1.158)	0.2825

(Continued)

TABLE 3 | Continued

Risk factor	Interpretation	n/N	Odds ratio	95% Confidence interval	*P*-value
Hypertension	Yes *vs.* no	289/821; 155/574	1.350	(1.052–1.732)	0.0184
Dyslipidemia	Yes *vs.* no	294/886; 150/509	0.956	(0.688–1.330)	0.7914
Proteinuria	Yes *vs.* no	62/148; 382/1,247	1.416	(0.988–2.030)	0.0585
Use of insulin	Yes *vs.* no	79/196; 365/1,199	1.284	(0.920–1.794)	0.1420
VII. Any positive symptom on at least 3 sites with at least two involving the lower limb					
Age	Every 1-yr increment	387/1,395	1.005	(0.993–1.017)	0.3975
Sex	Men *vs.* women	164/669; 223/726	0.715	(0.517–0.989)	0.0427
Diabetes duration	Every 1-yr increment	387/1,395	1.031	(1.014–1.047)	0.0003
Fasting glucose	Every 1-mg/dl increment	387/1,395	1.003	(1.001–1.005)	0.0070
Smoking	Yes *vs.* no	127/492; 260/903	1.131	(0.808–1.583)	0.4738
Obesity	Yes *vs.* no	270/966; 117/429	0.879	(0.671–1.152)	0.3505
Hypertension	Yes *vs.* no	255/821; 132/574	1.388	(1.070–1.801)	0.0135
Dyslipidemia	Yes *vs.* no	263/886; 124/509	1.038	(0.734–1.470)	0.8315
Proteinuria	Yes *vs.* no	54/148; 333/1,247	1.357	(0.937–1.964)	0.1060
Use of insulin	Yes *vs.* no	68/196; 319/1,199	1.199	(0.849–1.693)	0.3037
VIII. Any positive symptom on at least 4 sites with at least two involving the lower limb					
Age	Every 1-yr increment	331/1,395	1.006	(0.993–1.018)	0.3612
Sex	Men *vs.* women	138/669; 193/726	0.692	(0.491–0.977)	0.0362
Diabetes duration	Every 1-yr increment	331/1,395	1.033	(1.016–1.051)	0.0001
Fasting glucose	Every 1-mg/dl increment	331/1,395	1.002	(1.000–1.004)	0.0188
Smoking	Yes *vs.* no	108/492; 223/903	1.154	(0.808–1.648)	0.4315
Obesity	Yes *vs.* no	231/966; 100/429	0.868	(0.652–1.153)	0.3282
Hypertension	Yes *vs.* no	218/821; 113/574	1.334	(1.014–1.756)	0.0395
Dyslipidemia	Yes *vs.* no	232/886; 99/509	1.085	(0.749–1.570)	0.6662
Proteinuria	Yes *vs.* no	50/148; 281/1,247	1.515	(1.038–2.213)	0.0315
Use of insulin	Yes *vs.* no	61/196; 270/1,199	1.250	(0.875–1.787)	0.2202
IX. Any positive symptom on at least bilateral and symmetrical 2 sites involving the lower limb					
Age	Every 1-yr increment	201/1,395	1.006	(0.990–1.021)	0.4602
Sex	Men *vs.* women	81/669; 120/726	0.565	(0.365–0.875)	0.0105
Diabetes duration	Every 1-yr increment	201/1,395	1.049	(1.028–1.070)	<0.0001
Fasting glucose	Every 1-mg/dl increment	201/1,395	1.002	(0.999–1.004)	0.0908
Smoking	Yes *vs.* no	69/492; 132/903	1.500	(0.959–2.347)	0.0759
Obesity	Yes *vs.* no	137/966; 64/429	0.746	(0.529–1.053)	0.0956
Hypertension	Yes *vs.* no	142/821; 59/574	1.654	(1.171–2.336)	0.0043
Dyslipidemia	Yes *vs.* no	146/886; 55/509	1.473	(0.900–2.413)	0.1236
Proteinuria	Yes *vs.* no	34/148; 167/1,247	1.582	(1.025–2.443)	0.0385
Use of insulin	Yes *vs.* no	38/196; 163/1,199	1.139	(0.744–1.741)	0.5494

n, cases with sensory symptoms; N, cases studied.

prominent in female than in male rats (29, 30). Studies conducted in humans also suggested sex differences in the response to mechanical pressure pain (31) and cold pressor pain (32), which might be related to the differences in sex hormone levels between men and women and during different phases of menstruation cycle in women.

DPN may develop at an early stage of hyperglycemia including the prediabetes status (33). Therefore, the nerve damages caused by high glucose levels can develop insidiously during the long period of prediabetes status. The consistency of diabetes duration (but not age) and A1C in the association with SS (**Tables 3** and **4**) suggested that the SS might be diabetes-specific and related to glycemic control.

A recent study showed an association between pDPN and nondipping in blood pressure during midnight (34). Because the circadian change in blood pressure is controlled by autonomic nerve and diabetes patients with hypertension is highly associated with non-dipping (35), the link between hypertension and SS, but not other major atherosclerotic risk factors such as smoking, obesity, and dyslipidemia (**Tables 3** and **4**), suggested a potential involvement of autonomic neuropathy.

This study has several strengths. First, it was conducted in a population-based representative cohort of diabetes patients and might be more readily used for generalization of the findings. Second, the questionnaire covered three categories of symptoms on different anatomical sites for a better description of the clinical distributions of different symptoms. Third, by using the different definitions of SS, it was possible for us to evaluate the prevalence according to different definitions (**Table 2**) and to test the consistency of findings by using different definitions (**Tables 3** and **4**).

There are some limitations. First, this study was conducted in 1990s, and therefore, it remains unknown whether the prevalence might have changed significantly in recent years. However, a long-term follow-up of the patients would allow us to conduct studies in the near future to evaluate the impact of SS on the mortality of the patients by matching the national death certificates database in Taiwan. Second, because the study was conducted at a time when

TABLE 4 | Logistic regression evaluating risk factors of sensory symptoms according to different definitions (models with A1C).

Risk factor	Interpretation	n/N	Odds ratio	95% Confidence interval	*P*-value
I. Any positive symptom on at least 1 site					
Age	Every 1-yr increment	753/1,395	1.001	(0.991–1.012)	0.8035
Sex	Men *vs.* women	338/669; 415/726	0.702	(0.529–0.933)	0.0149
Diabetes duration	Every 1-yr increment	753/1,395	1.025	(1.010–1.041)	0.0015
A1C	Every 1% increment	753/1,395	1.095	(1.033–1.161)	0.0023
Smoking	Yes *vs.* no	262/492; 491/903	1.277	(0.952–1.712)	0.1022
Obesity	Yes *vs.* no	535/966; 218/429	1.059	(0.832–1.348)	0.6395
Hypertension	Yes *vs.* no	468/821; 285/574	1.267	(1.007–1.595)	0.0434
Dyslipidemia	Yes *vs.* no	489/886; 264/509	0.821	(0.604–1.117)	0.2090
Proteinuria	Yes *vs.* no	97/148; 656/1,247	1.483	(1.027–2.140)	0.0356
Use of insulin	Yes *vs.* no	126/196; 627/1,199	1.336	(0.957–1.866)	0.0884
II. Any positive symptom on at least 1 site involving the lower limb					
Age	Every 1-yr increment	581/1,395	1.011	(1.001–1.022)	0.0381
Sex	Men *vs.* women	257/669; 324/726	0.687	(0.512–0.922)	0.0125
Diabetes duration	Every 1-yr increment	581/1,395	1.023	(1.008–1.039)	0.0031
A1C	Every 1% increment	581/1,395	1.086	(1.025–1.152)	0.0055
Smoking	Yes *vs.* no	203/492; 378/903	1.318	(0.974–1.785)	0.0739
Obesity	Yes *vs.* no	406/966; 175/429	0.885	(0.692–1.133)	0.3328
Hypertension	Yes *vs.* no	371/821; 210/574	1.300	(1.029–1.644)	0.0282
Dyslipidemia	Yes *vs.* no	381/886; 200/509	0.835	(0.613–1.137)	0.2518
Proteinuria	Yes *vs.* no	83/148; 498/1,247	1.703	(1.193–2.431)	0.0034
Use of insulin	Yes *vs.* no	104/196; 477/1,199	1.463	(1.056–2.027)	0.0222
III. Any positive symptom on at least 2 sites with at least one involving the lower limb					
Age	Every 1-yr increment	495/1,395	1.009	(0.999–1.021)	0.0888
Sex	Men *vs.* women	220/669; 275/726	0.712	(0.525–0.964)	0.0281
Diabetes duration	Every 1-yr increment	495/1,395	1.028	(1.012–1.044)	0.0005
A1C	Every 1% increment	495/1,395	1.099	(1.035–1.167)	0.0019
Smoking	Yes *vs.* no	174/492; 321/903	1.307	(0.956–1.786)	0.0933
Obesity	Yes *vs.* no	340/966; 155/429	0.817	(0.635–1.052)	0.1172
Hypertension	Yes *vs.* no	317/821; 178/574	1.307	(1.027–1.665)	0.0298
Dyslipidemia	Yes *vs.* no	328/886; 167/509	1.000	(0.725–1.378)	0.9987
Proteinuria	Yes *vs.* no	66/148; 429/1,247	1.329	(0.930–1.900)	0.1186
Use of insulin	Yes *vs.* no	89/196; 406/1,199	1.378	(0.993–1.914)	0.0554
IV. Any positive symptom on at least 3 sites with at least one involving the lower limb					
Age	Every 1-yr increment	419/1,395	1.007	(0.996–1.019)	0.2268
Sex	Men *vs.* women	183/669; 236/726	0.700	(0.509–0.963)	0.0283
Diabetes duration	Every 1-yr increment	419/1,395	1.029	(1.013–1.046)	0.0004
A1C	Every 1% increment	419/1,395	1.097	(1.032–1.167)	0.0032
Smoking	Yes *vs.* no	145/492; 274/903	1.277	(0.920–1.773)	0.1434
Obesity	Yes *vs.* no	288/966; 131/429	0.813	(0.625–1.058)	0.1242
Hypertension	Yes *vs.* no	275/821; 144/574	1.416	(1.099–1.825)	0.0072
Dyslipidemia	Yes *vs.* no	284/886; 135/509	1.075	(0.766–1.510)	0.6743
Proteinuria	Yes *vs.* no	57/148; 362/1,247	1.307	(0.907–1.885)	0.1509
Use of insulin	Yes *vs.* no	73/196; 346/1,199	1.217	(0.867–1.709)	0.2567
V. Any positive symptom on at least 4 sites with at least one involving the lower limb					
Age	Every 1-yr increment	344/1,395	1.007	(0.995–1.020)	0.2436
Sex	Men *vs.* women	145/669; 199/726	0.694	(0.494–0.976)	0.0357
Diabetes duration	Every 1-yr increment	344/1,395	1.034	(1.017–1.051)	<0.0001
A1C	Every 1% increment	344/1,395	1.106	(1.036–1.180)	0.0026
Smoking	Yes *vs.* no	114/492; 230/903	1.189	(0.836–1.691)	0.3346
Obesity	Yes *vs.* no	240/966; 104/429	0.857	(0.647–1.136)	0.2843
Hypertension	Yes *vs.* no	227/821; 117/574	1.367	(1.042–1.792)	0.0238
Dyslipidemia	Yes *vs.* no	241/886; 103/509	1.083	(0.752–1.559)	0.6681
Proteinuria	Yes *vs.* no	51/148; 293/1,247	1.454	(0.997–2.120)	0.0518
Use of insulin	Yes *vs.* no	62/196; 282/1,199	1.197	(0.839–1.707)	0.3220
VI. Any positive symptom on at least 2 sites with at least two involving the lower limb					
Age	Every 1-yr increment	444/1,395	1.006	(0.995–1.018)	0.2641
Sex	Men *vs.* women	192/669; 252/726	0.713	(0.522–0.974)	0.0334
Diabetes duration	Every 1-yr increment	444/1,395	1.028	(1.012–1.044)	0.0007
A1C	Every 1% increment	444/1,395	1.106	(1.041–1.175)	0.0012
Smoking	Yes *vs.* no	150/492; 294/903	1.198	(0.868–1.653)	0.2717
Obesity	Yes *vs.* no	310/966; 134/429	0.878	(0.677–1.139)	0.3284

(Continued)

TABLE 4 | Continued

Risk factor	Interpretation	n/N	Odds ratio	95% Confidence interval	*P*-value
Hypertension	Yes *vs.* no	289/821; 155/574	1.370	(1.068–1.758)	0.0132
Dyslipidemia	Yes *vs.* no	294/886; 150/509	0.965	(0.694–1.342)	0.8330
Proteinuria	Yes *vs.* no	62/148; 382/1,247	1.400	(0.977–2.008)	0.0671
Use of insulin	Yes *vs.* no	79/196; 365/1,199	1.270	(0.909–1.774)	0.1608
VII. Any positive symptom on at least 3 sites with at least two involving the lower limb					
Age	Every 1-yr increment	387/1,395	1.006	(0.994–1.017)	0.3553
Sex	Men *vs.* women	164/669; 223/726	0.713	(0.515–0.986)	0.0410
Diabetes duration	Every 1-yr increment	387/1,395	1.030	(1.013–1.047)	0.0004
A1C	Every 1% increment	387/1,395	1.111	(1.043–1.183)	0.0011
Smoking	Yes *vs.* no	127/492; 260/903	1.134	(0.810–1.587)	0.4649
Obesity	Yes *vs.* no	270/966; 117/429	0.865	(0.660–1.134)	0.2929
Hypertension	Yes *vs.* no	255/821; 132/574	1.408	(1.085–1.827)	0.0100
Dyslipidemia	Yes *vs.* no	263/886; 124/509	1.044	(0.738–1.478)	0.8082
Proteinuria	Yes *vs.* no	54/148; 333/1,247	1.044	(0.923–1.937)	0.1241
Use of insulin	Yes *vs.* no	68/196; 319/1,199	1.186	(0.839–1.674)	0.3338
VIII. Any positive symptom on at least 4 sites with at least two involving the lower limb					
Age	Every 1-yr increment	331/1,395	1.006	(0.994–1.019)	0.3209
Sex	Men *vs.* women	138/669; 193/726	0.691	(0.490–0.975)	0.0356
Diabetes duration	Every 1-yr increment	331/1,395	1.033	(1.015–1.050)	0.0002
A1C	Every 1% increment	331/1,395	1.102	(1.032–1.178)	0.0037
Smoking	Yes *vs.* no	108/492; 223/903	1.156	(0.809–1.652)	0.4246
Obesity	Yes *vs.* no	231/966; 100/429	0.854	(0.642–1.136)	0.2776
Hypertension	Yes *vs.* no	218/821; 113/574	1.351	(1.027–1.778)	0.0316
Dyslipidemia	Yes *vs.* no	232/886; 99/509	1.089	(0.753–1.577)	0.6499
Proteinuria	Yes *vs.* no	50/148; 281/1,247	1.495	(1.023–2.185)	0.0376
Use of insulin	Yes *vs.* no	61/196; 270/1,199	1.239	(0.867–1.770)	0.2401
IX. Any positive symptom on at least bilateral and symmetrical 2 sites involving the lower limb					
Age	Every 1-yr increment	201/1,395	1.006	(0.991–1.022)	0.4356
Sex	Men *vs.* women	81/669; 120/726	0.565	(0.365–0.875)	0.0105
Diabetes duration	Every 1-yr increment	201/1,395	1.048	(1.028–1.069)	<0.0001
A1C	Every 1% increment	201/1,395	1.084	(1.001–1.175)	0.0474
Smoking	Yes *vs.* no	69/492; 132/903	1.504	(0.962–2.352)	0.0737
Obesity	Yes *vs.* no	137/966; 64/429	0.734	(0.520–1.036)	0.0786
Hypertension	Yes *vs.* no	142/821; 59/574	1.672	(1.185–2.360)	0.0035
Dyslipidemia	Yes *vs.* no	146/886; 55/509	1.479	(0.903–2.421)	0.1197
Proteinuria	Yes *vs.* no	34/148; 167/1,247	1.569	(1.016–2.423)	0.0423
Use of insulin	Yes *vs.* no	38/196; 163/1,199	1.134	(0.742–1.734)	0.5612

n, cases with sensory symptoms; N, cases studied.

the currently used tools such as LANSS (Leeds Assessment of Neuropathic Symptoms and Signs) (36) and DN4 (Douleur Neuropathique 4) (37) were not yet available, we used a questionnaire developed by ourselves. Third, we recognized that the validity and reliability of the questionnaire used in the study had not been tested. Therefore, we still need to take some effort to test the validity and reliability of this questionnaire and to examine its usefulness as a clinical tool for predicting the development of diabetes complications and mortality. The inclusion of neurological examinations and objective laboratory tests such as Achilles tendon reflex, vibration threshold and nerve conduction velocity, etc. in our future studies would be helpful for evaluating the usefulness of the questionnaire. Fourth, the cross-sectional nature of the study did not allow a direct interpretation of cause-effect relationship between the evaluated covariates and SS. Fifth, this study was not able to evaluate the prevalence of painless neuropathy, which is related to the development of diabetic foot (38). Sixth, symptom severity was not evaluated in the present study. Seventh, the abnormal sensation obtained from the interview might not be really due to DPN. Other diseases such as carpal tunnel syndrome or spinal stenosis could not be excluded because of similar sensory presentations.

In summary, the presentation of SS is very common in the Taiwanese diabetes patients. The prevalence may range from 14.4 to 54.0% by using the most stringent criterion to using the least stringent criterion. Women are more prone to report sensory abnormalities than men. SS may be diabetes-specific and related to female sex, diabetes duration, A1C, and hypertension. However, age, and other atherosclerotic risk factors such as smoking, obesity, and dyslipidemia are not associated with SS. Prospective cohort studies are required to explore the cause-effect relationship of some covariates.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because according to the Personal Information Protection Act enacted in Taiwan, individualized data cannot be released for the protection of privacy. Requests to access the datasets should be directed to ccktsh@ms6.hinet.net.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Department of Health, Taiwan. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

C-HT researched the data and wrote manuscript. C-KC designed the questionnaire and trained interviewers. J-JS designed the questionnaire and controlled the quality of the questionnaire. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Tai TY, Tseng CH, Sung SM, Huang RF, Chen CZ, Tsai SH. Retinopathy, neuropathy and nephropathy in non-insulin-dependent diabetic patients. *J Formosan Med Assoc* (1991) 90:936–40.
2. Spallone V, Greco C. Painful and painless diabetic neuropathy: one disease or two? *Curr Diabetes Rep* (2013) 13:533–49. doi: 10.1007/s11892-013-0387-7
3. Themistocleous AC, Ramirez JD, Shillo PR, Lees JG, Selvarajah D, Orengo C, et al. The Pain in Neuropathy Study (PiNS): a cross-sectional observational study determining the somatosensory phenotype of painful and painless diabetic neuropathy. *Pain* (2016) 157:1132–45. doi: 10.1097/j.pain.0000000000000491
4. Alleman CJ, Westerhout KY, Hensen M, Chambers C, Stoker M, Long S, et al. Humanistic and economic burden of painful diabetic peripheral neuropathy in Europe: A review of the literature. *Diabetes Res Clin Pract* (2015) 109:215–25. doi: 10.1016/j.diabres.2015.04.031
5. Sadosky A, Mardekian J, Parsons B, Hopps M, Bienen EJ, Markman J. Healthcare utilization and costs in diabetes relative to the clinical spectrum of painful diabetic peripheral neuropathy. *J Diabetes Complications* (2015) 29:212–7. doi: 10.1016/j.jdiacomp.2014.10.013
6. Eichholz M, Alexander AH, Cappelleri JC, Hlavacek P, Parsons B, Sadosky A, et al. Perspectives on the impact of painful diabetic peripheral neuropathy in a multicultural population. *Clin Diabetes Endocrinol* (2017) 3:12. doi: 10.1186/s40842-017-0051-2
7. Kim SS, Won JC, Kwon HS, Kim CH, Lee JH, Park TS, et al. Prevalence and clinical implications of painful diabetic peripheral neuropathy in type 2 diabetes: results from a nationwide hospital-based study of diabetic neuropathy in Korea. *Diabetes Res Clin Pract* (2014) 103:522–9. doi: 10.1016/j.diabres.2013.12.003
8. Win MMTM, Fukai K, Nyunt HH, Hyodo Y, Linn KZ. Prevalence of peripheral neuropathy and its impact on activities of daily living in people with type 2 diabetes mellitus. *Nurs Health Sci* (2019) 21:445–53. doi: 10.1111/nhs.12618
9. Yin L, Zhang D, Ren Q, Su X, Sun Z. Prevalence and risk factors of diabetic retinopathy in diabetic patients: A community based cross-sectional study. *Med (Baltimore)* (2020) 99:e19236. doi: 10.1097/MD.0000000000019236
10. Yokoyama H, Tsuji T, Hayashi S, Kabata D, Shintani A. Factors associated with diabetic polyneuropathy-related sensory symptoms and signs in patients with polyneuropathy: A cross-sectional Japanese study (JDDM 52) using a non-linear model. *J Diabetes Investig* (2020) 11:450–7. doi: 10.1111/jdi.13117
11. Sadosky A, Hopper J, Parsons B. Painful diabetic peripheral neuropathy: results of a survey characterizing the perspectives and misperceptions of patients and healthcare practitioners. *Patient* (2014) 7:107–14. doi: 10.1007/s40271-013-0038-8
12. Tsuji M, Yasuda T, Kaneto H, Matsuoka TA, Hirose T, Kawamori R, et al. Painful diabetic neuropathy in Japanese diabetic patients is common but underrecognized. *Pain Res Treat* (2013) 2013:318352. doi: 10.1155/2013/318352
13. Malik RA, Aldinc E, Chan SP, Deerochanawong C, Hwu CM, Rosales RL, et al. Perceptions of painful diabetic peripheral neuropathy in South-East Asia: results from patient and physician surveys. *Adv Ther* (2017) 34:1426–37. doi: 10.1007/s12325-017-0536-5
14. Tseng CH. Mortality and causes of death in a national sample of diabetic patients in Taiwan. *Diabetes Care* (2004) 27:1605–9. doi: 10.2337/diacare.27.7.1605
15. Tseng CH. Prevalence of lower-extremity amputation among patients with diabetes mellitus: Is height a factor? *CMAJ* (2006) 174:319–23. doi: 10.1503/cmaj.050680
16. Tseng CH. Waist-to-height ratio and coronary artery disease in Taiwanese type 2 diabetic patients. *Obesity* (2008) 16:2754–9. doi: 10.1038/oby.2008.430
17. Tseng CH, Chong CK, Tseng CP, Shau WY, Tai TY. Hypertension is the most important component of metabolic syndrome in the association with ischemic heart disease in Taiwanese type 2 diabetic patients. *Circ J* (2008) 72:1419–24. doi: 10.1253/circj.CJ-08-0009
18. Tseng CH. Body composition as a risk factor for coronary artery disease in Chinese type 2 diabetic patients in Taiwan. *Circ J* (2003) 67:479–84. doi: 10.1253/circj.67.479
19. Tseng CH. Sexual difference in the distribution of atherosclerotic risk factors and their association with peripheral arterial disease in Taiwanese type 2 diabetic patients. *Circ J* (2007) 71:1131–6. doi: 10.1253/circj.71.1131
20. World Health Organization. International Obesity Taskforce & International Association for the Study of Obesity. In: *The Asia–Pacific perspective: redefining obesity and its treatment*. Hong Kong: WHO, IOTF & IASO (2000).
21. Tseng CH. Lipid abnormalities associated with urinary albumin excretion rate in Taiwanese type 2 diabetic patients. *Kidney Int* (2005) 67:1547–53. doi: 10.1111/j.1523-1755.2005.00235.x
22. Tseng CH. Correlation of uric acid and urinary albumin excretion rate in patients with type 2 diabetes mellitus in Taiwan. *Kidney Int* (2005) 68:796–801. doi: 10.1111/j.1523-1755.2005.00459.x
23. Amandusson Å, Blomqvist A. Estrogenic influences in pain processing. *Front Neuroendocrinol* (2013) 34:329–49. doi: 10.1016/j.yfrne.2013.06.001
24. Wiesenfeld-Hallin Z. Sex differences in pain perception. *Gend Med* (2005) 2:137–45. doi: 10.1016/S1550-8579(05)80042-7

25. Sorge RE, Totsch SK. Sex differences in pain. *J Neurosci Res* (2017) 95:1271–81. doi: 10.1002/jnr.23841
26. Rosen S, Ham B, Mogil JS. Sex differences in neuroimmunity and pain. *J Neurosci Res* (2017) 95:500–8. doi: 10.1002/jnr.23831
27. Mapplebeck JC, Beggs S, Salter MW. Molecules in pain and sex: a developing story. *Mol Brain* (2017) 10:9. doi: 10.1186/s13041-017-0289-8
28. Payrits M, Sághy É, Cseko K, Pohóczky K, Bölcskei K, Ernszt D, et al. Estradiol sensitizes the Transient Receptor Potential Vanilloid 1 Receptor in pain responses. *Endocrinology* (2017) 158:3249–58. doi: 10.1210/en.2017-00101
29. Patil MJ, Ruparel SB, Henry MA, Akopian AN. Prolactin regulates TRPV1, TRPA1, and TRPM8 in sensory neurons in a sex-dependent manner: Contribution of prolactin receptor to inflammatory pain. *Am J Physiol Endocrinol Metab* (2013) 305:E1154–64. doi: 10.1152/ajpendo.00187.2013
30. Patil MJ, Green DP, Henry MA, Akopian AN. Sex-dependent roles of prolactin and prolactin receptor in postoperative pain and hyperalgesia in mice. *Neuroscience* (2013) 253:132–41. doi: 10.1016/j.neuroscience.2013.08.035
31. Kowalczyk WJ, Sullivan MA, Evans SM, Bisaga AM, Vosburg SK, Comer SD. Sex differences and hormonal influences on response to mechanical pressure pain in humans. *J Pain* (2010) 11:330–42. doi: 10.1016/j.jpain.2009.08.004
32. Kowalczyk WJ, Evans SM, Bisaga AM, Sullivan MA, Comer SD. Sex differences and hormonal influences on response to cold pressor pain in humans. *J Pain* (2006) 7:151–60. doi: 10.1016/j.jpain.2005.10.004
33. Callaghan BC, Cheng HT, Stables CL, Smith AL, Feldman EL. Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol* (2012) 11:521–34. doi: 10.1016/S1474-4422(12)70065-0
34. D'Amato C, Morganti R, Di Gennaro F, Greco C, Marfia GA, Spallone V. A novel association between nondipping and painful diabetic polyneuropathy. *Diabetes Care* (2014) 37:2640–2. doi: 10.2337/dc14-0528
35. Cardoso CR, Leite NC, Freitas L, Dias SB, Muxfeld ES, Salles GF. Pattern of 24-hour ambulatory blood pressure monitoring in type 2 diabetic patients with cardiovascular dysautonomy. *Hypertens Res* (2008) 31:865–72. doi: 10.1291/hypres.31.865
36. Bennett M. The LANSS Pain Scale: the Leeds assessment of neuropathic symptoms and signs. *Pain* (2001) 92:147–57. doi: 10.1016/S0304-3959(00)00482-6
37. Bouhassira D, Attal N, Alchaar H, Boureau F, Brochet B, Bruxelle J, et al. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain* (2005) 114:29–36. doi: 10.1016/j.pain.2004.12.010
38. Chantelau EA. Nociception at the diabetic foot, an uncharted territory. *World J Diabetes* (2015) 6:391–402. doi: 10.4239/wjd.v6.i3.391

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Association of Genetic Polymorphisms in MicroRNAs With Type 2 Diabetes Mellitus in a Chinese Population

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Introduction: MicroRNAs (miRNA) involved in the insulin signaling pathways deeply affect the pathogenesis of T2DM. The aim of this study was to assess the association between single nucleotide polymorphisms (SNP) of the related miRNAs (let-7f rs10877887, let-7a-1 rs13293512, miR-133a-1 rs8089787, miR-133a-2 rs13040413, and miR-27a rs895819) and susceptibility to type 2 diabetes mellitus (T2DM), and its possible mechanisms.

Methods: Five SNPs in miRNAs (let-7f rs10877887, let-7a-1 rs13293512, miR-133a-1 rs8089787, miR-133a-2 rs13040413, and miR-27a rs895819) involved in the insulin signaling pathways were selected and genotyped in a case-control study that enrolled 371 T2DM patients and 381 non-diabetic controls. The individual SNP association analyses, interaction analyses of SNP-SNP, SNP-environmental factors were performed. The effect the risk-associated polymorphism on regulating its mature miRNA expression was also evaluated.

Results: In overall analyses, miR-133a-2 rs13040413 and let-7a-1 rs13293512 were related to the susceptibility to T2DM. In stratified analyses, miR-133a-2 rs13040413, let-7a-1 rs13293512 and miR-27a rs895819 showed associations with T2DM in the age ≥ 60 years subgroup. Moreover, let-7a-1 rs13293512 and miR-27a rs895819 showed associations with T2DM in male subgroup. In SNP-environmental factors interaction analyses, there were interaction effects of miR-133a-2 rs13040413 with dyslipidemia, let-7a-1 rs13293512 with smoking, and let-7a-1 rs13293512 with dyslipidemia on T2DM. In SNP-SNP interaction analyses, there were also interaction effects of miR-133a-1 rs8089787 with let-7a-1 rs13293512, and miR-133a-1 rs8089787 with let-7f rs10877887 on T2DM. Furthermore, for miR-133a-2 rs13040413, the variant T allele showed a trend toward decreased miR-133a expression in comparison with the wild C allele. For let-7a-1 rs13293512, the variant C allele expressed a lower let-7a compared to the wild T allele.

Conclusion: MiRNAs polymorphisms involved in the insulin signaling pathways and the interaction effects of SNP-SNP, SNP-environmental factors were related to T2DM susceptibility in a Chinese population.

Keywords: microRNA, polymorphism, type 2 diabetes mellitus, insulin signaling pathway, Chinese population

INTRODUCTION

Type 2 diabetes mellitus (T2DM) posting one of the most common and serious chronic diseases, is characterized sustained hyperglycemia (1). Insulin resistance, involving a defect in insulin secretion, insulin action, or both, is central to the etiology of T2DM (2). The underlying molecular mechanism for insulin resistance is only partially understood. Nevertheless, increasing evidences have showed that various microRNAs (miRNA) involved in regulating the main protein cascades in the insulin signaling pathways that affect insulin resistance, and therefore, the pathogenesis of T2DM, such as let-7f with insulin growth factor-1 receptor (IGF1R), let-7a with phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), miR-133a with glucose transporter 4 (GLUT4), and miR-27a with mammalian target of rapamycin (mTOR) (3–6).

It has been showed that IGF1R was the target of let-7f. Let-7f mimics could suppress IGF1R expression, and that let-7f inhibitors could increase the expression level of IGF1R (3). Blood level of let-7f was down-regulated in T2DM subjects compared to controls (7). Moreover, let-7a is associated with PI3K/AKT signaling. Let-7a overexpression could decrease the expression levels of PI3K and p-AKT (4). Down-regulation of let-7a level was observed in T2DM subjects (8). Overexpression of miR-133a could decrease GLUT4 expression and reduced insulin-mediated glucose uptake (5). The circulatory miR-133a level was significantly higher in T2DM subjects than in controls. Further, there was a positive and significant correlation between miR-133a with fasting blood glucose (FBG) and glycated hemoglobin in the T2DM subjects (9). Elevated miR-27a could up-regulate mTOR phosphorylation level and enhance mTOR signaling (6). Increased blood level of miR-27a was identified in T2DM, and associated with measures of pancreatic β cell function (10). Collectively, let-7f, let-7a, miR-133a, and miR-27a have strong relationship with T2DM, both in underlying mechanisms and expression levels.

Single nucleotide polymorphisms (SNP) are variations in single nucleotides of genomic DNA sequence, which have modified potentials on gene functions (11). SNPs affect the miRNA binding efficiency, giving rise to increased or decreased miRNA regulation (12). We hypothesize that the SNPs in miRNA may impact the susceptibility to T2DM by miRNA dysregulation of mRNA degradation, translation, and expression. It has been showed that let-7f rs10877887 and let-7a-1 rs13293512 have relationships with stroke, depression, aneurysms and cancers (13–16). Especially, let-7a-1 rs13293512 have binding sites with interleukin-6 (IL-6), a contributor of T2DM. The variant genotypes of let-7a-1 rs13293512 were associated with increased IL-6 expression (17). MiR-133a-1 rs8089787 are associated with asthma (18). MiR-133a-2 rs13040413 variant could increase levels of miR-133a (19). However, there was no study concerning about the associations between let-7f rs10877887, let-7a-1 rs13293512, miR-133a-1 rs8089787 and miR-133a-2 rs13040413 and T2DM. Furthermore, the relationships of miR-27a rs895819 and T2DM were controversial and inconclusive, which need further investigation (20). In addition, only a part of T2DM heritability can be interpreted by a single miRNA polymorphism study. SNP-SNP and SNP-environmental factors interactions may account for another missing part of the heritability. But there are lacking studies on the interactions of miRNA SNPs, as well as with environmental factors in T2DM.

Thus, the aim of our study is comprehensively investigating the effects of variations in miRNA genes involving in insulin signaling pathways, let-7f rs10877887, let-7a-1 rs13293512, miR-133a-1 rs8089787, miR-133a-2 rs13040413, and miR-27a rs895819, the interactions of SNP-SNP, SNP-environmental factors on T2DM in a Chinese population, as well as the effect of the risk-associated polymorphism on regulating its mature miRNA expression.

MATERIALS AND METHODS

Study Subjects

A total of 752 participants were enrolled in this study, including 371 untreated T2DM patients and 381 non-diabetic controls. T2DM participants were recruited from inpatient and outpatient services at First Affiliated Hospital of China Medical University, and control participants from local community between August 2016 and December 2018. T2DM met the 1999 WHO criteria for diabetes: a FBG ≥ 7.0 mmol/l or a 2h blood glucose (2hBG) ≥ 11.1mmol/l (21). The non-diabetic controls were included as follows: a fasting glucose level < 6.1 mmol/l or 2h glucose level < 7.8 mmol/l, no past history of diabetes and no family history of T2DM. Exclusion criteria were participants with type 1 diabetes or other special types of diabetes, malignant disease, liver and kidney disease, acute infections, or myocardial infarction. All participants provided written informed consent after receiving a detailed description of the study. The study was approved by the Institutional Ethics Committee of China Medical University. This study was carried out in accordance with the standard biosecurity and institutional safety procedures of China Medical University.

Data Collection, Anthropometric Measure, and Laboratory Testing

Data of sex, age, body mass index (BMI), smoking status, and alcohol consumption were collected from questionnaires. Smokers were defined as having smoked at least one cigarette

per day for more than one year. Drinkers were defined as having consumed at least one alcoholic beverage a day for a minimum period of six months. Systolic blood pressure (SBP), diastolic blood pressure (DBP), blood glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglyceride (TG) were measured using standard laboratory procedures. Hypertension was defined as ≥ 140/90 mmHg or any antihypertensive treatment. Dyslipidemia was defined as TC ≥ 5.17 mmol/L, or TG ≥ 1.70 mmol/L, or LDL ≥ 2.58 mmol/L, or HDL ≤ 0.91 mmol/L or under taking hypolipidemic drugs.

SNP Selection and Genotyping

Five tag-SNPs were identified as the following steps (22). First, the candidate SNPs were screened in the 5′ and 3′ region, mature sequence, pri-miRNA sequence, pre-miRNA sequence of the miRNA genes. Second, the candidate SNPs were selected in the combinations provided by the HapMap database (http://www.HapMap.org) and Haploview software 4.0 (http://www.broadinstitute.org/mpg/haploview). Third, the potential functions of the candidate SNPs were predict by the web-based analysis tools (SNPinfo, http://snpinfo.niehs.nih.gov; PolymiRTS Database 3.0, http://compbio.uthsc.edu; TFSEARCH 1.3, http://www.cbrc.jp/research/db/TFSEARCH.html). Collectively, miR-133a-1 rs8089787, miR-133a-2 rs13040413, let-7a-1 rs13293512, let-7f rs10877887, and miR-27a rs895819 were selected for genotyping.

Genomic DNA was extracted using the standard phenol-chloroform method, and then diluted to a working concentration of 50ng/μl before genotyping (23). The assays, primer design, and genotyping of candidate gene polymorphisms were performed by Baygene Biotechnology Company Limited (Shanghai, China) using the KASP method with SNPLine platform (LGC, United Kingdom). For genotyping quality controlling, 5% samples were repeated genotyped and the results were 100% consistent.

Transfection and Real-Time PCR Reaction for miRNA Expression

The expression vectors pGCMV-rs13040413-C, pGCMV-rs13040413-T (site-specific mutagenesis from C to T), pGCMV-rs13293512-T, and pGCMV-rs13293512-C (site-specific mutagenesis from T to C) were synthesized by Genechem Company (Genechem Co. Ltd, Shanghai, China). The pGCMV-rs13040413-C, pGCMV-rs13040413-T, pGCMV-rs13293512-T, pGCMV-rs13293512-C, and empty plasmids were transfected into human embryonic kidney (HEK) 293T cells, respectively. After 48 h, the total RNA was extracted using TRIzol reagent (Invitrogen, USA). Real-time PCR was used to detect miR-133a and let-7a expression using TaqMan microRNA assay kits (ABI, USA). All experiments were carried out in triplicate.

Statistical Analysis

All statistical analyses were carried out with SPSS 16.0 software (SPSS, Chicago, IL, USA). Continuous variables were presented as mean ± SD and compared by Student's t test, and discrete variables, including the Hardy-Weinberg equilibrium (HWE) in control group, as frequencies and percentages and by Chi-square $\chi 2$ test. The association of SNPs and T2DM risk was assessed by the odds ratios (OR) with 95% confidence intervals (CI) using multiple logistic regression analysis after adjustment for potential risk factors (age, gender, smoking, drinking, hypertension, and dyslipidemia). The interaction effects (SNP-SNP, SNP-environment) were evaluated by the likelihood-ratio with a fully parameterized model. Differential expression of the wild type and mutant alleles was analyzed by Student's t test. A two-side *P*-value less than 0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

Demographic and clinical data are presented in **Table 1**. There were no significant differences in sex, age, BMI, smoking, and drinking between T2DM patients and controls ($P > 0.05$). T2DM patients had significant higher FBG, 2hBG, SBP, DBP, and TG, and lower HDL when compared with controls ($P < 0.05$). The dyslipidemia rate was higher in T2DM patients compared to controls ($P < 0.05$).

Association of MiRNA Polymorphisms With T2DM

All genotypes were distributed in accordance with Hardy–Weinberg equilibrium ($P > 0.05$). As shown in **Table 2**, miR-133a-2 rs13040413 variant genotypes were significantly associated with an increased risk of T2DM (TT: OR = 2.18, $P = 0.028$; CT+TT: OR = 1.38, $P = 0.040$). let-7a-1 rs13293512 variant genotypes were also associated with an increased risk of T2MD (CC: OR = 1.61, $P = 0.026$; TC+CC: OR = 1.41, $P = 0.029$). There was no overall genetic effect on T2DM for miR-133a-1 rs8089787, let-7f rs10877887, and miR-27a rs895819 ($P > 0.05$).

Moreover, stratified analyses were conducted to evaluate the associations between SNPs and T2DM by age and sex (**Table 3**). miR-133a-2 rs13040413 variant genotype was associated with an increased risk of T2MD in the age ≥ 60 years subgroup (TT: OR = 5.42, $P = 0.009$). let-7a-1 rs13293512 variant genotypes were associated with an increased risk of T2MD in both the age ≥ 60 years (TC: OR = 2.19, $P = 0.010$; CC: OR = 2.40, $P = 0.005$; TC+CC: OR = 3.20, $P = 0.002$) and male subgroup (CC: OR = 1.87, $P = 0.040$). miR-27a rs895819 variant genotypes were also associated with an increased risk of T2MD in both the age ≥ 60 years (GG: OR = 4.92, $P = 0.049$) and male subgroup (AG: OR = 1.53, $P = 0.049$; TC+CC: OR = 1.59, $P = 0.039$). No statistical significant differences were observed between miR-133a-1 rs8089787 or let-7f rs10877887 polymorphisms and T2DM ($P > 0.05$).

Interactions of MiRNA Polymorphisms and Environmental Factors in T2DM

Interaction effects of miRNA polymorphisms and environmental factors in T2DM were also explored. The models included SNPs

TABLE 1 | Demographics and clinical characteristics of type 2 diabetes mellitus cases and non-diabetic controls.

Variables	T2DM (N=371)	controls (N=381)	T/χ2	*P* value
Sex, M/F (%)	196/175 (52.8/47.2)	192/189 (50.4/49.6)	0.447	0.504
Age (years)	54.15 ± 10.55	54.71 ± 8.96	-0.778	0.437
Body mass index (kg/m2)	25.35 ± 3.74	25.38 ± 3.28	-0.070	0.944
Smoking, Y/N (%)	113/258 (30.4/69.6)	98/283 (25.5/74.5)	3.463	0.073
Drinking, Y/N (%)	75/296 (20.0/80.0)	68/313 (17.6/82.4)	0.689	0.407
Fasting blood glucose (mmol/L)	8.48 ± 2.77	5.34 ± 0.55	21.591	<0.001
2h blood glucose (mmol/L)	15.34 ± 5.29	6.29 ± 1.92	29.007	<0.001
Systolic blood pressure (mmHg)	138.301 ± 21.07	133.85 ± 19.96	-2.969	0.003
Diastolic blood pressure (mmHg)	87.49 ± 13.07	82.82 ± 13.27	-4.865	<0.001
Total cholesterol (mmol/L)	5.08 ± 1.15	5.03 ± 2.76	-0.332	0.740
Triglyceride (mmol/L)	2.33 ± 2.36	1.44 ± 1.03	6.659	<0.001
Low-density lipoprotein cholesterol (mmol/L)	3.20 ± 0.90	3.17 ± 0.96	-0.530	0.596
High-density lipoprotein cholesterol (mmol/L)	1.24 ± 0.54	1.40 ± 0.67	-3.452	0.001
Hypertension, Y/N (%)	157/214 (42.3/57.7)	140/241 (36.7/63.3)	2.466	0.116
Dyslipidemia, Y/N (%)	235/136 (63.5/36.5)	201/180 (52.8/47.2)	8.921	0.003

T2DM, type 2 diabetes mellitus.

TABLE 2 | Association of miRNA polymorphisms with type 2 diabetes mellitus.

Genotype			Crude models		Adjusted models	
			OR(95%CI)	*P* value	OR(95%CI)[a]	*P* value
miR-133a-1 rs8089787						
CT	vs	CC	0.90(0.63, 1.27)	0.540	0.91(0.64, 1.29)	0.583
TT	vs	CC	1.00(0.32, 3.15)	0.995	1.05(0.33, 3.32)	0.929
CT+TT	vs	CC	0.90(0.64, 1.27)	0.558	0.91(0.65, 1.29)	0.603
miR-133a-2 rs13040413						
CT	vs	CC	1.26(0.92, 1.75)	0.152	1.27(0.92, 1.76)	0.145
TT	vs	CC	2.19(1.10, 4.38)	0.026	2.18(1.09, 4.36)	0.028
CT+TT	vs	CC	1.38(1.01, 1.87)	0.041	1.38(1.02, 1.87)	0.040
let-7a-1 rs13293512						
TC	vs	TT	1.35(0.97, 1.88)	0.074	1.34(0.97, 1.88)	0.080
CC	vs	TT	1.61(1.06, 2.44)	0.026	1.61(1.06, 2.44)	0.026
TC+CC	vs	TT	1.42(1.04, 1.93)	0.028	1.41(1.04, 1.93)	0.029
let-7f rs10877887						
TC	vs	TT	1.22(0.90, 1.66)	0.197	1.21(0.89, 1.65)	0.219
CC	vs	TT	0.83(0.52, 1.31)	0.414	0.82(0.52, 1.30)	0.404
TC+CC	vs	TT	1.12(0.84, 1.49)	0.442	1.11(0.83, 1.48)	0.467
miR-27a rs895819						
AG	vs	AA	1.26(0.93, 1.71)	0.138	1.26(0.93, 1.71)	0.134
GG	vs	AA	1.27(0.72, 2.23)	0.408	1.26(0.72, 2.22)	0.421
AG+GG	vs	AA	1.26(0.94, 1.68)	0.116	1.26(0.94, 1.68)	0.117

[a]*ORs and 95%CI and corresponding P values were calculated by logistic regression analysis adjusted by sex, age, smoking, drinking, hypertension, and dyslipidemia; OR, odds ratio; CI, confidence interval.*

with smoking, drinking, hypertension, and dyslipidemia. As presented in **Table 4**, miR-133a-2 rs13040413 variant genotype had a positive interaction with dyslipidemia on T2DM (OR = 10.26, $P = 0.004$). let-7a-1 rs13293512 variant genotypes also had positive interactions with smoking (TC: OR = 2.04, $P = 0.049$; TC+CC: OR = 2.15, $P = 0.027$) and dyslipidemia (CC: OR = 1.93, $P = 0.040$; TC+CC: OR = 2.56, $P = 0.030$) on T2DM. However, there were no significant differences among other gene polymorphisms and environmental factors interactions ($P > 0.05$).

MiRNA SNP-SNP Interactions in T2DM

We further investigated miRNA SNP-SNP interaction effects in T2DM. The models included dominant genotypes of miRNA polymorphisms. It was showed that miR-133a-1 rs8089787 variant genotype and let-7a-1 rs13293512 variant genotype had a positive interaction effect in T2DM (OR = 3.79, $P = 0.037$). Moreover, there was a positive interaction effect of miR-133a-1 rs8089787 variant genotype and let-7f rs10877887 variant genotype in T2DM (OR = 3.61, P = 0.035) (**Table 5**). No significant differences were observed among other miRNA SNP-SNP interactions ($P > 0.05$).

The Effect of rs13040413 and rs13293512 on miR-133a and let-7a Expression

After 48 h, let-7a expression had statistical significance in HEK293T cell lines (**Figure 1B**). The variant C allele expressed a lower let-7a when compared to the wild T allele (2.51 ± 0.22 vs 3.11 ± 0.28, P = 0.047). While miR-133a expression did not reach

TABLE 3 | Stratified analysis of association between miRNA polymorphisms and type 2 diabetes mellitus.

Genotype			Male		Female		≥60		<60	
			OR(95%CI)[a]	*P* value	OR(95%CI)[a]	*P* value	OR(95%CI)[b]	*P* value	OR(95%CI)[b]	*P* value
miR-133a-1 rs8089787										
CT	vs	CC	0.76(0.45, 1.27)	0.289	1.05(0.65, 1.70)	0.850	1.31(0.70, 2.44)	0.402	0.74(0.48, 1.14)	0.165
TT	vs	CC	0.46(0.04, 5.07)	0.522	1.36(0.36, 5.19)	0.651	1.87(0.16, 21.35)	0.616	0.86(0.23, 3.26)	0.822
CT+TT	vs	CC	0.74(0.44, 1.23)	0.248	1.07(0.67, 1.71)	0.767	1.33(0.72, 2.45)	0.367	0.74(0.49, 1.13)	0.167
miR-133a-2 rs13040413										
CT	vs	CC	1.29(0.82, 2.02)	0.274	1.21(0.76, 1.95)	0.424	1.26(0.79, 2.010)	0.336	1.20(0.76, 1.89)	0.436
TT	vs	CC	2.27(0.76, 6.76)	0.141	2.13(0.86, 5.27)	0.103	5.42(1.52, 19.30)	0.009	1.22(0.49, 3.05)	0.672
CT+TT	vs	CC	1.38(0.90, 2.13)	0.145	1.31(0.85, 2.04)	0.226	1.50(0.96, 2.33)	0.077	1.19(0.78, 1.83)	0.425
let-7a-1 rs13293512										
TC	vs	TT	1.34(0.85, 2.11)	0.213	1.35(0.84, 2.17)	0.219	2.19(1.21, 3.97)	0.010	1.08(0.72, 1.60)	0.714
CC	vs	TT	1.87(1.03, 3.39)	0.040	1.38(0.76, 2.50)	0.287	2.40(1.37, 4.23)	0.005	1.25(0.76, 2.05)	0.375
TC+CC	vs	TT	1.47(0.95, 2.26)	0.084	1.36(0.87, 2.13)	0.179	3.20(1.42, 7.22)	0.002	1.12(0.77, 1.64)	0.545
let-7f rs10877887										
TC	vs	TT	1.17(0.77, 1.80)	0.466	1.24(0.80, 1.93)	0.336	1.44(0.82, 2.51)	0.200	1.14(0.79, 1.65)	0.486
CC	vs	TT	0.70(0.36, 1.36)	0.290	1.01(0.52, 1.94)	0.988	0.99(0.40, 2.46)	0.986	0.82(0.48, 1.41)	0.472
TC+CC	vs	TT	1.05(0.70, 1.57)	0.808	1.18(0.78, 1.79)	0.429	1.34(0.79, 2.27)	0.282	1.05(0.75, 1.49)	0.764
miR-27a rs895819										
AG	vs	AA	1.53(1.00, 2.34)	0.049	1.02(0.66, 1.58)	0.925	1.35(0.78, 2.34)	0.289	1.23(0.85, 1.77)	0.267
GG	vs	AA	1.50(0.69, 3.25)	0.308	1.01(0.44, 2.33)	0.977	4.92(1.01, 24.05)	0.049	0.95(0.50, 1.80)	0.874
AG+GG	vs	AA	1.59(1.07, 2.59)	0.039	1.02(0.67, 1.54)	0.942	1.53(0.90, 2.61)	0.119	1.17(0.83, 1.66)	0.366

[a]ORs and 95%CI and corresponding P values were calculated by logistic regression analysis adjusted by age, smoking, drinking, hypertension, and dyslipidemia; [b]ORs and 95%CI and corresponding P values were calculated by logistic regression analysis adjusted by sex, smoking, drinking, hypertension, and dyslipidemia; OR, odds ratio; CI, confidence interval.

TABLE 4 | SNP-environmental factors interaction effects on type 2 diabetes mellitus.

			Smoking (+) vs (-)		Drinking (+) vs (-)		Hypertension (+) vs (-)		Dyslipidemia (+) vs (-)	
			OR(95%CI)[a]	*P* value	OR(95%CI)[b]	*P* value	OR(95%CI)[c]	*P* value	OR(95%CI)[d]	*P* value
miR-133a-1 rs8089787										
CT	vs	CC	1.26(0.63, 2.56)	0.514	0.78(0.36, 1.68)	0.524	1.08(0.47, 2.49)	0.857	0.73(0.26, 2.04)	0.550
TT	vs	CC	4.77(0.32, 70.74)	0.257	1.05(0.33, 3.30)	0.935	1.87(0.12, 29.59)	0.656	1.05(0.33, 3.30)	0.935
CT+TT	vs	CC	1.36(0.68, 2.69)	0.385	0.89(0.42, 1.89)	0.756	1.13(0.50, 2.53)	0.775	0.73(0.26, 2.01)	0.538
miR-133a-2 rs13040413										
CT	vs	CC	1.02(0.54, 1.96)	0.946	1.42(0.70, 2.90)	0.333	1.82(0.86, 3.88)	0.120	0.87(0.38, 1.96)	0.730
TT	vs	CC	2.24(0.38, 13.14)	0.371	1.87(0.95, 3.67)	0.068	2.24(0.38, 13.14)	0.371	1.08(0.21, 5.46)	0.929
CT+TT	vs	CC	1.24(0.67, 2.29)	0.491	10.26(2.08, 50.56)	0.004	1.86(0.91, 3.82)	0.089	0.92(0.43, 1.97)	0.824
let-7a-1 rs13293512										
TC	vs	TT	2.04(1.00, 4.17)	0.049	1.75(0.91, 3.39)	0.096	1.06(0.51, 2.20)	0.882	0.74(0.32, 1.75)	0.498
CC	vs	TT	2.58(1.00, 6.68)	0.051	1.93(1.03, 3.60)	0.040	0.67(0.27, 1.66)	0.382	1.00(0.33, 3.04)	0.997
TC+CC	vs	TT	2.15(1.09, 4.23)	0.027	2.56(1.09, 6.01)	0.030	0.93(0.47, 1.84)	0.828	0.80(0.35, 1.80)	0.583
let-7f rs10877887										
TC	vs	TT	1.40(0.76, 2.58)	0.286	1.50(0.76, 2.97)	0.240	0.81(0.41, 1.61)	0.551	0.55(0.25, 1.21)	0.136
CC	vs	TT	1.74(0.69, 4.42)	0.241	2.33(0.81, 6.69)	0.116	1.11(0.38, 3.21)	0.850	1.46(0.46, 4.66)	0.520
TC+CC	vs	TT	1.46(0.82, 2.59)	0.202	1.64(0.86, 3.11)	0.134	0.88(0.46, 1.67)	0.689	0.69(0.33, 1.43)	0.318
miR-27a rs895819										
AG	vs	AA	1.11(0.61, 2.05)	0.730	0.97(0.49, 1.91)	0.926	1.18(0.59, 2.34)	0.640	1.30(0.59, 2.84)	0.518
GG	vs	AA	2.38(0.74, 7.67)	0.147	0.77(0.21, 2.81)	0.695	2.86(0.79, 10.43)	0.111	0.79(0.20, 3.12)	0.738
AG+GG	vs	AA	1.26(0.70, 2.24)	0.442	0.93(0.49, 1.78)	0.833	1.37(0.72, 2.63)	0.340	1.18(0.57, 2.48)	0.656

[a]ORs and 95%CI and corresponding P values were calculated by logistic regression analysis adjusted by age, sex, drinking, hypertension, and dyslipidemia; b, ORs and 95%CI and corresponding P values were calculated by logistic regression analysis adjusted by age, sex, smoking, hypertension, and dyslipidemia; [c]ORs and 95%CI and corresponding P values were calculated by logistic regression analysis adjusted by age, sex, smoking, drinking, and dyslipidemia; [d] ORs and 95%CI and corresponding P values were calculated by logistic regression analysis adjusted by age, sex, smoking, drinking, and hypertension; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

statistical significance in HEK293T cell lines (**Figure 1A**). The variant T allele showed a trend toward decreased miR-133a expression in comparison with the wild C allele (0.76 ± 0.10 vs 0.99 ± 0.12, P = 0.065).

DISCUSSION

Alterations in the miRNA involved in the insulin signaling pathways play important roles in the pathogenesis of T2DM.

TABLE 5 | SNP-SNP interaction effects on type 2 diabetes mellitus.

Genetic model			OR(95%CI)[a]	*P* value
miR-133a-2 rs13040413 CT+TT vs CC	×	miR-133a-1 rs8089787 CT+TT vs CC	0.78(0.38, 1.62)	0.510
miR-133a-2 rs13040413 CT+TT vs CC	×	let-7a-1 rs13293512 TC+CC vs TT	1.00(0.51, 1.95)	0.997
miR-133a-2 rs13040413 CT+TT vs CC	×	let-7f rs10877887 TC+CC vs TT	1.42(0.77, 2.63)	0.265
miR-133a-2 rs13040413 CT+TT vs CC	×	miR-27a rs895819 AG+GG vs AA	0.84(0.45, 1.56)	0.581
miR-133a-1 rs8089787 CT+TT vs CC	×	let-7a-1 rs13293512 TC+CC vs TT	3.79(1.60, 8.41)	0.037
miR-133a-1 rs8089787 CT+TT vs CC	×	let-7f rs10877887 TC+CC vs TT	3.61(2.01, 5.38)	0.035
miR-133a-1 rs8089787 CT+TT vs CC	×	miR-27a rs895819 AG+GG vs AA	1.07(0.54, 2.13)	0.844
let-7a-1 rs13293512 TC+CC vs TT	×	let-7f rs10877887 TC+CC vs TT	0.87(0.46, 1.62)	0.654
let-7a-1 rs13293512 TC+CC vs TT	×	miR-27a rs895819 AG+GG vs AA	0.78(0.42, 1.47)	0.443
let-7f rs10877887 TC+CC vs TT	×	miR-27a rs895819 AG+GG vs AA	1.25(0.70, 2.22)	0.458

[a]*ORs and 95%CI and corresponding P values were calculated by logistic regression analysis adjusted by age, sex, smoking, drinking, hypertension, and dyslipidemia; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.*

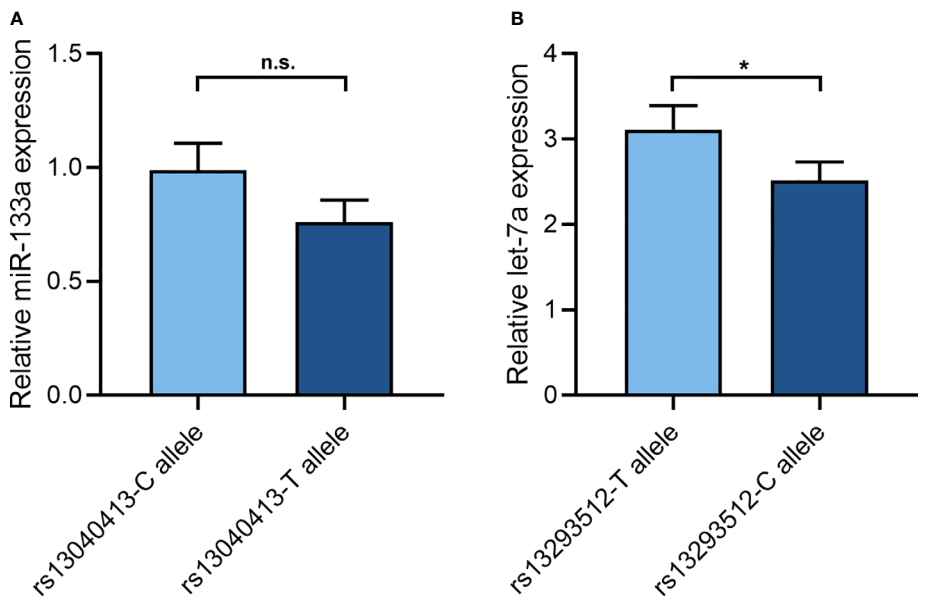

FIGURE 1 | The effect of rs13040413 and rs13293512 on miR-133a and let-7a expression. **(A)** The cell miR-133a expression by different miR-133a-2 rs13040413 plasmids. **(B)** The cell let-7a expression by different let-7a-1 rs13293512 plasmids. *$P < 0.05$. n.s., no significance.

SNPs in these miRNA may down-regulate or up-regulate miRNA expression in T2DM (24). The present study focused on the relation of SNPs of miRNA involved in the insulin signaling pathways, miR-133a-1 rs8089787, miR-133a-2 rs13040413, let-7a-1 rs13293512, let-7f rs10877887, and miR-27a rs895819, with T2DM, and the interaction effects of SNP-SNP and SNP-environmental factors on T2DM, as well as the effect of the risk-associated polymorphism on regulating its mature miRNA expression. To our knowledge, this is the first to comprehensively report the issues.

Our findings revealed that miR-133a-2 rs13040413 and let-7a-1 rs13293512 were associated with increased T2DM risk. For miR-133a-2 rs13040413, the variant T allele showed a trend toward decreased miR-133a expression in comparison with the

wild C allele. For let-7a-1 rs13293512, the variant C allele expressed a lower let-7a compared to the wild T allele. It has been suggested that miR-133a can affect IGF1R and GLUT4 expression in the insulin signaling pathways. Gong et al. verified that IGF1R gene is a direct target of miR-133a using luciferase reporter assays (25). MiR-133a can directly suppress the expression of IGF1R through translational repression. Moreover, miR-133a has been reported to act indirectly upon GLUT4 expression. MiR-133a can target the Krueppel-like factor 15 (Klf15) mRNA, inhibiting this transcriptional factor, which is an enhancer of GLUT4 expression, thus leading to the reduction in GLUT4 expression and in insulin stimulated glucose uptake (5). MiR-133a not only showed differential expression in blood level of T2DM, but also in tissue levels of T2DM complications, such as kidney, heart, sciatic nerve, and skeletal muscle (26–29). MiR-133a-2 rs13040413 variant could also increase levels of miR-133a (19). All the above contribute to the underlying mechanisms of the association between miR-133a-2 rs13040413 and T2DM. With regard to let-7a-1 rs13293512, it has been showed that the targets of let-7a is IGF1R, insulin receptor substrate 2 (IRS2) and PI3K/AKT of insulin signaling pathways. Exogenous expression of let−7a can suppress the expression of IGF1R (30). Meanwhile, inhibition of let-7a is sufficient to enhance the expression of IRS2 (31). Wang et al. has also showed that transfection of let−7a mimics can lead to the inhibition of the PI3K/AKT, and transfection of let−7a inhibitors may activate the PI3K/AKT through the increase in PI3K and AKT levels (4). IGF1R, IRS2, and PI3K/AKT are known to mediate both insulin signaling pathways and insulin-induced cell dysregulations. As a functional polymorphism, let-7a-1 rs13293512 can substantially reduce the transcription activity of let-7, as well as directly upregulate IL-6 expression (17). The deep understanding of the potential mechanisms will yield further insights into the relationship of let-7a-1 rs13293512 and T2DM.

Additionally, in the stratified analyses, miR-133a-2 rs13040413, let-7a-1 rs13293512, and miR-27a rs895819 were associated with increased T2DM risk in older subjects (age ≥ 60 years), suggesting that age effect was dominant cause of T2DM in older subjects. Accumulated exposure to insulin disturbance caused by these three SNPs and weak immune system were expected in older subjects with T2DM. Let-7a-1 rs13293512 and miR-27a rs895819 were associated with increased T2DM risk in male subjects. Hormonal differences between males and females may elucidate sex-specific variation in T2DM. There is evidence to show bidirectional signaling cross-talk between let-7a, miR-27a, and estrogen receptors (32, 33). A role for estrogen in the regulation of glucose metabolism provides a potential biologic explanation to our results (34).

Being a multifactorial disease, there might be complex interactions between the risk allele and confounding factors in T2DM. In our study, interaction effects on T2DM have been demonstrated between miR-133a-2 rs13040413 or let-7a-1 rs13293512 and dyslipidemia. It has been showed that the expression levels of miR-133a and let-7a were in a dysregulation pattern under high lipid condition (35). Evidence also showed that miR-133a and let-7a were also related to lipid accumulation (36, 37). Additionally, we observed there was interaction effect between let-7a-1 rs13293512 and smoking on T2DM. Banerjee A. showed that let-7a was differentially expressed between the smoking and nonsmoking subjects (38). Let-7a showed a correlation with haemoglobin adduct biomarkers of tobacco exposure. Moreover, besides miR-133a and let-7a, dyslipidemia and smoking have been known as high risk factors of T2DM. Collectively, we assumed that miR-133a and let-7a had cross-talk with dyslipidemia or smoking, which could explain the phenomenon of interaction effects between miR-133a-2 rs13040413 or let-7a-1 rs13293512 and dyslipidemia or smoking on T2DM.

In our study, we also detected interaction effects of miR-133a-1 rs8089787 and let-7a-1 rs13293512 or let-7f rs10877887 on T2DM. This phenomenon is defined as epistatic effect, which usually accounts for missing or underestimated heritability when one single gene is included in disease susceptibility (39). Likely, miR-133a rs8089787 had no effect on T2DM. In contrast, miR-133a-1 rs8089787 and let-7a-1 rs13293512 or let-7f rs10877887 worked together to generate interaction effects on T2DM. We assumed that the functional effect of miR-133a, let-7a, and let-7f in the insulin signaling pathway might account for the observed interaction effect. As described above, miR-133a and let-7a were involved in the insulin signaling pathway and the pathogenesis of T2DM. As for let-7f, it has been reported that let-7f could target multiple key genes related to insulin signaling pathway. Mellios et al. reported that let-7f could inhibit IGF1 expression, and inhibition of let-7f could significantly up-regulate levels of IGF1 mRNA (40). Hu et al. showed that let-7f mimics suppressed IGF1R expression, and let-7f inhibitors increased the expression level of IGF1R (3). Furthermore, Wang et al. revealed that insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) was potential target of let-7f. Let-7f could suppress IGF2BP1 expression (41). Combined, any genetic mutation in the insulin signaling pathway, like miR-133a-1 rs8089787, let-7a-1 rs13293512, and let-7f rs10877887, could potentially alter the action of each other so as to influence insulin function in pathogenesis of T2DM.

Several limitations of this study should be noted. First, the sample size in this study was relatively small, which might restrict the ability to explore weak genetic effect on T2DM. Prospective studies consisting of larger-scale sample and multicenter surveys are necessary to validate the findings of miRNA polymorphisms on T2DM. Second, only miRNA polymorphisms were included in this study. Further studies should involve SNPs in the miRNA binding site of potential targeted genes, which could deeply explored the SNP-SNP interactions in miRNA regulatory pathways. Third, considering the clinical significance of the study, the more comprehensive functional and molecular experiments of the mentioned miRNA polymorphisms on T2DM would be of great significance for clinical practice and may be an important future direction.

CONCLUSIONS

This study, for the first time, investigated the relationships of miRNA polymorphisms involving in insulin signaling pathways and T2DM, and reported that miR-133a-2 rs13040413, let-7a-1 rs13293512, and miR-27a rs895819 were related to the

susceptibility to T2DM in overall or stratified analyses in a Chinese population. For miR-133a-2 rs13040413, the variant T allele showed a trend toward decreased miR-133a expression in comparison with the wild C allele. For let-7a-1 rs13293512, the variant C allele expressed a lower let-7a compared to the wild T allele. Novel interaction effects on T2DM were revealed among miR-133a-2 rs13040413 with dyslipidemia, let-7a-1 rs13293512 with smoking, let-7a-1 rs13293512 with dyslipidemia, miR-133a-1 rs8089787 with let-7a-1 rs13293512, and miR-133a-1 rs8089787 with let-7f rs10877887. Future large-scale studies and more comprehensive mechanism experiments are required.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of China Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DS conceptualized and supervised the study. JY and CM designed the study together with DS, ZZ, YZ, and RB carried out genomic DNA extraction and SNP genotyping. RY and ZS participated in the clinical data and blood sample collections. DS and YZ did the statistical analyses. All authors contributed to data interpretation. DS, ZZ, and YZ wrote the paper with input from all authors. JY and CM revised the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Liu MH, Li Y, Han L, Zhang YY, Wang D, Wang ZH, et al. Adipose-derived stem cells were impaired in restricting CD4(+)T cell proliferation and polarization in type 2 diabetic ApoE(-/-) mouse. *Mol Immunol* (2017) 87:152–60.
2. Chiasson JL, Rabasa-Lhoret R. Prevention of type 2 diabetes: insulin resistance and beta-cell function. *Diabetes* (2004) 53 Suppl 3:S34–8.
3. Hu W, Li T, Hu R, Wu L, Li M, Meng X. MicroRNA let-7a and let-7f as novel regulatory factors of the sika deer (Cervus nippon) IGF-1R gene. *Growth Factors* (2014) 32:27–33.
4. Wang T, Zhu H, Yang S, Fei X. Let7a5p may participate in the pathogenesis of diabetic nephropathy through targeting HMGA2. *Mol Med Rep* (2019) 19:4229–37.
5. Horie T, Ono K, Nishi H, Iwanaga Y, Nagao K, Kinoshita M, et al. MicroRNA-133 regulates the expression of GLUT4 by targeting KLF15 and is involved in metabolic control in cardiac myocytes. *Biochem Biophys Res Commun* (2009) 389:315–20.
6. Zhang E, Chen Q, Wang J, Li D, Wan Z, Ju X. Protective role of microRNA-27a upregulation and HSP90 silencing against cerebral ischemia-reperfusion injury in rats by activating PI3K/AKT/mTOR signaling pathway. *Int Immunopharmacol* (2020) 86:106635.
7. Santovito D, De Nardis V, Marcantonio P, Mandolini C, Paganelli C, Vitale E, et al. Plasma exosome microRNA profiling unravels a new potential modulator of adiponectin pathway in diabetes: effect of glycemic control. *J Clin Endocrinol Metab* (2014) 99:E1681–5.
8. Liang Z, Gao KP, Wang YX, Liu ZC, Tian L, Yang XZ, et al. RNA sequencing identified specific circulating miRNA biomarkers for early detection of diabetes retinopathy. *Am J Physiol Endocrinol Metab* (2018) 315:E374–85.
9. Ghasemi H, Karimi J, Khodadadi I, Tavilani H. Correlation between miR-103 and miR-133a Expression and the Circulating ANGPTL8 in Type 2 Diabetic Patients and Healthy Control Subjects. *Clin Lab* (2019) 65:19436.
10. Nunez Lopez YO, Garufi G, Seyhan AA. Altered levels of circulating cytokines and microRNAs in lean and obese individuals with prediabetes and type 2 diabetes. *Mol Biosyst* (2016) 13:106–21.
11. Shen H, Li J, Zhang J, Xu C, Jiang Y, Wu Z, et al. Comprehensive characterization of human genome variation by high coverage whole-genome sequencing of forty four Caucasians. *PloS One* (2013) 8:e59494.
12. Ghaedi H, Bastami M, Zare-Abdollahi D, Alipoor B, Movafagh A, Mirfakhraie R, et al. Bioinformatics prioritization of SNPs perturbing microRNA regulation of hematological malignancy-implicated genes. *Genomics* (2015) 106:360–6.
13. Zhang L, Yang J, Xue Q, Yang D, Lu Y, Guang X, et al. An rs13293512 polymorphism in the promoter of let-7 is associated with a reduced risk of ischemic stroke. *J Thromb Thrombolysis* (2016) 42:610–5.
14. Liang Y, Zhao G, Sun R, Mao Y, Li G, Chen X, et al. Genetic variants in the promoters of let-7 family are associated with an increased risk of major depressive disorder. *J Affect Disord* (2015) 183:295–9.
15. Sima X, Sun H, Zhou P, You C. A Potential Polymorphism in the Promoter of Let-7 is Associated With an Increased Risk of Intracranial Aneurysm: A Case-Control Study. *Medicine* (2015) 94:e2267.
16. Liu J, Ni S. Association between genetic polymorphisms in the promoters of let-7 and risk of cervical squamous cell carcinoma. *Gene* (2018) 642:256–60.
17. Dong H, Huang Z, Zhang H, Xiao Z, Liu Q. Rs13293512 polymorphism located in the promoter region of let-7 is associated with increased risk of radiation enteritis in colorectal cancer. *J Cell Biochem* (2018) 119:6535–44.
18. Zhou PP, Li Y, Ma ZD, Li ZY, Chen FY, Jiang YX. Single nucleotide polymorphisms in the promoter region of mir-133a-1 and in pre-mir-152 rs1707 may contribute to the risk of asthma in a Chinese Han population. *Eur Rev Med Pharmacol Sci* (2016) 20:2642–9.
19. Ohanian M, Humphreys DT, Anderson E, Preiss T, Fatkin D. A heterozygous variant in the human cardiac miR-133 gene, MIR133A2, alters miRNA duplex processing and strand abundance. *BMC Genet* (2013) 14:18.
20. Li G, Liu B, Jiang Q, Zhang J, Xin S, Xu K. The association of two common polymorphisms in miRNAs with diabetes mellitus: A meta-analysis. *Medicine* (2019) 98:e17414.
21. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes

mellitus provisional report of a WHO consultation. *Diabet Med* (1998) 15:539–53.
22. Sun D, Sun L, Xu Q, Gong Y, Wang H, Yang J, et al. SNP-SNP Interaction between TLR4 and MyD88 in Susceptibility to Coronary Artery Disease in the Chinese Han Population. *Int J Environ Res Public Health* (2016) 13:278.
23. Xu Q, Yuan Y, Sun LP, Gong YH, Xu Y, Yu XW, et al. Risk of gastric cancer is associated with the MUC1 568 A/G polymorphism. *Int J Oncol* (2009) 35:1313–20.
24. Zhang Y, Bai R, Liu C, Ma C, Chen X, Yang J, et al. MicroRNA single-nucleotide polymorphisms and diabetes mellitus: A comprehensive review. *Clin Genet* (2019) 95:451–61.
25. Gong Y, Ren J, Liu K, Tang LM. Tumor suppressor role of miR-133a in gastric cancer by repressing IGF1R. *World J Gastroenterol* (2015) 21:2949–58.
26. Lee WC, Li LC, Ng HY, Lin PT, Chiou TT, Kuo WH, et al. Urinary Exosomal MicroRNA Signatures in Nephrotic, Biopsy-Proven Diabetic Nephropathy. *J Clin Med* (2020) 9.
27. Nandi SS, Zheng H, Sharma NM, Shahshahan HR, Patel KP, Mishra PK. Lack of miR-133a Decreases Contractility of Diabetic Hearts: A Role for Novel Cross Talk Between Tyrosine Aminotransferase and Tyrosine Hydroxylase. *Diabetes* (2016) 65:3075–90.
28. Chang LL, Wang HC, Tseng KY, Su MP, Wang JY, Chuang YT, et al. Upregulation of miR-133a-3p in the Sciatic Nerve Contributes to Neuropathic Pain Development. *Mol Neurobiol* (2020) 57:3931–42.
29. Frias Fde T, de Mendonca M, Martins AR, Gindro AF, Cogliati B, Curi R, et al. MyomiRs as Markers of Insulin Resistance and Decreased Myogenesis in Skeletal Muscle of Diet-Induced Obese Mice. *Front Endocrinol* (2016) 7:76.
30. Tian B, Huo N, Li M, Li Y, He Z. let-7a and its target, insulin-like growth factor 1 receptor, are differentially expressed in recurrent prostate cancer. *Int J Mol Med* (2015) 36:1409–16.
31. Gurung B, Muhammad AB, Hua X. Menin is required for optimal processing of the microRNA let-7a. *J Biol Chem* (2014) 289:9902–8.
32. Sun X, Qin S, Fan C, Xu C, Du N, Ren H. Let-7: a regulator of the ERalpha signaling pathway in human breast tumors and breast cancer stem cells. *Oncol Rep* (2013) 29:2079–87.
33. Ali HO, Arroyo AB, Gonzalez-Conejero R, Stavik B, Iversen N, Sandset PM, et al. The role of microRNA-27a/b and microRNA-494 in estrogen-mediated downregulation of tissue factor pathway inhibitor alpha. *J Thromb Haemost* (2016) 14:1226–37.
34. Weigt C, Hertrampf T, Flenker U, Hulsemann F, Kurnaz P, Fritzemeier KH, et al. Effects of estradiol, estrogen receptor subtype-selective agonists and genistein on glucose metabolism in leptin resistant female Zucker diabetic fatty (ZDF) rats. *J Steroid Biochem Mol Biol* (2015) 154:12–22.
35. Youssef EM, Elfiky AM, Soliman B, Abu-Shahba N, Elhefnawi MM. Expression profiling and analysis of some miRNAs in subcutaneous white adipose tissue during development of obesity. *Genes Nutr* (2020) 15:8.
36. Peng XP, Huang L, Liu ZH. miRNA-133a attenuates lipid accumulation via TR4-CD36 pathway in macrophages. *Biochimie* (2016) 127:79–85.
37. Kim YS, Nam HJ, Han CY, Joo MS, Jang K, Jun DW, et al. LXRalpha activation inhibits autophagy and lipophagy in hepatocytes by dysregulating ATG4B and Rab-8B, reducing mitochondrial fuel oxidation. *Hepatology* (2020).
38. Banerjee A, Waters D, Camacho OM, Minet E. Quantification of plasma microRNAs in a group of healthy smokers, ex-smokers and non-smokers and correlation to biomarkers of tobacco exposure. *Biomarkers* (2015) 20:123–31.
39. Eichler EE, Flint J, Gibson G, Kong A, Leal SM, Moore JH, et al. Missing heritability and strategies for finding the underlying causes of complex disease. *Nat Rev Genet* (2010) 11:446–50.
40. Mellios N, Woodson J, Garcia RI, Crawford B, Sharma J, Sheridan SD, et al. beta2-Adrenergic receptor agonist ameliorates phenotypes and corrects microRNA-mediated IGF1 deficits in a mouse model of Rett syndrome. *Proc Natl Acad Sci U S A* (2014) 111:9947–52.
41. Wang Y, Chen X, Zhang Y, Song J. Potential proteins targeted by let-7f-5p in HeLa cells. *Biosci Trends* (2017) 11:363–5.

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Combined Associations of Serum Ferritin and Body Size Phenotypes With Cardiovascular Risk Profiles: A Chinese Population-Based Study

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Background: Serum ferritin (SF) has been correlated with one or more metabolic syndrome features associated with an increased risk for cardiovascular disease (CVD). This study explored the associations between SF and CVD risk factors among different body size phenotypes that were based on metabolic status and body mass index (BMI) categories.

Methods: A cross-sectional study was performed using a cohort of 7,549 Chinese adults from the China Health and Nutrition Survey. Participants did not exhibit acute inflammation, were not underweight and were stratified based on their metabolic status and BMI categories. The metabolically at-risk status was defined as having two or more criteria of the Adult Treatment Panel-III metabolic syndrome definition, excluding waist circumference.

Results: Compared with individuals without high SF, subjects with high SF had an increased risk of diabetes in the metabolically at-risk normal-weight (MANW) and metabolically at-risk overweight/obesity (MAO) groups. The multivariate-adjusted odds ratios (ORs) were 1.52 [95% confidence interval (CIs): 1.02, 2.28] and 1.63 (95% CIs: 1.27, 2.09), respectively. Adjusted ORs for hyperuricemia from high SF in metabolically healthy normal-weight (MHNW), metabolically healthy overweight/obesity (MHO), MANW, and MAO phenotypes were 1.78 (95% CIs: 1.26, 2.53), 1.42 (95% CIs: 1.03, 1.95), 1.66 (95% CIs: 1.17, 2.36), and 1.42 (95% CIs: 1.17, 1.73), respectively. Similarly, positive correlations of high SF with triglycerides, non-high-density lipoprotein cholesterol, and apolipoprotein B100 were observed in all phenotypes. No association between high SF and elevated low-density lipoprotein cholesterol were observed among participants who were metabolically at-risk, regardless of their BMI categories. However, the ORs for elevated low-density lipoprotein cholesterol from high SF were 1.64 (95% CIs: 1.29, 2.08) in the MHNW group and 1.52 (95% CIs:1.22, 1.91) in the MHO group, significantly. This study demonstrated that the highest ORs were in MAO with a high SF group for all unfavorable CVD risk factors except low-density lipoprotein cholesterol (all $p < 0.001$).

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Conclusions: The associations of high SF with the prevalence of CVD risk factors, including diabetes, dyslipidemia, and hyperuricemia, vary in individuals among different body size phenotypes. In the MAO group, subjects with high SF levels exhibited worse CVD risk profiles than individuals without high SF.

Keywords: serum ferritin, iron overload, body mass index, metabolism, cardiovascular disease

INTRODUCTION

Serum ferritin (SF) is a storage protein that maintains excess iron in a safe and bioavailable form and correlates linearly with body iron stores (1). Accumulating evidence suggests that there is a correlation between SF levels and one or more metabolic syndrome characteristics (2–6), which are closely linked to insulin resistance (IR) and imply an increased risk for cardiovascular disease (CVD) (7–9). High SF in iron metabolism has been proposed as a component of IR due to its influence on insulin-producing cells as well as insulin-sensitive tissues (2, 10). A prospective cohort study has shown that a significant association between SF and metabolic disturbances remained even after adjusting for homeostatic model assessment of insulin resistance (HOMA-IR), which suggested that alternative underlying mechanisms might exist (11). Another recent study indicated that the SF level was significantly correlated with lipid profiles, independent of hyperglycemia or IR, but the significance of the associations of SF with diabetes and IR were weak or absent after adjusting for dyslipidemia (12). Elevated uric acid (UA) has also been reported to be associated with high SF and metabolic disorders and might be a possible predictor of CVD (13–15). Taken together, the strength of the correlations between SF and cardiometabolic risk factors, such as diabetes, dyslipidemia, and hyperuricemia, might vary due to metabolic status (metabolically at-risk or healthy) and because of disparities in their dependence on IR. Alternative mechanisms, in addition to IR, might be involved. On the other hand, evidence suggests that adipose tissue might play an important role in the process of glucose and lipid metabolism influenced by iron status. The CODAM study observed the association between adipose-tissue IR and iron overload in healthy volunteers (16). An experimental study in mice has reported that an iron-enriched diet led to iron accumulation and IR in visceral adipose tissue (17). Thus, concerning the relationships between SF and CVD risk factors, the different body mass index (BMI) categories (overweight/obesity or normal) and the metabolic status, must be taken into account.

Abbreviations: ApoA1, apolipoprotein A1; ApoB, apolipoprotein B100; BMI, body mass index; BP, blood pressure; CHNS, China Health and Nutrition Survey; CIs, confidence intervals; CVD, cardiovascular disease; FIN, fasting insulin; FPG, fasting plasma glucose; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, hypersensitive C-reactive protein; IR, insulin resistance; LDL-C, low density lipoprotein cholesterol; MANW, metabolically at-risk normal-weight; MAO, metabolically at-risk overweight/obesity; Met, metabolic status; MHNW, metabolically healthy normal-weight; MHO, metabolically healthy overweight/obesity; ORs, odds ratios; SF, serum ferritin; TC, total cholesterol; TG, triglycerides; non-HDL-C, non-high-density lipoprotein cholesterol; UA, uric acid.

The potential implications of the concomitant presence of metabolic status and BMI categories have triggered interest in comparing these associations with future CVD risk. Individuals were grouped into four different body size phenotypes, including metabolically at-risk overweight/obesity (MAO), metabolically at-risk normal weight (MANW), metabolically healthy overweight/obesity (MHO), and metabolically healthy normal weight (MHNW) (18, 19). Data showed that individuals with MAO experienced the detrimental metabolic profiles characterized by IR, hyperglycemia, hypertension, and atherogenic dyslipidemia, and suffered from an increased risk for CVD (18). Although the associations between the MHO phenotype and cardiometabolic outcomes were not consistent and definition dependent, it should not be considered as a benign status due to its increased risk of metabolic alterations (19).

The correlations between high SF and CVD risk factors mentioned above have not been well-established in different body size phenotypes. The objective of this study was therefore to determine the associations of SF with CVD risk factors according to different body size phenotypes and to assess the combined influence of an unfavorable SF, BMI, and metabolic status on the prevalence of CVD risk factors.

MATERIALS AND METHODS

Study Design and Participants

The China Health and Nutrition Survey (CHNS) is an ongoing prospective cohort survey that includes a total of 10 waves from 1989 to 2015. For each wave, the survey followed a stratified multistage, random cluster process to obtain samples from the provinces and autonomous cities or districts that included approximately half of the Chinese population and significant variation in geography, economic development, and health status. Details about the CHNS are available at http://www.cpc.unc.edu/projects/china/home.html or elsewhere (20). This survey was approved by the institutional review board of the University of North Carolina at Chapel Hill, the National Institute of Nutrition and Health, the China Center for Disease Control and Prevention, and the Human and Clinical Research Ethics Committee of China-Japan Friendship Hospital. Each subject provided written informed consent.

Data relating to 8,704 participants aged 18 years or older were selected from the CHNS 2009, since that was the 1st year blood samples were available. The exclusion criteria included pregnancy ($n = 62$), incomplete information on sex, weight, height, blood pressure (BP), medical history of diabetes and hypertension. We also excluded participants for whom data were missing for one or more of the following values, SF, total

cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B100 (apoB), apolipoprotein A1 (apoA1), UA, fasting plasma glucose (FPG), and fasting insulin (FIN) ($n = 251$). This study also excluded 349 subjects who exhibited hypersensitive C-reactive protein (hs-CRP) >10 mg/L, which implied the presence of acute inflammation (21). Subjects were categorized as underweight, normal weight, and overweight/obese (with BMI < 18.5, 18.5–22.9, and ≥ 23 kg/m^2, respectively) in accordance with the World Health Organization criteria for Asian people (22). All underweight individuals ($n = 493$) were removed from the study and a final number of 7,549 participants were included.

Measurements and Definitions

For each participant, information on age, gender, and medical history of diabetes and hypertension were collected using a structured questionnaire. The participants' weight, height, and resting BP were obtained following strict standard protocols. The BMI was calculated as weight (kg) divided by height squared (m^2).

Blood samples were collected after an overnight fast. The samples were preserved and analyzed at the national central laboratory in Beijing with standardized protocols. The biochemical parameters including FPG, UA, TC, TG, LDL-C, and HDL-C were measured enzymatically using a Hitachi 7600 automated analyzer (Hitachi Inc., Tokyo, Japan). The level of non-high-density lipoprotein cholesterol (non-HDL-C) was calculated as TC minus HDL-C. Apolipoproteins and hs-CRP were determined using the immunoturbidimetric method (Randox Laboratories Ltd., Crumlin, UK and Denka Seiken Ltd., Niigata, Japan, respectively). SF and FIN concentrations were assessed using radioimmunology (North Institute of Bio-Tech, Beijing, China) *via* an XH-6020 gamma counter. HOMA-IR was calculated as FIN (uIU/mL) × FPG (mmol/L)/22.5.

According to the metabolic syndrome definition of Adult Treatment Panel-III and previous studies (18, 23), individuals were classified as metabolically at-risk when they met two or more of the following criteria: (1) Systolic/diastolic BP ≥ 130/85 mmHg or taking antihypertensive medication. (2) FBG ≥ 5.6 mmol/l or taking antidiabetic medication. (3) HDL-C < 1.0/1.3 mmol/L for men or women, respectively. (4) TG ≥ 1.7 mmol/L. The criterion of waist circumference was not included due to its collinearity with BMI. Participants who met one or no criterion were classified as metabolically healthy. Based on combinations of metabolic status and BMI, subjects were grouped into four different body size phenotypes, MHNW, MHO, MANW, and MAO.

Elevated gender-specific SF levels were defined as SF ≥ the 75th percentile value (≥ 213.20/93.97 ug/L for men or women, respectively), consistent with a definition published in a previous study (24). Individuals were defined as having diabetes if FBG was ≥ 7.0 mmol/L or were previously diagnosed (25). Hypertension was defined as systolic/diastolic BP ≥ 140/90 mmHg or if it was previously diagnosed. Hyperuricemia was defined as having a UA ≥ 6.0/7.0 mg/dL for men or women, respectively (13, 26). Dyslipidemia was defined as TC ≥ 5.2 mmol/L, TG ≥ 1.7 mmol/L, HDL-C < 1.0/1.3 mmol/L for men or women, respectively, LDL-C ≥ 3.4 mmol/L, or non-HDL-C ≥ 4.1 mmol/L, based on the 2016 Chinese Guideline for Adults (27). The presence of unfavorable apolipoproteins was defined as apoB ≥ the 85th percentile value (≥ 1.17/1.19 g/L for men or women, respectively), and apoA1 < the 15th percentile value (0.85/0.90 g/L for men or women, respectively) (28). The inflammation status of participants was categorized as hs-CRP < 1, 1–3, and 4–10 mg/L (29).

Statistical Analysis

All statistical analyses were performed using SPSS 24.0 (SPSS Inc., Chicago, IL, USA). Data distribution was determined using the Kolmogorov-Smirnov test, and variables were described as medians (interquartile range) or percentages, where appropriate. Based on the cross-classification of the SF status within the four body size phenotypes, the participants were categorized into eight mutually exclusive groups. These specific groups included MAO with or without high SF, MANW with or without high SF, MHO with or without high SF, and MHNW with or without high SF. Differences among the groups were identified using Kruskal-Wallis one-way analysis for continuous variables and Cochran-Mantel-Haenszel chi-square statistics for categorical variables. When it was deemed necessary, a Bonferroni *post-hoc* analysis was used for further multiple comparisons. Logistic regression models were designed to determine the associations of high SF with CVD risk profiles. Multivariable odds ratios (ORs) and 95% confidence intervals (CIs) were adjusted for age, sex, inflammation status (hs-CRP), and HOMA-IR. A bar chart was constructed to delineate the concordance of the three status (metabolically at-risk, overweight/obesity, and high SF) within the whole population and samples with each unfavorable CVD risk factor. Venn diagrams were used to visually display the clustering of the three status in the accumulation of the unfavorable CVD risk factors diabetes, hyperuricemia, and dyslipidemia. Other logistic regression analyses were conducted to evaluate the combined influences of the SF status and body size phenotypes on CVD risk factors. A two-tailed $P < 0.05$ was considered to be statistically significant.

RESULTS

Characteristics of Study Subjects Stratified Based on SF Status and Body Size Phenotypes

The prevalence of a high level of SF in the MHNW, MHO, MANW, and MAO phenotypes was 15.9, 21.7, 31.2, and 37.6%, respectively. Sex ratios did not present any significant difference with respect to the SF status within the range of body size phenotypes ($P = 0.172$). Individuals with high SF exhibited higher UA, TG, TC, non-HDL-C, and apoB compared to subjects without high SF in all body size phenotypes (Bonferroni *post-hoc* analysis, $P < 0.001$). When subjects with and without high SF were compared, participants with high SF showed higher FBG for the MHO and MAO groups, higher LDL-C for the MHNW and MHO groups, and lower HDL-C for

the MANW group (Bonferroni *post-hoc* analysis $P < 0.001$). Moreover, the differences in FPG, TG, LDL-C, and HDL-C among individuals with and without high SF were significantly influenced by their body size phenotype (*P*-value for the interaction < 0.01). However, the systolic/diastolic BP and apoA1 showed no statistically significant relationship with the SF status for any body size phenotypes (Bonferroni *post-hoc* analysis $P > 0.05$) (**Table 1**).

Clustering of Unfavorable SF, BMI, and Metabolic Status

Figure 1 demonstrates the degree of coincidence for the metabolically at-risk status overweight/obesity, and high SF. Of the 7,549 participants, 71.04% were identified as having any of the three status, and 10.41% exhibited all three simultaneously. Similarly, this study found that the three status occurred together in 33.39% of the diabetic participants. For individuals with other unfavorable CVD risk factors, the proportion of participants that exhibited the simultaneous presence of all three status ranged from 12.84 to 25.05%, which was also higher than observed for the total population.

Figure 2 depicts the degree of clustering for the metabolically at-risk, overweight/obesity, and high SF status in the samples with 0–3 items of the following CVD risk factors, diabetes, hyperuricemia, and dyslipidemia. The degree of the simultaneous presence of these three status in subjects with 0, 1, 2, and 3 of the CVD risk factors was 3.3, 13.8, 27.4, and 52.4%, respectively. The overlap between the status increased with the presence of increased numbers of CVD risk factors.

Relationship Between SF and CVD Risk Profiles Among the Body Size Phenotypes

The comparative ORs for high SF and CVD risk profiles for each body size phenotype are shown in **Table 2**. The association of high SF with diabetes was significant for the MANW and MAO phenotypes. The multivariable-adjusted ORs were 1.52 (95% CI: 1.02, 2.28) and 1.63 (95% CI: 1.27, 2.09), respectively. However, no significance was observed for the association of high SF and diabetes for the MHNW and MHO groups ($P > 0.05$). Compared with individuals without high SF, subjects with high SF exhibited an increased prevalence of hyperuricemia and dyslipidemia (regarding TC, TG, non-HDL-C, and apoB) for all body phenotypes. The adjusted ORs for hyperuricemia in the MAO, MANW, MHO, and MHNW groups were 1.42 (95% CI: 1.17, 1.73), 1.66 (95% CI: 1.17, 2.36), 1.42 (95% CI: 1.03, 1.95), and 1.78 (95% CI: 1.26, 2.53), respectively. Similar results were obtained for dyslipidemia for all body size phenotypes (all $P < 0.05$) except TC (no significance was observed for high SF after adjusting for age, sex, hs-CRP, and HOMA-IR in the MANW group) ($p = 0.076$). There was a more prominent association of high SF with the prevalence of unfavorable non-HDL-C for the metabolically healthy status compared to metabolically at-risk individuals with normal weights (*P*-value for the interaction < 0.05). A high SF was associated with an elevated LDL-C only in the MHNW group (OR: 1.64; 95% CI: 1.29, 2.08) and MHO group (OR: 1.52; 95% CI: 1.22, 1.91). Subjects in this study with a high SF did not exhibit significant ORs for unfavorable systolic/diastolic BP, HDL-C, or apoA1 for any of the phenotypes compared to participants without high SF (all $P > 0.05$).

Joint Effects of SF Levels and Body Size Phenotypes on CVD Risk Profiles

The multivariable-adjusted ORs for CVD risk factors from high SF, overweight/obesity, metabolically at-risk individuals, and combined effects are presented in **Table 3**. FPG, UA, TG, TC, LDL-C, non-HDL-C, and apoB were chosen for further study based on their significant association with high SF for at least one body size phenotype. Relative to the MHNW without a high SF level, overweight/obesity, and high SF subjects exhibited increased ORs for diabetes when the subjects also were metabolically at-risk. However, if the subjects were not metabolically at-risk, no association was observed between being overweight/obese and having a high SF with a prevalence of diabetes. The status of being metabolically at-risk, overweight/obesity, and high SF increased the ORs for hyperuricemia and dyslipidemia (including TG, TC, LDL-C, non-HDL-C, and apoB). The highest ORs were observed in the MAO with a high SF group for all unfavorable CVD risk factors except LDL-C, and the increase in ORs was greater among participants with high SF.

DISCUSSION

This study has described the characteristics of participants classified according to SF status and body size phenotypes. When compared to subjects without high SF, those with high SF exhibited higher FBG in MHO and MAO groups, higher LDL-C in MHNW and MHO groups, lower HDL-C in the MANW group, and higher UA, TG, TC, non-HDL-C, and apoB in all body size phenotypes. Significant associations were observed for high SF with diabetes in MANW and MAO groups, with unfavorable LDL-C in MHNW and MHO groups, and with other dyslipidemia components (including TG, TC, non-HDL-C, and apoB) and hyperuricemia for all phenotypes. This was the first study to show the proportion of simultaneous presence of the three status, metabolically at-risk, overweight or obese, and high SF, which ranged from 12.84 to 33.39% in individuals with unfavorable CVD risk factors. The clustering of the three status increased with the accumulation of CVD risk factors. For all unfavorable CVD risk factors except LDL-C, the highest ORs were observed in MAO with a high SF group.

Although multiple epidemiological studies have reported correlations between elevated SF levels and CVD risk factors (4, 12, 13, 30, 31), the results are not entirely consistent. Sun et al. found an independent association between elevated SF and diabetes in a prospective study in a Chinese population (31). Similar findings have been reported from prospective cohort studies in Caucasian populations (32–34). The EPIC-Norfolk cohort study established that modest increases in SF could predict incident diabetes independent of confounding factors, especially inflammation indexes (35). The EPIC Postdam study demonstrated an association between SF and diabetic

TABLE 1 | Characteristics of the study participants, stratified by serum ferritin status and body size phenotypes.

	Metabolically healthy				Metabolically at-risk				P^a	P^b
	BMI < 23 kg/m^2		BMI ≥ 23 kg/m^2		BMI < 23 kg/m^2		BMI ≥ 23 kg/m^2			
	Without high SF	With high SF	Without high SF	With high SF	Without high SF	With high SF	Without high SF	With high SF		
N (%)	2186 (84.1)	414 (15.9)	1575 (78.3)	436 (21.7)	581 (68.8)	264 (31.2)	1307 (62.4)	786 (37.6)	-	-
Male, %	46.7	48.1	45.3	51.4	45.8	42	48.6	48	0.172	-
Age, years	44.8 (34.9, 56.5)	55.0 (41.7, 64.0)	47.2 (39.6, 56.7)	54.2 (44.0, 62.3)	55.1 (44.6, 66.4)	59.9 (52.9, 67.3)	53.5 (44.6, 62.9)	56.5 (46.7, 63.3)	<0.001	-
BMI, kg/m^2	21.0 (20.0, 21.9)	21.2 (20.0, 22.1)	25.0 (23.9, 26.6)	25.2 (24.0, 26.6)	21.4 (20.5, 22.2)	21.7 (20.5, 22.3)	25.9 (24.4, 28.0)	26.3 (24.6, 28.2)	<0.001	-
SF, ug/L	53.3 (25.1, 87.7)	238.3 (131.1, 408.7)	57.9 (28.9, 91.5)	248.2 (136.9, 490.7)	66.2 (34.6, 100.3)	232.1 (136.6, 440.7)	70.5 (42.7, 111.1)	268.6 (155.2, 509.5)	<0.001	-
SBP, mmHg	116.0 (108.0, 122.0)	120.0 (110.0, 126.0)	120.0 (110.0, 130.0)	120.0 (114.0, 130.0)	130.0 (120.0, 140.0)	130.0 (120.0, 141.0)	130.0 (120.0, 146.0)	130.0 (120.0, 146.0)	<0.001	0.783
DBP, mmHg	76.0 (70.0, 80.0)	78.0 (70.0, 80.0)	80.0 (74.0, 84.0)	80.0 (74.0, 86.0)	80.0 (76.0, 90.0)	82.0 (78.0, 90.0)	86.0 (80.0, 90.0)	86.0 (80.0, 92.0)	<0.001	0.952
FPG, mmol/L	4.87 (4.56, 5.21)	4.99 (4.62, 5.33)	4.94 (4.63, 5.28)	5.05 (4.69, 5.39)	5.64 (4.99, 6.06)	5.70 (5.04, 6.42)	5.63 (5.07, 6.21)	5.78 (5.25, 6.87)	<0.001	< 0.001
UA, mg/dL	4.47 (3.63, 5.45)	4.97 (4.10, 5.85)	4.67 (3.78, 5.63)	5.13 (4.37, 6.23)	4.99 (4.08, 6.20)	5.60 (4.63, 6.71)	5.55 (4.48, 6.70)	6.03 (4.97, 7.24)	<0.001	0.199
TG, mmol/L	0.91 (0.68, 1.23)	1.10 (0.84, 1,41)	1.09 (0.80, 1.44)	1.26 (0.99, 1.57)	1.85 (1.20, 2.59)	2.14 (1.61, 3.20)	2.10 (1.49, 2.92)	2.47 (1.83, 3.78)	<0.001	< 0.001
TC, mmol/L	4.49 (3.93, 5.08)	4.85 (4.22, 5.52)	4.73 (4.17, 5.35)	5.16 (4.53, 5.78)	4.80 (4.24, 5.55)	5.09 (4.41, 5.90)	4.98 (4.36, 5.61)	5.31 (4.59, 6.04)	<0.001	0.401
LDL-C, mmol/L	2.71 (2.20, 3.24)	3.01 (2.39, 3.97)	3.00 (2.49, 3.57)	3.29 (2.66, 3.98)	2.88 (2.27, 3.58)	2.95 (2.18, 3.75)	3.04 (2.43, 3.63)	3.08 (2.33, 3.83)	<0.001	< 0.001
HDL-C, mmol/L	1.52 (1.32, 1.77)	1.51 (1.33, 1.76)	1.43 (1.24, 1.64)	1.44 (1.26, 1.65)	1.27 (1.09, 1.55)	1.20 (1.04, 1.43)	1.17 (1.00, 1.37)	1.14 (0.97, 1.35)	<0.001	0.006
Non-HDL-C, mmol/L	2.91 (2.40, 3.46)	3.25 (2.69, 3.97)	3.28 (2.74, 3.84)	3.64 (3.07, 4.27)	3.49 (2.88, 4.16)	3.85 (3.14, 4.57)	3.77 (3.18, 4.36)	4.11 (3.46, 4.79)	<0.001	0.790
ApoB, g/L	0.77 (0.64, 0.92)	0.86 (0.73, 1.05)	0.87 (0.73, 1.04)	0.96 (0.80, 1.14)	0.91 (0.76, 1.11)	0.99 (0.79, 1.21)	0.99 (0.82, 1.16)	1.03 (0.86, 1.24)	<0.001	0.065
ApoA1, g/L	1.14 (1.0, 1.33)	1.17 (1.01, 1.34)	1.10 (0.97, 1.27)	1.13 (0.96, 1.29)	1.08 (0.94, 1.27)	1.08 (0.93, 1.30)	1.02 (0.88, 1.21)	1.01 (0.87, 1.21)	<0.001	0.447
Hs-CRP, mg/L	1.0 (0.0, 1.0)	1.0 (0.0, 2.0)	1.0 (1.0, 2.0)	1.0 (1.0, 3.0)	1.0 (0.0, 2.0)	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	2.0 (1.0, 4.0)	<0.001	0.007
HOMA-IR	1.87 (1.33, 2.61)	1.88 (1.36, 2.64)	2.22 (1.59, 3.20)	2.57 (1.84, 3.61)	2.74 (1.84, 4.09)	2.78 (1.84, 4.98)	3.32 (2.29, 5.42)	3.90 (2.54, 6.72)	<0.001	0.05

Data are median (interquartile range).

[a]P represents the difference across SF status within body size phenotypes, [b]P for interaction terms (SF status × body size phenotypes) were assessed by generalized linear models.

BMI, body mass index; SF, serum ferritin; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; UA, uric acid; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; ApoB, apolipoprotein B100; ApoA1, apolipoprotein A1; Hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homoeostasis model assessment of insulin resistance.

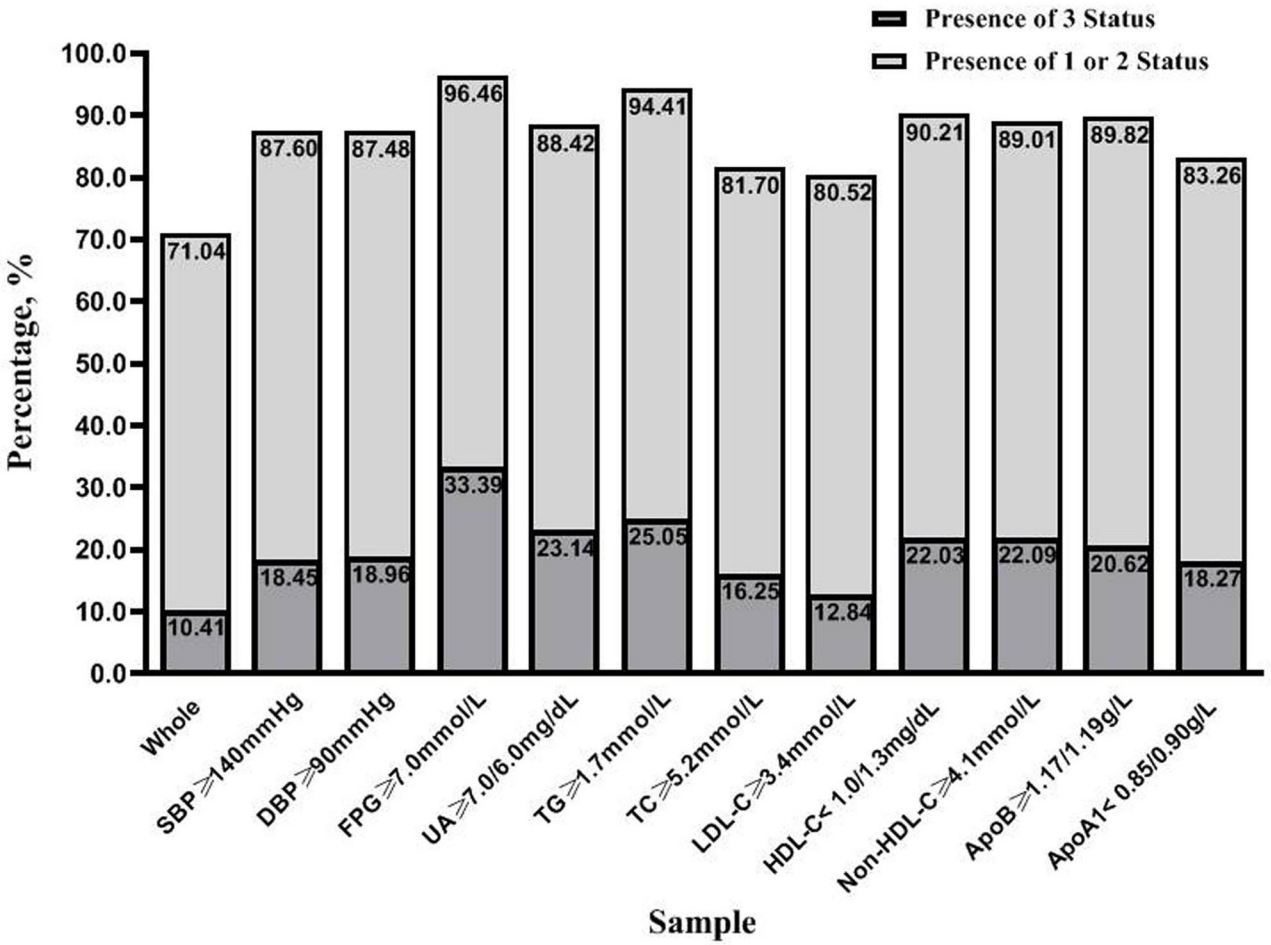

FIGURE 1 | The clustering of the three status (metabolically at-risk, overweight/obesity, and high serum ferritin) in the entire study population and samples with unfavorable risk factors for cardiovascular disease. Each sample was grouped based on the presence of three status and the presence of one or two status. SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; UA, uric acid; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; ApoB, apolipoprotein B100; ApoA1, apolipoprotein A1.

risk independent of biomarkers for hepatic fat accumulation, insulin resistance, and dyslipidemia (36). On the other hand, in the ARIC Study, Jehn et al. found that the significant association between SF and the incidence of diabetes was lost after the data were adjusted for BMI and metabolic syndrome components (37). The majority of studies have established associations between SF and CVD risk factors, as well as the predictive value of SF for risk. Nevertheless, few reports have explained whether or how the relationships have been influenced by the patient's metabolic status or BMI category. This study extended these previous findings by investigating associations based on body size phenotypes and evaluating the separate and combined associations of high SF, overweight/obesity, and those who are metabolically at-risk with CVD risk profiles. Notably, the associations of high SF with the prevalence of diabetes, dyslipidemia, and hyperuricemia varied among individuals within different body size phenotypes. The coincidence of all three status had the strongest associations with unfavorable CVD risk profiles except LDL-C.

Several mechanisms that form the basis for the relationship between SF and diabetes have been demonstrated. IR, as measured using the euglycemic-hyperinsulinemic clamp, has been correlated with body iron stores (10). Other studies have proposed that an iron overload, as reflected by an elevated SF likely induced IR through disturbing inhibition of hepatic glucose production by insulin, and insulin actions in adipose and muscle tissue (38). Furthermore, studies have confirmed the role of hepatic hormones and adipokines in inducing IR. Data from experiments with mice have shown that hepcidin, a key mediator of iron metabolism, could be up regulated by iron and activate the Jak2/STAT3 pathway, which in turn, induced production of suppressor of cytokine

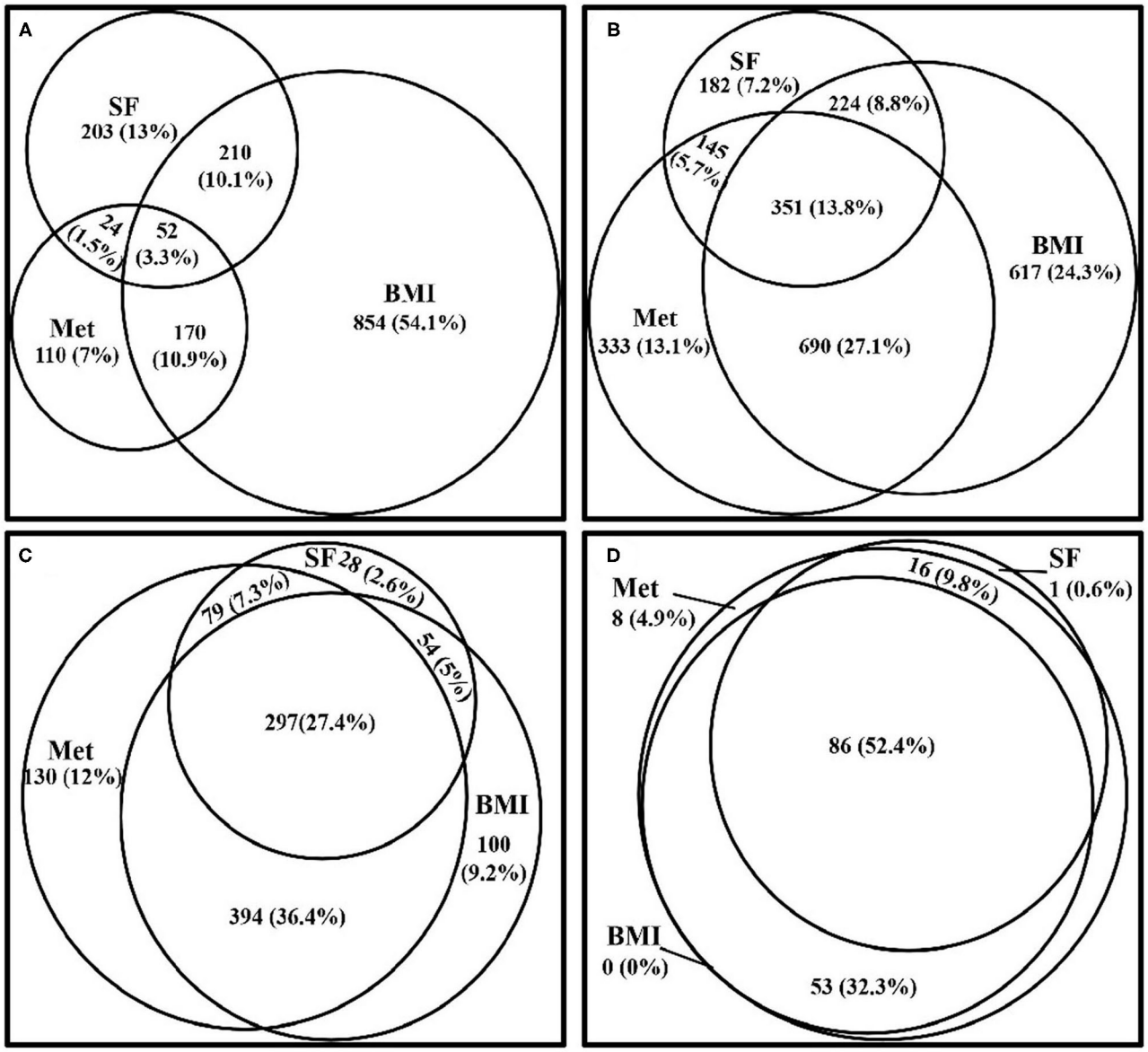

FIGURE 2 | Venn diagrams visually display how the three status (metabolically at-risk, overweight/obesity, and high serum ferritin) cluster together in the samples within 0–3 of the CVD risk factors, diabetes, hyperuricemia, and dyslipidemia, from the CVD risk profiles. **(A)** Venn diagram showing the clustering of the three status in subjects who did not exhibit any of the CVD risk factors. **(B)** Venn diagram showing the clustering of the three status in subjects who exhibited one of the CVD risk factors. **(C)** Venn diagram showing the clustering of the three status in subjects who exhibited two of the CVD risk factors. **(D)** Venn diagram showing the clustering of the three status in subjects who exhibited all three of the CVD risk factors. Met, metabolically at-risk; BMI, overweight/obesity; SF, high serum ferritin; CVD, cardiovascular disease.

signaling-3, which is an inhibitor of insulin signaling (17). Several other studies have demonstrated that induced downregulation of adiponectin, leptin, and upregulation of resistin *via* iron accumulation were causally related to IR (39). Excess iron in specific tissues may play a direct role in diabetes through other mechanisms, including oxidative stress, nitric oxide signaling, and inflammatory cytokines (40). It is worth noting that, in this study, a significant association between SF and diabetes only existed in subjects with a metabolically at-risk status, regardless of the BMI categories. Pancreatic β cells are particularly sensitive to oxygen radicals. Thus, the antioxidant property of SF is the potential reason for increased SF in β cells. The iron overload is not so severe as to induce the rapid apoptosis of β cells as seen with dysmetabolic-based iron overload, but it might have increased glucose levels by promoting β cell failure and reducing insulin synthesis and excretion (41). Although IR and β cell

failure contribute to overt diabetes in subjects with iron overload, it is known that metabolic disorders develop from an early stage that precedes β cell deficiency (38). Thus, iron overload might affect the development of diabetes that was tightly linked to a metabolically at-risk status. The present results from the adjusted model were stably independent of hs-CRP and HOMA-IR in metabolically at-risk subjects, which implied the inevitable role of β cell failure in the relationship, although residual confounding was still possible.

Similarly, the association between high SF and hyperuricemia was not entirely explained by IR or chronic low-grade inflammation since it was still significant after the adjustments for HOMA-IR and hs-CRP in all four phenotypes. Except for the induction of elevated UA synthesis by IR (42) and decreased UA excretion (43, 44), xanthine oxidase, a key enzyme in purine metabolism and UA production, has been hypothesized to be responsible for the association between SF and hyperuricemia. Studies have reported that iron overload was followed by elevation and activation of xanthine oxidase *in vivo* and *in vitro*, and tumor necrosis factor-α, interleukin-1, and interleukin-6, which are induced by oxidative stress, take part in the process (45, 46). As a major antioxidant, UA impedes oxidation reactions catalyzed by iron *via* forming a UA-Fe^{3+} complex, and inactivation of antioxidant enzymes such as superoxide dismutase, thus, it is a protective response to iron overload (47). Therefore, it appears that high SF is independently associated with hyperuricemia regardless of the BMI or metabolic status of the subject.

Inconsistencies have been observed for the links between iron overload and dyslipidemia, based on different samples. In a study of adolescents in Korea, positive correlations between SF and TC, LDL-C, and TG only occurred in boys, while a negative association between SF and HDL-C was observed for both genders (48). Other data from young non-obese adults in Korea showed the significant associations of SF with TG and HDL-C only for women (49). In both sexes of a Chinese population, SF was associated with lipid parameters (including TC, LDL-C, TG, and HDL-C) independent of diabetes and IR (12). The previous study using the same cohort confirmed positive correlations of SF with dyslipidemia and unfavorable lipid ratios, especially apoB and non-HDL-C, independent of HOMA-IR (50). Notably, apoB and non-HDL-C are proven independent risk factors for CVD (51). This study extended previously reported results by exploring how such associations performed in different body size phenotypes. It appears that SF was associated with abnormal TG, TC, non-HDL-C, and apoB in all phenotypes. The first proposed mechanism was that iron induced hyperinsulinemia and IR, which in turn contributed to unfavorable TG, LDL-C, TC, and non-HDL-C, and hepatic IR accelerated the synthesis and secretion of apoB through protein-tyrosine phosphatase 1B (52). However, the associations remained significant regardless of the subject's BMI or metabolic status after adjustment for age, sex, hs-CRP, and HOMA-IR. Therefore, alternative underlying mechanisms, except for IR and chronic low-grade inflammation, need to be considered. Studies using *in vivo* rat models suggested reactive oxygen species generated by iron could decrease fatty acid oxidation

TABLE 2 | Odds ratios (95% confidence intervals) for serum ferritin-related cardiovascular disease risk factors for each body size phenotype.

	Metabolically healthy				Metabolically at-risk				*P* for interaction	
	BMI < 23 kg/m^2		BMI ≥ 23 kg/m^2		BMI < 23 kg/m^2		BMI ≥ 23 kg/m^2			
	OR[a]	OR[b]	OR[a]	OR[b]	OR[a]	OR[b]	OR[a]	OR[b]	$P_{SF\times BMI}$	$P_{SF\times Met}$
SBP ≥ 140 mmHg	0.96 (0.67, 1.37)	0.60 (0.40, 0.89)	1.32 (1.01, 1.74)	0.97 (0.72, 1.31)	1.26 (0.93, 1.72)	1.04 (0.75, 1.46)	0.96 (0.80, 1.15)	0.83 (0.68, 1.00)	0.076	0.063
DBP ≥ 90 mmHg	1.10 (0.79, 1.54)	0.91 (0.64, 1.28)	1.06 (0.81, 1.40)	0.876 (0.66, 1.16)	1.13 (0.83, 1.55)	1.01 (0.73, 1.39)	0.96 (0.80, 1.15)	0.99 (0.83, 1.19)	0.919	0.699
FPG ≥ 7.0 mmol/L	1.52 (0.61, 3.78)	1.41 (0.55, 3.64)	1.36 (0.63, 2.95)	0.92 (0.40, 2.10)	1.66 (1.13, 2.43)	1.52 (1.02, 2.28)	1.83 (1.47, 2.28)	1.63 (1.27, 2.09)	0.581	0.884
UA ≥ 7.0/6.0 mg/dL (men/women)	1.89 (1.34, 2.67)	1.78 (1.26, 2.53)	1.60 (1.17, 2.17)	1.42 (1.03, 1.95)	1.76 (1.25, 2.49)	1.66 (1.17, 2.36)	1.50 (1.24, 1.81)	1.42 (1.17, 1.73)	0.328	0.825
TG ≥ 1.7 mmol/L	2.02 (1.44, 2.84)	2.12 (1.50, 3.00)	1.58 (1.18, 2.12)	1.57 (1.16, 2.13)	1.65 (1.21, 2.26)	1.87 (1.35, 2.61)	1.84 (1.49, 2.28)	1.89 (1.52, 2.36)	0.155	0.368
TC ≥ 5.2 mmol/L	1.99 (1.59, 2.49)	1.61 (1.29, 2.03)	2.27 (1.83, 2.81)	2.00 (1.60, 2.50)	1.54 (1.15, 2.07)	1.32 (0.97, 1.80)	1.73 (1.45, 2.07)	1.65 (1.37, 1.97)	0.252	0.270
LDL-C ≥ 3.4 mmol/L	2.01 (1.60, 2.53)	1.64 (1.29, 2.08)	1.73 (1.39, 2.15)	1.52 (1.22, 1.91)	1.17 (0.86, 1.60)	0.98 (0.71, 1.35)	1.18 (0.98, 1.42)	1.09 (0.90, 1.32)	0.566	0.012
HDL-C < 1.0/1.3 mg/dL (men/women)	0.70 (0.46, 1.06)	1.02 (0.65, 1.59)	0.57 (0.38, 0.85)	0.96 (0.62, 1.49)	1.14 (0.85, 1.53)	1.25 (0.90, 1.74)	1.11 (0.93, 1.32)	1.15 (0.95, 1.39)	0.429	0.482
Non-HDL-C ≥ 4.1 mmol/L	2.78 (2.11, 3.65)	2.30 (1.73, 3.05)	2.14 (1.68, 2.73)	1.84 (1.43, 2.36)	1.68 (1.24, 2.29)	1.49 (1.08, 2.05)	1.92 (1.60, 2.30)	1.81 (1.51, 2.17)	0.197	0.025
ApoB ≥ 1.17/1.19 g/L (men/women)	2.17 (1.52, 3.09)	1.80 (1.25, 2.59)	2.04 (1.55, 2.67)	1.74 (1.32, 2.30)	1.88 (1.35, 2.63)	1.65 (1.17, 2.33)	1.53 (1.25, 1.86)	1.43 (1.17, 1.75)	0.843	0.674
ApoA1 < 0.85/0.90 g/L (men/women)	0.83 (0.55, 1.25)	0.96 (0.63, 1.47)	0.91 (0.65, 1.29)	1.02 (0.72, 1.46)	1.07 (0.72, 1.60)	1.15 (0.76, 1.75)	1.09 (0.88, 1.34)	1.14 (0.92, 1.41)	0.908	0.538

[a]*Model was unadjusted,* [b]*Model was adjusted for age, sex, inflammation status (hs-CRP) and insulin resistance (HOMA-IR).*

P for interaction terms were assessed by logistic regression analyses.

BMI, body mass index; OR, odd ratio; SF, serum ferritin; Met, metabolic status; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; UA, uric acid; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; ApoB, apolipoprotein B100; ApoA1, apolipoprotein A1; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homoeostasis model assessment of insulin resistance.

TABLE 3 | Odds ratios (95% confidence intervals)[a] for joint associations of the SF status and body size phenotypes with the cardiovascular disease risk factors.

	MHNW	MHO	MANW	MAO
FPG ≥ 7.0 mmol/L				
Without high SF	1	1.55 (0.84, 2.86)	9.97 (5.93, 16.77)	11.55 (7.15, 18.68)
With high SF	1.44 (0.57, 3.63)	1.58 (0.68, 3.63)	15.49 (8.90, 26.97)	18.89 (11.60, 30.78)
UA ≥ 7.0/6.0 mg/dL (men/women)				
Without high SF	1	1.48 (1.17, 1.88)	2.77 (2.09, 3.67)	4.54 (3.66, 5.63)
With high SF	1.78 (1.26, 2.52)	2.09 (1.53, 2.87)	4.66 (3.35, 6.49)	6.50 (5.15, 8.21)
TG ≥ 1.7 mmol/L				
Without high SF	1	1.91 (1.52, 2.41)	28.12 (21.89, 36.11)	37.71 (30.26, 46.98)
With high SF	2.22 (1.58, 3.14)	3.06 (2.25, 4.17)	50.35 (35.98, 70.46)	72.45 (55.61, 94.38)
TC ≥ 5.2 mmol/L				
Without high SF	1	1.43 (1.23, 1.66)	1.63 (1.33, 2.00)	1.88 (1.61, 2.21)
With high SF	1.67 (1.33, 2.10)	2.88 (2.31, 3.58)	2.20 (1.68, 2.89)	3.07 (2.55, 3.68)
LDL-C ≥ 3.4 mmol/L				
Without high SF	1	1.65 (1.42, 1.92)	1.41 (1.15, 1.75)	1.53 (1.30, 1.80)
With high SF	1.66 (1.31, 2.10)	2.49 (1.99, 3.11)	1.42 (1.07, 1.89)	1.67 (1.38, 2.02)
Non-HDL-C ≥ 4.1 mmol/L				
Without high SF	1	1.86 (1.53, 2.27)	3.17 (2.50, 4.02)	4.24 (3.50, 5.13)
With high SF	2.41 (1.82, 3.19)	3.51 (2.72, 4.52)	4.73 (3.52, 6.35)	7.65 (6.20, 9.43)
ApoB ≥ 1.17/1.19 g/L (men/women)				
Without high SF	1	2.17 (1.71, 2.75)	3.21 (2.42, 4.25)	3.96 (3.15, 4.98)
With high SF	1.85 (1.29, 2.64)	3.82 (2.85, 5.13)	5.33 (3.83, 7.40)	5.63 (4.40, 7.20)

[a]*Adjusted for age, sex, high-sensitivity C-reactive protein and homoeostasis model assessment of insulin resistance.*

MHNW, metabolically healthy normal weight; MHO, metabolically healthy overweight/obese, MANW, metabolically at-risk normal weight; MAO, metabolically at-risk overweight/obese; SF, serum ferritin; FPG, fasting plasma glucose; UA, uric acid; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-high-density lipoprotein cholesterol; ApoB, apolipoprotein B100.

and enhance lipid transportation *via* suppressed expression of peroxisome proliferator-activated receptor-α and downstream genes (Nrf1, cpt-1α), which promoted hypercholesterolemia and hypertriglyceridemia (53, 54). Also, the up-regulation of microsomal triglyceride transfer protein in iron overloaded rats, which accelerated the processes of apoB production and lipid delivery to the apoB-containing lipoprotein particles, was causally related to the increased levels of apoB, TC, and TG (54, 55). Interestingly, in this study, high SF was associated with elevated LDL-C only in the MHNW and MHO phenotypes, and the associations of high SF with LDL-C and non-HDL-C were more pronounced in the MHNW phenotype compared to subjects with the MANW phenotype. This observation suggested that there were differences in the degree of influence of iron overload and having a metabolically at-risk status on unfavorable LDL-C and non-HDL-C. When a metabolically at-risk status played a more prominent role than iron overload, the iron overload might lose its significance in the relationship with unfavorable LDL-C and non-HDL-C in subjects who were metabolically at-risk. However, few previous studies have examined these underlying mechanisms with respect to this difference. Additional experimental explorations of mechanisms and epidemiological surveys using larger samples should be performed to assess these complex associations.

Although metabolically at-risk participants exhibited higher ORs for unfavorable UA, TG, non-HDL-C, and apoB compared with those with high SF, the presence of high SF was associated with worse CVD risk profiles within the same metabolic status. Clustering of all three status, metabolically at-risk, overweight/obesity, and high SF, had the strongest associations with glycemia, UA, and most lipid profiles. The overlap among the three status increased with the accumulation of CVD risk factors, which might help to identify the high-risk groups earlier. The fact that being metabolically at-risk, overweight/obese, and having high SF have synergistic associations with CVD risk factors suggests that reducing iron overload could be a potential target for the prevention of CVD in subjects with high risk. On the other hand, this study reported considerable dissociation between the three statuses. It is known that elevated BMI is related to the occurrence of insulin resistance and pro-inflammation; BMI can provide a general indication of obesity, but it does not easily distinguish adipose tissue distribution among visceral, ectopic, or subcutaneous accumulation (55). Individuals determined to be metabolically at-risk have been associated with visceral or ectopic adipose tissue distributions and a higher proportion of small adipose cells (56, 57). Therefore, exploring how these three statuses cluster together, and their unique mechanisms of action, could contribute to reducing the adverse effects of each status and scattering them as well.

There are several limitations in this study. First, a cross-sectional study design was not sufficient in drawing any conclusions about causal relationships. Second, a standard 75 g

oral glucose tolerance test was difficult to perform in this large-scale population-based survey, Therefore, it is likely that the prevalence of diabetes was underestimated. The use of a calculated HOMA-IR to assess IR was also a limitation.

CONCLUSIONS

The associations of high SF with the prevalence of CVD risk factors, including diabetes, dyslipidemia, and hyperuricemia, vary in individuals with different body size phenotypes. The various synergistic associations of high SF, metabolically at-risk, and overweight/obesity with CVD risk profiles suggest that specific strategies to ensure that the clustered status diverge could be particularly beneficial to prevent CVD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the University of North Carolina at Chapel Hill, National Institute of Nutrition and Health, China Center for Disease Control and Prevention, and the Human and Clinical Research Ethics Committee of China-Japan Friendship Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GY designed the research. BZ and SL conducted the data analyses. BZ wrote the draft. All authors approved the final manuscript.

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REFERENCES

1. Lipschitz DA, Cook JD, Finch CA. A clinical evaluation of serum ferritin as an index of iron stores. *N Engl J Med*. (1974) 290:1213–6. doi: 10.1056/NEJM197405302902201
2. Fernandez-Real JM, Ricart-Engel W, Arroyo E, Balanca R, Casamitjana-Abella R, Cabrero D, et al. Serum ferritin as a component of the insulin resistance syndrome. *Diabetes Care*. (1998) 21:62–8. doi: 10.2337/diacare.21.1.62
3. Jehn M, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in U.S. adults. *Diabetes Care*. (2004) 27:2422–8. doi: 10.2337/diacare.27.10.2422
4. Lee BK, Kim Y, Kim YI. Association of serum ferritin with metabolic syndrome and diabetes mellitus in the South Korean general population according to the Korean National Health and Nutrition Examination Survey 2008. *Metabolism*. (2011) 60:1416–24. doi: 10.1016/j.metabol.2011.02.008
5. Williams MJ, Poulton R, Williams S. Relationship of serum ferritin with cardiovascular risk factors and inflammation in young men and women. *Atherosclerosis*. (2002) 165:179–84. doi: 10.1016/S0021-9150(02)00233-2
6. Tuomainen TP, Nyyssonen K, Salonen R, Tervahauta A, Korpela H, Lakka T, et al. Body iron stores are associated with serum insulin and blood glucose concentrations. Population study in 1,013 eastern Finnish men. *Diabetes Care*. (1997) 20:426–8. doi: 10.2337/diacare.20.3.426
7. Malik S, Wong ND, Franklin SS, Kamath TV, L'Italien GJ, Pio JR, et al. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation*. (2004) 110:1245–50. doi: 10.1161/01.CIR.0000140677.20606.0E
8. Eckel RH, Alberti KG, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet*. (2010) 375:181–3. doi: 10.1016/S0140-6736(09)61794-3
9. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. (2009) 120:1640–5. doi: 10.1161/CIRCULATIONAHA.109.192644
10. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. *Diabetes*. (2002) 51:2348–54. doi: 10.2337/diabetes.51.8.2348
11. Park SK, Ryoo JH, Kim MG, Shin JY. Association of serum ferritin and the development of metabolic syndrome in middle-aged Korean men: a 5-year follow-up study. *Diabetes Care*. (2012) 35:2521–6. doi: 10.2337/dc12-0543
12. Li J, Bao W, Zhang T, Zhou Y, Yang H, Jia H, et al. Independent relationship between serum ferritin levels and dyslipidemia in Chinese adults: a population study. *PLoS ONE*. (2017) 12:e190310. doi: 10.1371/journal.pone.0190310
13. Li X, He T, Yu K, Lu Q, Alkasir R, Guo G, et al. Markers of iron status are associated with risk of hyperuricemia among Chinese adults: nationwide population-based study. *Nutrients*. (2018) 10:191. doi: 10.3390/nu10020191
14. Tsouli SG, Liberopoulos EN, Mikhailidis DP, Athyros VG, Elisaf MS. Elevated serum uric acid levels in metabolic syndrome: an active component or an innocent bystander? *Metabolism*. (2006) 55:1293–301. doi: 10.1016/j.metabol.2006.05.013
15. Baker JF, Krishnan E, Chen L, Schumacher HR. Serum uric acid and cardiovascular disease: recent developments, and where do they leave us? *Am J Med*. (2005) 118:816–26. doi: 10.1016/j.amjmed.2005.03.043
16. Wlazlo N, van Greevenbroek MM, Ferreira I, Jansen EH, Feskens EJ, van der Kallen CJ, et al. Iron metabolism is associated with adipocyte insulin resistance and plasma adiponectin: the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study. *Diabetes Care*. (2013) 36:309–15. doi: 10.2337/dc12-0505
17. Dongiovanni P, Ruscica M, Rametta R, Recalcati S, Steffani L, Gatti S, et al. Dietary iron overload induces visceral adipose tissue insulin resistance. *Am J Pathol*. (2013) 182:2254–63. doi: 10.1016/j.ajpath.2013.02.019
18. Hinnouho GM, Czernichow S, Dugravot A, Batty GD, Kivimaki M, Singh-Manoux A. Metabolically healthy obesity and risk of mortality: does the definition of metabolic health matter? *Diabetes Care*. (2013) 36:2294–300. doi: 10.2337/dc12-1654

19. Appleton SL, Seaborn CJ, Visvanathan R, Hill CL, Gill TK, Taylor AW, et al. Diabetes and cardiovascular disease outcomes in the metabolically healthy obese phenotype: a cohort study. *Diabetes Care*. (2013) 36:2388–94. doi: 10.2337/dc12-1971
20. Popkin BM, Du S, Zhai F, Zhang B. Cohort Profile: the China Health and Nutrition Survey–monitoring and understanding socio-economic and health change in China, 1989–2011. *Int J Epidemiol*. (2010) 39:1435–40. doi: 10.1093/ije/dyp322
21. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RR, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. (2003) 107:499–511. doi: 10.1161/01.CIR.0000052939.59093.45
22. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. (2004) 363:157–63. doi: 10.1016/S0140-6736(03)15268-3
23. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA*. (2001) 285:2486–97. doi: 10.1001/jama.285.19.2486
24. Suarez-Ortegon MF, Ensaldo-Carrasco E, Shi T, McLachlan S, Fernandez-Real JM, Wild SH. Ferritin, metabolic syndrome and its components: a systematic review and meta-analysis. *Atherosclerosis*. (2018) 275:97–106. doi: 10.1016/j.atherosclerosis.2018.05.043
25. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. (2014) 37(Suppl.1):S81–90. doi: 10.2337/dc14-S081
26. Veronese N, Stubbs B, Trevisan C, Bolzetta F, De Rui M, Maggi S, et al. Results of an observational cohort study of hyperuricemia as a predictor of poor physical performance in the elderly. *Arthritis Care Res*. (2017) 69:1238–44. doi: 10.1002/acr.23118
27. Zhao SP. Key points and comments on the 2016 Chinese guideline for the management of dyslipidemia in adults. *Zhonghua Xin Xue Guan Bing Za Zhi*. (2016) 44:827–9. doi: 10.3760/cma.j.issn.0253-3758.2016.10.003
28. Muntner P, Hamm LL, Kusek JW, Chen J, Whelton PK, He J. The prevalence of non-traditional risk factors for coronary heart disease in patients with chronic kidney disease. *Ann Intern Med*. (2004) 140:9–17. doi: 10.7326/0003-4819-140-1-200401060-00006
29. Roberts WL. CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease: application to Clinical and Public Health Practice: laboratory tests available to assess inflammation–performance and standardization: a background paper. *Circulation*. (2004) 110:e572–6. doi: 10.1161/01.CIR.0000148986.52696.07
30. Piperno A, Trombini P, Gelosa M, Mauri V, Pecci V, Vergani A, et al. Increased serum ferritin is common in men with essential hypertension. *J Hypertens*. (2002) 20:1513–8. doi: 10.1097/00004872-200208000-00013
31. Sun L, Zong G, Pan A, Ye X, Li H, Yu Z, et al. Elevated plasma ferritin is associated with increased incidence of type 2 diabetes in middle-aged and elderly Chinese adults. *J Nutr*. (2013) 143:1459–65. doi: 10.3945/jn.113.177808
32. Salonen JT, Tuomainen TP, Nyyssonen K, Lakka HM, Punnonen K. Relation between iron stores and non-insulin dependent diabetes in men: case-control study. *BMJ*. (1998) 317:727. doi: 10.1136/bmj.317.7160.727
33. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. *JAMA*. (2004) 291:711–7. doi: 10.1001/jama.291.6.711
34. Fumeron F, Pean F, Driss F, Balkau B, Tichet J, Marre M, et al. Ferritin and transferrin are both predictive of the onset of hyperglycemia in men and women over 3 years: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. *Diabetes Care*. (2006) 29:2090–4. doi: 10.2337/dc06-0093
35. Forouhi NG, Harding AH, Allison M, Sandhu MS, Welch A, Luben R, et al. Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. *Diabetologia*. (2007) 50:949–56. doi: 10.1007/s00125-007-0604-5
36. Montonen J, Boeing H, Steffen A, Lehmann R, Fritsche A, Joost HG, et al. Body iron stores and risk of type 2 diabetes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. *Diabetologia*. (2012) 55:2613–21. doi: 10.1007/s00125-012-2633-y
37. Jehn ML, Guallar E, Clark JM, Couper D, Duncan BB, Ballantyne CM, et al. A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Epidemiol*. (2007) 165:1047–54. doi: 10.1093/aje/kwk093
38. Fernandez-Real JM, Manco M. Effects of iron overload on chronic metabolic diseases. *Lancet Diabetes Endocrinol*. (2014) 2:513–26. doi: 10.1016/S2213-8587(13)70174-8
39. Rametta R, Fracanzani AL, Fargion S, Dongiovanni P. Dysmetabolic hyperferritinemia and dysmetabolic iron overload syndrome (DIOS): two related conditions or different entities? *Curr Pharm Des*. (2020) 26:1025–35. doi: 10.2174/1381612826666200131103018
40. Rajpathak SN, Crandall JP, Wylie-Rosett J, Kabat GC, Rohan TE, Hu FB. The role of iron in type 2 diabetes in humans. *Biochim Biophys Acta*. (2009) 1790:671–81. doi: 10.1016/j.bbagen.2008.04.005
41. Fernandez-Real JM, McClain D, Manco M. Mechanisms linking glucose homeostasis and iron metabolism toward the onset and progression of type 2 diabetes. *Diabetes Care*. (2015) 38:2169–76. doi: 10.2337/dc14-3082
42. Modan M, Halkin H, Karasik A, Lusky A. Elevated serum uric acid–a facet of hyperinsulinaemia. *Diabetologia*. (1987) 30:713–8. doi: 10.1007/BF00296994
43. Tang W, Fu Q, Zhang Q, Sun M, Gao Y, Liu X, et al. The association between serum uric acid and residual beta -cell function in type 2 diabetes. *J Diabetes Res*. (2014) 2014:709691. doi: 10.1155/2014/709691
44. Facchini F, Chen YD, Hollenbeck CB, Reaven GM. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. *JAMA*. (1991) 266:3008–11. doi: 10.1001/jama.266.21.3008
45. Martelin E, Lapatto R, Raivio KO. Regulation of xanthine oxidoreductase by intracellular iron. *Am J Physiol Cell Physiol*. (2002) 283:C1722–8. doi: 10.1152/ajpcell.00280.2002
46. Pfeffer KD, Hueckstead TP, Hoidal JR. Xanthine dehydrogenase and xanthine oxidase activity and gene expression in renal epithelial cells. Cytokine and steroid regulation. *J Immunol*. (1994) 153:1789–97.
47. Flais J, Bardou-Jacquet E, Deugnier Y, Coiffier G, Perdriger A, Chales G, et al. Hyperferritinemia increases the risk of hyperuricemia in HFE-hereditary hemochromatosis. *Joint Bone Spine*. (2017) 84:293–7. doi: 10.1016/j.jbspin.2016.05.020
48. Kim YE, Kim DH, Roh YK, Ju SY, Yoon YJ, Nam GE, et al. Relationship between serum ferritin levels and dyslipidemia in Korean adolescents. *PLoS ONE*. (2016) 11:e153167. doi: 10.1371/journal.pone.0153167
49. Yoo KD, Ko SH, Park JE, Ahn YB, Yim HW, Lee WC, et al. High serum ferritin levels are associated with metabolic risk factors in non-obese Korean young adults: Korean National Health and Nutrition Examination Survey (KNHANES) IV. *Clin Endocrinol*. (2012) 77:233–40. doi: 10.1111/j.1365-2265.2011.04248.x
50. Zhou B, Ren H, Zhou X, Yuan G. Associations of iron status with apolipoproteins and lipid ratios: a cross-sectional study from the China Health and Nutrition Survey. *Lipids Health Dis*. (2020) 19:140. doi: 10.1186/s12944-020-01312-9
51. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE. Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. *JAMA*. (2005) 294:326–33. doi: 10.1001/jama.294.3.326
52. Taghibiglou C, Rashid-Kolvear F, Van Iderstine SC, et al. Hepatic very low density lipoprotein-ApoB overproduction is associated with attenuated hepatic insulin signaling and overexpression of protein-tyrosine phosphatase 1B in a fructosefed hamster model of insulin resistance. *J Biol Chem*. (2002) 277:793–803. doi: 10.1074/jbc.M106737200
53. Bonomo LF, Silva M, Oliveira RP, Silva ME, Pedrosa ML. Iron overload potentiates diet-induced hypercholesterolemia and reduces liver PPAR-alpha expression in hamsters. *J Biochem Mol Toxicol*. (2012) 26:224–9. doi: 10.1002/jbt.21410
54. Silva M, Da CGJ, Sampaio AF, de Lima WG, Silva ME, Pedrosa ML. Iron dextran increases hepatic oxidative stress and alters expression of genes related to lipid metabolism contributing to hyperlipidaemia in murine model. *Biomed Res Int*. (2015) 2015:272617. doi: 10.1155/2015/272617
55. Walker GE, Marzullo P, Ricotti R, Bona G, Prodam F. The pathophysiology of abdominal adipose tissue depots in health and disease. *Horm Mol Biol Clin Investig*. (2014) 19:57–74. doi: 10.1515/hmbci-2014-0023

56. Wildman RP, Janssen I, Khan UI, Thurston R, Barinas-Mitchell E, El KS, et al. Subcutaneous adipose tissue in relation to subclinical atherosclerosis and cardiometabolic risk factors in midlife women. *Am J Clin Nutr*. (2011) 93:719–26. doi: 10.3945/ajcn.110.007153
57. McLaughlin T, Sherman A, Tsao P, Gonzalez O, Yee G, Lamendola C, et al. Enhanced proportion of small adipose cells in insulin-resistant vs. insulin-sensitive obese individuals implicates impaired adipogenesis. *Diabetologia*. (2007) 50:1707–15. doi: 10.1007/s00125-007-0708-y

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ESRα Promoter Methylation May Modify the Association Between Lipid Metabolism and Type 2 Diabetes in Chinese Farmers

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Objective: This study is aimed to explore the potential association among the estrogen receptor alpha (*ESRα*) promoter methylation, lipid metabolism and the risk of type 2 diabetes mellitus (T2DM).

Methods: A total of 1143 rural residents were recruited randomly from Henan Province, China. The circulating methylation levels in *ESRα* promoter region were determined by quantitative methylation-specific polymerase chain reaction. Serum high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), total cholesterol (TC) and fasting plasma-glucose (FPG) were measured.

Results: The *ESRα* promoter methylation levels were negatively associated with HDL-C levels whether gender stratification was performed ($P < 0.05$) and positively correlated with LDL-C in men ($P < 0.05$). Each unit standard deviation (SD) increment in TG was associated with a 43% increase (95% CI: 1.25, 1.64) in the risks of T2DM in all participants, a 36% increase (95% *CI*: 1.13, 1.64) in the risks of T2DM in men and a 49% increase (95% CI: 1.21, 1.83) in the risks of T2DM in women. Furthermore, each SD increment in HDL-C was associated with a reduction of 25% (OR = 0.75, 95% CI: 0.58, 0.97) in the risks of T2DM in men, and the risk of T2DM in men may be more susceptible to HDL-C than that in women (*P* for interaction < 0.05). Additionally, we found that the risk of T2DM in participants with lower methylation levels (≤4.07%) were more susceptible to HDL-C (*P* for interaction < 0.05).

Conclusions: These findings suggested that lipid metabolism was associated with *ESRα* promoter methylation levels and the risk of T2DM. Besides, the levels of *ESRα* promoter methylation and gender can modify the association of HDL-C and T2DM.

Keywords: estrogen receptor alpha, DNA methylation, lipid metabolism, diabetes mellitus, farmers

BACKGROUND

Type 2 diabetes mellitus (T2DM) poses a worldwide public health problem with a continuously increasing prevalence in both developing and developed countries (1–3). More than half of patients with T2DM suffer from dyslipidemia (4). Diabetic dyslipidemia is mainly a mixed dyslipidemia with higher triglycerides (TG) and low density lipoprotein cholesterol (LDL-C), and lower of high density lipoprotein cholesterol (HDL-C) which can be observed before the onset of diabetes (5–7). The use of lipid-lowing therapy can improve lipid metabolism and prevent T2DM (8). Consequently, exploring the molecular mechanisms underlying abnormal lipid metabolism and T2DM pathogenesis is critical to develop therapeutic strategies for T2DM.

Several evidences indicate that estrogens can regulate lipid metabolism and protect mouse from β-cell apoptosis (9, 10). Estrogens deficiency can contribute to metabolic dysfunction, and then cause obesity and insulin resistance (11, 12). Additionally, the estrogens therapy has been revealed to have various beneficial effects by decreasing fasting glucose, increasing insulin sensitivity and secretion and reduce T2DM incidence in postmenopausal women (13, 14). Notably, the metabolic effects of estrogens are mediated by estrogen receptor alpha (ESRα) (15). Animal studies revealed that mice were more resistant to insulin after *ESRα* knockout (11). Furthermore, Ribas et al. (16) found that ESRα deficiency can increase fasting insulin levels, impairs glucose tolerance and results in skeletal muscle insulin resistance. As an important epigenetic modification, DNA methylation is a key regulator of gene expression. Additionally, the methylation of *ESRα* promoter is reported to reduce the expression of ESRα (17). Furthermore, abnormal DNA methylation were found to be associated with lipid metabolism disorders (17, 18). However, whether the alteration of *ESRα* promoter methylation affects human lipid metabolism and the risk of T2DM has not been explored.

Given these, in this study, we conducted a cross-sectional study in rural areas of Henan Province, and recruited 1,143 Chinese farmers to identify the association of *ESRα* promoter methylation, lipid metabolism and T2DM. With the development of targeted interventions for DNA methylation (19), this study can provide a theoretical basis for the screening of diabetes-susceptible populations and future precision therapy.

METHODS

Study Participants

A cross-sectional study was conducted in Wuzhi County of Henan Province in China by random sampling in 2013. Participants were excluded as the following: (1) people with type 1 diabetes; (2) secondary diabetes (drug-induced, chemical-induced, exocrine pancreatic insufficiency, and genetic defects); (3) gestational diabetes and rare forms of diabetes. Finally, a total of 1,143 local permanent residents were recruited in this study. The project was approved by the Institutional Review Board at Zhengzhou University. All participants were informed of the purpose of the study and provided written informed consent.

Abbreviations: *ESRα*, estrogen receptor alpha; T2DM, type 2 diabetes mellitus; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglyceride; TC, total cholesterol; FPG, fasting plasma-glucose.

Outcome Variable

The diagnostic testing for T2DM was performed according to the criteria of World Health Organization (1999) (20) and the guidelines of American Diabetes Association (2002). All the nondiabetic people had normal glucose tolerance after an oral glucose tolerance test (OGTT).

Sampling and Data Collection

Trained investigators conducted a face-to-face interview with each participant using a standard questionnaire for obtaining information of demographic characteristics including age, gender, economic status, educational level, dietary habits and lifestyle (smoking and drinking, salt intake, physical activity, et, al). Among participants, those who had smoked at least 100 cigarettes in their lifetime were defined as "smoking"; those consuming any drink containing alcohol more than 12 times during the past 12 months were defined as "drinking." A total of 10 mL fasting blood samples (5 ml of anticoagulative and 5 ml of non-anticoagulative) were collected from each participant. Serum samples were isolated from non-anticoagulative blood after centrifugation (3,000 rpm for 15 min) at 4°C and frozen at −80°C for subsequent analyses.

Measurement of Biochemical Parameters

The concentration (mmol/L) of HDL-C, LDL-C, TG, total cholesterol (TC) and FPG in serum samples were measured with direct method of catalase clearance, direct method of surfactant removal, glycerol phosphate oxidase-peroxidase (GPO-PAP), cholesterol oxidase-peroxidase (GHOD-PAP) and glucose oxidative method, respectively (21). Finally, 120 serum samples were randomly selected for repeated measurements. All analyses were run on an automatic biochemical analyzer (Kehua Bio-engineering Co., Ltd, Shanghai, China).

Measurement of *ESRα* Promoter Methylation

The genomic DNA was extracted from whole blood samples using a BioTeke Magnetic beads kit (Bioteke Crporation, Beijing, China). The concentration of DNA samples was measured using a Nanodrop ND-2000 spectrophotometer (Thermo, MA, USA). Subsequently, the genomic DNA were treated with sodium bisulfite using an EZ DNA Methylation-Gold kit (Zymo Research, CA, USA). The putative promoter sequences of *ESRα* the sequence of the gene promoter region assumed to be 2,000 bp upstream from the *ESRα* start codon (22)] were obtained from UCSC/Ensembl, and then primer sequences (methylated specific primers: L, 5′-CGT AGG TTT ACG GTT AGA TCGG-3′; R, 5′-ATA CAA TAA CAT CAA CGA ACT CGAA-3′; unmethylated specific primers: L, 5′-ATG GTT AGA TTG GTT TTT TTT TAGG-3′; R, 5′-ACA TCA ACA AAC TCA AAA ACA CACT-3′) were designed using the methylation primer design software (Methyl Primer Express v1.0). The *ESRα*

methylation level was analyzed using quantitative methylation-specific PCR on a MX3000P real-time PCR system (Aglient, Santa Clara, CA, USA). PCR amplification was performed in a 15 μl reaction mixture contained 5.5 μl of diluted DNA template (100 ng), 7.5 μl of 2 × Power SYBR Green PCR Master Mixture (CWBIO, Beijing, China), 2 μl of primer with a concentration of 1.25 μmol/L each. The PCR cycling parameters were as follows: 95°C for 10 min; 40 cycles for degeneration at 94°C for 15 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s. Two negative controls (replace the DNA template with ddH_2O) were set for each plate. The level of DNA methylation was calculated according to the formula: $[1/(1+2^{-\Delta Ct})]\times 100\%$, where ΔCt =Ct(unmethylated)–Ct(methylated) (23). Ct is the threshold of PCR cycle number at which the increase in fluorescent signal reaches a critical point. Each sample was analyzed in duplicate.

Statistical Analysis

The Student's *t*-test, Wilcoxon test and Chi-square test were used to analyze the differences in normal/near-normal characteristics, other continuous variables and categorical variables between participants in T2DM group and non-diabetic group. We then utilized linear regression model to exam the association between *ESRα* methylation level and lipid metabolism. Besides, the association between T2DM and lipid metabolism (as well as *ESRα* methylation level) were estimated using binary logistic regression model. The linear trends across increasing quartiles of *ESRα* methylation level and lipid metabolism were estimated by treating the median of each quartile as a continuous variable. The quartiles of *ESRα* methylation and lipid metabolism are shown in **Supplementary Table 1**. After stratifying the participants according to the median of *ESRα* promoter methylation level, we analyzed the interactive effect of *ESRα* methylation and lipid metabolism on the risk of T2DM by adding an interaction term "*ESRα* methylation * lipid metabolism" to the logistic regression model. In addition, we performed other interactive analyses by adding an interaction term ("*ESRα* methylation*gender" or "lipid metabolism*gender") to each models. According to the characteristics of the participants and the previous reports, we adjusted a variety of potential confounding variables (including age, gender, BMI, educational level, smoking, drinking and household income) in this study.

All statistical analyses were performed by SPSS 22.0 (IBM Corp, Armonk, NY, USA). The *P*-values < 0.05 were considered statistically significant.

RESULTS

Distribution of Variables in Different Groups

The characteristics of the 1,143 residents are summarized in **Table 1**. As compared to the non-diabetic group, the T2DM group have a lower proportion of men/women, drinking and vegetables intake (≥500 g/day), and a higher proportion of illiteracy and family diabetes history ($P < 0.05$). Besides, the average age, BMI, TG and TC in T2DM group were higher than those in non-diabetic group ($P < 0.05$). The distributions of household incomes, *ESRα* promoter methylation, HDL-C and LDL-C are comparable between the two groups.

TABLE 1 | Characteristics of participants in T2DM group and Nondiabetic group[a].

Characteristics	T2DM group (*n* = 237)	Nondiabetic group (*n* = 906)	$t/\chi^2/Z$	*P*
Age (years)	57.58 ± 8.96	54.50 ±10.08	4.588	<0.001
Gender			6.195	0.013
Men	103 (43.5)	476 (52.5)		
Women	134 (56.5)	430 (47.5)		
BMI (kg/m^2)	26.35 ± 3.70	25.62 ± 3.58	2.785	0.005
Education level			10.951	0.012
Illiteracy	51 (21.6)	121 (13.4)		
Primary school	65 (27.5)	246 (27.2)		
Junior high school	93 (39.4)	406 (44.9)		
High school and above	27 (11.4)	131 (14.5)		
Smoking			1.875	0.171
Yes	75 (31.6)	330 (36.4)		
No	162 (68.4)	575 (63.6)		
Drinking			6.040	0.014
Yes	34 (14.3)	195 (21.5)		
No	203 (85.7)	711 (78.5)		
Vegetables intake (g/day)			8.728	0.003
≥ 500	56 (23.7)	306 (33.8)		
< 500	180 (76.3)	600 (66.2)		
Household income (RMB/year)			0.826	0.662
<6,000	182 (76.8)	670 (74.0)		
6,000–12,000	43 (18.1)	182 (20.0)		
>12,000	12 (5.1)	54 (6.0)		
Family diabetes history		5.869	0.015	
Yes	52 (22.2)	140 (15.6)		
No	182 (77.8)	760 (84.4)		
ESRα methylation (%)	4.58 ± 2.22	4.53 ± 2.21	0.338	0.735
FPG (mmol/L)	8.65 (6.68, 10.99)	4.81(4.33, 5.34)	20.08	<0.001
TG (mmol/L)	2.28 ± 1.67	1.72 ± 1.29	4.746	<0.001
TC (mmol/L)	4.77 ± 1.05	4.58 ± 0.97	2.558	0.011
HDL-C (mmol/L)	1.22 ± 0.31	1.24 ± 0.30	0.653	0.514
LDL-C (mmol/L)	2.59 ± 0.83	2.59 ± 0.75	0.099	0.922

RMB, China Yuan.

[a]Data are expressed as the mean ± SD or Median (P25, P75) for continuous variables and n (%) for categorical variables.

After stratifying the participants according to the tertiles of age, we found that found that the levels of *ESRα* methylation, FPG and LDL-C in the elderly were elevated (all $P < 0.05$). There is no statistically significant difference in the levels of TG, TC, HDL-C among different groups (**Supplementary Table 2**).

Association Between *ESRα* Methylation Level and Lipid Metabolism

As shown in **Table 2**, the HDL-C level showed a downward trend with the increase of the *ESRα* promoter methylation level regardless of gender stratification (*P* for trend < 0.05). Besides, we found that in the continuous analysis, the *ESRα* promoter

TABLE 2 | Association between *ESRα* methylation and lipid metabolism.

ESRα methylation (%)	All[a]		Men[b]		Women[b]		*P* for interaction[c]
	β (95% CI)	*P*	*β* (95% CI)	*P*	*β* (95% CI)	*P*	
TG (mmol/L)							
Quartile 1	Reference		Reference		Reference		
Quartile 2	−0.07(−0.31, 0.17)	0.580	−0.25(−0.63, 0.12)	0.185	0.17(−0.12, 0.46)	0.257	
Quartile 3	0.27(0.02, 0.51)	0.037	0.01(−0.37, 0.35)	0.974	0.48(0.15, 0.81)	0.005	
Quartile 4	−0.04(−0.26, 0.17)	0.007	−0.20(−0.55, 0.15)	0.262	0.06(−0.19, 0.31)	0.642	
Trend test		0.907		0.511		0.759	
Increase per SD	−0.03(−0.12, 0.05)	0.461	−0.07(−0.21, 0.06)	0.297	−0.02(−0.13,0.08)	0.659	0.258
TC (mmol/L)							
Quartile 1	Reference		Reference		Reference		
Quartile 2	−0.09(−0.27, 0.08)	0.297	−0.13(−0.38, 0.13)	0.321	0.01(−0.22, 0.25)	0.906	
Quartile 3	0.08(−0.10, 0.27)	0.379	0.02(−0.25, 0.28)	0.913	0.16(−0.09, 0.41)	0.220	
Quartile 4	−0.07(−0.24, 0.10)	0.425	−0.06(−0.33, 0.21)	0.651	−0.10(−0.32, 0.12)	0.381	
Trend test		0.781		0.952		0.411	
Increase per SD	−0.01(−0.07, 0.06)	0.838	0.01(−0.09, 0.10)	0.851	−0.04(−0.12, 0.04)	0.340	0.951
HDL-C (mmol/L)							
Quartile 1	Reference		Reference		Reference		
Quartile 2	−0.02(−0.07, 0.03)	0.336	−0.02(−0.09, 0.05)	0.575	−0.02(−0.10, 0.06)	0.604	
Quartile 3	−0.07(−0.12, −0.02)	0.009	−0.05(−0.12, 0.03)	0.197	−0.09(−0.17, −0.02)	0.015	
Quartile 4	−0.10(−0.15, −0.05)	<0.001	−0.10(−0.18, −0.03)	0.006	−0.10(−0.18, −0.03)	0.004	
Trend test		<0.001		0.001		0.002	
Increase per SD	−0.03(−0.05, −0.02)	<0.001	−0.04(−0.06, −0.01)	0.006	−0.03(−0.06, −0.01)	0.011	0.845
LDL-C (mmol/L)							
Quartile 1	Reference		Reference		Reference		
Quartile 2	−0.02(−0.16, 0.11)	0.732	0.01(−0.18, 0.19)	0.962	−0.01(−0.21, 0.19)	0.903	
Quartile 3	0.06(−0.08, 0.20)	0.420	0.09(−0.13, 0.30)	0.427	0.05(−0.15, 0.24)	0.626	
Quartile 4	0.06(−0.07, 0.20)	0.356	0.14(−0.07, 0.35)	0.180	−0.01(−0.20, 0.17)	0.877	
Trend test		0.167		0.079		0.997	
Increase per SD	0.04(−0.01, 0.09)	0.120	0.08(0.01, 0.15)	0.037	−0.00(−0.06, 0.06)	0.964	0.304

SD, standard deviation.

[a]*Adjusted for age, gender, BMI, educational level, smoking, drinking, household income.*

[b]*Adjusted for age, BMI, educational level, smoking, drinking, household income.*

[c]*The interaction between ESRα methylation and gender.*

methylation level was negatively associated with HDL-C levels whether gender stratification was performed (all $P < 0.05$). For each unit standard deviation (SD) increment in *ESRα* promoter methylation, the level of HDL-C decreased by 0.03 mmol/L in all participants, 0.04 mmol/L in men and 0.03 mmol/L in women. Besides, we observed an increase of 0.08 mmol/L of LDL-C in men with each unit SD increment in *ESRα* promoter methylation level (P=0.037).

Association Between Lipid Metabolism and T2DM

The association between lipid metabolism and T2DM is presented in **Table 3**. The risk of T2DM shown an upward trend with increasing quartiles of TG whether gender stratification was performed (all P for trend < 0.05). In continuous analysis, after adjusting for potential confounding factors, each unit SD increase in TG was associated with an increase of 43%, 36% and 49% in the risk of T2DM in all population, men and women respectively (all $P < 0.05$).

In addition, the risk of T2DM shown a downward trend with increasing levels of HDL-C in men (P for trend < 0.05). In continuous analysis, we observed a decrement of 25% in the risk of T2DM with each unit SD increase of HDL-C levels in men. Furthermore, the interactive effect between HDL-C and gender on the risk of T2DM was evaluated, and significant association was observed.

The Role of *ESRα* Methylation in the Association Between Lipid Metabolism and T2DM

We did not find any association between *ESRα* promoter methylation and FPG (**Supplementary Table 3**) or T2DM (**Table 4**). After stratifying the population into two groups by the *ESRα* promoter methylation level, we found that the risk

TABLE 3 | Association between lipid metabolism and T2DM.

Lipid metabolism (mmol/L)	T2DM						
	All[a]		Men[b]		Women[b]		*P* for interaction[c]
	OR (95% *CI*)	*P*	*OR* (95% *CI*)	*P*	OR (95% *CI*)	*P*	
TG							
Quartile 1	Reference		Reference		Reference		
Quartile 2	1.65(1.02, 2.69)	0.42	2.33(1.06, 5.11)	0.035	1.32(0.68, 2.54)	0.413	
Quartile 3	1.52(1.05, 2.21)	0.027	1.53(0.86, 2.74)	0.149	1.41(0.85, 2.33)	0.179	
Quartile 4	2.30(1.59, 3.33)	<0.001	2.64(1.49, 4.65)	0.001	1.91(1.16, 3.14)	0.011	
Trend test		<0.001		<0.001		0.011	
Increase per SD	1.43(1.25, 1.64)	<0.001	1.36(1.13, 1.64)	0.001	1.49(1.21, 1.83)	<0.001	0.549
TC							
Quartile 1	Reference		Reference		Reference		
Quartile 2	1.18(0.76, 1.83)	0.468	1.42(0.75, 2.70)	0.284	0.74(0.38, 1.43)	0.368	
Quartile 3	1.26(0.82, 1.95)	0.293	1.17(0.61, 2.24)	0.630	1.17(0.63, 2.16)	0.625	
Quartile 4	1.39(0.90, 2.13)	0.134	1.37(0.72, 2.62)	0.335	1.21(0.66, 2.22)	0.530	
Trend test		0.121		0.467		0.486	
Increase per SD	1.15(0.99, 1.33)	0.061	1.03(0.84, 1.29)	0.722	1.18(0.96, 1.45)	0.121	0.426
HDL-C							
Quartile 1	Reference		Reference		Reference		
Quartile 2	0.90(0.60, 1.34)	0.593	0.82(0.46, 1.45)	0.489	0.98(0.54, 1.77)	0.941	
Quartile 3	0.81(0.54, 1.24)	0.331	0.66(0.35, 1.22)	0.184	0.99(0.54, 1.81)	0.977	
Quartile 4	0.91(0.59, 1.41)	0.679	0.47(0.22, 0.97)	0.041	1.55(0.86, 2.81)	0.148	
Trend test		0.478		0.010		0.168	
Increase per SD	0.99(0.85, 1.15)	0.862	0.75(0.58, 0.97)	0.028	1.18(0.97, 1.44)	0.097	0.019
LDL-C							
Quartile 1	Reference		Reference		Reference		
Quartile 2	0.58(0.36, 0.92)	0.020	0.39(0.19, 0.81)	0.011	0.70(0.36, 1.36)	0.290	
Quartile 3	0.79(0.51, 1.22)	0.282	0.78(0.42, 1.43)	0.423	0.55(0.28, 1.09)	0.085	
Quartile 4	0.87(0.57, 1.34)	0.527	0.69(0.36, 1.32)	0.262	0.98(0.53, 1.82)	0.957	
Trend test		0.923		0.532		0.976	
Increase per SD	0.94(0.81, 1.10)	0.452	0.89(0.70, 1.12)	0.318	0.95(0.76, 1.17)	0.614	0.585

SD, standard deviation.

[a] *Adjusted for age, gender, BMI, educational level, smoking, drinking, household income.*

[b] *Adjusted for age, BMI, educational level, smoking, drinking, household income.*

[c] *The interaction between lipid metabolism and gender.*

of T2DM in participants with lower methylation ($\leq$ 4.07%) were more susceptible to HDL-C (*P* for interaction = 0.030), as manifested by a decreased of 22% in the risk of T2DM with the increment of each unit SD in HDL-C concentration (**Table 5**).

DISCUSSION

In the current study, we explored the demographic information of the demographic information and found that the prevalence of T2DM in men is significantly lower than in women. Several previous studies have also observed gender differences in the prevalence of T2DM (24–26). Among them, a large-scale epidemiological survey found that the risk of T2DM in male farmers in rural areas is lower than in female farmers (26). The physical activity has been reported to mitigate the impaired glucose tolerance caused by unhealthy lifestyle (such as sleep loss) (27) and prevent the occurrence of T2DM (28). Farmers were recruited as participants in our study. And the level of physical activity of male farmers is significantly higher than that of female farmers (29), which may lead to a lower prevalence of T2DM in men.

Besides, the association between *ESRα* promoter methylation and lipid metabolism was investigated. And negative correlations were observed between *ESRα* promoter methylation and HDL-C levels, whether gender stratification was performed. Considering that the *ESRα* promoter methylation can suppress the protein expression of ESRα (30), we speculate that the ESRα levels may relate positively to the HDL-C levels in adults. These findings are similar to the previous studies (12, 31). The expression of LDL receptor has been reported to depend on tyrosine kinase and protein kinase C activation, both signal pathways could be activated by estrogen (32). Additionally, as a regulator of LDL-C metabolism, *ESRα* can affect the gene expression of LDL-C receptors (33). Whereas Knopp et al. (34) observed a lesser change in low-density lipoprotein in women than men with high-carbohydrate or high-fat feeding. Here, we only observed

TABLE 4 | Association between *ESRα* methylation and T2DM.

ESRα methylation (%)	All[a]		Men[b]		Women[b]	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Quartile 1	Reference		Reference		Reference	
Quartile 2	1.20 (0.77, 1.86)	0.429	1.53 (0.80, 2.93)	0.200	1.14 (0.60, 2.17)	0.693
Quartile 3	1.01 (0.64, 1.58)	0.974	0.94 (0.47, 1.88)	0.851	1.05 (0.57, 1.96)	0.868
Quartile 4	0.94 (0.59, 1.49)	0.778	1.35 (0.64, 2.84)	0.427	0.70 (0.38, 1.30)	0.259
Trend test		0.276		0.896		0.105
Increase per SD	0.95 (0.81, 1.11)	0.486	1.10 (0.86, 1.41)	0.440	0.82 (0.67, 1.02)	0.070

SD, standard deviation.
[a]*Adjusted for age, gender, BMI, educational level, smoking, drinking, household income.*
[b]*Adjusted for age, BMI, educational level, smoking, drinking, household income.*

TABLE 5 | Interactive effects of *ESRα* methylation and lipid metabolism indexes on T2DM.

Lipid metabolism	Groups	T2DM[a]		
		OR (95%CI)	*P*	*P*-interaction[b]
TG	*ESRα* methylation			0.455
	≤4.07%	1.34(1.08, 1.65)	0.007	
	>4.07%	1.67(1.34, 2.09)	<0.001	
TC	*ESRα* methylation			0.757
	≤4.07%	1.10(0.89, 1.36)	0.390	
	>4.07%	1.22(0.98, 1.53)	0.082	
HDL-C	*ESRα* methylation			0.030
	≤4.07%	0.78(0.61, 1.00)	0.046	
	>4.07%	1.04(0.83, 1.31)	0.736	
LDL-C	*ESRα* methylation			0.273
	≤4.07%	1.02(0.81, 1.28)	0.878	
	>4.07%	0.88(0.69, 1.13)	0.322	

[a]*Adjusted for age, gender, BMI, educational level, smoking, drinking, household income.*
[b]*The interaction between lipid metabolism and ESRα methylation.*

a positive correlation between *ESRα* promoter methylation and LDL-C levels in men, whereas no association was observed in women, further suggesting that LDL-C may be affected by genetic factors in a gender-specific manner.

The concentrations of LDL-C in patients with T2DM are generally not significantly different from those in non-diabetic patients (35). Similarly, no significant association between the levels of LDL-C and the risk of T2DM was observed in the present study. This may be caused by the the management of LDL-C or the reduction of LDL-C catabolism in patients (8, 36). Besides, we here found that the risk of T2DM was positively associated with TG whether gender stratification was performed. Besides, we observed a negative correlation between HDL-C levels and the risk of T2DM in men, instead of women. As revealed in the previous study (37), the dominant lipid abnormality in diabetes is hypertriglyceridemia, which is commonly associated with a reduction in HDL-C. Our findings are similar to the previous study, suggesting that TG and HDL-C may be closely related to the risk of T2DM. Hanai et al. (38) found that the levels of HDL-C were associated with the progression of diabetic kidney disease in men but not in women. In addition, a greater difference in those with diabetes compared with those without diabetes were observed in women than in men for HDL-C (39). Combining these findings, we speculate that there may be gender difference in relationship between HDL-C and T2DM, and a relatively small alteration in HDL-C may trigger the occurrence of T2DM in male.

A previous study found that the level of *ESRα* promoter methylation in decidual tissue of Germans with gestational diabetes mellitus (GDM) is higher than that of Germans without GDM (40), indicating that *ESRα* promoter methylation may increase the risk of GDM. While in this study, we did not observe any association between *ESRα* promoter methylation and FPG or the risk of T2DM. These inconsistencies may be due to the obvious difference races and different types of diabetes mellitus. Notably, many reproducible studies found that the polymorphisms in the same site (rs1801282 in *PPARG* gene) is not significantly associated with the risk of GDM, while it can elevate the risk of T2DM (41), suggesting that the same genetic changes may have different associations

with different types of diabetes mellitus (42, 43). Finally, we observed a significant interactive effect of *ESRα* promoter methylation and HDL-C on the risk of T2DM, indicating that the level of *ESRα* methylation may modify the association between HDL-C and the risk of T2DM, and the risk of T2DM in participants with lower *ESRα* methylation is more susceptible to the alteration of HDL-C. Different DNA fragments located in the same gene may have different methylation levels and thus result in different biological effects (44). Consequently, further comprehensive methylation sequencing in large population may provide more clues for the pathogenesis of T2DM.

CONCLUSION

In summary, lipid metabolism was associated with the levels of *ESRα* promoter methylation and the risks of T2DM. Additionally, *ESRα* promoter methylation can modify the association of HDL-C and T2DM.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The project was approved by the Institutional Review Board of Zhengzhou University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LW, HH, WL, CW, and YB designed the research. GZ, XL, RS, and ZL collected the data. RS and LL performed the experiments. GZ and LL analyzed the data and wrote the manuscript. YB revised the language/article. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2021.578134/full#supplementary-material

REFERENCES

1. Wang Z, Sun J, Han R, Fan D, Dong X, Luan, Z, et al. Efficacy and safety of sodium-glucose cotransporter-2 inhibitors versus dipeptidyl peptidase-4 inhibitors as monotherapy or add-on to metformin in patients with type 2 diabetes mellitus: A systematic review and meta-analysis. *Diabetes, Obes Metab.* (2018) 20:113–20. doi: 10.1111/dom.13047
2. Zhang X, Liu J, Jin K, Xu H, Wang C, Zhang, Z. et al. Subcutaneous injection of hydrogen gas is a novel effective treatment for type 2 diabetes. *J Diabetes Investig.* (2018) 9:83–90. doi: 10.1111/jdi.12674
3. Vijayakumar G, Manghat S, Vijayakumar R, Simon L, Scaria LM, Vijayakumar, A, et al. Incidence of type 2 diabetes mellitus and prediabetes in Kerala, India: results from a 10-year prospective cohort. *BMC Public Health.* (2019) 19:140. doi: 10.1186/s12889-019-6445-6
4. Yan L, Xu MT, Yuan L, Chen B, Xu ZR, Guo, QH, et al. Prevalence of dyslipidemia and its control in type 2 diabetes: A multicenter study in endocrinology clinics of China. *J Clin Lipidol.* (2016) 10:150–60. doi: 10.1016/j.jacl.2015.10.009
5. Verges, B. Pathophysiology of diabetic dyslipidaemia: where are we? *Diabetologia.* (2015) 58:886–99. doi: 10.1007/s00125-015-3525-8
6. Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S, et al. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc Natl Acad Sci USA.* (2007) 104:12587–94. doi: 10.1073/pnas.0705408104
7. Opoku S, Gan Y, Fu W, Chen D, Addo-Yobo E, Trofimovitch D, et al. Prevalence and risk factors for dyslipidemia among adults in rural and urban China: findings from the China National Stroke Screening and prevention project (CNSSPP). *BMC Public Health.* (2019) 19:1500. doi: 10.1186/s12889-019-7827-5
8. Scicali R, Di Pino A, Ferrara V, Urbano F, Piro S, Rabuazzo AM, et al. New treatment options for lipid-lowering therapy in subjects with type 2 diabetes. *Acta Diabetologica.* (2018) 55:209–18. doi: 10.1007/s00592-017-1089-4
9. Khor VK, Dhir R, Yin X, Ahima RS, Song WC. Estrogen sulfotransferase regulates body fat and glucose homeostasis in female mice. *Am J Physiol Endocrinol Metab.* (2010) 299:E657–64. doi: 10.1152/ajpendo.00707.2009
10. Vogel H, Mirhashemi F, Liehl B, Taugner F, Kluth O, Kluge R, et al. Estrogen deficiency aggravates insulin resistance and induces β-cell loss and diabetes in female New Zealand obese mice. *Horm Metab Res.* (2013) 45:430–5. doi: 10.1055/s-0032-1331700
11. Gupte AA, Pownall HJ, Hamilton DJ. Estrogen: an emerging regulator of insulin action and mitochondrial function. J Diabetes Res. (2015) 2015:916585. doi: 10.1155/2015/916585
12. Khristi V, Ratri A, Ghosh S, Pathak D, Borosha S, Dai E, et al. Disruption of ESR1 alters the expression of genes regulating hepatic lipid and carbohydrate metabolism in male rats. *Mol Cell Endocrinol.* (2019) 490:47–56. doi: 10.1016/j.mce.2019.04.005
13. Mauvais-Jarvis F, Manson JE, Stevenson JC, Fonseca VA. Menopausal hormone therapy and type 2 diabetes prevention: evidence, mechanisms, and clinical implications. *Endocr Rev.* (2015) 38:173–88. doi: 10.1210/er.2016-1146
14. Mauvais-Jarvis F. Menopause, estrogens, and glucose homeostasis in women. *Adv Exp Med Biol.* (2017) 1043:217–25. doi: 10.1007/978-3-319-70178-3_11
15. Meyer MR, Clegg DJ, Prossnitz ER, Barton, M. Obesity, insulin resistance and diabetes: sex differences and role of oestrogen receptors. *Acta Physiologica.* (2011) 203:259–69. doi: 10.1111/j.1748-1716.2010.02237.x

16. Ribas V, Nguyen MT, Henstridge DC, Nguyen AK, Beaven SW, Watt MJ, et al. Impaired oxidative metabolism and inflammation are associated with insulin resistance in ERalpha-deficient mice. *Am J Physiol Endocrinol Metab.* (2010) 298:E304–19. doi: 10.1152/ajpendo.00504.2009
17. Maekawa R, Mihara Y, Sato S, Okada M, Tamura I, Shinagawa M, et al. Aberrant DNA methylation suppresses expression of estrogen receptor 1 (ESR1) in ovarian endometrioma. *J Ovarian Res.* (2019) 12:14. doi: 10.1186/s13048-019-0489-1
18. Yang BT, Dayeh TA, Kirkpatrick CL, Taneera J, Kumar R, Groop L, et al. Insulin promoter DNA methylation correlates negatively with insulin gene expression and positively with HbA(1c) levels in human pancreatic islets. *Diabetologia.* (2011) 54:360–7. doi: 10.1007/s00125-010-1967-6
19. Papikian A, Liu W, Gallego-Bartolomé J, Jacobsen SE. Site-specific manipulation of Arabidopsis loci using CRISPR-Cas9 SunTag systems. *Nat Commun.* (2019) 10:729. doi: 10.1038/s41467-019-08736-7
20. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic Med. (1998) 15:539–53. doi: 10.1002/(sici)1096-9136(199807)15:7<539::aid-dia668>3.0.co;2-s
21. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst.* (1972) 97:142–5. doi: 10.1039/an9729700142
22. Yu Z, Armant O, Fischer R. Fungi use the SakA (HogA) pathway for phytochrome-dependent light signalling. *Nat Microbiol.* (2016) 1:16019. doi: 10.1038/nmicrobiol.2016.19
23. Lu L, Katsaros D, Rigault de la Longrais IA, Sochirca O, Yu H. Hypermethylation of let-7a-3 in epithelial ovarian cancer is associated with low insulin-like growth factor-II expression and favorable prognosis. *Cancer Res.* (2007) 67:10117–22. doi: 10.1158/0008-5472.can-07-2544
24. Choi SE, Liu M, Palaniappan LP, Wang EJ, Wong ND. Gender and ethnic differences in the prevalence of type 2 diabetes among Asian subgroups in California. *J Diabetes Complications.* (2013) 27:429–35. doi: 10.1016/j.jdiacomp.2013.01.002
25. Chang H-Y, Hsu C-C, Pan W-H, Liu W-L, Cheng JY-C, Tseng C-H, et al. Gender differences in trends in diabetes prevalence from 1993 to 2008 in Taiwan. *Diabetes Res Clin Pract.* (2010) 90:358–64. doi: 10.1016/j.diabres.2010.09.032
26. Liu X, Mao Z, Li Y, Wu W, Zhang X, Huo W, et al. Cohort profile: the henan rural cohort: a prospective study of chronic non-communicable diseases. *Int J Epidemiol.* (2019) 48:1756. doi: 10.1093/ije/dyz039
27. Saner NJ, Lee MJC, Kuang J, Pitchford NW, Roach GD, Garnham A, et al. Exercise mitigates sleep loss-induced changes in glucose tolerance, mitochondrial function, sarcoplasmic protein synthesis, and diurnal rhythms. *Mol Metab.* (2020) 43:101110. doi: 10.1016/j.molmet.2020.101110
28. Baik I, Park SI. Associations of alcohol consumption and physical activity with lean type 2 diabetes mellitus among Korean adults: a prospective cohort study. *PLoS ONE.* (2020) 15:e0238641. doi: 10.1371/journal.pone.0238641
29. Maruyama S, Nakamura, S. Why are women slimmer than men in developed countries? *Econ Hum Biol.* (2018) 30:1–13. doi: 10.1016/j.ehb.2018.04.002
30. Tsuboi K, Nagatomo T, Gohno T, Higuchi T, Sasaki S, Fujiki N, et al. Single CpG site methylation controls estrogen receptor gene transcription and correlates with hormone therapy resistance. *J Steroid Biochem Mol Biol.* (2017) 171:209–17. doi: 10.1016/j.jsbmb.2017.04.001
31. Pynadath TI, Chanapai S. Elevation of serum HDL and HDL cholesterol in cholesterol in cholesterol-FED male rabbits treated with estrogen. *Atherosclerosis.* (1981) 38:255–65.
32. Distefano E, Marino M, Gillette JA, Hanstein B, Pallottini V, Brüning J, et al. Role of tyrosine kinase signaling in estrogen-induced LDL receptor gene expression in HepG2 cells. *Biochim Biophys Acta.* (2002) 1580:145–9. doi: 10.1016/s1388-1981(01)00197-4
33. Brüning JC, Lingohr P, Gillette J, Hanstein B, Avci H, Krone W, et al. Estrogen receptor-α and Sp1 interact in the induction of the low density lipoprotein-receptor. *J Steroid Biochem Mol Biol.* (2003) 86:113–21. doi: 10.1016/s0960-0760(03)00263-2
34. Knopp RH, Paramsothy P, Retzlaff BM, Fish B, Walden C, Dowdy A, et al. Gender differences in lipoprotein metabolism and dietary response: Basis in hormonal differences and implications for cardiovascular disease. *Curr Atheroscler Rep.* (2005) 7:472–9. doi: 10.1007/s11883-005-0065-6
35. Krentz AJ. Lipoprotein abnormalities and their consequences for patients with Type 2 diabetes. Diabetes Obes Metab. (2003)5:S19–27. doi: 10.1046/j.1462-8902.2003.0310.x
36. Verges B. Lipid modification in type 2 diabetes: the role of LDL and HDL. *Fundam Clin Pharmacol.* (2009) 23:681–5. doi: 10.1111/j.1472-8206.2009.00739.x
37. Quispe R, Martin SS, Jones SR. Triglycerides to high-density lipoprotein-cholesterol ratio, glycemic control and cardiovascular risk in obese patients with type 2 diabetes. *Curr Opin Endocrinol Diabetes Obes.* (2016) 23:150–6. doi: 10.1097/MED.0000000000000241
38. Hanai K, Babazono T, Yoshida N, Nyumura I, Toya K, Hayashi T, et al. Gender differences in the association between HDL cholesterol and the progression of diabetic kidney disease in type 2 diabetic patients. *Nephrol Dial Transplantation.* (2012) 27:1070–5. doi: 10.1093/ndt/gfr417
39. Howard BV, Cowan LD, Go O, Welty TK, Robbins DC, Lee ET. Adverse effects of diabetes on multiple cardiovascular disease risk factors in women. Strong heart study. *Diabetes Care.* (1998) 21:1258–65. doi: 10.2337/diacare.21.8.1258
40. Knabl J, Hiden U, Hüttenbrenner R, Riedel C, Hutter S, Kirn V, et al. GDM alters expression of placental estrogen receptor α in a cell type and gender-specific manner. *Reprod Sci.* (2015) 22:1488–95. doi: 10.1177/1933719115585147
41. Mao H, Li Q, Gao S. Meta-analysis of the relationship between common type 2 diabetes risk gene variants with gestational diabetes mellitus. *PLoS ONE.* (2012) 7:e45882. doi: 10.1371/journal.pone.0045882
42. Anna M, Lluis G, Jose-Manuel FR, Joan V, Inmaculada S, Cristina G, et al. Mannose-binding lectin gene polymorphisms are associated with gestational diabetes mellitus. J Clin Endocrinol Metab. (2004) 89:5081–7. doi: 10.1210/jc.2004-0211
43. Gu HF. Genetic variation screening and association studies of the adenylate cyclase activating polypeptide 1 (ADCYAP1) gene in patients with type 2 diabetes. *Hum Mutat.* (2010) 19:572–6. doi: 10.1002/humu.9034
44. Fabrizio FP, Sparaneo A, Centra F, Trombetta D, Storlazzi CT, Graziano P, et al. Methylation density pattern of KEAP1 gene in lung cancer cell lines detected by quantitative methylation specific PCR and pyrosequencing. *Int J Mol Sci.* (2019) 20:E2697. doi: 10.3390/ijms20112697

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Intermuscular Fat Content in Young Chinese Men With Newly Diagnosed Type 2 Diabetes: Based on MR mDIXON-Quant Quantitative Technique

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Objective: Skeletal muscle fat content is one of the important contributors to insulin resistance (IR), but its diagnostic value remains unknown, especially in the Chinese population. Therefore, we aimed to analyze differences in skeletal muscle fat content and various functional MRI parameters between diabetic patients and control subjects to evaluate the early indicators of diabetes. In addition, we aimed to investigate the associations among skeletal muscle fat content, magnetic resonance parameters of skeletal muscle function and IR in type 2 diabetic patients and control subjects.

Methods: We enrolled 12 patients (age:29-38 years, BMI: 25-28 kg/m^2) who were newly diagnosed with type 2 diabetes (intravenous plasma glucose concentration≥11.1mmol/l or fasting blood glucose concentration≥7.0mmol/l) together with 12 control subjects as the control group (age: 26-33 years, BMI: 21-28 kg/m^2). Fasting blood samples were collected for the measurement of glucose, insulin, 2-hour postprandial blood glucose (PBG2h), and glycated hemoglobin (HbAlc). The magnetic resonance scan of the lower extremity and abdomen was performed, which can evaluate visceral fat content as well as skeletal muscle metabolism and function through transverse relaxation times (T2), fraction anisotropy (FA) and apparent diffusion coefficient (ADC) values.

Results: We found a significant difference in intermuscular fat (IMAT) between the diabetes group and the control group (p<0.05), the ratio of IMAT in thigh muscles of diabetes group was higher than that of control group. In the entire cohort, IMAT was positively correlated with HOMA-IR, HbAlc, T2, and FA, and the T2 value was correlated with HOMA-IR, PBG2h and HbAlc (p<0.05). There were also significant differences in T2 and FA values between the diabetes group and the control group (p<0.05). According to the ROC, assuming 8.85% of IMAT as the cutoff value, the sensitivity and specificity of IMAT were 100% and 83.3%, respectively. Assuming 39.25ms as the cutoff value, the

sensitivity and specificity of T2 value were 66.7% and 91.7%, respectively. All the statistical analyses were adjusted for age, BMI and visceral fat content.

Conclusion: Deposition of IMAT in skeletal muscles seems to be an important determinant for IR in type 2 diabetes. The skeletal muscle IMAT value greater than 8.85% and the T2 value greater than 39.25ms are suggestive of IR.

Keywords: type 2 diabetes, insulin resistance, skeletal muscle, intermuscular fat, MRI

INTRODUCTION

Human organs that can cause insulin resistance (IR) by fat deposition mainly include the heart, liver, skeletal muscles and fat (1) Skeletal muscle fat, which includes subcutaneous adipose tissue (SAT), subfascial adipose tissue (SFAT), intermuscular adipose tissue (IMAT), and intramyocellular lipids (IMCL) (2), is one of the major target tissues of insulin and is important for glucose and lipid uptake and utilization in the human body. Unlike visceral fat, muscle fat and muscle metabolism in relation to the risk of type 2 diabetes has been understudied (3, 4).

Among various fat components of skeletal muscle, IMAT is widely defined as fat infiltration in muscles. Retrospective studies suggest that IMAT may be related to IR and that the increased IMAT may impair muscle blood flow, reduce the ability of insulin to spread, and increase the local concentration of fatty acids (5–7). Under conditions of excess lipid supply, lipid deposition in skeletal muscle is associated with the development of IR, which may even impair insulin signaling (8, 9) However, the previous studies measured the skeletal muscle fat content mainly by DXA, CT or T1-weighted magnetic resonance imaging (T1WI-MRI), which are not accurate enough because of the limited resolution and non-uniformity of the magnetic field (10). The chemical displacement-based water lipid separation (MR Dixon-Quant) is more accurate in measuring fat content (11). Dixon technology is now available for most types of MRI, even for various types of clinical scanning needs (12). In addition, the technology can separate the MRI signals of intramuscular fat, thereby overcome the major limitations of T1WI-MRI (13).

In addition to skeletal muscle fat content, functional MRI indicators also have important value in indicating muscle metabolism. Hence, the relationship between skeletal muscle and magnetic resonance parameters, and their contribution to the risk of type 2 diabetes is important for understanding the pathophysiology of diabetes. Fractional anisotropy (FA) value can detect the water migration rate and limitation in the tissue, and is used to evaluate the microstructure of the tissue (14). Apparent diffusion coefficient (ADC) value can be used to distinguish the difference between normal and pathological skeletal muscles (15). The transverse relaxation times (T2) value can replace the routine laboratory test to quantitatively analyze the skeletal muscle injury (16). However, the associations between the functional MRI muscle indicators (FA, ADC and T2 values) and type 2 diabetes have rarely been studied. The relationships between muscle fat content and functional MRI muscle indicators are also not well understood.

Therefore, we aimed to investigate the association of diabetic biomarker with the skeletal muscle fat content and functional muscle MRI indicators, test the association between muscle fat content and functional MRI muscle indicator, and explore the diagnostic value of skeletal muscle fat content and functional MRI muscle indicators in type 2 diabetes.

MATERIAL AND METHODS

Subjects and Experimental Design

A total of 24 male subjects were recruited from December 2017 to December 2018 for this cross-sectional study. Of them, twelve newly diagnosed type 2 diabetic patients (intravenous plasma glucose concentration ≥11.1mmol/l or fasting blood glucose concentration ≥7.0mmol/l) were recruited from the First Endocrinology Clinic of Shengjing Hospital, Shenyang, China. Twelve control young adults were also recruited from the geographic area of Shenyang, China. The inclusion criteria were: aged 25 to 40 years; body mass index (BMI) was between 21 to 29 kg/m^2; weight was stable within the past 3 months; not following a special exercise program within the past 3 months; non-smoking; no acute illness; no history of diabetes and related family history; no hypertension, cerebrovascular disease, coronary heart disease, chronic heart failure or high uric academia; and have no contraindications for magnetic resonance imaging.

Participants started fasting at 8 pm before the testing day, and had venous blood samples for the measurement of fasting blood glucose, fasting insulin, 2-hour postprandial blood glucose (PBG2h) and glycated hemoglobin (HbAlc) at 7-8 am and 9-10 am at the test of the day. The lower extremity magnetic resonance scans were performed at 12 am. Of notes, for the patients with type 2 diabetes, the magnetic resonance scans of the lower extremity were performed within the first three weeks of diagnosis.

The China Medical University Institutional Review Board approved the study protocols and all subjects provided written and informed consent for their participation. The current study is registered under 2019PS443K.

Anthropometric Measurements

Subjects' height (± 0.1cm) and weight (± 0.1kg) were measured using a wall-mounted stadiometer and a digital balance scale, respectively. These measurements were used to calculate BMI (kg/m^2).

Laboratory Assays

All the laboratory assays were processed at the Central Laboratory of Shengjing Hospital of Shenyang, China. Fasting blood glucose and 2-hour postprandial blood glucose were measured within 2 hours after centrifugation (glucose oxidase assay, Olympus 400 automatic biochemical analyzer). HbA1c was detected on the same day by high performance liquid chromatography, Bio Rad D-10, Bole company of the United States. Radioimmunoassay was used to determine fasting plasma insulin (FINS). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as: fasting blood glucose × fasting insulin/22.5.

Magnetic Resonance Imaging and Image Analyses

MRI scans (sagittal T2W1, coronal T2W1, functional imaging T2-mapping, DTI, mDixon-Quant sequences) were performed by Philip Intera Achieva 3.0T scanner to evaluate the cross-sectional areas of the visceral fat of the abdomen (14 cases, 7 newly diagnosed type 2 diabetic patients and 7 controls) and the skeletal muscle fat of the thigh, and the magnetic resonance parameter values (T2 value, FA value, and ADC value) of the skeletal muscle were measured.

Axial images of the mid-L2 vertebral level in MRI data were selected for VAT measurements. The location method was also assisted by the coronal plane in MRI. Then, the relevant single slice Digital Imaging and Communications in Medicine files were imported into Mimics Research software version 21.0 (Materialise, Leuven, Belgium) for image segmentation of VAT (**Figure 1**). The lower extremity scans were obtained at femoral neck, proximal to the terminal end of the femur. This site was chosen because it is the region with the largest skeletal muscle fat, and there is little variability across persons (17). The scan time was approximately 20-25 minutes (**Table 1**). Images of cross-sectional areas of the muscle and fat tissue were analyzed by using Philips Research Imaging Development Environment (PRIDE) software (version 4.1.V3).

TABLE 1 | Magnetic resonance sequence acquisition parameters.

Scanning parameter	T1	T2	DTI	T2 mapping	mDIXON-quant
TR	260	5384	3357	2010	9.1
TE	15	100	84	40/60/80/100	1.33
FOV	400	400	410	414	420
SNR	1.00	1.00	1.00	1.00	1.00
NSA	3	2	2	2	2
Thickness/Pitch	4.0/0.4	4.0/0.4	4.0/5.0	4.0/15	6.0/-3.0
Scan time	2:09	2:10	3:13	2:13	0:09

TR, time of repetition; TE, time of echo; FOV, field of view; SNR, signal to noise ratio; NSA, number of signal averaged.

By drawing ROI, the thigh MRI image can be segmented into bone and soft tissue measures. For this current study, we measured 1) the percentage of SFAT, IMAT, and SAT in skeletal muscle; 2) T2 value, FA value and ADC value in skeletal muscle (**Figure 2**).

Statistical Analysis

All data were checked for normality using the Shapiro-Wilk's test. The measurement data was expressed as mean ± standard deviation (SD). The magnetic resonance parameters and the fat content of the skeletal muscle were compared between the diabetes group and the control group by Binary logistic. Partial variance correlation analysis was carried out to test the associations between magnetic resonance parameters (T2, ADC and FA values) and type 2 diabetes markers (HOMA-IR, PBG2h and HbAlc). Multiple linear regression models were used to assess the association of IMAT and SFAT and SAT with type 2 diabetes markers (HOMA-IR, PBG2h and HbAlc) and magnetic resonance parameters (T2, ADC and FA values). The missing values of the visceral fat content were replaced with the average values in each group. All analyses were adjusted for age, BMI and visceral fat content. The ROC curve was

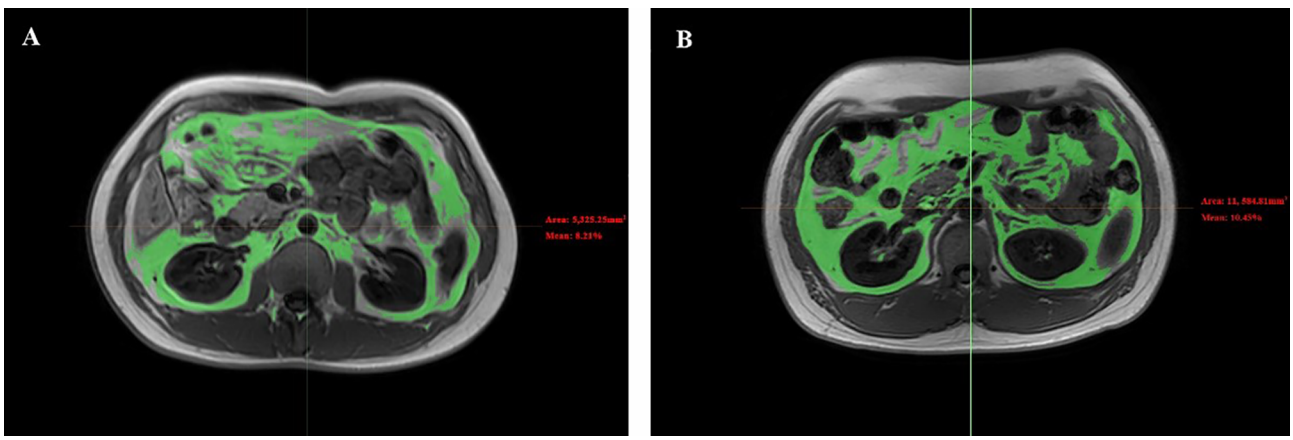

FIGURE 1 | Representative abdomen MRI images of VAT between diabetes group and control group. Representative abdomen MRI images in a healthy volunteer **(A)** and a patient with diabetes **(B)**. Results for the percentage of the VAT were 8.21% for the healthy volunteer and 10.45% for the patient with diabetes. VAT, visceral fat.

drawn to analyze the value of the above indicators for the diagnosis of type 2 diabetes alone. $p < 0.05$ was considered statistically significant. All analyses were performed using SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

General Characteristics of the Subjects

A total of 24 subjects were included in the study. Subjects in the diabetes group were older, and had higher levels of HOMA-IR, HbA1c, and PBG2h compared to the ones in the control group (p<0.05) (**Table 2**).

TABLE 2 | General characteristics.

	Diabetes group	Control group	*p*-value
N	12	12	—
Age (years)	34.33 ± 3.98	28.92 ± 3.52	0.002
BMI (kg/m^2)	26.73 ± 1.98	24.78 ± 3.27	0.091
HOMA-IR	5.74 ± 3.28	1.83 ± 0.75	0.003
HbAlc (mg/dl)	6.67 ± 1.35	5.20 ± 0.21	0.001
PBG2h (mg/dl)	11.11 ± 2.58	4.960.91	<0.001
Visceral fat (%)	8.36 ± 2.37	6.86 ± 3.58	0.238

BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; HbAlc, glycosylated hemoglobin; PBG2h, 2-hour postprandial blood glucose.

Comparison of Muscle Magnetic Resonance Parameters and Muscle Fat Components Between Diabetic and Control Subjects

Subjects in the diabetes group had higher levels of IMAT, T2, and ADC compared to the ones in the control group (p<0.05) (**Table 3**).

Correlation Between Muscle Magnetic Resonance Parameters and Type 2 Diabetes Markers

Among the three selected magnetic resonance parameters (T2, FA, and ADC), muscle T2 value and FA value were associated with HOMA-IR, PBG2h and HbAlc (p<0.05). ADC value was associated with PBG2h (p<0.05) (**Table 4**).

Associations Between Thigh Fat Composition and Muscle Magnetic Resonance Parameters and Type 2 Diabetes Markers

In the models including IMAT, SFAT and SAT, IMAT was significantly associated with type 2 diabetes markers (HOMA-IR, PBG2h and HbAlc) and magnetic resonance parameters (T2, ADC and FA values) (p<0.05), and SFAT was significantly

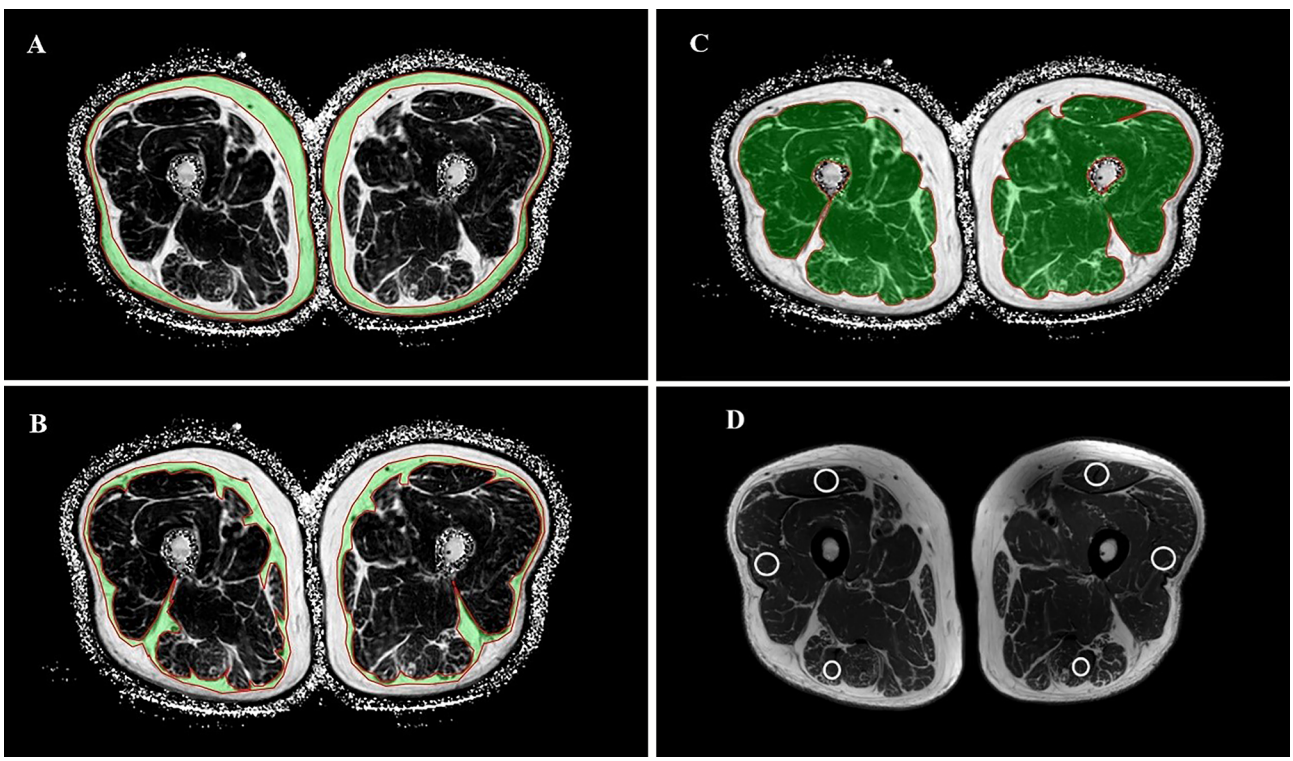

FIGURE 2 | Selection and measurement of mDixon-Quant sequence, T2mapping and DTI sequence ROI. Regions of interest for SAT **(A)**, SFAT **(B)**, IMAT **(C)**, and the positions of the ROI on the rectus femoris, the biceps femoris and the lateral femoral muscle in T2 sequence **(D)**.

TABLE 3 | Comparison of muscle magnetic resonance parameters and muscle fat components in diabetes group (N = 12) and control group (N = 12).

	Diabetes group	Control group	OR	95%CI	*p*-value
IMAT (%)	10.08 ± 1.37	7.06 ± 2.02	3.27	1.14-9.35	0.03
SFAT (%)	3.69 ± 0.60	3.51 ± 0.79	1.07	0.16-7.44	0.94
SCAT (%)	20.13 ± 6.43	25.19 ± 6.56	0.94	0.77-1.13	0.50
Total fat (%)	33.91 ± 6.35	35.78 ± 7.24	1.02	0.82-1.26	0.86
FA	0.56 ± 0.05	0.51 ± 0.04	2.38	0.89-6.41	0.09
T2 (ms)	40.67 ± 3.75	37.25 ± 1.94	1.75	1.00-3.05	0.05
ADC (10^-3mm^2/s)	1.20 ± 0.97	0.96 ± 0.26	1.12	1.01-1.23	0.02

All estimates were adjusted for age, BMI and visceral fat content. IMAT, Intermuscular Fat; SFAT, subfascial adipose tissue; SAT, subcutaneous adipose tissue; T2, transverse relaxation times; FA, fractional anisotropy; ADC, apparent diffusion coefficient; OR, odds ratio; CI, confidence interval.

associated with HOMA-IR (p<0.05). However, SAT was not associated with type 2 diabetes markers (HOMA-IR, PBG2h and HbAlc) and magnetic resonance parameters (T2, ADC and FA values) (p>0.05) (**Table 5**).

The Diagnostic Value of IMAT, FA, and T2 Values for Type 2 Diabetes

Figure 2 presents the ROC curve of type 2 diabetes based on T2, FA and IMAT, which showed significant associations with HOMA-IR, PBG2h and HbAlc values of type 2 diabetes. The selection of the cutoff value was based on the maximization of the Youden's index. The cut-off value of IMAT was 8.85%, its sensitivity to type 2 diabetes was 100%, specificity was 83.3%. The cutoff value of T2 value was 39.25ms, and its diagnostic sensitivity was 66.7% and specificity was 91.7%. The cutoff value of FA value was 0.58, and its diagnostic sensitivity was 41.7% and specificity was 91.8%. The area under the ROC (AUROC) for the diagnosis of type 2 diabetes by IMAT, T2 and FA values were 0.917, 0.840 and 0.774, respectively. Among these indicators, IMAT appeared to be the best predictor, followed by T2 and FA (**Figure 3**).

TABLE 4 | Correlation between muscle magnetic resonance parameters and type 2 diabetes markers (N = 24).

	HOMA-IR	HbAlc (mg/dl)	PBG2h (mg/dl)
	p-value	*p*-value	*p*-value
T2 (ms)	<0.001	<0.001	0.020
ADC (10^-3mm^2/s)	0.071	0.052	0.045
FA	0.005	0.001	0.001

All estimates were adjusted for age, BMI and visceral fat content. T2, transverse relaxation times; FA, fractional anisotropy; ADC, apparent diffusion coefficient; HOMA-IR, homeostatic model assessment of insulin resistance; HbAlc, glycosylated hemoglobin; PBG2h, 2-hour postprandial blood glucose.

DISCUSSION

In this study, we show that IMAT is associated with IR among young adults, independent of age, BMI and visceral fat content. However, SAT, which makes up the highest proportion of muscle fat, is not associated with IR. The T2 and FA values are also positively correlated with IR, which provides new evidence for the relationship between the muscle magnetic resonance index and IR. In addition, IMAT discriminates type 2 diabetic patients from control subjects with a sensitivity of 100%, and a specificity of 83.3%.

The mechanism underlying the relationship between skeletal muscle fat and IR is still not clear. Previous studies have shown that IMAT may be related to multiple metabolic factors (18–21), this association is often attributed to the close relationship between IMAT and BMI, Some studies also suggested that due

TABLE 5 | Association between thigh fat composition and muscle magnetic resonance parameters and type 2 diabetes markers (N = 24).

Model		HOMA-1R		HbAlc (mg/dl)		PBG2h (mg/dl)		T2 (ms)		FA	
		β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value
1	IMAT	1.025	<0.001	0.587	<0.001	1.190	0.001	1.464	<0.001	0.019	0.002
2	SFAT	1.082	0.018	0.441	0.275	1.100	0.416	2.207	0.123	0.027	0.216
3	SAT	-0.087	0.060	-0.096	0.058	-0.232	0.070	-0.335	0.244	-0.002	0.391
4	IMAT	0.511	<0.001	0.614	<0.001	1.213	0.004	1.390	0.001	0.018	0.008
	SFAT	0.527	0.046	-0.255	0.454	-0.216	0.849	0.699	0.520	0.008	0.680
5	IMAT	0.573	<0.001	0.581	<0.001	1.096	0.010	1.607	<0.001	0.021	0.005
	SAT	0.004	0.891	-0.004	0.924	-0.058	0.629	0.089	0.444	0.001	0.487
6	IMAT	0.485	<0.001	0.623	<0.001	1.106	0.020	1.528	0.002	0.020	0.014
	SFAT	0.563	0.047	-0.269	0.465	-0.063	0.958	0.501	0.664	0.001	0.827
	SAT	-0.013	0.640	0.005	0.901	-0.056	0.665	0.073	0.554	0.005	0.559

Multivariate linear regression analysis was used; All estimates were adjusted for age, BMI and visceral fat content. Type 2 diabetes markers (HOMA-IR, PBG2h and HbAlc) and magnetic resonance parameters (T2, ADC and FA values) were dependent variables. IMAT, SFAT and SAT were independent variables of model 1-3, respectively. The independent variables of model 4-5 were paired by IMAT, SFAT, and SAT. The independent variables of model 6 included IMAT, SFAT and SAT. IMAT, intermuscular adipose tissue; SFAT, subfascial adipose tissue; SAT, subcutaneous adipose tissue; HOMA-IR, homeostatic model assessment of insulin resistance; HbAlc, glycosylated hemoglobin; PBG2h, 2-hour postprandial blood glucose; T2, transverse relaxation times; FA, fractional anisotropy.

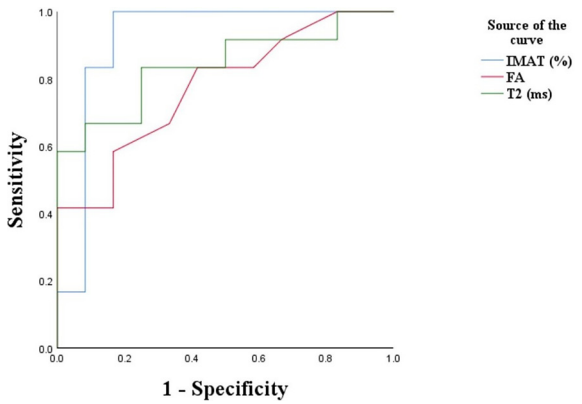

FIGURE 3 | ROC plot of each type of indicator for diagnosis of type 2 diabetes. IMAT, intermuscular adipose tissue; FA, fractional anisotropy; T2, transverse relaxation times.

to the adjacent anatomical structure of skeletal muscle and IMAT, IMAT may affect the direct contact between fat and muscle cells through the secreted adipokines or locally affect the muscles through the shared micro-vessels (22, 23). The infiltration of IMAT in skeletal muscle may also increase the local inflammation of muscle fibers, which may lead to increased oxidative stress in the muscles. This could result in decreased insulin-stimulated tyrosine phosphorylation and decreased activity of downstream signaling molecules, which in turn causes insulin resistance (24, 25). Recent research indicates that IMAT may regulate muscle insulin sensitivity by secreting inflammatory cytokines and extracellular matrix proteins, as well as increasing the concentration of free fatty acids (FFA) in the body (26). Our study found that IMAT was statistically different between type 2 diabetic patients and control subjects. There was no significant difference in SFAT or SAT between the two groups. Moreover, we showed that IMAT was associated with higher HOMA-IR, HbA1c, and PBG2h. Our findings are consistent with the results of Goodpaster et al. (18, 27). They evaluated the adipose tissue of thighs in 65 Americans by CT and DXA and suggested that IMAT and SFAT might be markers of IR in type 2 diabetes. Another study found that high levels of thigh subcutaneous adipose tissue and low levels of thigh IMAT might maintain good insulin metabolism in early postmenopausal American women (19). In addition, Kim et al. (20) reported that among 75 middle-aged and older adults, IMAT might have a negative influence on fasting glucose concentration. These findings collectively indicate that IMAT may increase metabolic risk in IR and type 2 diabetes. In summary, we speculate that IMAT may increase in the skeletal muscle of Chinese male patients with type 2 diabetes, which could affect the muscle mass and muscle function.

There are a few studies regarding the correlation between skeletal muscle MR parameters and IR, as well as type 2 diabetes (28, 29), and whether it can be used as an index to predict or diagnoses type 2 diabetes still remains unclear. Their findings indicate that fasting hyperinsulinemia (insulin resistance) and dyslipidemia have independent and additional contributions to increased tissue magnetic resonance T2 values, and T2 values should be early screened for metabolic dysfunction to prevent diabetes and cardiovascular disease (30). Others have shown that biomarkers associated with water T2 can provide evidence for the pathophysiology of metabolic syndrome and early metabolic disorders prior to the occurrence of type 2 diabetes and cardiovascular disease (31). DTI sequence and its parameters (ADC and FA values) can be used to evaluate the difference of fibrous tissue in diseased and control muscle tissues, and as one of the commonly used methods. It can also provide an in-depth understanding of muscle dynamics, which may apply the analysis and research of muscle tissue metabolism (32, 33). Among muscle fat components, IMAT has a positive correlation with T2 and FA values, which indicates that IMAT may affect muscle metabolism and participate in the process of early IR in type 2 diabetes.

In recent years, various types of diabetes prediction models have been proposed (34–37), but none of those have been constructed or validated in skeletal muscle fat content or magnetic resonance parameters in the Chinese population. Moreover, our model was based on MR mDIXON-quant quantitative technique, and IMAT and T2 value that may affect the incidence of the disease were included in the regression model. Our results showed that among the equal magnetic resonance parameters, T2 and FA values were associated with increased HOMA-IR and HbA1c. It is indicated that T2 can detect abnormalities in skeletal muscle signals in newly diagnosed diabetic patients. IMAT discriminated type 2 diabetic patients from control subjects with the sensitivity of 100% and the specificity of 83.3% when setting the cutoff value of IMAT as 8.85%. Muscle T2 value might also be used to detect early skeletal muscle changes in type 2 diabetes patients, assuming 39.25ms as the cutoff value, the sensitivity and specificity were 66.7% and 91.7% respectively. In our study, the IMAT and SFAT levels of one control subject were higher than the cut-off value, but the HOMA-IR, PBG2h and HbAlc values

were normal. During 2-year follow up period, his fasting insulin and fasting blood glucose were found to have exceeded the normal value. After clinical consultation, treatment options including medication and exercise therapy were developed for this subject.

At current stage, various weight loss guidelines rarely consider specific organs (e.g., for skeletal muscle and pancreas) (38, 39). MR imaging provides a unique, non-radiative and non-invasive diagnostic platform that can directly quantify the physiological and biochemical variables of skeletal muscle (40). Using more accurate MR analysis to analyze the content of IMAT and the measurement of muscle magnetic resonance signals may provide clinicians with more specific strategies for treating skeletal muscle fat infiltration, and even predict the long-term risk of type 2 diabetes in obese patients.

The employment of MR mDIXON-quant quantitative technique to quantify IMAT is a strength of this study. This technique is able to directly measure IMAT and more sensitive than computed tomography (CT), which indirectly measures IMAT (41). Type 2 diabetes has many confounding factors such as medications, but the patients in our study were all newly diagnosed with type 2 diabetes and not on medications, which is another strength.

There are several limitations in our study. First, our sample size is modest, and we did not calculate our sample size before conducting this study. However, as a *post hoc* analysis, we found that the average IMAT was 10.08% with an SD of 1.37 in the diabetes group, and the average was 7.06% with an SD of 2.02 in the control group. Based on our data, this study had a power of 98.24% to detect the difference in IMAT between the diabetes group and the control group at 5% significance level with a total of 24 samples equally allocated between the two groups. In other words, despite the modest sample size, we were still able to identify the significant differences between the two groups. Second, this study is cross-sectional, and no causal relationship can be derived. Prospective follow-up studies with an adequate sample size are needed to validate our findings. Third, because the mDIXON-quant technique scan image used was susceptible to motion artifacts, and image post-processing only by manually delineating ROI to measure the content of various fat components in muscles, there could be measurement errors. Fourth, the missing values of the visceral fat content, when used as a confounding factor in the statistical analyses, might cause biased results. Fifth, our participants are all Chinese males, such that the findings cannot be generalized to other populations. Last, MRI scanning is costly, and not widely used yet. To the best of our knowledge, the relationship between the fat components of muscles and the prevalence of type 2 diabetes is understudied (42). Thus, at this stage, mDIXON-Quant, a robust noninvasive quantitative method available, would be well suited for the accurate measurement of the content of IMAT and muscle magnetic resonance signals in this regard.

In conclusion, IMAT in skeletal muscles is associated with IR, and T2 value can detect the metabolic irregularity of skeletal muscle in Chinese male patients with newly diagnosed type 2 diabetes. The skeletal muscle IMAT value greater than 8.85% and the T2 value greater than 39.25ms are suggestive of type 2 diabetes in Chinese males.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by 2019PS443K. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FY, SP, and HZ conceived and designed research. FY performed experiments, analyzed data and drafted manuscript. BH and FW provided clinical support for the experiment. LC, HZ, and YD edited and revised manuscript. SP approved the final version of manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *Int J Med Sci* (2014) 11(11):1185–200. doi: 10.7150/ijms.10001
2. Addison O, Marcus RL, Lastayo PC, Ryan AS. Intermuscular fat: a review of the consequences and causes. *Int J Endocrinology* (2014) 2014:309570. doi: 10.1155/2014/309570
3. Garg SK, Lin F, Kandula N, Ding J, Carr J, Allison M, et al. Ectopic Fat Depots and Coronary Artery Calcium in South Asians Compared With Other Racial/

Ethnic Groups. *J Am Heart Assoc* (2016) 5(11):e004257. doi: 10.1161/JAHA.116.004257
4. Neeland IJ, Turer AT, Ayers CR, Powell-Wiley TM, Vega GL, Farzaneh-Far R, et al. Dysfunctional adiposity and the risk of prediabetes and type 2 diabetes in obese adults. *Jama* (2012) 308(11):1150–9. doi: 10.1001/2012.jama.11132
5. Zoico E, Rossi A, Di Francesco V, Sepe A, Olioso D, Pizzini F, et al. Adipose tissue infiltration in skeletal muscle of healthy elderly men: relationships with body composition, insulin resistance, and inflammation at the systemic and tissue level. *J Gerontol A Biol Sci Med Sci* (2010) 65(3):295–9. doi: 10.1093/gerona/glp155
6. Hamrick MW, McGee-Lawrence ME, Frechette DM. Fatty Infiltration of Skeletal Muscle: Mechanisms and Comparisons with Bone Marrow Adiposity. *Front Endocrinology* (2016) 7:69. doi: 10.3389/fendo.2016.00069
7. Khan IM, Perrard XY, Brunner G, Lui H, Sparks LM, Smith SR, et al. Intermuscular and perimuscular fat expansion in obesity correlates with skeletal muscle T cell and macrophage infiltration and insulin resistance. *Int J Obes (2005)* (2015) 39(11):1607–18. doi: 10.1038/ijo.2015.104
8. Bosma M, Kersten S, Hesselink MK, Schrauwen P. Re-evaluating lipotoxic triggers in skeletal muscle: relating intramyocellular lipid metabolism to insulin sensitivity. *Prog Lipid Res* (2012) 51(1):36–49. doi: 10.1016/j.plipres.2011.11.003
9. Dirks ML, Wall BT, van de Valk B, Holloway TM, Holloway GP, Chabowski A, et al. One Week of Bed Rest Leads to Substantial Muscle Atrophy and Induces Whole-Body Insulin Resistance in the Absence of Skeletal Muscle Lipid Accumulation. *Diabetes* (2016) 65(10):2862–75. doi: 10.2337/db15-1661
10. Hollingsworth KG, Garrood P, Eagle M, Bushby K, Straub V. Magnetic resonance imaging in Duchenne muscular dystrophy: longitudinal assessment of natural history over 18 months. *Muscle Nerve* (2013) 48(4):586–8. doi: 10.1002/mus.23879
11. Zhang Y, Zhou Z, Wang C, Cheng X, Wang L, Duanmu Y, et al. Reliability of measuring the fat content of the lumbar vertebral marrow and paraspinal muscles using MRI mDIXON-Quant sequence. *Diagn Interventional Radiol (Ankara Turkey)* (2018) 24(5):302–7. doi: 10.5152/dir.2018.17323
12. Johnson BL, Schroeder ME, Wolfson T, Gamst AC, Hamilton G, Shiehmorteza M, et al. Effect of flip angle on the accuracy and repeatability of hepatic proton density fat fraction estimation by complex data-based, T1-independent, T2*-corrected, spectrum-modeled MRI. *J Magnetic Resonance Imaging JMRI* (2014) 39(2):440–7. doi: 10.1002/jmri.24153
13. Idilman IS, Tuzun A, Savas B, Elhan AH, Celik A, Idilman R, et al. Quantification of liver, pancreas, kidney, and vertebral body MRI-PDFF in non-alcoholic fatty liver disease. *Abdominal Imaging* (2015) 40(6):1512–9. doi: 10.1007/s00261-015-0385-0
14. Glaser C. New techniques for cartilage imaging: T2 relaxation time and diffusion-weighted MR imaging. *Radiologic Clinics North America* (2005) 43 (4):641–53, vii. doi: 10.1016/j.rcl.2005.02.007
15. Sigmund EE, Novikov DS, Sui D, Ukpebor O, Baete S, Babb JS, et al. Time-dependent diffusion in skeletal muscle with the random permeable barrier model (RPBM): application to normal controls and chronic exertional compartment syndrome patients. *NMR Biomedicine* (2014) 27(5):519–28. doi: 10.1002/nbm.3087
16. Hooijmans MT, Damon BM, Froeling M, Versluis MJ, Burakiewicz J, Verschuuren JJ, et al. Evaluation of skeletal muscle DTI in patients with duchenne muscular dystrophy. *NMR Biomedicine* (2015) 28(11):1589–97. doi: 10.1002/nbm.3427
17. Goodpaster BH, Chomentowski P, Ward BK, Rossi A, Glynn NW, Delmonico MJ, et al. Effects of physical activity on strength and skeletal muscle fat infiltration in older adults: a randomized controlled trial. *J Appl Physiol (Bethesda Md 1985)* (2008) 105(5):1498–503. doi: 10.1152/japplphysiol.90425.2008
18. Goodpaster BH, Thaete FL, Kelley DE. Thigh adipose tissue distribution is associated with insulin resistance in obesity and in type 2 diabetes mellitus. *Am J Clin Nutr* (2000) 71(4):885–92. doi: 10.1093/ajcn/71.4.885
19. Goss AM, Gower BA. Insulin sensitivity is associated with thigh adipose tissue distribution in healthy postmenopausal women. *Metabolism: Clin Experimental* (2012) 61(12):1817–23. doi: 10.1016/j.metabol.2012.05.016
20. Kim JE, Dunville K, Li J, Cheng JX, Conley TB, Couture CS, et al. Intermuscular Adipose Tissue Content and Intramyocellular Lipid Fatty Acid Saturation Are Associated with Glucose Homeostasis in Middle-Aged and Older Adults. *Endocrinol Metab (Seoul Korea)* (2017) 32(2):257–64. doi: 10.3803/EnM.2017.32.2.257
21. Vella CA, Allison MA. Associations of abdominal intermuscular adipose tissue and inflammation: The Multi-Ethnic Study of Atherosclerosis. *Obes Res Clin Practice* (2018) 12(6):534–40. doi: 10.1016/j.orcp.2018.08.002
22. Kelley DE, Goodpaster BH. Stewing in Not-So-Good Juices: Interactions of Skeletal Muscle With Adipose Secretions. *Diabetes* (2015) 64(9):3055–7. doi: 10.2337/db15-0403
23. Yim JE, Heshka S, Albu J, Heymsfield S, Kuznia P, Harris T, et al. Intermuscular adipose tissue rivals visceral adipose tissue in independent associations with cardiovascular risk. *Int J Obes (2005)* (2007) 31(9):1400–5. doi: 10.1038/sj.ijo.0803621
24. Beasley LE, Koster A, Newman AB, Javaid MK, Ferrucci L, Kritchevsky SB, et al. Inflammation and race and gender differences in computerized tomography-measured adipose depots. *Obes (Silver Spring Md)* (2009) 17 (5):1062–9. doi: 10.1038/oby.2008.627
25. Evans JL, Maddux BA, Goldfine ID. The molecular basis for oxidative stress-induced insulin resistance. *Antioxidants Redox Signaling* (2005) 7(7-8):1040–52. doi: 10.1089/ars.2005.7.1040
26. Sachs S, Zarini S, Kahn DE, Harrison KA, Perreault L, Phang T, et al. Intermuscular adipose tissue directly modulates skeletal muscle insulin sensitivity in humans. *Am J Physiol Endocrinol Metab* (2019) 316(5):E866–e79. doi: 10.1152/ajpendo.00243.2018
27. Miljkovic-Gacic I, Gordon CL, Goodpaster BH, Bunker CH, Patrick AL, Kuller LH, et al. Adipose tissue infiltration in skeletal muscle: age patterns and association with diabetes among men of African ancestry. *Am J Clin Nutr* (2008) 87(6):1590–5. doi: 10.1093/ajcn/87.6.1590
28. Boersma GJ, Johansson E, Pereira MJ, Heurling K, Skrtic S, Lau J, et al. Altered Glucose Uptake in Muscle, Visceral Adipose Tissue, and Brain Predict Whole-Body Insulin Resistance and may Contribute to the Development of Type 2 Diabetes: A Combined PET/MR Study. *Hormone Metab Res = Hormon- und Stoffwechselforschung = Hormones Metabolisme* (2018) 50(8):627–39. doi: 10.1055/a-0643-4739
29. Ferrannini E, Iozzo P, Virtanen KA, Honka MJ, Bucci M, Nuutila P. Adipose tissue and skeletal muscle insulin-mediated glucose uptake in insulin resistance: role of blood flow and diabetes. *Am J Clin Nutr* (2018) 108 (4):749–58.doi: 10.1093/ajcn/nqy162
30. Robinson MD, Mishra I, Deodhar S, Patel V, Gordon KV, Vintimilla R, et al. Water T2 as an early, global and practical biomarker for metabolic syndrome: an observational cross-sectional study. *J Trans Med* (2017) 15(1):258. doi: 10.1186/s12967-017-1359-5
31. Mishra I, Jones C, Patel V, Deodhar S, Cistola DP. Early detection of metabolic dysregulation using water T2 analysis of biobanked samples. *Diabetes Metab Syndrome Obes Targets Ther* (2018) 11:807–18. doi: 10.2147/DMSO.S180655
32. Rockel C, Akbari A, Kumbhare DA, Noseworthy MD. Dynamic DTI (dDTI) shows differing temporal activation patterns in post-exercise skeletal muscles. *Magma (New York NY)* (2017) 30(2):127–38. doi: 10.1007/s10334-016-0587-7
33. Kermarrec E, Budzik JF, Khalil C, Le Thuc V, Hancart-Destee C, Cotten A. In vivo diffusion tensor imaging and tractography of human thigh muscles in healthy subjects. *AJR Am J Roentgenology* (2010) 195(5):W352–6. doi: 10.2214/AJR.09.3368
34. Ban HJ, Heo JY, Oh KS, Park KJ. Identification of type 2 diabetes-associated combination of SNPs using support vector machine. *BMC Genet* (2010) 11:26. doi: 10.1186/1471-2156-11-26
35. Baan CA, Ruige JB, Stolk RP, Witteman JC, Dekker JM, Heine RJ, et al. Performance of a predictive model to identify undiagnosed diabetes in a health care setting. *Diabetes Care* (1999) 22(2):213–9. doi: 10.2337/diacare.22.2.213
36. Lindstrom J, Tuomilehto J. The diabetes risk score: a practical tool to predict type 2 diabetes risk. *Diabetes Care* (2003) 26(3):725–31. doi: 10.2337/diacare.26.3.725
37. Gao WG, Dong YH, Pang ZC, Nan HR, Wang SJ, Ren J, et al. A simple Chinese risk score for undiagnosed diabetes. *Diabetic Med J Br Diabetic Assoc* (2010) 27(3):274–81. doi: 10.1111/j.1464-5491.2010.02943.x
38. Gepner Y, Shelef I, Schwarzfuchs D, Zelicha H, Tene L, Yaskolka Meir A, et al. Effect of Distinct Lifestyle Interventions on Mobilization of Fat Storage Pools: CENTRAL Magnetic Resonance Imaging Randomized Controlled Trial. *Circulation* (2018) 137 (11):1143–57. doi: 10.1161/CIRCULATIONAHA.117.030501
39. Hausman GJ, Basu U, Du M, Fernyhough-Culver M, Dodson MV. Intermuscular and intramuscular adipose tissues: Bad vs. good adipose tissues. *Adipocyte* (2014) 3(4):242–55. doi: 10.4161/adip.28546
40. Sun H, Xu MT, Wang XQ, Wang MH, Wang BH, Wang FZ, et al. Comparison Thigh Skeletal Muscles between Snowboarding Halfpipe Athletes and Healthy Volunteers Using Quantitative Multi-Parameter Magnetic Resonance

Imaging at Rest. *Chin Med J* (2018) 131(9):1045–50. doi: 10.4103/0366-6999.230740

41. Grimm A, Meyer H, Nickel MD, Nittka M, Raithel E, Chaudry O, et al. Repeatability of Dixon magnetic resonance imaging and magnetic resonance spectroscopy for quantitative muscle fat assessments in the thigh. *J Cachexia Sarcopenia Muscle* (2018) 9(6):1093–100. doi: 10.1002/jcsm.12343
42. Granados A, Gebremariam A, Gidding SS, Terry JG, Carr JJ, Steffen LM, et al. Association of abdominal muscle composition with prediabetes and diabetes: The CARDIA study. *Diabetes Obes Metab* (2019) 21(2):267–75. doi: 10.1111/dom.13513

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Does Universal Screening for Gestational Diabetes Mellitus Improve Neonatal Outcomes in a Socially Vulnerable Population: A Prospective Study in French Guiana

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Aims/Introduction: French Guiana has a high prevalence of metabolic diseases, which are risk factors for gestational diabetes mellitus. Despite routine screening for gestational diabetes, treatment is still challenging because of health inequalities and different cultural representations of disease and pregnancy. This study was conducted to assess the role of early and universal GDM screening on obstetrical and neonatal complications in a socially deprived population.

Materials and Methods: A prospective study was conducted, in the level III maternity in French Guiana. Of 2136 deliveries, 223 had gestational diabetes mellitus, 110 of whom were followed-up for 6 month to detail their social and laboratory parameters.

Results: The prevalence of gestational diabetes in French Guiana (Cayenne Hospital) was estimated at 10.3%. The study population was very precarious with 70% of patients on welfare (universal health coverage or state medical assistance). The following obstetrical complications were observed: cesarean delivery (32%), history of miscarriage (26%) and preeclampsia (7.4%). Nevertheless, neonatal complications were rarely present and included hypoglycemia (2.8%) and macrosomia (2.8%).

Conclusion: In French Guiana, gestational diabetes mellitus is very common. However, in a context of widespread poverty and diverse cultural representations, universal screening and monitoring limited the risk of macrosomia.

Keywords: French Guiana, gestational diabetes mellitus, macrosomia, poverty, systematic screening procedure

INTRODUCTION

French Guiana—a French territory between Brazil and Suriname —has one of the highest birth rates in Latin America and in France [https://www.insee.fr/fr/statistiques/3309060]. There is a very high prevalence of preterm delivery and preeclampsia (1–3). In addition, the prevalence of obesity and diabetes in French Guiana are very high (respectively 17% (4) and 10% (5), notably among women). In a context of massive immigration, geographical isolation, and low medical density, social inequalities of health are widespread (6), notably for obstetrical problems (2). The Level III maternity (managing high risk pregnancies) in French Guiana is located in Cayenne Hospital. Patients from isolated areas and, especially, patients with gestational diabetes mellitus (GDM) are usually transferred to the Level III maternity unit from the 8th month of pregnancy onward for monitoring, due to the geographic distances. The risk factors most often associated with GDM include being overweight (body mass index [BMI] >25), age (>35 years), history of GDM, familial diabetes, and ethnicity (7, 8). Obstetrical and neonatal complications of GDM include macrosomia, preeclampsia, prematurity, shoulder dystocia, neonatal hypoglycemia, and cesarean delivery (8). The diagnostic and screening criteria for patients with GDM vary between different countries. In 2011, Vignoles et al. reported a GDM prevalence of 5.1% in Guadeloupe (French overseas department) using the World Health Organization (WHO) criteria (7, 9); while in Mainland France the GDM prevalence was found between 5 and 8%, but a single center study detected during a systematic screening a prevalence of 14% based on the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria (10). Some studies have found differences in GDM prevalence that are linked to ethnicity; In 2012, a study conducted in the United States by Kim et al. found variable GDM prevalence, with Pacific Islanders being at a relatively high risk (9.9%) (11).

Unlike in mainland France, since 2015 screening for GDM is routinely undertaken. This was implemented by the perinatal network –a network of obstetricians and midwives disseminating best practices in French Guiana— in order to have a standardized attitude because evaluating risk factors for gestational diabetes is challenging in the most isolated areas of the territory, and because the prevalence of obesity and diabetes are among the greatest in France, notably in women younger than 35 years (5). The selection criteria for screening for GDM in France are not adapted to French Guiana, which has many different ethnic groups, as described in the study by Cosson et al. (12)

Abbreviations: AME, Aide médicale d'état (state medical assistance); CGSS, Caisse générale de sécurité sociale (general social security fund); CMU, Couverture maladie universelle (universal health coverage); CMUc, Couverture maladie universelle complémentaire (complementary universal health coverage); DIM, Department of Medical Information; GDM, Gestational Diabetes Mellitus; HbA1c, glycated hemoglobin; IADPSG, International Association of Diabetes and Pregnancy Study Groups; INSEE, Institut National de la Statistique et des Etudes Economiques (French National Institute of Statistics and Economic Studies); WHO, World Health Organization.

Although the incidence of macrosomia and caesarian section in GDM are higher in low and middle-income countries, the literature is scarce about the specific role played by poverty and social deprivation in obstetrical and neonatal complications in gestational diabetes mellitus. Furthermore, the impact of universal screening, early treatment and monitoring of GDM is unknown in particular in the most deprived populations.

The principal objective of this cross-sectional study is to analyze the role of early and universal GDM screening on obstetrical and neonatal complications in a socially deprived population.

Furthermore, it is necessary to highlight the usefulness of systematic screening and hospitalization in the obstetrical wards for women with obstetrical complications or difficult access to care from the 8th gestational month. The geographic isolation and poverty of the population in this region makes it essential to implement a locally appropriate, graduated care policy for the treatment of GDM.

MATERIALS AND METHODS

Study Site

This single-center, prospective descriptive study took place at Cayenne Hospital over a 6-month period (December 1, 2017 through June 1, 2018) with continuous data collection. Patients were admitted to the adult diabetes outpatient, the short-stay unit, or Cayenne Hospital's maternity ward for complicated pregnancies. There are 3 public hospitals in French Guiana, one in each main coastal city and two private hospitals. The healthcare infrastructure also comprises 17 health centers and 15 maternal and child-care centers. There is only 1 Level III maternity in French Guiana located in Cayenne hospital. Because of geographic distances, patients from isolated areas –especially patients with diabetes— are usually transferred to the Level III maternity unit from the 8th month of pregnancy onward.

Inclusion and Exclusion Criteria

The inclusion criterion was a diagnosis of Gestational Diabetes Mellitus according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria (13) in female patients (age 18–46 years) hospitalized at Cayenne Hospital during the study period. The exclusion criteria were: refusal to provide written informed consent, preexisting type 1 or 2 diabetes, or failing to meet the inclusion criteria.

Definition of GDM

GDM was confirmed when fasting glycemia reached or exceeded 92 mg/dL (5.1 mmol/L). Furthermore, pregnant patients undergo an oral glucose tolerance test with a 75-g glucose load between 24 and 28 gestational weeks, and are diagnosed as GDM if at least one plasma glucose measurement reaches or exceeds the following thresholds: glycemia at t0 of >92 mg/dL (5.1 mmol/L) and/or 1-hour plasma glucose >180 mg/dL (10.0 mmol/L) and/or 2-hour plasma glucose >153 mg/dL (8.5 mmol/L) (14, 15).

Study Conduct and Judgment Criteria

The total number of deliveries during the study period was 2136. Among these, 223 patients were hospitalized and diagnosed with GDM during the study period (data collected by the Department of Medical Information [DIM]). Prevalence of GDM in Cayenne was hence obtained by dividing the number of GDM cases by the number of deliveries during the study period. Among the 223 women with GDM, a subsample of 110 women seen at the diabetology day hospital –where they had the time, assistance and if needed translation services to fill-in the social questionnaire—were enrolled for further data collection and laboratory testing (**Figure 1** and **Table 3**). Laboratory blood test were taken on the day of enrollment. When diagnosed with GDM, patients are referred to our outpatient diabetology unit, which allows them to access health education, dietitian and diabetologist consultations; they are then monitored externally by a nurse, in particular when they receive insulin, and by a midwife trained in diabetes management. They are followed (consultation) every 15 days by education nurses and are required to send their daily blood sugar self-control to the diabetology department.

Clinical and laboratory data as well as obstetrical and neonatal complications were collected after the first medical contact from electronic hospital and laboratory records. To estimate precariousness, a binary variable was created to differentiate between patients with a monthly income >1000 or <1000 euros, a threshold chosen to fit the definition of the poverty line in French Guiana. A second variable was created to differentiate between patients with monthly income >500 and <500 euros, a threshold chosen to look for extreme poverty (16). Finally, unemployed patients were differentiated from patients who were students or employed.

In addition, we differentiated patients who received state-sponsored medical assistance (Aide Médicale Etat [AME]), which is an emergency assistance for undocumented foreign patients without health insurance. Moreover, we categorized patients receiving universal health coverage (Couverture Maladie Universelle [CMU]; low-income patients (French or legal residents) receiving social insurance benefits) and other patients with normal health insurance.

Statistical Analysis

Quantitative variables were expressed as median and interquartile range. Qualitative variables were expressed as frequencies and percentages. The means were compared using Student's *t*-test. Qualitative variables were compared with the chi-square test. Variables related to the outcome on univariate analysis were subsequently analyzed in a multivariate logistic regression model. A p-value <0.05 was considered significant. All statistical analyses were conducted using SPSS® software, version 24.

RESULTS

Overall, during the 6-month study period, the medical information system showed that GDM prevalence was 10.3% (223/2136), (95% confidence interval (95%CI) = 9.17-11.8%). Among the subsample who received the social questionnaire, the mean age was 32.8 years [21-45 years], and the mother tongue was mostly a foreign language and 40% of the women were single; Most were unemployed and lived under poverty line (<1000 euros per month) and half had a monthly income <500 euros; 70% benefited from universal health insurance (CMU) or state medical aid (AME). The socioeconomic and language characteristics are described in **Table 1**.

The overall mean number of pregnancies was 4.1 per woman and the mean number of children was 2.3 (range 1-8). Patients

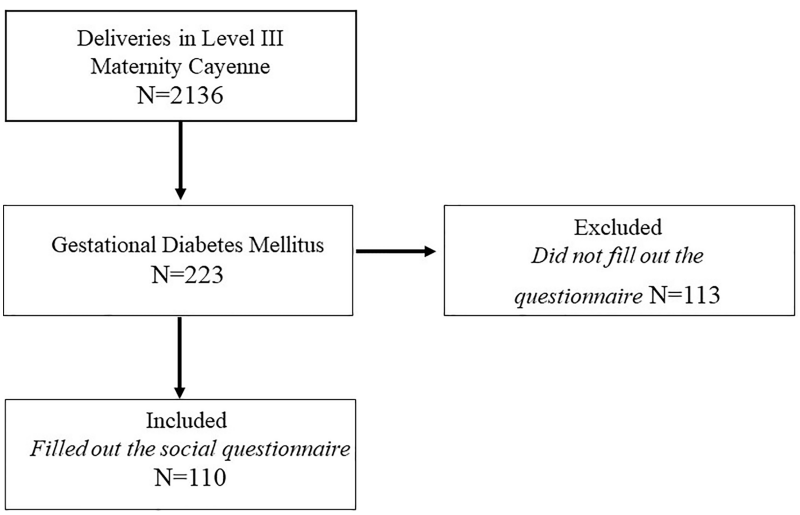

FIGURE 1 | Flow-chart Gestational Diabetes Mellitus patients included during the study period.

TABLE 1 | Medical and social criteria.

	Number *n*	Percentages (%)
Risk factors for gestational diabetes (GDM)		
Family history of diabetes	49	44.5
History of GDM	18	16.5
History of macrosomia	11	10
Age > 35 years	55	50
Body mass index > 25 kg/m² (before pregnancy)	26 *	74
Socioeconomic data		
Unemployed	76	69
CMU/CMUc/AME	75	68
3 meals per day	93	85
Income < 1000 euros per month	64	58
Cardiovascular risk factors		
History of high blood pressure	10	9
Dyslipidemia	1	1
Obstetric history		
Miscarriages	26	24
Abortion	28	25.5
Cesarean	13	12
Ectopic pregnancy	2	2
Abortion for medical reasons	2	2
Spoken languages		
French	83	75
Brazilian Portuguese	10	9
Haitian Creole	49	45
Guianese Creole	26	23.6
English	10	9
Spanish	12	11
Bushinengue	5	4.5

Macrosomia: newborn weighing more than 4kg, CMU, Couverture maladie universelle (universal health coverage).
CMUc Couverture maladie universelle complémentaire (complementary universal health coverage) AME, Aide médicale d'état (state medical assistance).
**on 35 available data.*

TABLE 2 | Obstetrical and neonatal complications.

	Number *n*	Percentages (%)
Obstetric complications	31	28.9
Cesarean	36	32.7
Macrosomia	3	2.8
Premature membrane rupture	6	5.6
Retroplacental hematoma	2	1.9
Uterine rupture	2	1.9
Preeclampsia	8	7.4
Fetal heart arrythmia	12	11.2
Prematurity	14	12.7
Neonatal complications	17	15.5
Hypoglycemia	3	2.8
Respiratory distress	13	12.3
Maternal-fetal infection	8	7.4
Patent ductus arteriosus	2	1.9
Shoulder dystocia	1	0.9

with a history of multiple abortions were found to be at significantly higher risk for obstetrical complications than those without abortions (**Table 3**). Obstetrical history is described in **Table 1**. Half of the women reported a family history of diabetes, and 18 (16.5%) said they previously had GDM. Two-thirds of patients (67%) had a history of one or more obstetrical complications. Many women had a history of obstetrical complication—most often in their country of origin (80% in Haiti).

The mean gestational duration was 38 weeks and 1 day. Fifty percent of patients were on a diet alone and 50% were on insulin (detemir or glargine).

Present obstetric complications were: cesarean section (32.7%), prematurity (12.7%), fetal heart arrhythmia (11.2%), pre-eclampsia (7.4%), premature membrane rupture (5.6%), and only 3 (2.8%) macrosomia (more than 4kg) (**Table 2**). Seventeen newborns had complications (detailed in **Table 2**), the most frequent was respiratory distress. One neonatal death, and one loss to follow-up were reported. The mean infant length, head circumference (HC), and weight were 48 cm, 34.1 cm, and 3086 g, respectively.

Nearly 77% of women had anemia (hemoglobin <11.5 g/dL; n=82), and 6 patients (5.7%) had thrombocytopenia.

The glycated hemoglobin (HbA1c) and fructosamine assays were abnormal (>5.9% and >285 µmol/L, respectively) in 20 (20.8%) and 38 (39%) patients, respectively, of the 96 patients who were tested. Eighty-four patients underwent microalbuminuria assay, 28.5% had abnormal results (>20 mg/L); of these 24 patients, 2 (8%) had a history of chronic hypertension and 2 (8%) preeclampsia.

Patients with a history of multiple abortions were found to be at significantly higher risk for obstetrical complications than those without abortions (**Table 3**). We found no significant differences with regard to the occurrence of obstetrical complications in patients with a history of GDM, familial diabetes, macrosomia, preeclampsia, or abnormal values for HbA1c, fructosamine or microalbuminuria (**Table 3**).

Important Negative Results

The cross tabulation of socioeconomic data with obstetrical complications clearly showed a higher risk of complications in French-speaking and English-speaking patients (**Table 3**). Patients speaking Haitian Creole and/or with universal health coverage, complementary universal health coverage, or state medical assistance had significantly fewer obstetrical complications (**Table 3**). There were no significant differences for age, marital status, type of coverage, monthly income, or occupation (**Table 3**).

No significant differences were found with regard to neonatal complications based on the patient's type of coverage, age, monthly income, occupation, or a history of GDM, familial diabetes, macrosomia, or preeclampsia. Abnormal HbA1c, microalbuminuria, or elevated fructosamine were not associated with a significantly higher rate of neonatal complications. Boys had significantly fewer neonatal complications (p=0.01) (**Table 3**).

DISCUSSION

We found that the prevalence in Cayenne (one of the two maternity hospitals in the area) of gestational diabetes was

TABLE 3 | Bivariate analysis of socioeconomic and medical data in relation to onset of obstetric or neonatal complications.

	Obstetric complications n (%)			Neonatal complications (%)		
	Yes	**No**	**p-value**	**Yes**	**No**	**p-value**
Socioeconomic data						
AME	3 (15)	17 (85)	0,127	3 (15)	17 (85)	0.979
CMU/CMUc/AME	21 (28.7)	52 (71)	0.945	13 (17.8)	60 (82.2)	0.206
CMU/CMUc/AME + Haitian creole	8 (17.7)	37 (82.2)	0.03*	8 (17.8)	37 (82.2)	0.464
French-speaking	29 (36.2)	51 (63.7)	0.004*	14 (17.2)	67 (82.7)	0.211
Haitian creole-speaking	9 (18)	41 (82)	0.019*	8 (16)	42 (84)	0.748
English-speaking	6 (60)	4 (40)	0.023*	0 (0)	10 (100)	0.166
Income < 1000 euros	18 (28.5)	45 (71.4)	0.939	10 (62.5)	6 (37.5)	0.915
Unemployed	19 (26)	54 (73.9)	0.325	11(68.7)	5 (31.2)	0.864
History and risk factors						
History of gestational diabetes (GD)	3 (16.6)	15 (83.3)	0.18	2 (11.1)	16 (88.9)	0.592
Family history of diabetes	16 (33.3)	32 (66.6)	0.256	7 (14.5)	41 (85.4)	1
Macrosomia	2 (18.1)	9 (81.8)	0.418	1 (9)	10 (90.9)	0.601
History of abortion	13 (46.4)	15 (53.5)	0.015*	2 (20)	8 (80)	0.979
History of preeclampsia	5 (50)	5 (50)	0.113	4 (14.2)	24 (85.7)	0.586
Lab tests						
Fructosamine (> 285 µmol/L)	4 (11.4)	31 (88.5)	0.024*	2 (5.6)	34 (94.4)	0.072
Microalbuminuria (> 20 mg>/L)	7 (29.1)	17 (70.8)	0.517	6 (25)	18 (75)	0.082
HbA1c (> 5.9%)	4 (20)	10 (80)	0.207	3 (15)	17 (85)	0.160

CMU, Couverture maladie universelle (universal health coverage).
**significant (p-value < 0.05).*

high at 10.3% of pregnancies. Despite variations between studies with different diagnostic criteria, the observed prevalence is twice the GDM prevalence in mainland France (hospital database) (10, 14, 17, 18). As in the West Indies and Reunion Islands, prevalence of metabolic diseases is double of what is observed in mainland France (4, 5, 19). Although, there are no published studies focused on GDM prevalence and characteristics in French Guiana, a general study on hospital deliveries between 2013 and 2014 observed 0.5% of preexisting diabetes, and a 4.7% of gestational diabetes (615 on 12983 births) but probably underestimated because less screened (realized before the implementation of systematic screening, in 2015) (2). There are only two maternity units where patients with gestational diabetes or type two diabetes give birth, which limits selection bias.

We showed that 81 patients (73.6%) had one or more risk factors for developing GDM. The main known risk factors were: age > 35 years (50%), family history of diabetes in a first or second-degree relative (50%), history of GDM (16%) and ethnic characteristic (black, and hispanic) (20). Several studies demonstrated the role of maternal age as a risk factor within the framework of GDM screening (7, 8, 21). The mean age in our study was indeed similar to reports from France and worldwide (13, 14). Although the risk of GDM in women older than 35 is often underestimated (21), universal screening in French Guiana –the only French territory to do so (22)—allows to avoid missing cases of GDM.

Sixty percent of women with GDM in Cayenne had a history of obstetrical complications, 10% of macrosomia, and we highlighted a frequent history of miscarriages in comparison with other French territories, much higher than the 12% reported in mainland France (23, 24). This high frequency miscarriages, especially in Western French Guiana (43%), emphasizes the challenges associated with monitoring difficult pregnancies.

Despite the frequent history of a variety of adverse obstetrical outcomes, the initial interview showed that most of these complications in fact occurred in their country of origin (most often Haiti). The literature on obstetrical and neonatal complications in Haiti is very poor, but there is a significant morbidity and mortality rate, particularly related to acute complications during pregnancy (25, 26).

Health issues in French Guiana are shaped by intense immigration and poverty, a shortage of health professionals, and logistical constraints to ensuring continuity of care, particularly in isolated areas. Most women included in the study came from a foreign country.

Of the 110 patients having answered the social questionnaire, nearly 1/3 had obstetrical complications. The rate of cesarean delivery was higher for pregnancies complicated by GDM (36%) than for normal pregnancies (27) or among women with GDM in mainland France (27.8%) (8). Contrarily to what has been reported elsewhere, there was no difference in cesarean section rate between precarious and non-precarious women (28).

All complications cannot be attributed to GDM, in particular pre-eclampsia, because the causes are often multifactorial, and several metabolic problems and hypertension are intertwined before pregnancy (2).

Half of patients in our study were receiving insulin. The high prevalence of prepartum obesity in French Guiana may partially explain the greater proportion of GDM patients treated with insulin relative to mainland France. This frequent insulin prescription is consistent with reports that the proportion of patients treated with insulin increased with the patient's BMI (18). Moreover, insulin may be the only solution in difficult social contexts where dietary modifications –the first treatment of choice for GDM—is financially impossible (anti diabetic drugs as being completely covered by the health insurance).

GDM increases obstetrical and neonatal complications and early screening and management is necessary to prevent them (29–31). The biological markers for determining GDM fructosamine and, more rarely, HbA1c were elevated in 39% and 11% of patients, respectively. No significant difference was shown for HbA1c in relation to the risk of onset of obstetrical and/or neonatal complications. The interest of the HbA1c assay during pregnancy is controversial but most agree that its interest is limited compared to screening according to the IASPDG criteria (32); indeed, it represents the average blood sugar level from the previous 2 or 3 months, and it increases during iron deficiency with or without anaemia (33). The cut off corresponding to the level of HbA1c which must be considered as predictor of adverse obstetric outcomes during pregnancy has not been established, and varies between 5.55 (34) and 5.9% (35). Moreover, fructosamine is not a reliable biological marker in screening for GDM because it lacks sensitivity (36, 37). Very few studies have evaluated its connection with neonatal outcomes, and the results are inconsistent (17, 38). Nevertheless, recent studies showed that fructosamine is a better predictor of birthweight and large-for-gestational age infants than HbA1c (17).

Several studies showed that microalbuminuria at the end of the second trimester of a nondiabetic pregnancy could increase the risks of preterm labor, preeclampsia, intrauterine growth restriction, and premature membrane rupture (39–41). In our study however microalbuminuria was not associated with higher risk of neonatal or obstetrical complication.

Fifteen and a half-percent of newborns from mothers with GDM had one or more complications. The commonest complications were respiratory distress and feto-maternal infections; Hypoglycemia, macrosomia and shoulder dystocia were very rare. GDM treatment decreases the risk of macrosomia, large for gestational age births, and shoulder dystocia but not neonatal hypoglycemia, preterm births, pre-eclampsia, or caesarean section (27). Macrosomia has been reported in 15% of newborns from a cohort of GDM mothers in France, with a 3.6% rate of respiratory distress at birth (14). In contrast, in French Guiana, the prevalence of macrosomia (2.6%), corrected for age and sex, was low, despite frequent social precariousness (42). In Cuba, Cruz et al. found macrosomia rates of 10% to 20% (7). This difference between our study and the prevalence of macrosomia in neighboring countries or in South America, where precariousness is also frequent, is probably linked with the routine hospital admission of high-risk pregnancies in French Guiana during the last 2 months and/or weekly follow-up in multidisciplinary consultations for patients with easy mobility.

Many of our patients had previously given birth in a country with limited healthcare resources and difficulties in access to care, and screening was late. In French Guiana, these women benefitted from stringent monitoring because they are specifically at high risk of complications. In Caribbean and Latin American countries, complications are higher than in French Guiana despite comparable precariousness and gestational diabetes (43). In 2015, a French study carried out on a precarious population, revealed a more precocious onset of gestational death, more shoulder dystocia and more large for gestational age infants (44). However, women from Surinam or Guyana –but not Haiti—had more obstetrical complications than other patients; Perhaps this is due to language difficulties, cultural representations and social obstacles to regular care and monitoring.

Although the prevalence estimation had a narrow confidence interval, our descriptive study's weakness was linked to its small sample size and lack of statistical power. Moreover, there was no postpartum follow-up, which is an essential part of metabolic screening. Patients were contacted again after delivery, but postpartum data collection was difficult because, most patients had not had their fasting glucose levels checked or could not be reached, which is another characteristic of vulnerable populations in French Guiana.

CONCLUSION

Gestational diabetes is very common in French Guiana, where screening is universal. The population in our study was poor and culturally diverse. Cesarean delivery and preeclampsia were the main obstetrical complications identified, while the main neonatal complications were prematurity and respiratory distress. The rate of macrosomia, however, was very low in our population. GDM screening and intensive follow-up of psychosocial deprivation pregnancies in French Guiana is a major advantage, and clearly makes it possible to limit common GDM complications.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the General Data Protection Regulations (GDPR) and in compliance with both French and European regulations (EU 2016/679), and reported to the National Institute of Health Data (INDS) under registry number MR3014270220. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LL, HK, MN, and NS participated in study protocol development, data collection, and data analysis and co-authored

the manuscript. ML-M participated in data collection writing, and analysis. LT, MD, and RM participated in data collection. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Article - Bulletin Épidémiologique Hebdomadaire. Available at: http://beh.santepubliquefrance.fr/beh/2020/2-3/2020_2-3_3.html (Accessed April 8, 2020).
2. Leneuve-Dorilas M, Favre A, Carles G, Louis A, Nacher M. Risk Factors for Premature Birth in French Guiana: The Importance of Reducing Health Inequalities. *J Matern-Fetal Neonatal Med* (2019) 32(8):1388–96. doi: 10.1080/14767058.2017.1403578
3. Mhiri R, Mvogo A, Kamga A, Yassinguezo S, Fagla H, Dotou D, et al. Epidemiology and Maternal Prognosis of Hypertension Disorders of Pregnancy in French Guiana. *Pregnancy Hypertens* (2020) 20:96–101. doi: 10.1016/j.preghy.2020.03.010
4. Daigre J-L, Atallah A, Boissin J-L, Jean-Baptiste G, Kangambega P, Chevalier H, et al. The Prevalence of Overweight and Obesity, and Distribution of Waist Circumference, in Adults and Children in the French Overseas Territories: The PODIUM Survey. *Diabetes Metab* (2012) 38(5):404–11. doi: 10.1016/j.diabet.2012.03.008
5. Sabbah N, Massicard M, Mathieu N. Specificities of the Diabetic Population in French Guiana: The Health Barometer Survey. *Curr Diabetes Rev* (2021) 1:1 doi: 10.2174/1573399817666210129103506
6. Van Melle A, Cropet C, Parriault M-C, Adriouch L, Lamaison H, Sasson F, et al. Renouncing Care in French Guiana: The National Health Barometer Survey. *BMC Health Serv Res* (2019) 19(1):99. doi: 10.1186/s12913-019-3895-6
7. Cruz-Hernández J, Hernández-García P, Lang-Prieto J, Yanes-Quesada M, Iglesias-Marichal I, Márquez-Guillén A. Controversies in Screening and Diagnosis of Gestational Diabetes: Cuba's Position. *MEDICC Rev* (2016) 18(3):35–9. doi: 10.37757/MR2016.V18.N3.9
8. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational Diabetes Mellitus. *Nat Rev Dis Primer* (2019) 5(1):47. doi: 10.1038/s41572-019-0098-8
9. Vignoles P, Gire C, Mancini J, Bretelle F, Boubli L, Janky E, et al. Gestational Diabetes: A Strong Independent Risk Factor for Severe Neonatal Respiratory Failure After 34 Weeks. *Arch Gynecol Obstet* (2011) 284(5):1099–104. doi: 10.1007/s00404-010-1810-9
10. Senat M-V, Deruelle P. Gestational Diabetes Mellitus. *Gynecol Obstet Fertil* (2016) 44(4):244–7. doi: 10.1016/j.gyobfe.2016.01.009
11. Kim SY, Sappenfield W, Sharma AJ, Wilson HG, Bish CL, Salihu HM, et al. Racial/Ethnic Differences in the Prevalence of Gestational Diabetes Mellitus and Maternal Overweight and Obesity, by Nativity, Florida, 2004-2007. *Obes Silver Spring Md* (2013) 21(1):E33–40. doi: 10.1002/oby.20025
12. Cosson E, Cussac-Pillegand C, Benbara A, Pharisien I, Jaber Y, Banu I, et al. The Diagnostic and Prognostic Performance of a Selective Screening Strategy for Gestational Diabetes Mellitus According to Ethnicity in Europe. *J Clin Endocrinol Metab* (2014) 99(3):996–1005. doi: 10.1210/jc.2013-3383
13. International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy. *Diabetes Care* (2010) 33(3):676–82. doi: 10.2337/dc09-1848
14. Billionnet C, Mitanchez D, Weill A, Nizard J, Alla F, Hartemann A, et al. Gestational Diabetes and Adverse Perinatal Outcomes From 716,152 Births in France in 2012. *Diabetologia* (2017) 60(4):636–44. doi: 10.1007/s00125-017-4206-6
15. Ducarme G, Desroys Du Roure F, Le Thuaut A, Grange J, Dimet J, Crepin-Delcourt I. Efficacy of Maternal and Biological Parameters At the Time of Diagnosis of Gestational Diabetes Mellitus in Predicting Neonatal Morbidity. *Eur J Obstet Gynecol Reprod Biol* (2018) 221:113–8. doi: 10.1016/j.ejogrb.2017.12.036
16. Available at: https://www.insee.fr/fr/statistiques/4623886.
17. Mendes N, Alves M, Andrade R, Ribeiro RT, Papoila AL, Serrano F. Association Between Glycated Albumin, Fructosamine, and HbA1c With Neonatal Outcomes in a Prospective Cohort of Women With Gestational Diabetes Mellitus. *Int J Gynaecol Obstet* (2019) 146(3):326–32. doi: 10.1002/ijgo.12897
18. Huet J, Beucher G, Rod A, Morello R, Dreyfus M. Joint Impact of Gestational Diabetes and Obesity on Perinatal Outcomes. *J Gynecol Obstet Hum Reprod* (2018) 47(9):469–76. doi: 10.1016/j.jogoh.2018.08.003
19. Rouget F, Lebreton J, Kadhel P, Monfort C, Bodeau-Livinec F, Janky E, et al. Medical and Sociodemographic Risk Factors for Preterm Birth in a French Caribbean Population of African Descent. *Matern Child Health J* (2013) 17(6):1103–11. doi: 10.1007/s10995-012-1112-x
20. Dooley SL, Metzger BE, Cho N, Liu K. The Influence of Demographic and Phenotypic Heterogeneity on the Prevalence of Gestational Diabetes Mellitus. *Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet* (1991) 35(1):13–8. doi: 10.1016/0020-7292(91)90057-c
21. Kuo C-H, Chen S-C, Fang C-T, Nien F-J, Wu E-T, Lin S-Y, et al. Screening Gestational Diabetes Mellitus: The Role of Maternal Age. *PLoS One* (2017) 12(3):e0173049. doi: 10.1371/journal.pone.0173049
22. Legardeur H, Girard G, Mandelbrot L. [Screening of Gestational Diabetes Mellitus: A New Consensus]? *Gynecol Obstet Fertil* (2011) 39(3):174–9. doi: 10.1016/j.gyobfe.2010.12.009
23. Delabaere A, Huchon C, Deffieux X, et al. Available at: https://www.em-consulte.com/en/article/942050.
24. Beucher G. Available at: https://www.em-consulte.com/en/article/250684.
25. Gomes DJ, Kaufman B, Aluisio AR, Kendall S, Thomas V, Bloem C. Assessment of Acute Obstetrical Needs and the Potential Utility of Point-Of-Care Ultrasound in the North East Region of Haiti: A Cross-Sectional Study. *Ann Glob Health* (2020) 86(1):72. doi: 10.5334/aogh.2597
26. Valcin J, Jean-Charles S, Malfa A, Tucker R, Dorcélus L, Gautier J, et al. Mortality, Morbidity and Clinical Care in a Referral Neonatal Intensive Care Unit in Haiti. *PLoS One* (2020) 15(10):e0240465. doi: 10.1371/journal.pone.0240465
27. Poolsup N, Suksomboon N, Amin M. Effect of Treatment of Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis. *PLoS One* (2014) 9(3):e92485. doi: 10.1371/journal.pone.0092485
28. Wang Z, Kanguru L, Hussein J, Fitzmaurice A, Ritchie K. Incidence of Adverse Outcomes Associated With Gestational Diabetes Mellitus in Low- and Middle-Income Countries. *Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet* (2013) 121(1):14–9. doi: 10.1016/j.ijgo.2012.10.032
29. Basri NI, Mahdy ZA, Ahmad S, Karim AKA, Shan LP, Manaf MRA, et al. The World Health Organization (WHO) Versus The International Association of Diabetes and Pregnancy Study Group (IADPSG) Diagnostic Criteria of Gestational Diabetes Mellitus (GDM) and Their Associated Maternal and Neonatal Outcomes. *Horm Mol Biol Clin Investig* (2018) 34:1–9. doi: 10.1515/hmbci-2017-0077
30. Buchanan TA, Xiang AH, Page KA. Gestational Diabetes Mellitus: Risks and Management During and After Pregnancy. *Nat Rev Endocrinol* (2012) 8(11):639–49. doi: 10.1038/nrendo.2012.96
31. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of Treatment of Gestational Diabetes Mellitus on Pregnancy Outcomes. *N Engl J Med* (2005) 352(24):2477–86. doi: 10.1056/NEJMoa042973
32. Siricharoenthai P, Phupong V. Diagnostic Accuracy of HbA1c in Detecting Gestational Diabetes Mellitus. *J Matern Fetal Neonatal Med* (2020) 33(20):3497–500. doi: 10.1080/14767058.2019.1576169
33. Mendes N, Tavares Ribeiro R, Serrano F. Beyond Self-Monitored Plasma Glucose and HbA1c: The Role of Non-Traditional Glycaemic Markers in Gestational Diabetes Mellitus. *J Obstet Gynaecol J Inst Obstet Gynaecol* (2018) 38(6):762–9. doi: 10.1080/01443615.2017.1412409
34. Kwon SS, Kwon J-Y, Park Y-W, Kim Y-H, Lim J-B. HbA1c for Diagnosis and Prognosis of Gestational Diabetes Mellitus. *Diabetes Res Clin Pract* (2015) 110(1):38–43. doi: 10.1016/j.diabres.2015.07.014

35. Mañé L, Flores-Le Roux JA, Benaiges D, Rodríguez M, Marcelo I, Chillarón JJ, et al. Role of First-Trimester HbA1c as a Predictor of Adverse Obstetric Outcomes in a Multiethnic Cohort. *J Clin Endocrinol Metab* (2017) 102 (2):390–7. doi: 10.1210/jc.2016-2581
36. Agarwal MM, Dhatt GS, Othman Y, Ljubisavljevic MR. Gestational Diabetes: An Evaluation of Serum Fructosamine as a Screening Test in a High-Risk Population. *Gynecol Obstet Invest* (2011) 71(3):207–12. doi: 10.1159/000318705
37. Nasrat HA, Ajabnoor MA, Ardawi MS. Fructosamine as a Screening-Test for Gestational Diabetes Mellitus: A Reappraisal. *Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet* (1991) 34(1):27–33. doi: 10.1016/0020-7292(91)90534-c
38. Gingras V, Rifas-Shiman SL, Switkowski KM, Oken E, Hivert M-F. Mid-Pregnancy Fructosamine Measurement-Predictive Value for Gestational Diabetes and Association With Postpartum Glycemic Indices. *Nutrients* (2018) 10:1–11. doi: 10.3390/nu10122003
39. Bomback AS, Rekhtman Y, Whaley-Connell AT, Kshirsagar AV, Sowers JR, Chen S-C, et al. Gestational Diabetes Mellitus Alone in the Absence of Subsequent Diabetes Is Associated With Microalbuminuria: Results From the Kidney Early Evaluation Program (Keep). *Diabetes Care* (2010) 33 (12):2586–91. doi: 10.2337/dc10-1095
40. Zen M, Padmanabhan S, Cheung NW, Kirby A, Jesudason S, Alahakoon TI, et al. Microalbuminuria as an Early Predictor of Preeclampsia in the Pre-Gestational Diabetic Population: A Prospective Cohort Study. *Pregnancy Hypertens* (2019) 15:182–8. doi: 10.1016/j.preghy.2019.01.010
41. Bahasadri S, Kashanian M, Khosravi Z. Comparison of Pregnancy Outcome Among Nulliparas With and Without Microalbuminuria At the End of the Second Trimester. *Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet* (2011) 115(1):34–6. doi: 10.1016/j.ijgo.2011.04.012
42. Kramer MS, Demissie K, Yang H, Platt RW, Sauvé R, Liston R. The Contribution of Mild and Moderate Preterm Birth to Infant Mortality. Fetal and Infant Health Study Group of the Canadian Perinatal Surveillance System. *JAMA* (2000) 284(7):843–9. doi: 10.1001/jama.284.7.843
43. Zar HJ, Pellowski JA, Cohen S, Barnett W, Vanker A, Koen N, et al. Maternal Health and Birth Outcomes in a South African Birth Cohort Study. *PLoS One* (2019) 14(11):e0222399. doi: 10.1371/journal.pone.0222399
44. Cosson E, Bihan H, Reach G, Vittaz L, Carbillon L, Valensi P. Psychosocial Deprivation in Women With Gestational Diabetes Mellitus is Associated With Poor Fetomaternal Prognoses: An Observational Study. *BMJ Open* (2015) 5(3):e007120. doi: 10.1136/bmjopen-2014-007120

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CASE REPORT
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Case Report: Diabetes in Chinese Bloom Syndrome

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Bloom syndrome (BS) is a rare autosomal recessive disorder that causes several endocrine abnormalities. So far, only one BS pedigree, without diabetes, has been reported in the Chinese population. We presented the first case of BS with diabetes in the Chinese population and explored the clinical spectrum associated with endocrine. Possible molecular mechanisms were also investigated. Our study indicated that BS may be one rare cause of diabetes in the Chinese population. We also found a new pathogenic sequence variant in *BLM* (BLM RecQ like helicase gene)(NM_000057.4) c.692T>G, which may expand the spectrum of *BLM* variants.

Keywords: Bloom syndrome, diabetes, short stature, azoospermia, leptin

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INTRODUCTION

Bloom syndrome (BS) was first reported by Bloom, a dermatologist, in 1954, and there are currently no more than 300 cases of BS reported worldwide. BS is a rare cause of diabetes, and no cases of diabetes in the Chinese population have been reported. BS can lead to clinical phenotypes including many other endocrine abnormalities, which required attention from endocrinologists. This study aimed to improve the understanding of BS from the perspective of endocrinologists.

CASE REPORT

The patient was a 19-year-old male. He was born in full-term, with a weight of 1.8 kilograms (kg) and a height of 40 centimeters (cm). Apart from growth failure, no developmental delay was presented. He reached his final height of 143 cm at his 14s and his maximum weight was 42 kg at his 15s. The patient repeatedly experienced infection of the upper respiratory tract during his childhood, and he was diagnosed with "acute type A hepatitis" at his 1.5 years. He was also diagnosed with "cytomegalovirus pneumonia" at 8 years old. The patient showed facial erythema since he was 2 years old. One year ago, the patient developed well-demarcated patchy areas of hypopigmentation and hyperpigmentation. In 2013, when he was 13 years old, the patient started to experience polydipsia, polyphagia, and polyuria. His fasting blood glucose (FBG) was revealed to be 19 mmol/L, and the urinary ketone body was positive. C-peptide level and type 1 diabetes-related auto-antibodies were unknown. Though with 14 units insulin aspart for each meal and 21 units glargine insulin a day, his blood glucose was not controlled well (FBG 8-14 mmol/L, and 2 hours postprandial glucose 10-18 mmol/L). Four years after diabetes onset, his fasting C-peptide (FCP) was 1.70 ng/mL and 2 hours postprandial C peptide (2hCP) was 3.19 ng/mL, with simultaneous

FBG 13 mmol/L and 2 hours postprandial glucose (2hPG) 27 mmol/L. Glycated hemoglobin (HbA1c) was 10%. In January 2019, a total of 36 U insulin aspart and insulin glargine 38 U per day still failed to control his glucose. In June 2019, the patient went to our department.

The proband's parents denied consanguinity. His grandmother was diagnosed with type 2 diabetes (T2D) in her 60s. His father was also diagnosed with T2D when he was 43 years old, with a body mass index (BMI) of 33.1 kg/m^2. Physical examination revealed a height of 143 cm and a weight of 37 kg (BMI 18.09 kg/m^2), his waist circumference was only 60 cm. Symmetrical erythema can be seen on his face (**Figure 1**), areas of hypopigmentation and hyperpigmentation scattered. Tanner stage was V, with a length of penis 6 cm and the volume of bilateral testes was 8 ml, respectively. No other abnormalities discovered. BS was suspected and further clinical investigation was implemented.

His glycated albumin (GA%) was 22.4% and HbA1c was 10.2%, which suggested poor glycemic control with a high dose of insulin (2 U/kg·d, 74 units per day). FCP was 0.43 ng/mL, and 2hCP was 1.91 ng/mL, while synchronous FBG was 9.3 mmol/L and 2hPG was 15.7 mmol/L, indicating islet dysfunction. Hyperlipidemia did not exist: total cholesterol (TC) 2.85 mmol/L, triglyceride (TG) 1.06 mmol/L, high density lipoprotein cholesterol (HDL-C) 1.06 mmol/L, low density lipoprotein cholesterol (LDL-C) 1.41 mmol/L. However, an abdominal ultrasound indicated fatty liver. The level of leptin was only 0.5 ng/mL. Quantitative MRI of fat did not suggest a reduction or abnormal distribution of fat (**Figure 2**). Metformin 0.5g tid and pioglitazone 30mg qd were added, while the total amount of insulin reduced from 74 units to 64 units per day. His FBG was 5-8 mmol/L and 2hPG was 8-12 mmol/L. Though the patient seemed normal in puberty development, his level of follicle-stimulating hormone (FSH) was as high as 19.36 IU/L, with normal luteinizing hormone (LH) of 7.17 IU/L and testosterone (T) of 4.64 ng/ml. No sperms were detected in his semen. No abnormality of hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-thyroid were revealed. Growth hormone (GH) 0.3 ng/ml and insulin-like growth factor 1 (IGF1) 36 ng/ml were normal. Blood parathyroid hormone, calcium and phosphorus were all within the normal range.

This study was approved by the Ethics Committee of PUMCH (Approval number is JS-1233) and informed consent was obtained from the proband and his parents. The genomic DNA of the proband and his parents were extracted from peripheral blood using the DNA extraction kit of Beijing Tiangen Biochemical Technology Co., Ltd. The PCR product was identified by 1.5% agarose gel electrophoresis, purified and sequenced using ABI 3730 automatic sequencer (Applied Biosystems, USA). The results were analyzed by DNAStar6.0 software (Lasergene, USA) subroutine SeqMan. The standard sequence was based on the NCBI database candidate gene reference sequence.

The proband had heterozygous *BLM* (BLM RecQ like helicase gene) variants (NM_000057.4), c.692T>G (p.Leu231*) and c.1544delA (p.Asn515Metfs*16) heterozygous variants, inherited from their parents, respectively (**Figures 3** and **4**). *BLM* c.1544delA (p.Asn515Metfs*16) has been reported to be pathogenic (1). *BLM* c.692T>G (p.Leu231*), which results in a truncated protein, was neither found in ExAC nor 1000G. The sequence variant was assigned to be pathogenic (PVS1+PP3+PP4) according to the guidelines of the American Society of Medical Genetics and Genomics (ACMG).

DISCUSSION

BS (OMIM 210900) is an autosomal recessive hereditary disease caused by a sequence variant in the *BLM* on chromosome 15q26.1, which encodes RecQ helicase (RECQL3). RecQ helicase is an important DNA helicase during DNA replication and repairmen, maintaining DNA stability when DNA double strands are unfolded. BS is rare, with no more than 300 cases reported globally and about one-third of the patients are German Jews (1). At present, only one family of BS without diabetes is reported in China (2). Carbohydrate and lipid metabolism, as well as growth and development disorder, may be involved in BS. However, a detailed description of endocrine system involvement from an endocrinologist perspective is scarce.

BS is a rare cause of diabetes. According to the BS registration system (https://www.ncbi.nlm.nih.gov/books/NBK1398/), 16% of patients with BS have T2D. However, different from typical T2D, patients with BS developed diabetes at a younger age and have a lower BMI. The pathogenesis of diabetes in patients with BS is unclear. Diaz et al. suggested that insulin resistance plays an

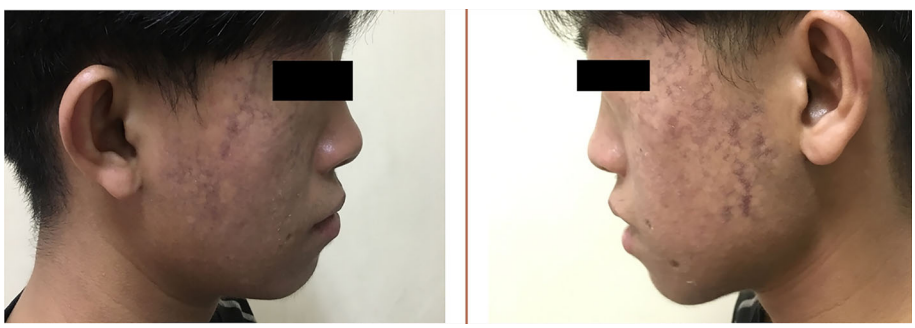

FIGURE 1 | Symmetrical erythema on the patient's face.

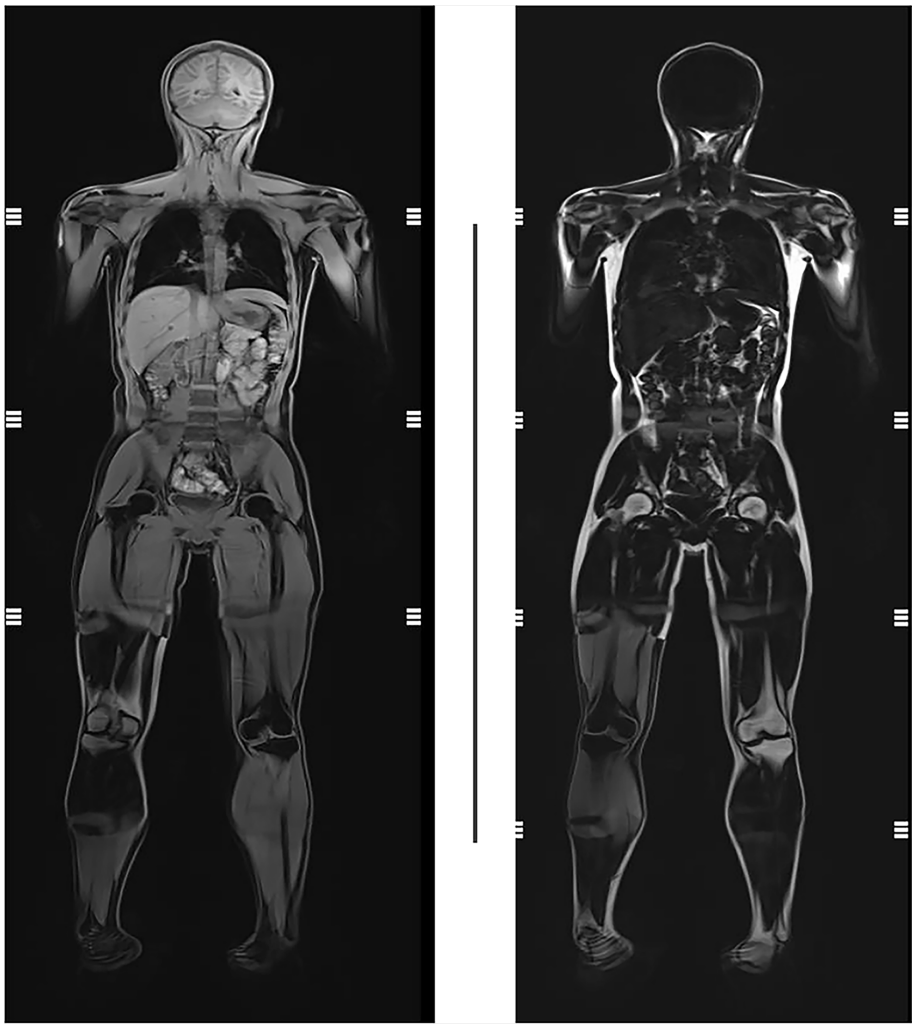

FIGURE 2 | Quantitative MRI of fat of the proband. No reduction or abnormal distribution of fat was presented.

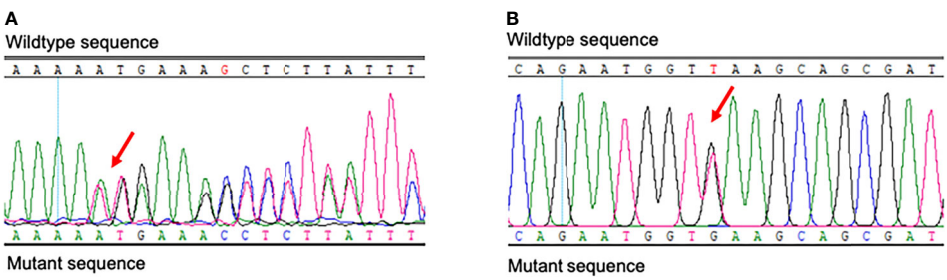

FIGURE 3 | Results of Sanger sequencing of *BLM* of the proband. The red arrows indicate the c.1544delA (p.Asn515Metfs*16) **(A)** and c.692T>G (p.Leu231*) **(B)** heterozygous variants.

important role in the glucose metabolism of BS (3). In our case, a high dose of insulin failed to control blood glucose, which indicated that the patient had significant insulin resistance. However, it is worth noting that, similar to our patient, none of the BS patients had acanthosis nigricans. As we have known, insulin resistance is related to abnormal secretion of adipokines (1). His leptin level was 0.5 ng/mL, which suggested that abnormal adipokines secretion might be involved in the pathogenesis of his diabetes.

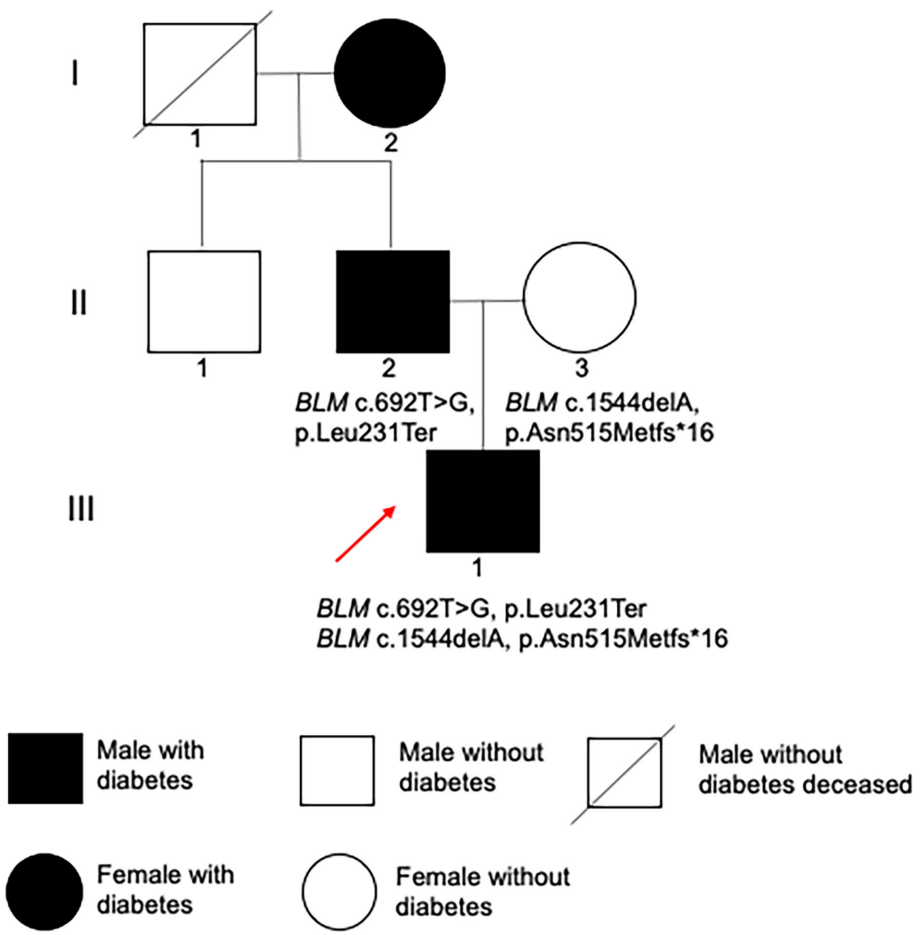

FIGURE 4 | Family pedigree. Filled black symbols represent individuals with diabetes. The red arrow indicates the proband.

However, unlike diabetes resulted from lipoatrophy, no significant dyslipidemia was presented in our patient, and quantification and distribution of fat were still within the normal range. It is also worth noting that our patient occurred ketosis at the onset of diabetes, and his islet function was poorer compared with classical T2D, suggesting that islet failure may also be involved in patients with BS. Our finding supports the conclusion from Kondo et al. that insulin-dependent diabetes can also be developed in early adulthood in BS (4). In a word, insulin resistance and islet β-cell dysfunction may both be involved in the development of diabetes in patients with BS. By combining metformin and pioglitazone based on insulin, our patient's blood glucose was well controlled. Moreover, the risk of cancers in BS is high, resulting in their short lifetime. As metformin is beneficial for reducing hyperglycemia-induced genome instability (5), which might make it count in improving clinical outcomes of BS patients. In addition to diabetes, patients with BS can also have other endocrine abnormalities. BS patients often come to an endocrinologist because of their short stature. It is extremely important for an endocrinologist to recognize BS and then avoid GH treatment. As for patients with hypogonadism or azoospermia, BS should be included in the differential diagnosis.

BLM is the only known gene that causes BS. For now, less than 80 pathogenic sequence variants have been identified (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=BLM). The homozygote at the 2281 position of the 6 bp deletion and the 7 bp insertion is referred to as blm^{Ash}. The presence of blm^{Ash} in over 95% of the BS chromosomes is due to the co-founder's ancestry inheritance. The *BLM* sequence variants so far identified have also suggested additional founder sequence variants. Among 64 different sequence variants that were reported by German et al, 19 were recurrent (6). No recurrent sequence variant has been found in Chinese BS. In the only reported Chinese BS pedigree, the proband carried a reported heterozygous sequence variant c.772_773delCT and a new sequence variant c.959 +2T>A (2). In our proband, *BLM* c.1544delA has been reported, while *BLM* c.692T>G is a new sequence variant. Our research expanded the BS genetic spectrum. However, functional tests *in vivo* and *vitro* are necessary to further establish pathogenicity of the new variant, and sequencing of genomic DNA in more family members may help to deepen our understanding of *BLM* variants.

In conclusion, we present the first case of BS with diabetes in the Chinese population, indicating BS may be one rare cause of diabetes in the Chinese population. In this study, the sequence variant of *BLM* c.1544delA has been reported, while *BLM* c.692T>G is a new sequence variant, which may expand the spectrum of *BLM* variants.

ETHICS STATEMENT

This study was approved by the Ethics Committee of Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All the authors have contributed significantly. MD collected the clinical data, wrote the manuscript. RJ summarized the relevant literature. KF and XX give suggestions about clinical investigations. All the work was done under the instructions of MY. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Cunniff C, Bassetti JA, Ellis NA. Bloom's Syndrome: Clinical Spectrum, Molecular Pathogenesis, and Cancer Predisposition. *Mol Syndromology* (2017) 8:4–23. doi: 10.1159/000452082
2. Wu ML, Wang XM, Li J, Ding Y, Chen Y, Chang GY, et al. Clinical and Molecular Analysis of Two Chinese Siblings With Bloom Syndrome. *Chin J Pediatr* (2018) 56:373–6. doi: 10.3760/cma.j.issn.0578-1310.2018.05.013
3. Diaz A, Vogiatzi MG, Sanz MM, German J. Evaluation of Short Stature, Carbohydrate Metabolism and Other Endocrinopathies in Bloom's Syndrome. *Hormone Res* (2006) 66:111–7. doi: 10.1159/000093826
4. Kondo N, Asano J, Kimura S, Asano T, Orii T. Insulin-Dependent Diabetes Developed in a Young Man With Bloom's Syndrome. *Clin Genet* (1991) 40:251–2. doi: 10.1111/j.1399-0004.1991.tb03088.x
5. Attia SM, Helal GK, Alhaider AA. Assessment of Genomic Instability in Normal and Diabetic Rats Treated With Metformin. *Chem Biol Interact* (2009) 180:296–304. doi: 10.1016/j.cbi.2009.03.001
6. German J, Sanz MM, Ciocci S, Ye TZ, Ellis NA. Syndrome-Causing Mutations of the BLM Gene in Persons in the Bloom's Syndrome Registry. *Hum Mutat* (2007) 28:743–53. doi: 10.1002/humu.20501

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ORIGINAL RESEARCH
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Peripheral Nerve Conduction And Sympathetic Skin Response Are Reliable Methods to Detect Diabetic Cardiac Autonomic Neuropathy

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Aim: This study aimed to investigate the role of nerve conduction studies (NCS) and sympathetic skin response (SSR) in evaluating diabetic cardiac autonomic neuropathy (DCAN).

Methods: DCAN was diagnosed using the Ewing test combined with heart rate variability analysis. NCS and SSR were assessed by electrophysiological methods. The association between NCS/SSR and DCAN was assessed *via* multivariate regression and receiver-operating characteristic analyses.

Results: The amplitude and conduction velocity of the motor/sensory nerve were found to be significantly lower in the DCAN+ group (all $P < 0.05$). A lower amplitude of peroneal nerve motor fiber was found to be associated with increased odds for DCAN (OR 2.77, $P < 0.05$). The SSR amplitude was lower while the SSR latency was longer in the DCAN+ group than in the DCAN– group. The receiver-operating characteristic analysis revealed that the optimal cutoff points of upper/lower limb amplitude of SSR to indicate DCAN were 1.40 mV (sensitivity, 61.9%; specificity, 66.3%, $P < 0.001$) and 0.85 mV (sensitivity, 66.7%; specificity, 68.5%, $P < 0.001$), respectively. The optimal cutoff points of upper/lower limb latency to indicate DCAN were 1.40 s (sensitivity, 61.9%; specificity, 62%, $P < 0.05$) and 1.81 s (sensitivity, 69.0%; specificity, 52.2%, $P < 0.05$), respectively.

Conclusions: NCS and SSR are reliable methods to detect DCAN. Abnormality in the peroneal nerve (motor nerve) is crucial in predicting DCAN. SSR may help predict DCAN.

Keywords: diabetic cardiac autonomic neuropathy, Ewing test, heart rate variability, nerve conduction, sympathetic skin response, T2DM

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INTRODUCTION

Diabetic cardiac autonomic neuropathy (DCAN) is a serious long-term complication of diabetes mellitus (DM). It results from chronic damage to autonomic nerve fibers that innervate the heart and blood vessels, in turn causing abnormalities in heart rate and vascular dynamics. It significantly increases the morbidity and mortality of patients with diabetes (1, 2). Early diagnosis of DCAN may

reduce the risk of painless myocardial ischemia, myocardial infarction, and sudden cardiac death associated with DCAN (3). Therefore, the American Diabetes Association (ADA) recommends that the clinical screening for DCAN should be routinely performed in patients with diabetes (4).

Screening for DCAN is important because it is a frequently overlooked complication of diabetes, especially in the early stage. The Ewing test and heart rate variability (HRV) analysis are recommended by the ADA as the diagnostic tests for DCAN (5). The Ewing test, which assesses autonomic reflexes by measuring heart rate and blood pressure during activities such as the Valsalva, deep breathing, or standing, is a classic clinical test for cardiac autonomic neuropathy. However, it is a semi-quantitative method and relies on patients' cooperation, and its interpretation is operator dependent. HRV analysis provides indirect insight into autonomic nervous system tone and plays a well-established role as a marker of cardiovascular risk (6).

HRV is composed of time-domain and frequency-domain components, but it does not estimate cardiac reflexes (7, 8). Some prior studies have demonstrated that the diagnostic specificity can be increased by combining the results of the Ewing test and HRV (5, 9), which was the approach used in this study.

Objective examinations that can identify DCAN during its early stage are critical. Considering that Ewing test and HRV analysis tests are time-consuming and require a high degree of cooperation from patients, it is difficult to screen for every diabetic patient in clinical work. Therefore, it is important to find a more convenient method to screen DCAN. Nerve conduction studies (NCS) and the sympathetic skin response (SSR) are routinely employed in the diagnosis of diabetic peripheral neuropathy (10, 11), which may be useful in identifying patients at high risk for DCAN. The present study was designed to investigate the role of NCS and SSR in the evaluation of DCAN in type 2 diabetes mellitus (T2DM).

MATERIALS AND METHODS

Study Design

This cross-sectional, open-label, controlled clinical study was performed to explore the role of nerve conduction studies (NCS) and sympathetic skin response (SSR) in evaluating diabetic cardiac autonomic neuropathy (DCAN). Participants were recruited from the inpatients at the Department of Endocrinology of Nanfang Hospital. This study was reviewed and received ethical approval from the Ethics Committee of Nanfang Hospital, Southern Medical University. Written consent was obtained from all study participants at the time of enrollment. The Chinese Clinical Trials Registration Number is ChiCTR1900020491.

The inclusion criteria were as follows: age more than 18 years and current diagnosis of T2DM.

The exclusion criteria were as follows: inability to undertake the examination, neurological or autonomic disorders caused by other diseases (Guillain–Barre syndrome, Shy–Drager syndrome, and so forth), history of coronary artery disease (such as myocardial infarction or angina), arrhythmia, severe heart failure, malignant tumor, limb trauma, chronic rheumatic disease, thyroid function abnormalities, severe psychiatric disorders that interfered with the patient's ability to complete study procedures, severe hepatic or renal dysfunction [GFR < 30 mL/(min · 1.73 m^2)], alcohol abuse (women 14 units per week and men 21 units per week in the last year), intake of neurotoxic medications or beta-blockers, vitamin B12 deficiency, and pregnancy.

The participants' clinical data, including sex, age, duration of diabetes, height, body weight, body mass index (BMI), and smoking history, were recorded. Laboratory measurements, including triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), fasting plasma glucose (FPG), postprandial blood glucose (PBG), glycosylated hemoglobin (HbA1c), fasting C-peptide (FCP), and fasting insulin (FINS), were obtained after an 8-h fast.

Ewing Test and HRV Analysis

The presence of DCAN was first assessed by measuring the four cardiovascular reflexes as described by Ewing in 1970: heart rate variation with deep breathing with an assessment of expiration to inspiration (E/I) ratio, heart rate analysis in the standing position (the 30 s/15 s ratio), Valsalva ratio, and blood pressure response to positional changes from lying to standing (orthostatic hypotension, OH) (12, 13). Three of these tests assessed the parasympathetic functions, such as E/I ratio, 30 s/15 s ratio, and Valsalva ratio, while OH assessed the sympathetic function (14) primarily. An E/I ratio more than the age-specific reference value, a Valsalva ratio ≥1.21, a posture ratio ≥1.04, and a systolic blood pressure reduction in response to the standing of ≤10 mm Hg were considered normal. An E/I ratio below the age-adjusted values, a Valsalva ratio ≤1.10, a posture ratio ≤1.00, and a systolic blood pressure fall in response to the standing of ≥20 mm Hg were considered abnormal. Values that fell between normal and abnormal were considered borderline. Each of these four tests was assigned a score of 0 for normal, 0.5 for borderline, and 1 for abnormal results; the sum of these four scores made up the Ewing score, which was used to assess the severity of DCAN. An Ewing score ≥2 was considered abnormal. Participants were instructed to avoid food and particular pharmacological agents (antidepressants, neuroleptics, nicotine, and caffeine) for 12 h preceding the examination.

HRV was evaluated according to the European Society of Cardiology guidelines (15) using a 24-h Holter monitor. Time-domain HRV and frequency-domain HRV indexes were analyzed. Time-domain HRV indexes included the standard deviation (SD) of all normal-to-normal (NN) intervals (SDNN), SD of the average NN intervals calculated over 5-min periods of the entire recording (SDANN), root mean square successive difference in the R-R interval (rMSSD, ms), and percentage of adjacent RR intervals with a difference of duration greater 50 ms (PNN50, %). The frequency-domain HRV indexes included low-frequency power (LF, ms^2) and high-frequency power (HF, ms^2). HRV was considered

abnormal if at least two of the following six abnormal parameters were met: SDNN <50 ms, SDANN <40 ms, PNN50 <0.75%, rMSSD <15 ms, LF <300 ms^2, and HF <300 ms^2 (15, 16).

In this study, the presence of DCAN (DCAN+) was defined as having both an abnormal Ewing score (≥2) and an abnormal HRV analysis (≥2 abnormal parameters).

Electrophysiologic Evaluation

NCS were performed using the Viking Quest (Nicolet VIASYS Healthcare, USA). Skin temperature was maintained above 32°C in the upper limbs and above 31°C in the lower limbs. The filtering frequency was 20 Hz to 10 kHz. The compound muscle action potential (CMAP) amplitude, latency, and motor conduction velocity (CV) of the median, ulnar, posterior tibial, and peroneal nerves were recorded. The CMAP amplitude was recorded from peak to peak by a supramaximal stimulation. The supramaximal stimulation was defined as 10% addition of stimulation charge after CMAP amplitude reaches its maximum. The measurements of sensory nerve action potential (SNAP) amplitude, latency, and sensory conduction velocity (CV) of the median, ulnar, and sural nerves were recorded also by supramaximal stimulation. The mean value of 20 results was taken for the sensory NCS. For each individual, the mean of the motor nerve amplitude was calculated using the following formula: Amplitude of motor nerve = (Amplitude of median nerve M + Amplitude of ulnar nerve M+ Amplitude of posterior tibial nerve M + Amplitude of peroneal nerve M)/4. The means of motor nerve CV, sensory nerve amplitude, and sensory nerve CV were calculated using the same method.

In the present study, diabetic peripheral neuropathy (DPN) was defined as the present of one or more abnormal nerve conduction result (amplitude or CV) in at least two different peripheral nerve (10).

Sympathetic Skin Response

The SSR was studied using the standard method (17). The room temperature was maintained at 25°C–26°C. A standard electromyographic active electrode was attached to the right palm and sole and the reference electrode to the dorsum of the hand and foot. The stimulus used was a single electrical stimulus at the right wrist of 10 mA for 100ms duration. This stimulation procedure was standardized in previous studies on fibromyalgia and correlated with symptoms (18). Stimuli were delivered unexpectedly and at random intervals between 30 and 60 s. Five consecutive stimuli were delivered. The latency was measured from the onset of the stimulus artifact to the onset of the first negative deflection and expressed in seconds. The amplitude was measured from the baseline to the maximal negative peak and expressed in mV. The response was considered absent if no consistent voltage change occurred using a sensitivity of 50 mV per division after three trials at maximum stimulus intensity. Response latencies were considered pathological when exceeding two SD more than the mean latency in the control group. The SSR habituation was considered as the percent rate of the maximal amplitude change between the fifth and the first response. A value less than 1 indicated habituation.

Statistical Analysis

For continuous variables, the results were presented as the mean ± SD if normally distributed, and median (interquartile range, IQR) if nonnormally distributed. The Student t test (normally distributed data) and the Kruskal–Wallis test (nonparametric data) were employed to examine differences between groups. Categorical data were analyzed using Pearson's χ^2 test. Multivariate logistic regression was performed to determine which nerve parameter had the greatest predictive value for the diagnosis of DCAN. Receiver-operating characteristic (ROC) analysis was performed to assess the optimal SSR cutoff for indicating DCAN. A two-sided $P < 0.05$ was assumed to be statistically significant, while $P < 0.001$ was taken as highly significant. All statistical analyses were performed using SPSS statistics software (version 20.0, 2011; IBM, USA).

RESULTS

Clinical Characteristics of Participants

From January 2019 to June 2019, 172 patients with T2DM were screened for inclusion in this study. Of these, 38 patients were excluded based on the listed exclusion criteria, and the remaining 134 participants were enrolled. Further, 42 patients (31.34%) were diagnosed with DCAN as described early (both positive Ewing test and positive HRV analysis). The average test results in each group are shown in **Table 1**. The baseline clinical and laboratory characteristics of the patients are also shown in **Table 1**. Patients with DCAN were more likely to be male compared with controls ($P < 0.05$), and were older ($P < 0.001$). Participants with DCAN also had a slightly lower FPG ($P < 0.05$). Diabetes duration, smoking, BMI, blood pressure, TG, TC, HDL, LDL, PBG, HbA1c, FCP, and FINS were similar between the two groups.

Ewing Test Parameters Between DCAN+ and DCAN– Groups

Statistically significant differences were observed in E/I ratio ($P < 0.001$), Valsalva ratio ($P < 0.001$), 30 s/15 s ratio ($P < 0.001$), OH ($P < 0.05$), and Ewing score ($P < 0.001$) between DCAN+ group and DCAN– group (**Supplementary Table 1**).

HRV Parameters Between DCAN+ and DCAN– Groups

Statistically significant differences were found in SDANN ($P < 0.05$), LF ($P < 0.001$), and HF ($P < 0.001$) between DCAN+ and DCAN– groups, while SDNN, rMSSD, and pNN50 were similar between the two groups (**Supplementary Table 2**).

NCS Parameters Between DCAN+ and DCAN– Groups

There are 78.57% (33/42) patients diagnosed as DPN (based on NCS test) in DCAN group, while 59.78% (55/92) in non-DCAN group ($P < 0.05$). The averaged NCS results of different motor and sensory nerves were compared in **Table 2**. The amplitude (7.96 ± 2.66 mV *vs.* 9.60 ± 2.22 mV, $P < 0.001$) and CV (44.22 ± 5.10 m/s *vs.*

TABLE 1 | Clinical characteristics of patients with T2DM and DCAN *versus* those without DCAN.

	DCAN+ (*n* = 42)	DCAN– (*n* = 92)	*P*
Sex (M/F)	30/12	48/44	<0.05*
Age (year)	63.86 ± 10.57	55.35 ± 12.63	<0.001**
Duration (year)	10.13 ± 7.59	8.25 ± 6.79	n.s.
Smoking (%)	26.5%	32.5%	n.s.
BMI (kg/m^2)	24.22 ± 3.51	23.20 ± 3.09	n.s.
SBP (mm Hg)	148.38 ± 26.73	140.07 ± 24.11	n.s.
DBP (mm Hg)	81.95 ± 12.66	82.70 ± 11.45	n.s.
TG (mmol/L)	2.08 ± 1.83	1.82 ± 1.27	n.s.
TC (mmol/L)	4.55 ± 1.83	4.95 ± 1.57	n.s.
HDL (mmol/L)	0.97 ± 0.29	1.03 ± 0.28	n.s.
LDL (mmol/L)	2.85 ± 1.28	3.08 ± 0.99	n.s.
FPG (mmol/L)	8.45 ± 2.98	8.66 ± 3.34	<0.05*
PBG (mmol/L)	15.27 ± 7.30	14.97 ± 5.63	n.s.
HbA1c(%)	8.83 ± 2.74	9.62 ± 2.65	n.s.
FCP (ng/mL)	2.96 (1.07–3.69)	1.69 (1.02–2.73)	n.s.
FINS (μU/mL)	8.81 (3.60–15.44)	6.69 (3.49–13.79)	n.s.

Values were expressed as mean ± SD for normally distributed data and median with interquartile range for nonnormally distributed data, or n (%). Differences between the groups were analyzed using independent-sample t test for normally distributed values and using the Kruskal–Wallis test for nonparametric values. Pearson's χ^2 test was employed to analyze categorical data. BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FCP, fasting C-peptide; FINS, fasting insulin; FPG, fasting plasma glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PBG, postprandial blood glucose; TG, triglycerides; TC, total cholesterol.

**P < 0.05, **P < 0.001.*

n.s., Not significant.

46.62 ± 3.96 m/s, $P < 0.05$) of the motor nerve were lower in the DCAN+ group than in the DCAN– group. Similarly, the sensory amplitude (17.90 ± 13.66 mV *vs.* 26.24 ± 11.92 mV, $P < 0.001$) and CV (48.13 ± 7.27 m/s *vs.* 51.16 ± 6.23 m/s, $P < 0.05$) were significantly lower in the DCAN+ group than in the DCAN– group (**Table 2**).

Nerve Conduction for DCAN in Multivariate Logistic Regression

For logistic regression, the continuous variables were transformed into grade variables according to the reference value. Multivariate logistic regression analysis was performed with DCAN as dependent variables and the amplitude/CV of motor nerve for the median, ulnar, posterior tibial, and peroneal nerves as independent variables. Similarly, multivariate logistic regression analysis was performed with DCAN as dependent variables and the amplitude/CV of sensory nerve for the median, ulnar, and sural nerves as independent variables. As shown in **Table 3**, a lower amplitude of motor fiber of the peroneal nerve was found to demonstrate an increased risk for DCAN (OR 2.77, 95% CI 1.20–6.44, $P < 0.05$).

TABLE 2 | Nerve conduction of patients with T2DM and DCAN *versus* those without DCAN.

		DCAN+ (*n* = 42)	DCAN– (*n* = 92)	*P*
Motor nerve	Amp (mV)	7.96 ± 2.66	9.60 ± 2.22	<0.001**
	CV (m/s)	44.22 ± 5.10	46.62 ± 3.96	<0.05*
Sensory nerve	Amp (mV)	17.90 ± 13.66	26.24 ± 11.92	<0.001**
	CV (m/s)	48.13 ± 7.27	51.16 ± 6.23	<0.05*

Data were presented as mean ± SD. Differences between the groups were analyzed using unpaired-sample t test.

Amp, Amplitude; CV, conduction velocity.

**P < 0.05, **P < 0.001.*

SSR Value in DCAN+ and DCAN– Groups

The SSR amplitude of the upper limb [0.99 (0.16–2.70) mV *vs.* 2.18 (1.19–3.48) mV, $P < 0.001$] and lower limb [0.44 (0.13–1.38) mV *vs.* 1.17 (0.73–1.97) mV, $P < 0.001$] were lower in the DCAN + group than in the DCAN– group. The SSR latency of the upper limb [1.49 (1.32–2.21) s*vs.* 1.36 (1.23–1.46) s, $P < 0.05$] and lower limb [1.99 (1.76–3.26) s *vs.* 1.79 (1.62–1.93) s, $P < 0.001$] were higher in the DCAN+ group than in the DCAN– group (**Table 4**).

ROC Analysis of SSR to Predict DCAN in Patients With T2DM

ROC analysis revealed that the cutoff points of upper limb amplitude and lower limb amplitude, which maximized both the sensitivity and specificity to indicate DCAN, were 1.40 mV (AUC = 0.70; 95% CI: 0.59–0.80; sensitivity, 61.9%; specificity, 66.3%, $P < 0.001$) and 0.85 mV (AUC = 0.70; 95% CI: 0.60–0.80; sensitivity, 66.7%; specificity, 68.5%, $P < 0.001$), respectively. Similarly, the optimal cutoff points of upper limb latency and lower limb latency to indicate DCAN were 1.40 s (AUC = 0.67; 95% CI: 0.57–0.77; sensitivity, 61.9%; specificity, 62%, $P < 0.05$) and 1.81 s (AUC = 0.66; 95% CI: 0.55–0.77; sensitivity, 69.0%; specificity, 52.2%, $P < 0.05$), respectively (**Figure 1**).

TABLE 3 | Nerve conduction for DCAN in multivariate logistic regression.

Variables	Odds ratio (95% CI)	*P* value
Peroneal nerve M Amp		
>2.6 mV	1 (Ref.)	
<2.6 mV	2.77 (1.20–6.44)	<0.05*

M, motor nerve; Amp, Amplitude.

**P < 0.05.*

TABLE 4 | SSR of patients with T2DM and DCAN *versus* those without DCAN.

		DCAN+ (*n* = 42)	DCAN– (*n* = 92)	*P*
Upper limb	Amp (mV)	0.99 (0.16–2.70)	2.18 (1.19–3.48)	<0.001**
	Laten (s)	1.49 (1.32–2.21)	1.36 (1.23–1.46)	<0.05*
Lower limb	Amp (mV)	0.44 (0.13–1.38)	1.17 (0.73–1.97)	<0.001**
	Laten (s)	1.99 (1.76–3.26)	1.79 (1.62–1.93)	<0.05*

Amp, Amplitude; Laten, latency.
**P < 0.05, **P < 0.001.*

DISCUSSION

DCAN results from damage to nerve fibers within the parasympathetic and sympathetic nervous systems. It is one of the leading causes of heart arrhythmias and is an independent risk factor for cardiovascular mortality among patients with diabetes. Early diagnosis and intervention in patients with T2DM can reduce DCAN progression. Recent guidelines strongly recommend screening for DCAN in patients with diabetes because the progression of cardiovascular denervation is partly reversible or can be slowed in the early stages of the disease (19).

In the present study, the overall prevalence of DCAN in a sample of patients with diabetes was 31.34%. The prevalence reported in previous studies ranged from 25% to 75% in patients with T2DM (1, 20, 21). The significant variability between studies might be attributed to the lack of uniform diagnostic criteria and underdiagnosis due to inadequate screening (1, 22). In this study, DCAN was diagnosed using the Ewing test combined with HRV analysis to increase diagnostic specificity.

The association between diabetes duration and the development of DCAN has not been established. Interestingly, in the present study, older age was associated with DCAN but diabetes duration was not. A recent study also suggested that the duration of DM had no significant association with HRV measurements (23). However, a previous review reported that the prevalence of DCAN in patients with T2DM was associated with increased age and duration of diabetes (24). Pop-Busui et al. showed that DCAN was associated with poor glycemic control, increased age, and duration of disease, and diastolic blood pressure (25). Most likely, DCAN started in the early stages of diabetes (26). The discrepancy in results between studies might be due to inconsistent DCAN diagnostic criteria.

In the present study, the Valsalva ratio, E/I ratio, 30 s/50s ratio, and OH were different between DCAN+ and DCAN– groups. Except for the rMSSD and PNN50, all the parameters of HRV analysis were lower in the DCAN+ group than in the

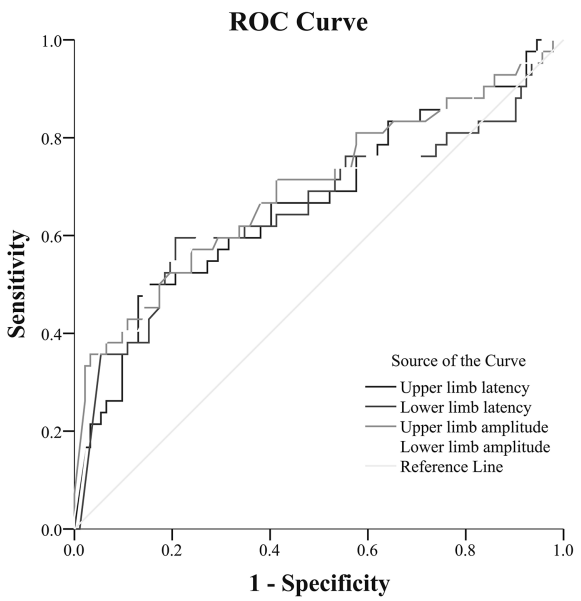

FIGURE 1 | Receiver-operating characteristic (ROC) analysis of SSR to predict DCAN in patients with T2DM [(upper limb amplitude: AUC = 0.70; 95% CI: 0.59–0.80; sensitivity, 61.9%; specificity, 66.3%, cut-point 1.40 mV, $P < 0.001$) (lower limb amplitude: AUC = 0.70; 95% CI: 0.60–0.80; sensitivity, 66.7%; specificity, 68.5%, cut-point 0.85 mV, $P < 0.001$) (upper limb latency: AUC = 0.67; 95% CI: 0.57–0.77; sensitivity, 61.9%; specificity, 62%, cut-point 1.40 s, $P < 0.05$) [lower limb latency: AUC = 0.66; 95% CI: 0.55–0.77; sensitivity, 69.0%; specificity, 52.2%, cut-point 1.81 s, $P < 0.05$)].

DCAN– group, indicating that both parasympathetic and sympathetic dysfunction existed during the development of CAN in patients with diabetes. This conclusion was consistent with that of multiple previous studies (27, 28). The results of the present study extended and confirmed these findings and suggested that T2DM was a metabolic disease responsible for cardiac autonomic neuropathy due to damage to both sympathetic and parasympathetic fibers.

The proportion of DPN was higher in the DCAN+ group than that in the DCAN – group. This finding was consistent with previous research (29). The amplitude and CV of the motor and sensory nerves were lower in the DCAN+ group than in the DCAN– group. Patients with severe DCAN had significantly reduced nerve CV and amplitude of peripheral nerves (30); it appeared that patients with diabetes and DCAN suffered more frequently from peripheral neuropathy compared with those without DCAN (31). A positive association existed between low HRV during deep breathing and large nerve fiber neuropathy, the latter documented with electrophysiology and clinical examination (32). Although some studies did not agree with these findings (33), a possible explanation was that CAN diagnosis was not based on a unanimous definition.

The present study found that DCAN was closely related to impaired peripheral nerve conduction. Moreover, NCS abnormality in the peroneal nerve (motor nerve) was more important in predicting DCAN. This was in line with previous studies. For example, parallel involvement of peripheral neuropathy and DCAN may principally affect the more vulnerable large nerve fibers (34). Parallel development of DCAN and peripheral neuropathy is thought to involve vulnerable large nerve fibers, with recent evidence pointing to an association between parasympathetic nervous system impairment and reduced peroneal motor nerve CV (35).

Peripheral NCS examine large-fiber sensory and motor nerve conduction. However, SSR is a simple, noninvasive method for evaluating small-fiber sudomotor function (36, 37). The sudomotor function is primarily mediated by the stimulation of the post sympathetic cholinergic fibers, which reflect the sweat nerve glands (unmyelinated C fiber) function or density (38). SSR latency measurements reflect the function of unmyelinated C fibers; SSR amplitude measurements reflect the density of spontaneously activable sweat glands (39). SSR appears to be useful for assessing autonomic neuropathy in patients with diabetes (40). In the present study, both the latency and the amplitude of SSR were different between the DCAN+ and DCAN– groups. The ROC analysis in the present study indicated that patients with T2DM should be alert for DCAN if SSR had upper limb amplitude lower than 1.40 mV, lower limb amplitude lower than 0.85 mV, upper limb latency longer than 1.40 s, or lower limb latency longer than 1.81 s.

In the present study, the results suggested that a lower amplitude of peroneal nerve motor fiber, as well as a lower amplitude and longer latency of SSR, were associated with increased risk of DCAN. Therefore, NCS and SSR can be used to predict and assist in DCAN diagnosis Nevertheless, the small sample size of the study is a limitation. Prospective studies with a larger sample size and long-term follow-up are required to confirm this conclusion.

CONCLUSIONS

The present study examined the role of NCS and sympathetic SSR in the evaluation of DCAN in diabetic patients. Consequently, this study revealed that a peroneal nerve (motor nerve) abnormality is crucial for predicting DCAN, wherein SSR may help. As DCAN is difficult to diagnose and predict, the data presented herein may be clinically useful.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Nanfang Hospital, Southern Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Each author has made an important scientific contribution to the study and is thoroughly familiar with the primary data. XL and CC carried out the clinical studies, participated in the statistical analysis and drafted the manuscript. YL, YP, ZC, and HH carried out the data acquisition, participated in the manuscript preparation and literature research. LX conceived of the study, and participated in its design and helped to review the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.709114/full#supplementary-material

REFERENCES

1. Dimitropoulos G, Tahrani AA, Stevens MJ. Cardiac Autonomic Neuropathy in Patients With Diabetes Mellitus. *World J Diabetes* (2014) 5(1):17–39. doi: 10.4239/wjd.v5.i1.17
2. Maser RE, Lenhard JM, Decherney SG. Cardiovascular Autonomic Neuropathy: The Clinical Significance of Its Determination. *Endocrinologist* (2000) 10(1):27–33. doi: 10.1097/00019616-200010010-00006
3. Balcıoğlu AS, Müderrisoğlu H. Diabetes and Cardiac Autonomic Neuropathy: Clinical Manifestations, Cardiovascular Consequences, Diagnosis and Treatment. *World J Diabetes* (2015) 6(1):80–91. doi: 10.4239/wjd.v6.i1.80
4. Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, et al. Diabetic Neuropathies: A Statement by the American Diabetes Association. *Diabetes Care* (2005) 28(4):956–62. doi: 10.2337/diacare.28.4.956
5. Lin K, Wei L, Huang Z, Zeng Q. Combination of Ewing Test, Heart Rate Variability, and Heart Rate Turbulence Analysis for Early Diagnosis of Diabetic Cardiac Autonomic Neuropathy. *Medicine* (2017) 96(45):e8296. doi: 10.1097/md.0000000000008296
6. Cygankiewicz I, Zareba W. Heart Rate Variability. *Handb Clin Neurol* (2013) 117:379–93. doi: 10.1016/b978-0-444-53491-0.00031-6
7. Mathias CJ. Autonomic Diseases: Clinical Features and Laboratory Evaluation. *J Neurol Neurosurg Psychiatry* (2003) 74Suppl 3(Suppl 3):iii31–41. doi: 10.1136/jnnp.74.suppl_3.iii31
8. Ziegler D. Diabetic Cardiovascular Autonomic Neuropathy: Prognosis, Diagnosis and Treatment. *Diabetes Metab Rev* (1994) 10(4):339–83. doi: 10.1002/dmr.5610100403
9. Mala S, Potockova V, Hoskovcova L, Pithova P, Brabec M, Kulhankova J, et al. Cardiac Autonomic Neuropathy May Play a Role in Pathogenesis of Atherosclerosis in Type 1 Diabetes Mellitus. *Diabetes Res Clin Pract* (2017) 134:139–44. doi: 10.1016/j.diabres.2017.10.002
10. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, et al. Diabetic Neuropathies: Update on Definitions, Diagnostic Criteria, Estimation of Severity, and Treatments. *Diabetes Care* (2010) 33(10):2285–93. doi: 10.2337/dc10-1303
11. Braffett BH, Gubitosi-Klug RA, Albers JW, Feldman EL, Martin CL, White NH, et al. Risk Factors for Diabetic Peripheral Neuropathy and Cardiovascular Autonomic Neuropathy in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study. *Diabetes* (2020) 69(5):1000–10. doi: 10.2337/db19-1046
12. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, et al. Cardiovascular Autonomic Neuropathy in Diabetes: Clinical Impact, Assessment, Diagnosis, and Management. *Diabetes Metab Res Rev* (2011) 27(7):639–53. doi: 10.1002/dmrr.1239
13. Pafili K, Trypsianis G, Papazoglou D, Maltezos E, Papanas N. Simplified Diagnosis of Cardiovascular Autonomic Neuropathy in Type 2 Diabetes Using Ewing's Battery. *Rev Diabetic Stud RDS* (2015) 12(1-2):213–9. doi: 10.1900/rds.2015.12.213
14. Pafili K, Trypsianis G, Papazoglou D, Maltezos E, Papanas N. Cardiovascular Autonomic Neuropathy and Distal Symmetric Sensorimotor Polyneuropathy: These Two Diabetic Microvascular Complications Do Not Invariably Co-Exist. *Curr Vasc Pharmacol* (2020) 18(1):50–6. doi: 10.2174/1570161116666180829120101
15. Heart Rate Variability: Standards of Measurement, Physiological Interpretation and Clinical Use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* (1996) 93(5):1043–65.
16. Metelka R. Heart Rate Variability–Current Diagnosis of the Cardiac Autonomic Neuropathy. *A Rev Biomed Pap Med Fac Univ Palacky Olomouc Czech* (2014) 158(3):327–38. doi: 10.5507/bp.2014.025
17. Shahani BT, Halperin JJ, Boulu P, Cohen J. Sympathetic Skin Response–a Method of Assessing Unmyelinated Axon Dysfunction in Peripheral Neuropathies. *J Neurol Neurosurg Psychiatry* (1984) 47(5):536–42. doi: 10.1136/jnnp.47.5.536
18. Ulas UH, Unlu E, Hamamcioglu K, Odabasi Z, Cakci A, Vural O. Dysautonomia in Fibromyalgia Syndrome: Sympathetic Skin Responses and RR Interval Analysis. *Rheumatol Int* (2006) 26(5):383–7. doi: 10.1007/s00296-005-0007-1
19. Sztanek F, Jebelovszki É, Gaszner B, Zrínyi M, Páll D, Kempler P, et al. [Diagnosis of Diabetic Cardiac Autonomic Neuropathy]. *Orvosi Hetilap* (2019) 160(35):1366–75. doi: 10.1556/650.2019.31491
20. Agashe S, Petak S. Cardiac Autonomic Neuropathy in Diabetes Mellitus. *Methodist DeBakey Cardiovasc J* (2018) 14(4):251–6. doi: 10.14797/mdcj-14-4-251
21. Vinik AI, Ziegler D. Diabetic Cardiovascular Autonomic Neuropathy. *Circulation* (2007) 115(3):387–97. doi: 10.1161/circulationaha.106.634949
22. Ziegler D, Gries FA, Spüler M, Lessmann F. The Epidemiology of Diabetic Neuropathy. Diabetic Cardiovascular Autonomic Neuropathy Multicenter Study Group. *J Diabetes Complications* (1992) 6(1):49–57. doi: 10.1016/1056-8727(92)90049-q
23. Khandoker AH, Al-Angari HM, Khalaf K, Lee S, Almahmeed W, Al Safar HS, et al. Association of Diabetes Related Complications With Heart Rate Variability Among a Diabetic Population in the UAE. *PloS One* (2017) 12(1):e0168584. doi: 10.1371/journal.pone.0168584
24. Vinik AI, Erbas T, Casellini CM. Diabetic Cardiac Autonomic Neuropathy, Inflammation and Cardiovascular Disease. *J Diabetes Invest* (2013) 4(1):4–18. doi: 10.1111/jdi.12042
25. Pop-Busui R. Cardiac Autonomic Neuropathy in Diabetes: A Clinical Perspective. *Diabetes Care* (2010) 33(2):434–41. doi: 10.2337/dc09-1294
26. Fakhrzadeh H, Yamini-Sharif A, Sharifi F, Tajalizadekhoob Y, Mirarefin M, Mohammadzadeh M, et al. Cardiac Autonomic Neuropathy Measured by Heart Rate Variability and Markers of Subclinical Atherosclerosis in Early Type 2 Diabetes. *ISRN Endocrinol* (2012) 2012:168264. doi: 10.5402/2012/168264
27. Benichou T, Pereira B, Mermillod M, Tauveron I, Pfabigan D, Maqdasy S, et al. Heart Rate Variability in Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. *PloS One* (2018) 13(4):e0195166. doi: 10.1371/journal.pone.0195166
28. Oakley I, Emond L. Diabetic Cardiac Autonomic Neuropathy and Anesthetic Management: Review of the Literature. *AANA J* (2011) 79(6):473–9.
29. Kim SH, Lee KA, Jin HY, Baek HS, Park TS. Relationship Between the Korean Version Survey of the Autonomic Symptoms Score and Cardiac Autonomic Neuropathy Parameters in Patients With Diabetic Peripheral Neuropathy. *Diabetes Metab J* (2014) 38(5):349–55. doi: 10.4093/dmj.2014.38.5.349
30. Haq T, Ahmed T, Latif ZA, Sayeed MA, Ashrafuzzaman SM. Cardiac Autonomic Neuropathy in Patients With Type 2 Diabetes Mellitus Having Peripheral Neuropathy: A Cross-Sectional Study. *Diabetes Metab Syndr* (2019) 13(2):1523–8. doi: 10.1016/j.dsx.2019.03.015
31. Voulgari C, Psallas M, Kokkinos A, Argiana V, Katsilambros N, Tentolouris N. The Association Between Cardiac Autonomic Neuropathy With Metabolic and Other Factors in Subjects With Type 1 and Type 2 Diabetes. *J Diabetes Complications* (2011) 25(3):159–67. doi: 10.1016/j.jdiacomp.2010.06.001
32. Orlov S, Bril V, Orszag A, Perkins BA. Heart Rate Variability and Sensorimotor Polyneuropathy in Type 1 Diabetes. *Diabetes Care* (2012) 35(4):809–16. doi: 10.2337/dc11-1652
33. Töyry JP, Partanen JV, Niskanen LK, Länsimies EA, Uusitupa MI. Divergent Development of Autonomic and Peripheral Somatic Neuropathies in NIDDM. *Diabetologia* (1997) 40(8):953–8. doi: 10.1007/s001250050773
34. Pafili K, Trypsianis G, Papazoglou D, Maltezos E, Papanas N. Correlation of Cardiac Autonomic Neuropathy With Small and Large Peripheral Nerve Function in Type 2 Diabetes Mellitus. *Diabetes Res Clin Pract* (2019) 156:107844. doi: 10.1016/j.diabres.2019.107844
35. Lange-Maia BS, Newman AB, Jakicic JM, Cauley JA, Boudreau RM, Schwartz AV, et al. Relationship Between Sensorimotor Peripheral Nerve Function and Indicators of Cardiovascular Autonomic Function in Older Adults From the Health, Aging and Body Composition Study. *Exp Gerontol* (2017) 96:38–45. doi: 10.1016/j.exger.2017.04.007
36. Gerawarapong C. Association of Peripheral Autonomic Neuropathy and Sympathetic Skin Response in the Patients With Diabetic Polyneuropathy: A Pilot Study in Thailand. *J Med Assoc Thailand = Chotmaihet Thangphaet* (2015) 98(12):1222–30.
37. Hoitsma E, Reulen JP, de Baets M, Drent M, Spaans F, Faber CG. Small Fiber Neuropathy: A Common and Important Clinical Disorder. *J Neurol Sci* (2004) 227(1):119–30. doi: 10.1016/j.jns.2004.08.012
38. Stanley EF. Single Calcium Channels and Acetylcholine Release at a Presynaptic Nerve Terminal. *Neuron* (1993) 11(6):1007–11. doi: 10.1016/0896-6273(93)90214-c

39. Levy DM, Reid G, Rowley DA, Abraham RR. Quantitative Measures of Sympathetic Skin Response in Diabetes: Relation to Sudomotor and Neurological Function. *J Neurol Neurosurg Psychiatry* (1992) 55(10):902–8. doi: 10.1136/jnnp.55.10.902
40. Spitzer A, Lang E, Birklein F, Claus D, Neundörfer B. Cardiac Autonomic Involvement and Peripheral Nerve Function in Patients With Diabetic Neuropathy. *Funct Neurol* (1997) 12(3-4):115–22.

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Association Between Age at Diagnosis of Type 2 Diabetes and Cardiovascular Diseases: A Nationwide, Population-Based, Cohort Study

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Objectives: Nationwide studies focusing on the impact of early-onset type 2 diabetes and obesity on the development of cardiovascular diseases (CVD) are limited in China. We aimed to investigate the association between age at diagnosis of type 2 diabetes and the risk of CVD, and to further examine the modifying effect of obesity on this association among Chinese adults.

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Methods: This study included 23,961 participants with previously diagnosed diabetes from a large nationwide population-based cohort study across mainland China. With an interviewer-assisted questionnaire, we collected detailed information on CVDs. Logistic regression analysis was used to evaluate the risk of CVDs associated with age at diagnosis of diabetes.

Results: Compared with patients with late-onset diabetes (≥60 years), those with earlier-onset diabetes had increased risks for CVD, with adjusted ORs (95% CIs) of 1.72 (1.36-2.17), 1.52 (1.31-1.75) and 1.33 (1.19-1.48) for patients diagnosed aged <40, 40-49 and 50-59 years, respectively. Each 5-year earlier age at diagnosis of type 2 diabetes was significantly associated with 14% increased risk of CVD (OR, 1.14; 95%CI, 1.11-1.18). This association was more prominent for patients with obesity than those with normal body mass index (BMI). Significant interaction was detected between age at diagnosis and BMI categories on CVD risk (*P* for interaction=0.0457).

Conclusion: Early-onset type 2 diabetes was significantly associated with higher risk of CVD, and this association was more prominent among patients with obesity.

Keywords: cardiovascular disease, type 2 diabetes, early-onset diabetes, age at diagnosis of diabetes, obesity

INTRODUCTION

Type 2 diabetes has become a major health burden worldwide. In the past few decades, the prevalence of diabetes in China has increased dramatically, with 12.8% of adults of diabetes and 35.2% of prediabetes in 2017 (1, 2). Although type 2 diabetes was conventionally recognized as a disease of the middle-aged and elderly, a rapid growth of type 2 diabetes has been observed among younger adults and even adolescents (3, 4), especially in developing countries. In Asia, one in five patients with diabetes was diagnosed before 40 years (5). Early-onset type 2 diabetes was reported to be associated with poor metabolic control and accelerated development of complications (5–12). Although the impact of age at diagnosis of type 2 diabetes on the risk of cardiovascular disease (CVD) has been investigated in previous studies (8, 9, 13–17), the results remain inconclusive. Some studies proposed inverse association between age at diagnosis of diabetes and risk of CVD (8, 14, 15), whereas others suggested positive (16, 17) or null association (9, 18) in prospective analyses.

During the past decades, China has experienced a rapid transition to western lifestyles with more sedentary behavior and a high energy/fat diet (19), leading to the escalating rates of obesity and related metabolic diseases, including type 2 diabetes. The rise in the prevalence of obesity in early and middle-aged population is likely to increase the incidence of early-onset type 2 diabetes (20). Previous studies have reported a strong relationship between obesity in youth and subsequent onset of type 2 diabetes (20–23). Obesity was also an independent risk factor for CVD, which is the leading cause of premature death in China (24). However, to the best of our knowledge, studies investigating the effect of obesity on the association between age at diagnosis of type 2 diabetes and the risk of CVD are limited.

Therefore, using the comprehensive data from the Risk Evaluation of cAncers in Chinese diabeTic Individuals: a lONgitudinal (REACTION) study, we aim to investigate the association between age at diagnosis of type 2 diabetes and the risk of CVDs, and to further evaluate the modifying effect of BMI on this association among Chinese adults.

MATERIALS AND METHODS

Study Population

The study population was derived from the REACTION study, including 259,657 participants from 25 communities across mainland China. The details of the study population were described previously (25, 26). Briefly, community dwelling adults aged 40 years or older were invited to participate the baseline survey during 2011-2012. There was no restriction on sex or ethnicity. Participants who signed informed consents were recruit and scheduled for a comprehensive clinical examination (including physical and biochemical measurements) and a structured questionnaire interview. Among 259,657 participants in the REACTION study, 26,795 individuals reported having ever been diagnosed as type 2 diabetes by professional physicians. We further excluded participants with missing information about the time of diagnosis (n=2797) or those diagnosed before 18 years (n=37). Finally, 23,961 individuals were included in the current analysis.

The REACTION study was approved by the ethic committee of Ruijin hospital affiliated to Shanghai Jiaotong University School of Medicine. All participants provided written informed consent.

Data Collection

During the personal interview, information on sociodemographic characteristics, lifestyle factors (including smoking or drinking status, physical activity, and dietary habits), and medical history was collected using a standard questionnaire. Education levels

were divided into less than high school versus high school education or above. Current smoking was defined as smoking cigarettes one per day or seven per week regularly during the past 6 months. People who consumed alcohol once per week regularly during the past 6 months was considered current drinker. Physical activity was estimated using the short form of the International Physical Activity Questionnaire. Participants were asked about the questions on the intensity, duration, and frequency of physical activity at leisure time. According to this information, total metabolic equivalents were calculated to measure the physical activity levels. Moderate and vigorous physical activity refers to moderate intensity activity ≥ 150 min/wk, or vigorous intensity or combination activity ≥ 75 min/wk. A dietary questionnaire was used to collect the information on the frequency and quantity of major food items (such as red meat, fruits and vegetables, dairy, soy, and Chinese traditional food) over the past 12 months. According to the recommendation of the American Heart Association (27), a healthy diet was defined as a diet score ≥ 3, including the following 4 components: fruits and vegetables ≥ 4.5 cups/d, fish ≥ 198 g/wk, sweets/sugar-sweetened beverages ≤ 450 kcal/wk, and soy protein ≥ 25 g/d. Probable depression was accessed using the patient health questionnaire (PHQ)–9 with the total score of ≥10 (28).

All participants underwent measurements on height, weight, and blood pressure by experienced nurses. Height and weight were measured using standard protocol with participants wearing lightweight clothes and no shoes. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Blood pressure was tested three times consecutively at 1-min intervals with an automated electronic device (OMRON Model HEM-725 FUZZY, Omron Company, Dalian, China). The average of the three readings for systolic or diastolic blood pressure was used to analyze.

After 10 hours of overnight fasting, a blood sample was collected and aliquoted into 0.5-mL Eppendorf tubes within 2 hours. Sera was then shipped by air in dry ice to the central laboratory of the study located at Shanghai Institute of Endocrine and Metabolic Diseases, which is certificated by the U.S. National Glycohemoglobin Standardization Program and passed the Laboratory Accreditation Program of the College of American Pathologists. The level of HbA1c was determined by the method of high-performance liquid chromatography (VARIANT™ II and D-10™ Systems, BIO-RAD, Hercules, CA, USA) in the central laboratory. Total cholesterol and triglycerides were measured with an autoanalyzer (ARCHITECT c16000 System, Abbott Laboratories, IL, USA).

Classification and Definition

The date of diagnosis was recorded for each participant who reported having type 2 diabetes. Age at diagnosis of type 2 diabetes was categorized as < 40 years, 40-49 years, 50-59 years and ≥ 60 years. Obesity was defined as a BMI of ≥ 28.0 kg/m^2, and overweight was defined as a BMI of 24.0-27.9 kg/m^2.

We collected information on CVDs with an interviewer-assisted questionnaire. An open-ended question was asked: "Has a doctor or other health professional ever told you that you have myocardial infarction, coronary heart disease (CHD), or stroke?" Total CVD in the analysis referred to the composite of the three CVDs (reported myocardial infarction, CHD, or stroke). Validation of the self-reported CVDs from questionnaire was performed in Shanghai Youyi Community, 1 of the 25 communities in the REACTION study. Two physicians who were blind to the self-reported data reviewed the related hospitalization records, and classified the cases as definite, questionable, or misdiagnosed. The validation rate of CVDs was 91.1% (29, 30).

Statistical Analysis

Continuous variables are presented as means ± SDs or medians (IQRs) and categorical variables are presented as number (proportions). The P_{trend} for general characteristics according to age at diagnosis of diabetes was calculated using linear regression analyses for continuous variables and logistic regression for categorical variables. Multivariable adjusted logistic regression analyses were performed to estimate the association of age at diagnosis of type 2 diabetes on the risk of CVDs, with the group of the latest-onset type 2 diabetes (≥60 years) as the reference. Model 1 was adjusted for age and sex; Model 2 was adjusted for age, sex, education, smoking status, drinking status, physical activity, healthy dietary, BMI; Model 3 was further adjusted for systolic blood pressure, total cholesterol, triglycerides and HbA1c. Age at diagnosis of type 2 diabetes was also analyzed as continuous variables and the association between every 5-year earlier age at diabetes diagnosis and CVD risk was estimated.

Stratified analysis was conducted according to BMI categories. To explore the possible interaction between age at diagnosis of diabetes and BMI in the development of CVDs, we used the cross-products of age at diagnosis of diabetes and BMI categories as the interaction terms. The likelihood ratio test was used to analyze the potential interaction by comparing the full model including the interaction item with the simplified model without the interaction item. We further examined the combined effects of BMI and age at diagnosis of diabetes on the risk of CVDs.

All analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC). A two-tailed $p<0.05$ was considered statistically significant.

RESULTS

Characteristics of the Study Participants

This study included 23,961 people with previously diagnosed type 2 diabetes. Overall, the mean (± SD) age at diagnosis of diabetes was 54.60 ± 9.48 years. A total of 3653 (15.25%) participants have reported CVD in this study. Characteristics of the study population according to age at diagnosis of type 2 diabetes were presented in **(Table 1)**. On average, patients with earlier age at diagnosis were much younger, and they are more likely to have poor glycemic control, probable depression, and high triglycerides, but lower BMI and systolic blood pressure. Besides, Individuals with earlier age at diagnosis of diabetes have higher percentage of insulin-user, while lipid-lowering treatment and antihypertensive drugs are less used (all P for trend < 0.05, **Table 1**).

TABLE 1 | Baseline characteristics according to age at diagnosis of diabetes.

	Age at diagnosis of diabetes (years)				
	18-39	40-49	50-59	≥60	P_{trend}
No. of participants (%)	1413 (5.90)	6103 (25.47)	9827 (41.01)	6618 (27.62)	
Age at baseline, years	50.81 ± 8.43	54.82 ± 7.02	61.46 ± 5.83	70.59 ± 5.76	<.0001
Male gender, n (%)	669 (47.35)	2653 (43.47)	3814 (38.81)	2593 (39.18)	<.0001
BMI, kg/m^2	25.28 ± 3.66	25.43 ± 3.65	25.53 ± 3.62	25.60 ± 3.60	0.0006
BMI < 24kg/m^2, n(%)	573 (40.55)	2225 (36.46)	3469 (35.30)	2240 (33.85)	<.0001
BMI 24-27.9 kg/m^2, n(%)	559 (39.56)	2693 (44.13)	4299 (43.75)	2942 (44.45)	0.0325
BMI ≥28 kg/m^2, n(%)	281 (19.89)	1185 (19.42)	2059 (20.95)	1436 (21.70)	0.0022
High school or above education, n (%)	693 (49.04)	2644 (43.32)	3411 (34.71)	2109 (31.87)	<.0001
Current cigarette smoking, n (%)	291 (20.59)	1083 (17.75)	1273 (12.95)	576 (8.70)	<.0001
Current alcohol drinking, n (%)	143 (10.12)	578 (9.47)	732 (7.45)	379 (5.73)	<.0001
Moderate and vigorous physical activity, n (%)	181 (12.81)	831 (13.62)	1387 (14.11)	877 (13.25)	0.4462
Healthy diet, n (%)	171 (14.12)	785 (14.69)	1179 (13.84)	700 (12.22)	0.0005
Triglycerides, mmol/L	2.02 ± 1.81	2.01 ± 1.69	1.92 ± 1.47	1.80 ± 1.23	<.0001
Total cholesterol, mmol/L	4.95 ± 1.23	4.93 ± 1.18	4.95 ± 1.19	4.90 ± 1.18	0.1024
Systolic blood pressure, mmHg	132.98 ± 20.22	135.42 ± 20.24	139.77 ± 20.47	144.42 ± 20.65	<.0001
HbA1c, %	7.80 (6.80-9.40)	7.40 (6.60-8.80)	7.10 (6.40-8.10)	6.90 (6.30-7.80)	<.0001
Glucose-lowering treatment, n (%)	1169 (82.73)	4882 (79.99)	7762 (78.99)	5077 (76.72)	<.0001
Insulin therapy, n (%)	561 (39.70)	1632 (26.74)	1784 (18.15)	820 (12.39)	<.0001
Oral hypoglycemic drugs use, n (%)	861 (60.93)	4068 (66.66)	6877 (69.98)	4677 (70.67)	<.0001
Lipid-lowering treatment, n (%)	18 (1.27)	138 (2.26)	239 (2.43)	170 (2.57)	0.0136
Antihypertensive drugs use, n (%)	230 (16.28)	1220 (19.99)	2523 (25.67)	2234 (33.76)	<.0001
Probable depression, n (%)	34 (2.41)	119 (1.95)	151 (1.54)	95 (1.44)	0.0019

Association of Age at Diagnosis of Diabetes With CVD

The association between age at diagnosis of type 2 diabetes and the risk of CVD is presented in **Table 2**. In age and sex-adjusted model, compared with late-onset diabetes (diagnosed ≥60 years), young-onset diabetes (diagnosed <40 years of age) was associated with 46%, 56%, 77%, and 38% increased risk for total CVD, stroke, myocardial infarction, and CHD, respectively. Further adjustment for lifestyle factors and metabolic measurements did not change the estimates significantly. Compared with patients diagnosed ≥60 years, the fully adjusted ORs (95%CIs) of total CVD for patients with adjusted ORs (95% CIs) of 1.72 (1.36-2.17), 1.52 (1.31-1.75) and 1.33 (1.19-1.48) for patients diagnosed aged <40, 40-49 and 50-59 years, respectively. Each 5-year younger age at diagnosis of type 2 diabetes was significantly associated with an increased risk of CVD (OR, 1.14; 95%CI, 1.11-1.18). Results of individual CVD components were similar. Earlier age at diagnosis of diabetes was also strongly associated with stroke, myocardial infarction, and CHD, with the ORs (95% CIs) for per 5-year earlier of age at diagnosis of type 2 diabetes

TABLE 2 | Odds ratio (95% CI) for cardiovascular disease risks according to age at diagnosis of diabetes.

	Age at diagnosis of diabetes (years)				
	≥60	50-59	40-49	18-39	Per 5-years earlier
Cardiovascular disease, case (%)	1337 (20.20)	1475 (15.01)	692 (11.34)	149 (10.54)	
Model 1	1.00 (reference)	1.21 (1.10-1.33)	1.28 (1.13-1.46)	1.46 (1.19-1.79)	1.09 (1.07-1.12)
Model 2	1.00 (reference)	1.31 (1.18-1.46)	1.45 (1.26-1.67)	1.61 (1.28-2.02)	1.12 (1.09-1.16)
Model 3	1.00 (reference)	1.33 (1.19-1.48)	1.52 (1.31-1.75)	1.72 (1.36-2.17)	1.14 (1.11-1.18)
Stroke, case (%)	350 (5.29)	365 (3.71)	174 (2.85)	37 (2.62)	
Model 1	1.00 (reference)	1.24 (1.04-1.47)	1.39 (1.11-1.76)	1.56 (1.07-2.28)	1.09 (1.04-1.14)
Model 2	1.00 (reference)	1.26 (1.05-1.52)	1.42 (1.11-1.82)	1.50 (0.99-2.28)	1.10 (1.05-1.15)
Model 3	1.00 (reference)	1.26 (1.05-1.52)	1.47 (1.14-1.89)	1.61 (1.05-2.45)	1.11 (1.06-1.17)
Myocardial infarction, case (%)	124 (1.87)	137 (1.39)	84 (1.38)	20 (1.42)	
Model 1	1.00 (reference)	1.15 (0.88-1.52)	1.49 (1.05-2.11)	1.77 (1.03-3.02)	1.13 (1.06-1.21)
Model 2	1.00 (reference)	1.26 (0.94-1.71)	1.59 (1.09-2.32)	2.22 (1.26-3.91)	1.18 (1.09-1.27)
Model 3	1.00 (reference)	1.25 (0.92-1.69)	1.52 (1.03-2.24)	2.14 (1.21-3.79)	1.17 (1.08-1.26)
Coronary heart disease, case (%)	1021 (15.43)	1142 (11.62)	525 (8.60)	113 (8.00)	
Model 1	1.00 (reference)	1.20 (1.07-1.33)	1.23 (1.06-1.41)	1.38 (1.09-1.74)	1.08 (1.05-1.11)
Model 2	1.00 (reference)	1.32 (1.17-1.48)	1.43 (1.22-1.67)	1.54 (1.19-2.00)	1.12 (1.08-1.15)
Model 3	1.00 (reference)	1.35 (1.20-1.52)	1.49 (1.27-1.75)	1.65 (1.27-2.15)	1.13 (1.10-1.17)

Model 1, adjusted for age and sex; Model 2: adjusted for age, sex, education, smoking status, drinking status, physical activity, healthy dietary, body mass index; Model 3, further adjusted for systolic blood pressure, total cholesterol, triglycerides and HbA1c.

were 1.11 (1.06-1.17), 1.17 (1.08-1.26), and 1.13 (1.10-1.17), respectively, in fully adjusted analyses. We did not observe sex differences for the association between age at diagnosis of diabetes and risk of CVD (*P* for interaction >0.05, **Supplementary Table 1**).

Sensitivity analysis was performed to evaluate the modification of the medication use on the association. After further adjustment for insulin therapy and oral hypoglycemic drugs, the estimates of CVD risk moderated but remained significant, with the OR (95%CI) of 1.25 (1.09-1.79), 1.32 (1.13-1.54), 1.39 (1.09-1.79) for individuals diagnosed at 50-59, 40-49 and 18-39 years, respectively, compared with participants diagnosed ≥60 years. Each 5-year younger age at diagnosis of diabetes was significantly associated with 10% increased risk of CVD (OR, 1.10; 95%CI, 1.17-1.14). Further adjustment for lipid-lowering treatment, antihypertensive drugs use and probable depression, the estimates did not change significantly (**Supplementary Table 2**).

The Interaction Effect of BMI on the Association of Age at Diagnosis of Diabetes With CVD

In the BMI stratified analysis, earlier age at diagnosis of type 2 diabetes was associated with higher risk of total CVD, and the association was more pronounced among participants with obesity (**Table 3**). After multivariable adjustment, every 5 years earlier of age at diagnosis of diabetes was significantly associated with the ORs (95%CIs) of CVD of 1.09 (1.03-1.15), 1.14 (1.09-1.19), and 1.24 (1.17-1.33) among participants with normal weight, overweight, and obesity, respectively. Significant interaction was found between age at diagnosis of diabetes and BMI categories for risk of total CVD (*P* for interaction= 0.0457). Significant increased risk of stroke was observed for those with earlier age at diagnosis of type 2 diabetes among people with overweight or obesity, while no significant association was observed among patients with normal weight (*P* for interaction= 0.0205). The association between age at diagnosis of diabetes and the risk of myocardial infarction or CHD was directionally consistent with the combined analysis of total CVD, although the interactions were not significant (*P* for interaction = 0.1931 for myocardial infarction, and *P* for interaction = 0.1568 for CHD).

We further explored the combined effects of BMI and age at diagnosis of diabetes on the risk of CVD (**Figure 1**). Multivariate-adjusted logistic regression analysis revealed that compared with the reference group (who were diagnosed ≥60 years and with normal weight), those with earlier age at diagnosis and with obesity or overweight had significantly increased risk of total CVD or individual CVD components. Overall, the ORs (95%CI) for risk of total CVD, stroke, myocardial infarction, and CHD associated early age at diagnosis of diabetes and obesity (diagnosed <40 years and with obesity *vs* diagnosed ≥60 years and with normal weight) were 3.57 (2.37-5.37), 2.45 (1.18-5.10), 4.58 (1.76-11.96), and 3.29 (2.05-5.29), respectively.

DISCUSSION

In this large population-based study, we found that age at diagnosis of type 2 diabetes was associated with the risk of total CVD, stroke, myocardial infarction, and CHD among Chinese adults. The association of age at diagnosis of type 2 diabetes with risk of CVD was more pronounced among patients with obesity compared to those with normal weight, indicating

TABLE 3 | Association between age at diagnosis of diabetes and cardiovascular disease risk by BMI categories.

	Cardiovascular disease		Stroke		Myocardial infarction		Coronary heart disease	
	Case (%)	OR (95%CI)	Case (%)	OR (95%CI)	Case (%)	OR (95%CI)	Case (%)	OR (95%CI)
BMI< 24kg/m^2								
≥60	366 (16.34)	1.00 (reference)	107 (4.78)	1.00 (reference)	36 (1.61)	1.00 (reference)	270 (12.05)	1.00 (reference)
50-59	399 (11.50)	1.29 (1.06-1.57)	107 (3.08)	1.02 (0.73-1.41)	27 (0.78)	0.83 (0.46-1.51)	304 (8.76)	1.39 (1.11-1.74)
40-49	175 (7.87)	1.30 (1.002-1.69)	47 (2.11)	1.09 (0.70-1.70)	17 (0.76)	0.99 (0.46-2.13)	128 (5.75)	1.29 (0.96-1.75)
18-39	49 (8.55)	1.81 (1.21-2.69)	10 (1.75)	1.01 (0.46-2.23)	5 (0.87)	1.70 (0.58-4.98)	38 (6.63)	1.89 (1.20-2.96)
Per 5-years earlier		1.09 (1.03-1.15)		1.01 (0.92-1.10)		1.09 (0.95-1.26)		1.10 (1.03-1.16)
BMI 24-27.9 kg/m^2								
≥60	614 (20.87)	1.00 (reference)	163 (5.54)	1.00 (reference)	59 (2.01)	1.00 (reference)	462 (15.70)	1.00 (reference)
50-59	684 (15.91)	1.34 (1.15-1.56)	167 (3.88)	1.37 (1.04-1.80)	74 (1.72)	1.33 (0.86-2.06)	530 (12.33)	1.35 (1.14-1.61)
40-49	325 (12.07)	1.49 (1.21-1.84)	86 (3.19)	1.63 (1.13-2.35)	46 (1.71)	1.66 (0.95-2.90)	237 (8.80)	1.39 (1.10-1.76)
18-39	59 (10.55)	1.53 (1.07-2.19)	16 (2.86)	1.90 (1.02-3.54)	8 (1.43)	2.01 (0.84-4.81)	46 (8.23)	1.49 (1.003-2.23)
Per 5-years earlier		1.14 (1.09-1.19)		1.13 (1.05-1.22)		1.18 (1.06-1.32)		1.13 (1.07-1.18)
BMI ≥ 28kg/m^2								
≥60	357 (24.86)	1.00 (reference)	80 (5.57)	1.00 (reference)	29 (2.02)	1.00 (reference)	289 (20.13)	1.00 (reference)
50-59	392 (19.04)	1.44 (1.16-1.79)	91 (4.42)	1.45 (0.97-2.16)	36 (1.75)	1.74 (0.94-3.24)	308 (14.96)	1.40 (1.11-1.77)
40-49	192 (16.20)	1.99 (1.48-2.68)	41 (3.46)	1.78 (1.03-3.08)	21 (1.77)	2.13 (0.95-4.81)	160 (13.50)	2.12 (1.54-2.91)
18-39	41 (14.59)	2.30 (1.45-3.67)	11 (3.91)	2.30 (1.003-5.29)	7 (2.49)	3.33 (1.10-10.10)	29 (10.32)	1.96 (1.16-3.31)
Per 5-years earlier		1.24 (1.17-1.33)		1.24 (1.12-1.38)		1.25 (1.07-1.45)		1.22 (1.14-1.30)

Adjusted for age, sex, education, smoking status, drinking status, physical activity, healthy dietary, systolic blood pressure, total cholesterol, triglycerides and HbA1c. P for interaction for cardiovascular disease = 0.0457; p for interaction for stroke = 0.0205; p for interaction for myocardial infarction= 0.1931; p for interaction for coronary heart disease = 0.1568. BMI, body mass index.

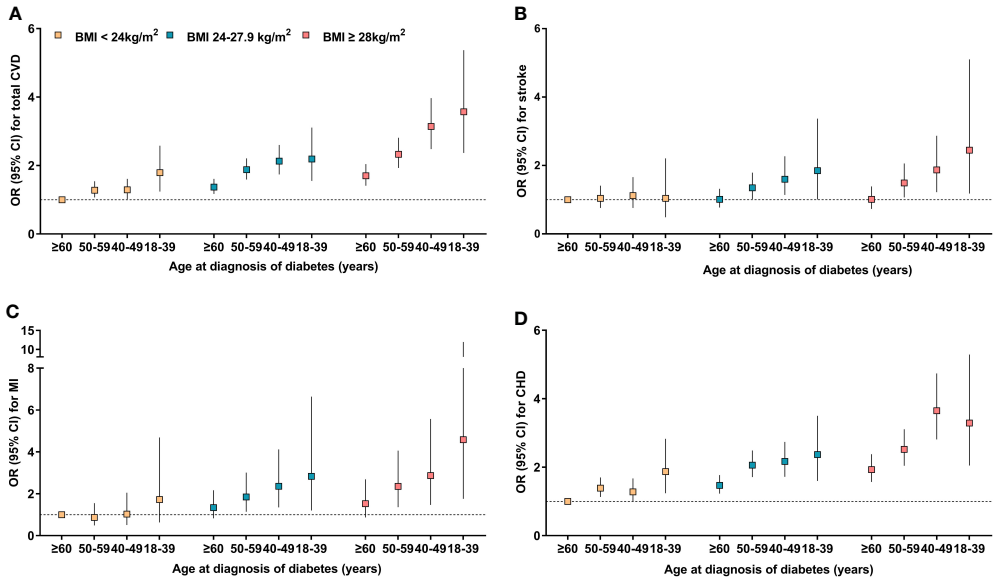

FIGURE 1 | Adjusted odds ratios for cardiovascular disease risk according to combinations of BMI and age at diagnosis of diabetes. **(A)** cardiovascular disease; **(B)** Stroke; **(C)** Myocardial infarction; **(D)** Coronary heart disease. ORs (95% CIs) were adjusted for age, sex, education, smoking status, drinking status, physical activity, healthy dietary, systolic blood pressure, total cholesterol, triglycerides and HbA1c.

that obesity might modify the association between age at diagnosis of type 2 diabetes and CVD risk. To the best of our knowledge, this is the first nation-wide cohort study to explore the modifying effect of obesity on the association between diabetes onset age and the CVD risk. These findings have important clinical implications.

Young-onset type 2 diabetes has been associated with an increased CVD risk in previous epidemiology studies (3, 10, 11, 13, 31, 32). Our study is consistent with some previous studies (8, 13–15), emphasized that those with earlier-onset type 2 diabetes might be associated with worse macrovascular outcomes than later-onset type 2 diabetes. In the cross-sectional study from the China National HbA1c Surveillance System, patients with early-onset type 2 diabetes had an increased risk of non-fatal CVD than people with late-onset diabetes after further adjustment for diabetes duration (8). A study including 7,844 newly diagnosed diabetes reported that the hazard of macrovascular complication in early-onset type 2 diabetic patients was twice as high in usual-onset diabetes compared with age-matched control subjects (14). Similarly, a recent meta-analysis including 26 observational studies comprising 1,325,493 individuals confirmed that age at diagnosis of diabetes was inversely associated with risk of macrovascular disease, with each 1-year increase in age at diabetes diagnosis associated with a 3% decreased risk of macrovascular disease (OR 0.97, 95%CI 0.96- 0.98) (15). However, some studies suggested there was a positive (16, 17) or null association (9, 18) between age at diagnosis of diabetes and CVD risk. A cross-sectional study from the 2015 Australian National Diabetes Audit (16) suggested that 1 year increase of age at diagnosis of diabetes was associated with increased risk of macrovascular complications (OR, 1.03, 95%CI 1.02-1.04). A 7-years prospective analysis concluded that the higher risk of CVD in patients with young-onset diabetes was driven by longer diabetes duration (9). Results from the Da Qing Diabetes Study indicated that the risk of CVD events did not differ significantly between those age <45 years with diabetes and those age ≥45 years with normal glucose tolerance (HR 1.25, 95% CI 0.93-1.69) (18). The inconsistent findings might be partially explained by the wide variety in population characteristics and the study design. Our study provided evidence from the nation-wide community-dwelling Chinese population, emphasized that those with early-onset diabetes might be associated with worse macrovascular outcomes than late-onset diabetes.

More importantly, we contribute new knowledge about the modifying effect of BMI on the association between early-onset diabetes and CVD risk. Obesity was emphasized to be an important risk factor of diabetes and cardiometabolic disease (33). Over the past four decades, the prevalence of obesity in China has increased steadily, especially in young people. The emerging prevalence of obesity in childhood and adolescent is associated with emergence of comorbidities previously considered to be "elderly" diseases including type 2 diabetes mellitus (34, 35). It was reported that obesity was a more general feature of early-onset type 2 diabetes than it is in later-onset type 2 diabetes (3, 36). A prospective study including 1,462,362 adolescents confirmed that severe obesity significantly increased the risk for incidence of type 2 diabetes in young (20). Importantly, we found that the association of age at diagnosis of diabetes with risk of CVD was more pronounced among patients with obesity or overweight than those with

normal weight. Thus, obesity may play an important role in the development of the young-onset phenotype. Interventions of obesity may be effective for the early prevention of CVD among people with early-onset type 2 diabetes.

Potential pathogenetic mechanisms leading to young-onset type 2 diabetes as an aggressive disease compared to late-onset diabetes including the rapid onset of beta cell failure and insulin resistance, and the obesity-related mechanisms (3, 36). Prevailing evidence suggests that young-onset type 2 diabetes has an accelerated decline of beta cell function, especially in second-phase insulin secretion (37, 38). Loss of glycemic control and other metabolic profiles also play essential roles in excessive risk of macrovascular or microvascular complications (5). Recent analysis from Hong Kong found that individuals with early-onset type 2 diabetes had poorly controlled hyperglycemia throughout their life *versus* usual-onset type 2 diabetes (32). Results from the Treatment Options for type 2 Diabetes in Adolescents and Youth Study confirmed that young adults with type 2 diabetes had poor glycemic control regardless of health care coverage (6). Similarly, in our study, those with earlier-onset diabetes tend to have higher HbA1c and triglycerides than patients with later-onset diabetes. Although patients with early-onset diabetes receive more glucose-lowering treatment, they have poor glycemic control. Thus, it is important to improve the effectiveness of blood glucose management for individuals with early-onset diabetes. Besides, younger-onset diabetes often usually has a longer diabetes duration, which was a noticeable traditional risk factor for diabetes complications (16, 17, 39–41).

The study has several strengths. First, the study population was from the REACTION study, which was a multicenter population-based cohort study representing middle-aged and elderly population from various geographical regions in mainland China. Besides, we have comprehensive physical and biochemical measurements, and detailed lifestyle information for the confounding adjustment. However, several potential limitations should be discussed. First, in the current study, the collection of CHD, stroke, and myocardial infarction status was based on self-reported questionnaire. Although we did a validation study of CVD in one of the communities and the validation rate is about 91.1% (29, 30), we still can't exclude recall bias. Second, the cross-sectional nature may underestimate the risk of CVD associated with early-onset diabetes. Since patients with earlier-onset type 2 diabetes would be more vulnerable to premature death (42). Long-term follow-up study could provide more information in the future. In addition, our study population is limited to 40 years or older. Thus, patients with early-onset diabetes with a relatively short duration were not included in the study.

In conclusion, this large population-based cohort study indicates that age at diagnosis of type 2 diabetes is related to the risk of CVD in patients with type 2 diabetes in China, and this association is more prominent among patients with obesity. The excess risk related to early-onset diabetes mandates more attention to preventive strategies and management guidelines in the Chinese population, and underscore the importance for weight management among early-onset type 2 diabetes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethic committee of Ruijin hospital Affiliated to Shanghai Jiaotong University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CH, LL, JL, YC, WW, and GN contributed to the concept and design. CH and LL contributed to the acquisition and analysis of data. CH drafted the manuscript. LL, JL and DL edited the manuscript. YJZ, YZ, SYW, JZ, HQ, ML, YYZ, YH, QW, YQ, RH, LS, QS, XY, LY, GQ, XT, GC, MX, YX, TW, ZZ, ZG, GW, FS, ZL, LC, QL, ZY, YFZ, CL, YW, SLW, TY, HD, LLC, TZ, JJZ, YM, YB, WW, GN, YC and JL collected data. All authors made important contributions to critically revising the manuscript for important intellectual content. GN, WW, YB, YC and JL guarantee this work and have full access to the data and take responsibility for the integrity of the data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.717069/full#supplementary-material

REFERENCES

1. Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, et al. Prevalence of Diabetes Recorded in Mainland China Using 2018 Diagnostic Criteria From the American Diabetes Association: National Cross Sectional Study. *BMJ* (2020) 369:m997. doi: 10.1136/bmj.m997
2. Xu Y, Wang L, He J, Bi Y, Li M, Wang T, et al. Prevalence and Control of Diabetes in Chinese Adults. *JAMA* (2013) 310(9):948–59. doi: 10.1001/jama.2013.168118
3. Magliano DJ, Sacre JW, Harding JL, Gregg EW, Zimmet PZ, Shaw JE. Young-Onset Type 2 Diabetes Mellitus - Implications for Morbidity and Mortality. *Nat Rev Endocrinol* (2020) 16(6):321–31. doi: 10.1038/s41574-020-0334-z
4. Viner R, White B, Christie D. Type 2 Diabetes in Adolescents: A Severe Phenotype Posing Major Clinical Challenges and Public Health Burden. *Lancet* (2017) 389(10085):2252–60. doi: 10.1016/S0140-6736(17)31371-5
5. Yeung RO, Zhang Y, Luk A, Yang W, Sobrepena L, Yoon KH, et al. Metabolic Profiles and Treatment Gaps in Young-Onset Type 2 Diabetes in Asia (the JADE Programme): A Cross-Sectional Study of a Prospective Cohort. *Lancet Diabetes Endocrinol* (2014) 2(12):935–43. doi: 10.1016/S2213-8587(14)70137-8
6. Group TS. Health Care Coverage and Glycemic Control in Young Adults With Youth-Onset Type 2 Diabetes: Results From the TODAY2 Study. *Diabetes Care* (2020) 43(10):2469–77. doi: 10.2337/dc20-0760
7. Fang M, Echouffo-Tcheugui JB, Selvin E. Burden of Complications in U.S. Adults With Young-Onset Type 2 or Type 1 Diabetes. *Diabetes Care* (2020) 43(4):e47–9. doi: 10.2337/dc19-2394
8. Huo X, Gao L, Guo L, Xu W, Wang W, Zhi X, et al. Risk of non-Fatal Cardiovascular Diseases in Early-Onset *Versus* Late-Onset Type 2 Diabetes in China: A Cross-Sectional Study. *Lancet Diabetes Endocrinol* (2016) 4(2):115–24. doi: 10.1016/S2213-8587(15)00508-2
9. Chan JC, Lau ES, Luk AO, Cheung KK, Kong AP, Yu LW, et al. Premature Mortality and Comorbidities in Young-Onset Diabetes: A 7-Year Prospective Analysis. *Am J Med* (2014) 127(7):616–24. doi: 10.1016/j.amjmed.2014.03.018
10. Morton JI, Liew D, McDonald SP, Shaw JE, Magliano DJ. The Association Between Age of Onset of Type 2 Diabetes and the Long-Term Risk of End-Stage Kidney Disease: A National Registry Study. *Diabetes Care* (2020) 43(8):1788–95. doi: 10.2337/dc20-0352
11. Kim SM, Lee G, Choi S, Kim K, Jeong SM, Son JS, et al. Association of Early-Onset Diabetes, Prediabetes and Early Glycaemic Recovery With the Risk of All-Cause and Cardiovascular Mortality. *Diabetologia* (2020) 63(11):2305–14. doi: 10.1007/s00125-020-05252-y
12. Constantino MI, Molyneaux L, Limacher-Gisler F, Al-Saeed A, Luo C, Wu T, et al. Long-Term Complications and Mortality in Young-Onset Diabetes: Type 2 Diabetes is More Hazardous and Lethal Than Type 1 Diabetes. *Diabetes Care* (2013) 36(12):3863–9. doi: 10.2337/dc12-2455
13. Zhao M, Song L, Sun L, Wang M, Wang C, Yao S, et al. Associations of Type 2 Diabetes Onset Age With Cardiovascular Disease and Mortality: The Kailuan Study. *Diabetes Care* (2021) 44(6):1426–32. doi: 10.2337/DC20-2375
14. Hillier TA, Pedula KL. Complications in Young Adults With Early-Onset Type 2 Diabetes: Losing the Relative Protection of Youth. *Diabetes Care* (2003) 26(11):2999–3005. doi: 10.2337/diacare.26.11.2999
15. Nanayakkara N, Curtis AJ, Heritier S, Gadowski AM, Pavkov ME, Kenealy T, et al. Impact of Age at Type 2 Diabetes Mellitus Diagnosis on Mortality and Vascular Complications: Systematic Review and Meta-Analyses. *Diabetologia* (2020) 64(2):275–87. doi: 10.1007/s00125-020-05319-w
16. Nanayakkara N, Ranasinha S, Gadowski A, Heritier S, Flack JR, Wischer N, et al. Age, Age at Diagnosis and Diabetes Duration Are All Associated With Vascular Complications in Type 2 Diabetes. *J Diabetes Complicat* (2018) 32(3):279–90. doi: 10.1016/j.jdiacomp.2017.11.009
17. Zoungas S, Woodward M, Li Q, Cooper ME, Hamet P, Harrap S, et al. Impact of Age, Age at Diagnosis and Duration of Diabetes on the Risk of Macrovascular and Microvascular Complications and Death in Type 2 Diabetes. *Diabetologia* (2014) 57(12):2465–74. doi: 10.1007/s00125-014-3369-7
18. Hui Y, Wang J, An Y, Gong Q, Li H, Zhang B, et al. Premature Death and Risk of Cardiovascular Disease in Young-Onset Diabetes: A 23-Year Follow-Up of the Da Qing Diabetes Study. *Endocrine* (2019) 65(1):46–52. doi: 10.1007/s12020-019-01928-7
19. Li Y, Wang DD, Ley SH, Vasanti M, Howard AG, He Y, et al. Time Trends of Dietary and Lifestyle Factors and Their Potential Impact on Diabetes Burden in China. *Diabetes Care* (2017) 40(12):1685–94. doi: 10.2337/dc17-0571
20. Twig G, Zucker I, Afek A, Cukierman-Yaffe T, Bendor CD, Derazne E, et al. Adolescent Obesity and Early-Onset Type 2 Diabetes. *Diabetes Care* (2020) 43(7):1487–95. doi: 10.2337/dc19-1988
21. Bjerregaard LG, Jensen BW, Angquist L, Osler M, Sorensen TIA, Baker JL. Change in Overweight From Childhood to Early Adulthood and Risk of Type 2 Diabetes. *N Engl J Med* (2018) 378(14):1302–12. doi: 10.1056/NEJMoa1713231
22. Stokes A, Collins JM, Grant BF, Scamuffa RF, Hsiao CW, Johnston SS, et al. Obesity Progression Between Young Adulthood and Midlife and Incident Diabetes: A Retrospective Cohort Study of U.S. Adults. *Diabetes Care* (2018) 41(5):1025–31. doi: 10.2337/dc17-2336
23. Tirosh A, Shai I, Afek A, Dubnov-Raz G, Ayalon N, Gordon B, et al. Adolescent BMI Trajectory and Risk of Diabetes *Versus* Coronary Disease. *N Engl J Med* (2011) 364(14):1315–25. doi: 10.1056/NEJMoa1006992
24. Zhao D, Liu J, Wang M, Zhang X, Zhou M. Epidemiology of Cardiovascular Disease in China: Current Features and Implications. *Nat Rev Cardiol* (2019) 16(4):203–12. doi: 10.1038/s41569-018-0119-4
25. Hu C, Zhang Y, Zhang J, Huo Y, Wan Q, Li M, et al. Age at Menarche, Ideal Cardiovascular Health Metrics, and Risk of Diabetes in Adulthood: Findings From the REACTION Study. *J Diabetes* (2020) 13(6):458–68. doi: 10.1111/1753-0407.13128
26. Bi Y, Lu J, Wang W, Mu Y, Zhao J, Liu C, et al. Cohort Profile: Risk Evaluation of Cancers in Chinese Diabetic Individuals: A Longitudinal (REACTION) Study. *J Diabetes* (2014) 6(2):147–57. doi: 10.1111/1753-0407.12108
27. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, et al. Defining and Setting National Goals for Cardiovascular Health Promotion and Disease Reduction: The American Heart Association's Strategic Impact Goal Through 2020 and Beyond. *Circulation* (2010) 121(4):586–613. doi: 10.1161/CIRCULATIONAHA.109.192703
28. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: Validity of a Brief Depression Severity Measure. *J Gen Intern Med* (2001) 16(9):606–13. doi: 10.1046/j.1525-1497.2001.016009606.x
29. Lu J, Mu Y, Su Q, Shi L, Liu C, Zhao J, et al. Reduced Kidney Function Is Associated With Cardiometabolic Risk Factors, Prevalent and Predicted Risk of Cardiovascular Disease in Chinese Adults: Results From the REACTION Study. *J Am Heart Assoc* (2016) 5(7):e003328. doi: 10.1161/JAHA.116.003328
30. Du R, Zheng R, Xu Y, Zhu Y, Yu X, Li M, et al. Early-Life Famine Exposure and Risk of Cardiovascular Diseases in Later Life: Findings From the REACTION Study. *J Am Heart Assoc* (2020) 9(7):e014175. doi: 10.1161/JAHA.119.014175
31. Koye DN, Ling J, Dibato J, Khunti K, Montvida O, Paul SK. Temporal Trend in Young-Onset Type 2 Diabetes-Macrovascular and Mortality Risk: Study of U.K. Primary Care Electronic Medical Records. *Diabetes Care* (2020) 43(9):2208–16. doi: 10.2337/dc20-0417
32. Ke C, Stukel TA, Shah BR, Lau E, Ma RC, So WY, et al. Age at Diagnosis, Glycemic Trajectories, and Responses to Oral Glucose-Lowering Drugs in Type 2 Diabetes in Hong Kong: A Population-Based Observational Study. *PloS Med* (2020) 17(9):e1003316. doi: 10.1371/journal.pmed.1003316
33. Bendor CD, Bardugo A, Pinhas-Hamiel O, Afek A, Twig G. Cardiovascular Morbidity, Diabetes and Cancer Risk Among Children and Adolescents With Severe Obesity. *Cardiovasc Diabetol* (2020) 19(1):79. doi: 10.1186/s12933-020-01052-1
34. Perng W, Oken E, Dabelea D. Developmental Overnutrition and Obesity and Type 2 Diabetes in Offspring. *Diabetologia* (2019) 62(10):1779–88. doi: 10.1007/s00125-019-4914-1
35. Lobstein T, Jackson-Leach R, Moodie ML, Hall KD, Gortmaker SL, Swinburn BA, et al. Child and Adolescent Obesity: Part of a Bigger Picture. *Lancet* (2015) 385(9986):2510–20. doi: 10.1016/S0140-6736(14)61746-3
36. Lascar N, Brown J, Pattison H, Barnett AH, Bailey CJ, Bellary S. Type 2 Diabetes in Adolescents and Young Adults. *Lancet Diabetes Endocrinol* (2018) 6(1):69–80. doi: 10.1016/S2213-8587(17)30186-9
37. Druet C, Tubiana-Rufi N, Chevenne D, Rigal O, Polak M, Levy-Marchal C. Characterization of Insulin Secretion and Resistance in Type 2 Diabetes of

Adolescents. *J Clin Endocrinol Metab* (2006) 91(2):401–4. doi: 10.1210/jc.2005-1672
38. Mohan V, Amutha A, Ranjani H, Unnikrishnan R, Datta M, Anjana RM, et al. Associations of Beta-Cell Function and Insulin Resistance With Youth-Onset Type 2 Diabetes and Prediabetes Among Asian Indians. *Diabetes Technol Ther* (2013) 15(4):315–22. doi: 10.1089/dia.2012.0259
39. Huo L, Magliano DJ, Ranciere F, Harding JL, Nanayakkara N, Shaw JE, et al. Impact of Age at Diagnosis and Duration of Type 2 Diabetes on Mortality in Australia 1997-2011. *Diabetologia* (2018) 61(5):1055–63. doi: 10.1007/s00125-018-4544-z
40. Yeap BB, McCaul KA, Flicker L, Hankey GJ, Almeida OP, Golledge J, et al. Diabetes, Myocardial Infarction and Stroke are Distinct and Duration-Dependent Predictors of Subsequent Cardiovascular Events and All-Cause Mortality in Older Men. *J Clin Endocrinol Metab* (2015) 100(3):1038–47. doi: 10.1210/jc.2014-3339
41. Noh M, Kwon H, Jung CH, Kwon SU, Kim MS, Lee WJ, et al. Impact of Diabetes Duration and Degree of Carotid Artery Stenosis on Major Adverse Cardiovascular Events: A Single-Center, Retrospective, Observational Cohort Study. *Cardiovasc Diabetol* (2017) 16(1):74. doi: 10.1186/s12933-017-0556-0
42. Al-Saeed AH, Constantino MI, Molyneaux L, D'Souza M, Limacher-Gisler F, Luo C, et al. An Inverse Relationship Between Age of Type 2 Diabetes Onset and Complication Risk and Mortality: The Impact of Youth-Onset Type 2 Diabetes. *Diabetes Care* (2016) 39(5):823–9. doi: 10.2337/dc15-0991

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Waist-To-Height Ratio Is a More Accurate Tool for Predicting Hypertension Than Waist-To-Hip Circumference and BMI in Patients With Type 2 Diabetes: A Prospective Study

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Background: Anthropometric measures [i.e., body mass index (BMI), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR)] have been used as prediction factors for incident hypertension. However, whether any of these measures is superior to another in the matter of accuracy in predicting hypertension in diabetic patients has been controversial. The present prospective study aimed to determine whether WHtR is a more accurate tool for predicting hypertension than WHR and BMI in patients with type 2 diabetes.

Methods: The study population consisted of 1,685 normotensive patients with type 2 diabetes. BMI, WHR, and WHtR were assessed at baseline and followed up for hypertension incidence for a mean of 4.8 years. A cox regression analysis was performed to assess the association between anthropometric measures (i.e., BMI, WHR, and WHtR) and incident hypertension during the follow-up period. The area under the ROC curve analysis was performed and optimal cutoff values were calculated for each anthropometric measure for hypertension prediction.

Results: WHtR and BMI were significantly associated with an increased incidence of hypertension (HR = 3.296 (0.936–12.857), $P < 0.001$, and HR = 1.050 (1.030–1.070), $P < 0.001$, respectively). The discriminative powers for each anthropometric index for hypertension were 0.571 (0.540–0.602) for BMI, 0.518 (0.486–0.550) for WHR, and 0.609 (0.578–0.639) for WHtR. The optimal cutoff points for predicting hypertension in patients with type 2 diabetes were 26.94 (sensitivity = 0.739, specificity = 0.380)

for BMI, 0.90 (sensitivity = 0.718, specificity = 0.279) for WHR, and 0.59 (sensitivity = 0.676, specificity = 0.517) for WHtR.

Conclusion: WHtR was a more accurate tool for predicting hypertension compared to WHR and BMI in patients with type 2 diabetes.

Keywords: hypertension, type 2 diabetes, body mass index, waist-to-height ratio, waist-to-hip ratio

INTRODUCTION

Diabetes mellitus is among the most common diseases worldwide that significantly affects the cardiovascular system. The main cause of death among patients with diabetes is cardiovascular diseases (CVD) (1–3). Diabetes mellitus is associated with several major cardiovascular complications, including coronary artery disease (myocardial infarction and angina pectoris), heart failure, stroke, and peripheral artery disease. Besides, cardiovascular risk factors increase the risk of developing these complications. Hypertension (HTN), a highly prevalent cardiovascular risk factor in patients with diabetes (present in 35% of men and 46% of women with diabetes), causes three-quarters of the cardiovascular deaths in these patients (4). The hypertension diagnosis is confirmed in a gradual process, requiring multiple careful blood pressure assessments at different times (5). Late diagnosis of hypertension extends the harmful effects of high blood pressure on the cardiovascular system, promoting the onset of irrecoverable sequelae (6).

Since obesity, particularly abdominal obesity, plays a significant role in the etiology of hypertension, using anthropometric indicators of adiposity may help screen patients who are at a higher risk for developing hypertension. The identified individuals could be referred to health centers with better hypertension monitoring systems earlier, and therefore, the early diagnosis of hypertension optimizes the opportunity for secondary prevention measures (7).

Body mass index (BMI), the standard general obesity measure, is the most commonly used indicator in hypertension prediction and screening. The adipose tissue distribution is a significant factor in developing cardiovascular diseases compared to total body fat. Thus, several measures that consider the distribution of adipose tissue, including WHR (waist-to-hip ratio) and WHtR (waist-to-height ratio) have been developed (8). Researchers have conducted numerous studies to assess the associations between the various obesity indicators and hypertension in recent decades. Cuban and Japanese studies have suggested BMI as the best single predictor of hypertension (9, 10). In contrast, an Iranian cross-sectional study suggested WHR combined with BMI as the best predictor of hypertension in women (11). A 2006 meta-analysis reported the superiority of centralized obesity measures, especially WHtR over BMI for CVD risk detection (12). However, another meta-analysis in 2008 reported that none of the anthropometric variables were systematically better than the others at hypertension discrimination (13). Therefore, there is no consensus in the literature over the most accurate measure for hypertension prediction. The present prospective study aimed to determine whether WHtR is a more accurate tool for predicting HTN than WHR and BMI in patients with type 2 diabetes.

Abbreviations: HTN, Hypertension; T2DM, Type 2 Diabetes; BMI, Body mass index; WHtR, Waist-to-height ratio; WHR, Waist-to-hip ratio; SBP, Systolic blood pressure;DBP, Diastolic blood pressure; TC, Total cholesterol; TG, Triglycerides; HDL, High density lipoprotein; LDL, Low density lipoprotein; FPG, Fasting plasma glucose;2hpp, 2-hour post prandial; HbA1c, Hemoglobin A1c; HTN, Hypertension; CAD, Coronary artery disease.

MATERIALS AND METHODS

Participants

Four thousand fifty-five patients who were previously diagnosed with type 2 diabetes participated in this study from April 2002 to January 2020. All patients attended the endocrinology clinic of Vali-Asr Hospital, a tertiary center affiliated with Tehran University of Medical Sciences. After excluding the patients with previously diagnosed hypertension (N = 2,362), the ones who failed to attend monthly follow-up, or had missing data on systolic blood pressure, weight, height, or waist circumference (N = 8), the final study population consisted of 1,685 normotensive patients with T2DM (**Figure 1**). The patients' anthropometric measures, including BMI, WHR, and WHtR, were assessed at baseline. Moreover, each participant was followed-up for hypertension for a mean follow-up period of 4.8 years at some point between April 2002 to January 2020. This study was in full compliance with the Declaration of Helsinki and was reviewed and approved by the Ethics Committee of Tehran University of Medical Sciences.

Data Collection

At baseline, all participants were interviewed by a trained interviewer. Data collection at baseline consisted of the following parameters: identification and sociodemographic characteristics, including age, gender, education, marital status, lifestyle habits such as smoking, self-reported health conditions, and specifically, the use of antihypertensive drugs, past medical history, including any physical or mental conditions, and objective measures including, blood pressure and body weight. Data collection during the follow-up period was limited to the following parameters: identification (only age and gender), self-reported health conditions (use of antihypertensive medications), and objective measures (only blood pressure). In this study, current smokers were defined as individuals who had smoked a minimum of 100 cigarettes in their lifetime and continued smoking during the follow-up period. A team of trained and closely observed researchers using standard equipment was responsible for data collection at all times.

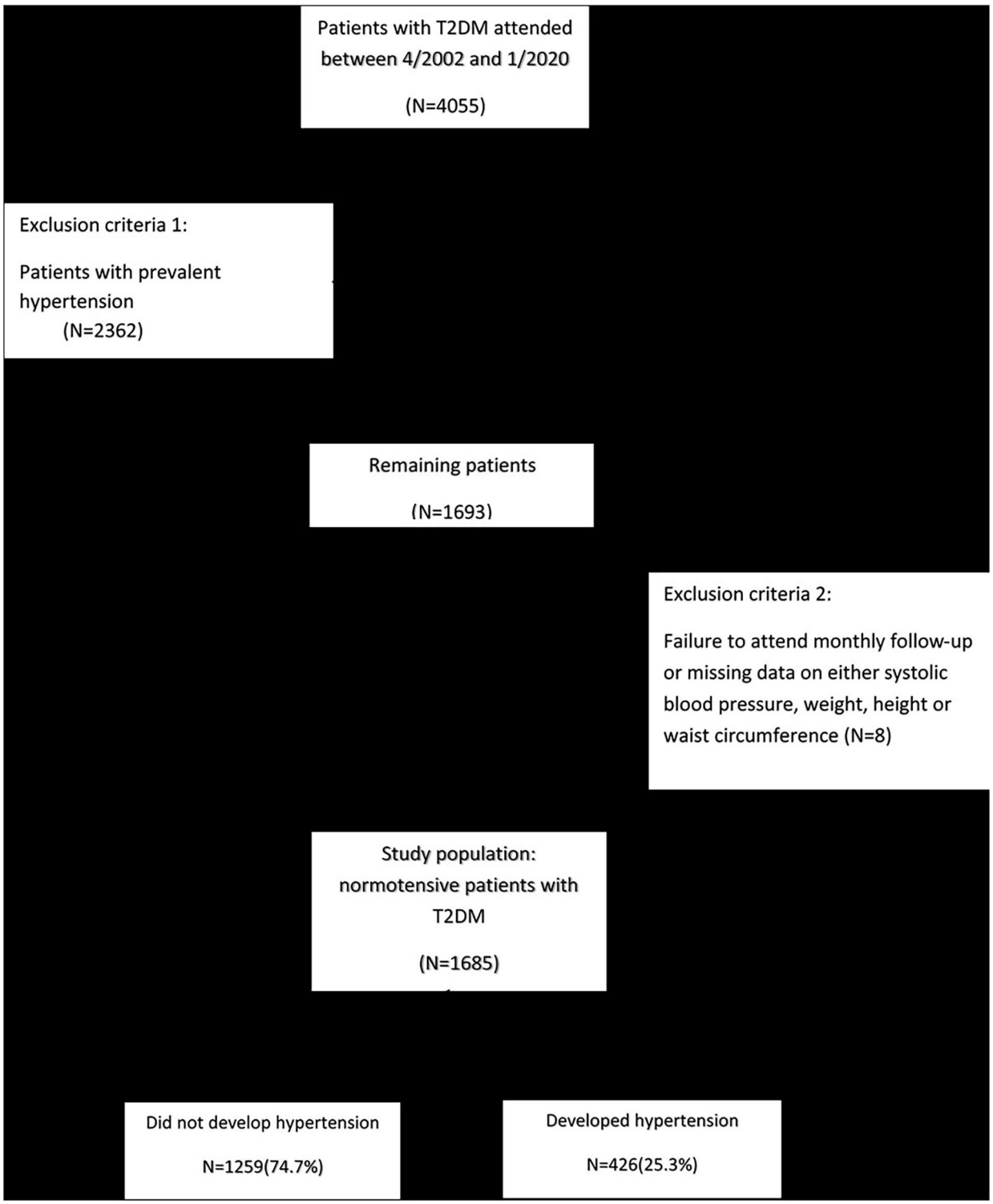


FIGURE 1 | Study population selection flow chart; T2DM = type 2 diabetes mellitus.

Anthropometric Measurements

Weight was measured using a portable digital scale with a precision of 0.1 kg in minimally clothed participants without shoes. Height was measured using a standard protocol with a tape measure. The body mass index (BMI) was calculated as weight (kilograms) divided by squared height (m^2). The BMI values

were categorized according to the World Health Organization (WHO)'s classification: < 18.5 kg/m^2 (underweight), 18.5 to 24.9 kg/m^2 (normal weight), 25 to 29.9 kg/m^2 (overweight) and $\geq$30 kg/m^2 (obese). Waist circumference (WC) was measured at the end of a normal expiration. The tape was positioned horizontally at the midway between the iliac crest and the inferior border of the last rib according to standard WHO protocol (14). Likewise, Hip circumference was measured at the widest circumference of buttock while the tape was horizontal and parallel to the ground. During these procedures, the subjects were standing and wearing minimal clothing. All the measurements were performed with a precision of 0.1 cm. In order to minimize observer error, all measurements were carried out by the same technician. The waist-to-height (WHtR) and waist-to-hip ratio (WHR) were calculated by dividing waist (cm) by height (cm) and hip circumference (cm), respectively.

Laboratory Measurements

From each subject, 10 mL of venous blood was drawn after 12–14 h of overnight fasting. The samples were kept in cold biochemistry tubes with a temperature of 4–8°C and sent to the representative calibrating laboratories within 4 h, where the samples were immediately centrifuged (1,500 RPM, for 10 min at standard room temperature 21°C). Then, the extracted serum was used for laboratory evaluations. Enzymatic calorimetric methods using the glucose oxidase test were implemented to measure the fasting plasma glucose (FPG) and 2 h post-prandial glucose (2 hPPG). Glycated hemoglobin (HbA1c) was measured using high-performance liquid chromatography. By using enzymatic methods, the serum concentrations of triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and total cholesterol were determined. The commercially-available kits, distributed by the central reference laboratory (Tehran, Iran), have passed all the quality control procedures.

Definition of Hypertension

Blood pressure (BP) was measured on the left arm by using a mercury sphygmomanometer (Reishter, Germany) with an appropriate cuff size according to the subjects' arm circumference. The procedure was performed in the sitting position after a 10-min resting period, and all the subjects were asked not to smoke or consume caffeine 30 min preceding the procedure commencement. BP was measured twice, with a 10-min interval between the two measurements. During this interval, the patients were asked to remain seated with their left hand placed at the heart level and their palm in the upward-facing position. The mean of the two measured values were considered as the patient's BP. Auscultated systolic blood pressure (SBP) was defined as the first Korotkoff sound heard during the cuff deflation, while diastolic blood pressure (DBP) was marked by the disappearance of the last Korotkoff sound. Patients using antihypertensive drugs or subjects with an SBP higher than 140 mmHg or a DBP higher than 90 mmHg were classified as hypertensive (15).

Statistical Analysis

SPSS version 24.0 (SPSS Inc., Chicago, IL, USA) was employed for statistical analysis. We used Kolmogorov–Smirnov and Shapiro-Wilk normality tests, P-P plot, and histogram to test the normality of our study population. The null hypothesis was rejected for all the variables; thus, they were normal. To assess the association between different variables and incident hypertension, uni-variable analysis of potential continuous and categorical risk factors was performed using *t*-test and chi-square, respectively. The data were reported as mean ± standard deviation (SD) for continuous variables and as proportions for categorical variables. Cox regression analysis was performed to assess the association between anthropometric measures (i.e., BMI, WHR, and WHtR) and incident hypertension during the follow-up period. Hazard ratios (HR) were adjusted for the confounding variables (i.e., age, gender, history of coronary artery disease (CAD), SBP, DBP, FPG, and family history of hypertension). The Cox regression analysis was also used to calculate HRs of hypertension and their 95% CIs with reference to a normal BMI and the first quartile of WHR and WHtR. The adjustment was performed for age, gender, history of CAD, SBP, DBP, FPG, history of CAD, and family history of hypertension. The discriminative powers of BMI, WHR, and WHtR were assessed by the area under the ROC curve analysis in the group with incident hypertension. The area under the ROC curve of the baseline BMI, WHR, WHtR, and their 95% confidence intervals (CIs) were reported. Optimal cutoff values were calculated for each aforementioned anthropometric measure for the diagnosis of hypertension. Youden index was employed for calculating the optimal cutoff value. The sensitivity and specificity of each of the anthropometric measures were determined based on the calculated cutoff values. The risk of incident hypertension was compared among the BMI categories (i.e., underweight, normal, overweight, and obese) and the quartiles of WHR and WHtR. A two-sided p-value < 0.05 was considered necessary to reject the null hypothesis.

RESULTS

Baseline Characteristics

A total of 1,685 participants were enrolled in this study. The mean follow-up period was 4.8 years, and throughout this period, 426 participants (25.3%) developed hypertension. **Table 1** summarizes the baseline characteristics of all participants based on the development of HTN. A comparison of study subgroups at baseline revealed higher means for age, SBP, DBP, 2-hpp, CAD prevalence, and anthropometric measures, as well as a higher frequency of HTN in family members of the subjects who developed HTN. Furthermore, the two groups differed significantly in terms of BMI and WHtR. The frequency of obese subjects was significantly higher in HTN group (42.8 vs. 37%). Also, a higher proportion of patients who developed HTN were in the fourth quartiles of WHtR (37.6 vs. 24.9%) (**Table 1**).

TABLE 1 | Baseline characteristics of the study population, based on hypertension status at the time of follow-up.

		Did not develop hypertension ($N = 1259$)	Developed hypertension ($N = 426$)	*P*-value
Age(yr)		54.82 (± 11.12)	58.95 (± 9.15)	<0.001
Gender (M)		614 (48.8%)	152 (35.7%)	<0.001
Waist/height ratio		0.60 (± 0.07)	0.63 (± 0.07)	<0.001
Waist/hip ratio		0.93 (± 0.06)	0.94 (± 0.06)	0.130
Weight (kg)		77.66 (± 14.85)	77.92 (± 14.86)	0.756
BMI (kg/m^2)		29.04 (± 4.96)	30.24 (± 5.23)	<0.001
SBP (mmHg)		124.92 (± 13.53)	130.01 (± 9.65)	0.041
DBP (mmHg)		77.99 (± 6.31)	79.18 (± 9.00)	0.038
TC (mg/dL)		183.03 (± 41.96)	181.14 (± 47.97)	0.468
TG (mg/dL)		173.03 (± 109.95)	182.64 (± 98.95)	0.110
HDL (mg/dL)		45.30 (± 12.00)	45.46 (± 13.79)	0.823
LDL (mg/dL)		104.14 (± 33.42)	101.38 (± 37.07)	0.152
FPG (mg/dL)		156.54 (± 52.51)	163.59 (± 52.70)	0.170
2hpp (mg/dL)		215.77 (± 84.37)	236.85 (± 86.05)	<0.001
HbA1c (mg/dL)		7.74 (± 4.97)	7.78 (± 1.50)	0.874
Current smokers, *n* (%)*		28 (2.2)	36 (12.1)	0.033
Family history of HTN, yes, *n* (%)		434 (34.7)	240 (56.7)	<0.001
History of CAD, yes		234 (18.6)	120 (28.2)	<0.001
BMI categorized *n* (%)	Underweight	5 (0.4)	1 (0.2)	<0.001
	Normal	239 (19.0)	49 (11.5)	
	Overweight	558 (44.3)	186 (43.7)	
	Obese	457 (36.3)	190 (44.6)	
Waist-to-hip ratio quartiles *n* (%)	Q1	93 (7.4)	27 (6.3)	0.873
	Q2	296 (23.5)	105 (24.6)	
	Q3	408 (32.4)	136 (31.9)	
	Q4	462 (36.7)	157 (37.1)	
Waist-to-height ratio quartiles *n* (%)	Q1	188 (14.9)	33 (7.7)	<0.001
	Q2	392 (31.1)	94 (22.1)	
	Q3	366 (29.1)	139 (32.6)	
	Q4	313 (24.9)	160 (37.6)	

**n(%): Number of patients who matched each category and the percentage in each patient group. BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; TC, Total cholesterol; TG, Triglycerides; HDL, High density lipoprotein; LDL, Low density lipoprotein; FPG, Fasting plasma glucose; 2 hpp, 2 h post prandial; HbA1c, Hemoglobin A1c; HTN, Hypertension; CAD, Coronary artery disease.*

Development of Hypertension in Patients With T2DM According to WHtR, WHR, and BMI

After adjusting for age, gender, history of CAD, SBP, DBP, FPG, and family history of HTN, WHtR and BMI were found to be significantly associated with an increased risk of hypertension (HR = 3.296, 95% CI: 0.936–12.857, $P < 0.001$, and HR = 1.050, 95%CI: 1.030–1.070, $P < 0.001$, respectively). However, this association was not significant for WHR (**Table 2**). In another adjusted cox regression model assessing the association between incident hypertension and different categories of BMI (i.e., underweight, normal, overweight, and obese), and four quartiles of WHR and WHtR, a higher WHtR at the baseline, was positively and significantly associated with the development of hypertension in a ratio-dependent manner (highest vs. lowest quartile HR 1.936, 95%CI: 1.306–2.871) (**Table 3**).

TABLE 2 | Cox regression analysis determining the association between BMI, waist-to-hip ratio, and waist-to-height ratio with incident hypertension during the follow-up period; results are adjusted for age, gender, history of CAD, SBP, DBP, FPG and family history of HTN.

	HR (95% CI)	*P*-value
BMI	1.050 (1.030–1.070)	<0.001
Waist-to-hip ratio	2.604 (0.605–10.477)	0.159
Waist-to-height ratio	3.296 (0.936–12.857)	<0.001

BMI, Body mass index.

ROC Analysis of WHtR, WHR, and BMI for Prediction of Hypertension in Patients With T2DM

Figure 2 shows the areas under the ROC curve (AUC) of the anthropometric measurements for the prediction of HTN. All anthropometric indices, especially WHtR, exhibited a predictive power in detecting the incidence of HTN in patients with T2DM. The discriminative powers of each anthropometric index for HTN were 0.571 (95% CI: 0.540–0.602) for BMI, 0.518 (95% CI: 0.486–0.550) for WHR, and 0.609 (95% CI: 0.578–0.639) for WHtR. The optimal cutoff points for predicting HTN in patients with T2DM were 26.94 (sensitivity = 0.739, specificity = 0.380) for BMI, 0.90 (sensitivity = 0.718, specificity = 0.279) for WHR, and 0.59 (sensitivity = 0.676, specificity = 0.517) for WHtR.

DISCUSSION

More than 4.5 million Iranian adults were diagnosed with diabetes in 2011; this number is projected to rise to 9.2 million by 2030. This increase emphasizes the significance of type 2 diabetes, most importantly, when considering the burden of diabetes-related complications (16). Several studies have reported high prevalence of hypertension in patients with diabetes (17–22). The existence of hypertension in patients with diabetes significantly increases the risk of stroke, coronary artery disease, retinopathy, and nephropathy (23). Therefore, identifying the risk factors and predictors of hypertension is of high importance to diagnose, prevent, control, and treat this condition as soon as possible. Our results indicated higher means for age, TG, CAD prevalence, smoking status, and anthropometric measures, as well as a higher frequency of HTN in family members of the subjects who developed HTN. Additionally, the frequency of obese subjects was significantly higher in the HTN group. These results are in line with the data from previous studies on the associated risk factors of hypertension (24).

TABLE 3 | Hazard ratio of incident hypertension based on waist-to-height ratio, waist-to-hip ratio and BMI during the follow-up period; Data are presented as HR (95% CI); Results are adjusted for age, gender, history of CAD, SBP, DBP, FPG and family history of HTN.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*-value
Waist-to-hip ratio	Ref	1.247 (0.812–1.914)	1.039 (0.680–1.587)	1.246 (0.810–1.917)	0.328
Waist- to-height ratio	Ref	1.354 (0.905–2.025)	1.534 (1.034–2.276)	1.936 (1.306–2.871)	0.003
	Underweight	**Normal**	**Overweight**	**Obese**	**P-value**
BMI	0.664 (0.091–4.867)	Ref	1.394 (1.013–1.919)	1.793 (1.294–2.486)	0.002

BMI, Body mass index.

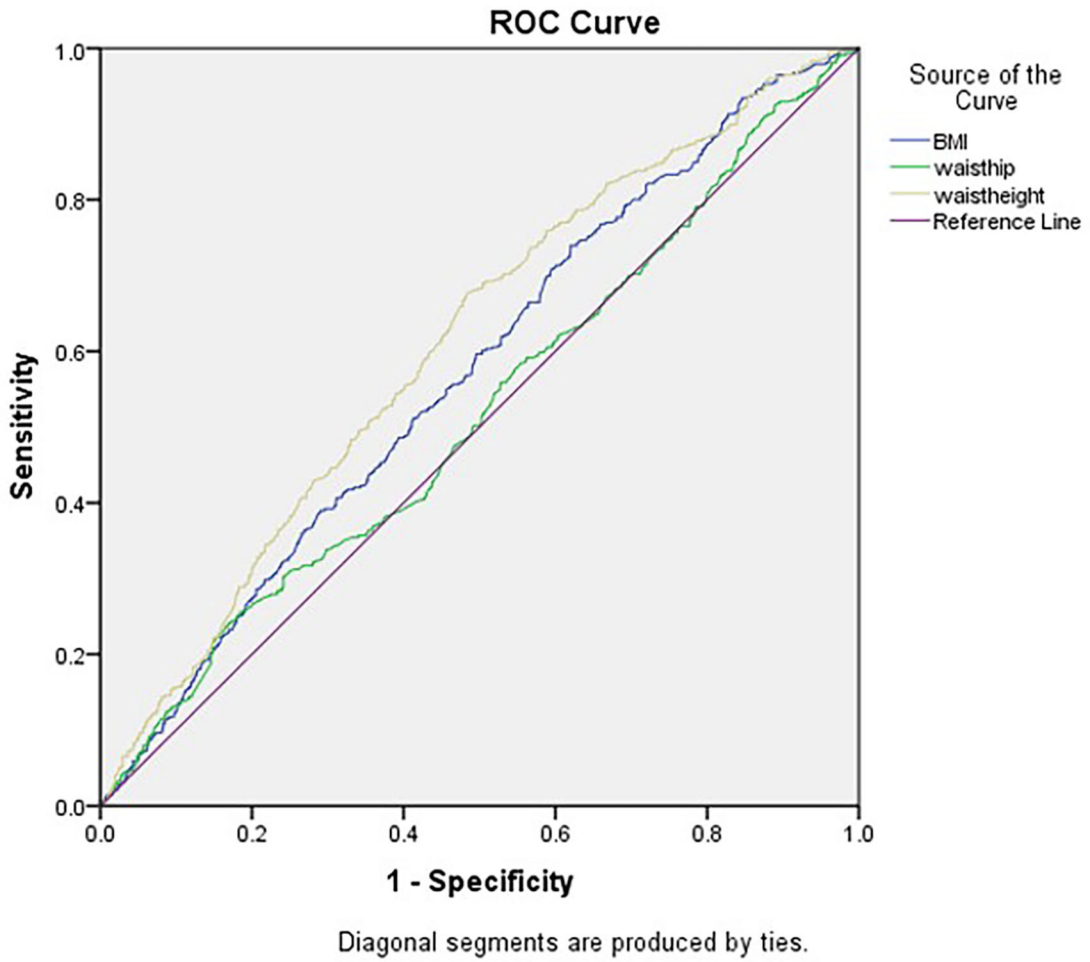
Furthermore, multivariate analysis in this study revealed that WHtR, unlike BMI and WHR, was significantly associated with increased risk of developing hypertension, subsequent to adjustment for age, gender, history of CAD, SBP, FBS, and TG. Moreover, the ROC analysis of anthropometric indices for prediction of hypertension demonstrated that all indices, especially WHtR, exhibited a statistically significant predictive power in detecting the incidence of HTN in patients with T2DM. Therefore, according to our data, WHtR is a better screening tool for HTN than WHR and BMI in Iranian men with type 2 diabetes. This finding is similar to the results of various studies on different ethnicities worldwide (7, 25–30). Three meta-analyses also supported WHtR as the most accurate anthropometric index for predicting hypertension (12, 31, 32).

WHtR can be considered as the best anthropometric index for screening for health risks. Height can predict hypertension and diabetes (33, 34), and the percentage of body fat is an independent risk factor for CVD (35). People with shorter stature have noticeably higher amounts of body fat than taller individuals with the same BMI (36). Individuals with similar WCs might not have a similar percentage of body fat if they are unequal in height. Therefore, WHtR can be a helpful predictor of hypertension by considering the impact of waist circumference and height on body fat composition (29).

In the current study, optimal cutoff for WHtR was calculated as 0.59. A recent Iranian study on anthropometric indices for obesity reported cutoff of 0.49–0.51 among adults (37). A cutoff of 0.5 for WHtR was first mentioned in a study by McCarthy HD et al. in 2006 on UK adolescents and children over two decades supporting the simple message: "Keep your waist circumference to less than half your height" (38). Hsieh et al. recommended a WHtR cutoff of 0.5 for Japanese men and women (39). Tseng et al. also reported a cutoff of 0.48–0.52 in both sexes and suggested that this is the clinical importance of WHtR as the same cutoff could be applied to different sexes and ethnicities (29). WHtR is the least expensive and a simple measure that has a strong relationship with cardiovascular diseases, with a similar optimal cutoff at 0.5 in both sexes and various ethnic groups. Due to these features, WHtR can be of great value to public health and can be utilized as a standard screening tool for more accurate epidemiological data comparisons between studies. The cutoff value of 0.5 can be simply memorized and instantly employed to explain the risk of cardio-metabolic diseases to the patients to determine if the WC is less than half of the height. Thus, no more complicated calculations are needed (as required for BMI) (29).

On the other hand, Cuban and Japanese studies suggested BMI as the single best indicator for developing hypertension (9, 10). Li et al. reported BMI and WC can predict incident hypertension more accurately than WHR, skinfold thickness, and WHtR in the Chinese population (8). A study on the Iranian population also reported that BMI is the best predictor of hypertension in men; BMI combined with WHR were the best predictors of hypertension in women (11). Another Iranian cross-sectional study also suggested WHR combined with BMI as the best predictor of hypertension in women (11). Rezende et al. suggested that both central obesity (WC and WHtR) and overall obesity (BMI) anthropometric indicators could be used in the Brazilian population to evaluate the risk of incident hypertension (40). Another Brazilian study, as well as a 2008 meta-analysis reported that all indices had similar performances in hypertension detection (13, 41). These differences might be due to the populations' varying characteristics, differences in sampling strategies, data collection quality, and the differences in operational definitions for abdominal and general obesity (42).

A screening measure should be both efficient and practical. BMI is calculated using weight and height. This measure does not reflect the distribution of individual's fat (general vs. abdominal obesity). Studies have shown that patients with the same BMI may have different waist circumferences. Since, people with abdominal obesity are more prone to cardiovascular diseases and hypertension (43), it is crucial to employ a measure that reflects WC and either WHR or WHtR. When comparing WHR with WHtR; WHtR seems to have several advantages over WHR. Firstly, as mentioned earlier people with shorter stature have noticeably higher amounts of body fat than taller individuals with the same BMI (36). Secondly, studies have reported that self-assessment of height is more accurate than weight (43). Thirdly, height has usually been shown to have inverse associations with cardiometabolic morbidity and mortality and this is probably because height, as well as having a major genetic component, can also reflect general early life exposures and WHR does not reflect body height (32, 44, 45). Furthermore, in women with T2DM, WHtR has been shown to be independently and better associated with elevated urinary albumin excretion rate, a common cardiovascular risk



	AUC (95% CI)	P-value	Calculated cutoff
BMI	0.571 (0.540-0.602)	<0.001	26.94
Waist to hip ratio	0.518(0.486-0.550)	0.263	0.90
Waist to height ratio	0.609(0.578-0.639)	<0.001	0.59

FIGURE 2 | Receiver operating characteristics curve analysis for comparison of BMI, waist to hip ratio, and waist to height ratio as an index for incident hypertension.

factor in diabetes (46), than WC, WHR, and BMI (47). It has also been reported to have the superiority of independent association with coronary artery disease and the highest magnitude of association than WHR, and BMI in patients with T2DM (48).

Hsieh et al. argued that among the Japanese men in the third quartile of WC (84.5 - 89 cm), shorter individuals were more prone to hypertension than the taller individuals (49). Furthermore, it is harder to measure hip circumference than WC, since there is a need to accurately identify the point of

maximal protrusion of the buttocks in obese people (14). A former study argued that the area under the ROC for WHR for hypertension was reported as the lowest among anthropometric indices (50). This finding is in line with the results of our study. Most importantly, WHR and WC are not suitable measures due to the variously reported cutoffs for the two sexes (45, 51).

The current study had several strengths. Firstly, to the best of our knowledge, this is the first prospective cohort study comparing obesity anthropometric indices in patients with diabetes. Secondly, it used a prospective cohort design as well as a large population-based sample. Thus, there is a clear causality between incident hypertension and obesity anthropometric indices. Thirdly, professional interviewers performed several interviews. Anthropometric data were recorded through repeated measurements in accordance with a standard protocol. Such procedures helped to reduce bias. This study had several limitations. Firstly, since our study population was limited to the Iranian ethnicity, caution should be taken when extrapolating the results to other ethnicities. Secondly, since the participant's diet details were not available, we couldn't adjust for hypertension-related covariates, such as salt and fat intake. Thirdly, repeated measures of the anthropometric indices and other variables measured at baseline, were not available for all the patients, so we could not analyze the role of time-varying indices and adjust for the time-varying confounders.

CONCLUSION

The present prospective study showed that WHtR is a more accurate screening tool for HTN than WHR and BMI in patients with type 2 diabetes. Our data reinforce the significance of including the most accurate anthropometric indices in the public health strategies to prevent and control the obesity epidemic and address the risk of developing hypertension. Therefore, WHtR could be recommended as a useful and accurate screening tool to predict hypertension due to its high discriminative power.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Tehran University of Medical Sciences. The patients/participants provided their written informed consent to participate in this study.

CONSENT FOR PARTICIPATE

All participants voluntarily agreed to participate in this research study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Gill G, Woodward A, Pradhan S, Wallymahmed M, Groves T, English P, et al. Intensified treatment of type 2 diabetes—positive effects on blood pressure, but not glycaemic control. *QJM.* (2003) 96:833–6. doi: 10.1093/qjmed/hcg143
2. Stanciu I, Peralta MI, Emanuele MA, Emanuele NV. Clinical trial evidence for cardiovascular risk reduction in type 2 diabetes. *J Cardiovasc Nurs.* (2002) 16:24–43. doi: 10.1097/00005082-200201000-00004
3. Hu G, Jousilahti P, Barengo NC, Qiao Q, Lakka TA, Tuomilehto J. Physical activity, cardiovascular risk factors, and mortality among finnish adults with diabetes. *Diabetes Care.* (2005) 28:799–805. doi: 10.2337/diacare.28.4.799
4. White F, Wang L, Jelinek HF. Management of hypertension in patients with diabetes mellitus. *Exp Clin Cardiol.* (2010) 15:5.
5. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension.* (2003) 42:1206–52. doi: 10.1161/01.HYP.0000107251.49515.c2
6. Mendis S, Puska P, Norrving B, World Health Organization. *Global Atlas On Cardiovascular Disease Prevention And Control*: World Health Organization (2011).
7. Caminha TC, Ferreira HS, Costa NS, Nakano RP, Carvalho RES, Xavier AF, et al. Waist-to-height ratio is the best anthropometric predictor of hypertension: a population-based study with women from a state of northeast of Brazil. *Medicine.* (2017) 96:e5874. doi: 10.1097/MD.0000000000005874
8. Liu K, Huang X, Cui S, Ye X, Zhou Y, Song J, et al. Voxel-based morphometry reveals regional reductions of gray matter volume in school-aged children with short-term type 1 diabetes mellitus. *Neuroreport.* (2019) 30:516–21. doi: 10.1097/WNR.0000000000001238
9. Barbosa AR, Munaretti DB, Coqueiro RDS, Borgatto AF. Anthropometric indexes of obesity and hypertension in elderly from Cuba and Barbados. *J Nutr Health Aging.* (2011) 15:17–21. doi: 10.1007/s12603-011-0007-7
10. Sakurai M, Miura K, Takamura T, Ota T, Ishizaki M, Morikawa Y, et al. Gender differences in the association between anthropometric indices of obesity and blood pressure in Japanese. *Hypertens Res.* (2006) 29:75–80. doi: 10.1291/hypres.29.75
11. Faramarzi E, Ostadrahimi A, Nikniaz Z, Jafarabadi MA, Fakhari A, Somi M. Determination of the best anthropometric index of obesity for prediction of prehypertension and hypertension in a large population - based - study; the azar- cohort. *Iran Red Crescent Med J.* (2018) 20:e59911. doi: 10.5812/ircmj.59911
12. De Simone G, Chinali M. Is central obesity a better discriminator of the risk of hypertension than body mass index in ethnically diverse populations? *J Hypertens.* (2008) 26:169–81. doi: 10.1097/HJH.0b013e3282f16ad3

13. Lee CMY, Huxley RR, Wildman RP, Woodward M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. *J Clin Epidemiol.* (2008) 61:646–53. doi: 10.1016/j.jclinepi.2007.08.012
14. Organization WH. *Waist Circumference And Waist-Hip Ratio: Report Of A Who Expert Consultation,* Geneva (2011).
15. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, et al. 2020 International society of hypertension global hypertension practice guidelines. *Hypertension.* (2020) 75:1334–57. doi: 10.1161/hypertensionaha.120.15026
16. Esteghamati A, Larijani B, Aghajani MH, Ghaemi F, Kermanchi J, Shahrami A, et al. Diabetes in Iran: prospective analysis from first nationwide diabetes report of national program for prevention and control of diabetes (NPPCD-2016). *Sci Rep.* (2017) 7:1–10. doi: 10.1038/s41598-017-13379-z
17. Sowers JR. Treatment of hypertension in patients with diabetes. *Arch Intern Med.* (2004) 164:1850–7. doi: 10.1001/archinte.164.17.1850
18. Sowers JR, Williams M, Epstein M, Bakris G. Hypertension in patients with diabetes: strategies for drug therapy to reduce complications. *Postgrad Med.* (2000) 107:47–60. doi: 10.3810/pgm.2000.04.990
19. Akalu Y, Belsti Y. Hypertension and its associated factors among type 2 diabetes mellitus patients at debre tabor general hospital, northwest Ethiopia. *Diabetes Metab Syndr Obes.* (2020) 13:1621. doi: 10.2147/DMSO.S254537
20. Mengesha AY. Hypertension and related risk factors in type 2 diabetes mellitus (DM) patients in Gaborone City Council (GCC) clinics, Gaborone, Botswana. *Afr Health Sci.* (2007) 7:244–5.
21. Kabakov E, Norymberg C, Osher E, Koffler M, Tordjman K, Greenman Y, et al. Prevalence of hypertension in type 2 diabetes mellitus: impact of the tightening definition of high blood pressure and association with confounding risk factors. *J Cardiometab Syndr.* (2006) 1:95–101. doi: 10.1111/j.1559-4564.2006.05513.x
22. Mansour AA. Prevalence and control of hypertension in iraqi diabetic patients: a prospective cohort study. *Open Cardiovasc Med J.* (2012) 6:68–71. doi: 10.2174/1874192401206010068
23. Govindarajan G, Sowers JR, Stump CS. Hypertension and diabetes mellitus. *J Hypertens Diabetes Mellit.* (2006) 2:1–7. doi: 10.15420/ECR.2006.1.1a
24. Singh S, Shankar R, Singh GP. Prevalence and associated risk factors of hypertension: a cross-sectional study in urban Varanasi. *Int J Hypertens.* (2017) 2017:5491838. doi: 10.1155/2017/5491838
25. Kawamoto R, Kikuchi A, Akase T, Ninomiya D, Kumagi T. Usefulness of waist-to-height ratio in screening incident hypertension among Japanese community-dwelling middle-aged and elderly individuals. *Clin Hypertens.* (2020) 26:1–9. doi: 10.1186/s40885-020-00142-2
26. Khader Y, Batieha A, Jaddou H, El-Khateeb M, Ajlouni K. The performance of anthropometric measures to predict diabetes mellitus and hypertension among adults in Jordan. *BMC Public Health.* (2019) 19:1416. doi: 10.1186/s12889-019-7801-2
27. Lee J-W, Lim N-K, Baek T-H, Park S-H, Park H-Y. Anthropometric indices as predictors of hypertension among men and women aged 40–69 years in the Korean population: the Korean Genome and epidemiology study. *BMC Public Health.* (2015) 15:1–7. doi: 10.1186/s12889-015-1471-5
28. Saeed AA, Al-Hamdan NA. Anthropometric risk factors and predictors of hypertension among Saudi adult population–a national survey. *J Epidemiol Glob Health.* (2013) 3:197–204. doi: 10.1016/j.jegh.2013.08.004
29. Tseng C-H, Chong C-K, Chan T-T, Bai C-H, You S-L, Chiou H-Y, et al. Optimal anthropometric factor cutoffs for hyperglycemia, hypertension and dyslipidemia for the Taiwanese population. *Atherosclerosis.* (2010) 210:585–9. doi: 10.1016/j.atherosclerosis.2009.12.015
30. Mansour AA, Al-Jazairi MI. Cut-off values for anthropometric variables that confer increased risk of type 2 diabetes mellitus and hypertension in Iraq. *Arch Med Res.* (2007) 38:253–8. doi: 10.1016/j.arcmed.2006.09.014
31. Deng G, Yin L, Liu W, Liu X, Xiang Q, Qian Z, et al. Associations of anthropometric adiposity indexes with hypertension risk: a systematic review and meta-analysis including PURE-China. *Medicine.* (2018) 97:e13262. doi: 10.1097/MD.0000000000013262
32. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obes Rev.* (2012) 13:275–86. doi: 10.1111/j.1467-789X.2011.00952.x
33. Lara-Esqueda A, Aguilar-Salinas CA, Velazquez-Monroy O, Gómez-Pérez FJ, Rosas-Peralta M, Mehta R, et al. The body mass index is a less-sensitive tool for detecting cases with obesity-associated co-morbidities in short stature subjects. *Int J Obes Relat Metab Disord.* (2004) 28:1443–50. doi: 10.1038/sj.ijo.0802705
34. Fuchs FD, Gus M, Moreira LB, Moraes RS, Wiehe M, Pereira GM, et al. Anthropometric indices and the incidence of hypertension: a comparative analysis. *Obes Res.* (2005) 13:1515–7. doi: 10.1038/oby.2005.184
35. Tseng CH. Body composition as a risk factor for coronary artery disease in Chinese type 2 diabetic patients in Taiwan. *Circ J.* (2003) 67:479–84. doi: 10.1253/circj.67.479
36. López-Alvarenga JC, Montesinos-Cabrera RA, Velázquez-Alva C, González-Barranco J. Short stature is related to high body fat composition despite body mass index in a Mexican population. *Arch Med Res.* (2003) 34:137–40. doi: 10.1016/S0188-4409(03)00002-X
37. Ramezankhani A, Ehteshami-Afshar S, Hasheminia M, Hajebrahimi MA, Azizi F, Hadaegh F. Optimum cutoff values of anthropometric indices of obesity for predicting hypertension: more than one decades of follow-up in an Iranian population. *J Hum Hypertens.* (2018) 32:838–48. doi: 10.1038/s41371-018-0093-7
38. McCarthy HD, Ashwell M. A study of central fatness using waist-to-height ratios in UK children and adolescents over two decades supports the simple message–'keep your waist circumference to less than half your height'. *Int J Obes.* (2006) 30:988–92. doi: 10.1038/sj.ijo.0803226
39. Hsieh S, Yoshinaga H, Muto T. Waist-to-height ratio, a simple and practical index for assessing central fat distribution and metabolic risk in Japanese men and women. *Int J Obes.* (2003) 27:610–6. doi: 10.1038/sj.ijo.0802259
40. Rezende AC, Souza LG, Jardim TV, Perillo NB, Araújo YCL, de Souza SG, et al. Is waist-to-height ratio the best predictive indicator of hypertension incidence? A cohort study. *BMC Public Health.* (2018) 18:281. doi: 10.1186/s12889-018-5177-3
41. de Souza APA, Rodrigues PRM, Muraro AP, Moreira NF, Sichieri R, Pereira RA, et al. Cut-off points of anthropometric markers associated with hypertension in the Brazilian population: national health survey, 2013. *Public Health Nutr.* (2019) 22:2147–54. doi: 10.1017/S1368980019000533
42. Wai WS, Dhami RS, Gelaye B, Girma B, Lemma S, Berhane Y, et al. Comparison of measures of adiposity in identifying cardiovascular disease risk among Ethiopian adults. *Obesity.* (2012) 20:1887–95. doi: 10.1038/oby.2011.103
43. Fan H, Li X, Zheng L, Chen X, Lan Q, Wu H, et al. Abdominal obesity is strongly associated with cardiovascular disease and its risk factors in elderly and very elderly community-dwelling Chinese. *Sci Rep.* (2016) 6:21521. doi: 10.1038/srep21521
44. Browning LM, Hsieh SD, Ashwell M. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0·5 could be a suitable global boundary value. *Nutr Res Rev.* (2010) 23:247–69. doi: 10.1017/S0954422410000144
45. Li W-C, Chen I-C, Chang Y-C, Loke S-S, Wang S-H, Hsiao K-Y. Waist-to-height ratio, waist circumference, and body mass index as indices of cardiometabolic risk among 36,642 Taiwanese adults. *Eur J Nutr.* (2013) 52:57–65. doi: 10.1007/s00394-011-0286-0
46. Donnelly R, Yeung JM, Manning G. Microalbuminuria: a common, independent cardiovascular risk factor, especially but not exclusively in type 2 diabetes. *J Hypertens Suppl.* (2003) 21:S7–12.
47. Tseng C-H. Waist-to-height ratio is independently and better associated with urinary albumin excretion rate than waist circumference or waist-to-hip ratio in Chinese adult type 2 diabetic women but not men. *Diabetes Care.* (2005) 28:2249. doi: 10.2337/diacare.28.9.2249
48. Tseng C-H. Waist-to-height ratio and coronary artery disease in taiwanese type 2 diabetic patients. *Obesity.* (2008) 16:2754–9. doi: 10.1038/oby.2008.430

49. Hsieh SD, Yoshinaga H. Do people with similar waist circumference share similar health risks irrespective of height? *Tohoku J Exp Med.* (1999) 188:55–60. doi: 10.1620/tjem.188.55
50. Gupta S, Kapoor S. Optimal cut-off values of anthropometric markers to predict hypertension in North Indian population. *J Community Health.* (2012) 37:441–7. doi: 10.1007/s10900-011-9461-8
51. Ashwell M, Hsieh SD. Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. *Int J Food Sci Nutr.* (2005) 56:303–7. doi: 10.1080/09637480500195066

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Hypoglycemia Among Type 1 Diabetes Patients After Insulin Use in Southwest Ethiopia

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Introduction: Glycemic control is a valuable goal for people with diabetes; however, the greatest challenge to achieving tight glycemic control is hypoglycemia. Hypoglycemic events are probably common in type 1 diabetes; however, little is known about hypoglycemia in Ethiopia. Therefore, this study aimed to assess the prevalence and the associated factors of hypoglycemia among type 1 diabetes (T1D) patients after insulin use at Metu Karl Referral Hospital in southwest Ethiopia.

Materials and Methods: A hospital-based cross-sectional study was conducted among 242 T1D patients at Metu Karl Referral Hospital in southwest Ethiopia. The prevalence of hypoglycemia was assessed by a structured questionnaire through a face-to-face interview in which all the possible symptoms of hypoglycemia were included. If the patients reported that they had experienced the symptoms at least two times in a month and the symptoms were relieved upon consuming sugar/candy/honey, such cases were considered to have had a hypoglycemic episode. Binary logistic regression analysis was done to identify the factors associated with the occurrence of hypoglycemia.

Results: Out of 242 T1D patients interviewed, 114 (47.1%) had self-reported hypoglycemia. The most reported symptom of hypoglycemia was sweating (91.7%), followed by dizziness and hunger and nausea with a prevalence of 24.8 and 14.5%, respectively. The study also found that educational level with reading and writing skills up to primary level [adjusted odds ratio, AOR = 0.41; 95% confidence interval, CI (0.19–0.88)] and secondary level and above [AOR = 0.32, 95% CI (0.14–0.70)], poor knowledge of diabetes [AOR = 2.26, 95% CI (1.06–4.84)], good knowledge of insulin self-administration [AOR = 0.54, 95% CI (0.30–0.99)], and duration of insulin use ≥5 years [AOR = 3.93, 95% CI (1.44–10.7)] were factors associated with hypoglycemia.

Conclusions: The prevalence of hypoglycemia was found remarkable. We can conclude that hypoglycemia is of public health importance among T1D patients. Since the study assesses hypoglycemia after insulin injection, this prevalence may be due to the poor practice of insulin injection. Therefore, imparting education on the proper technique of insulin administration should be considered at each follow-up visit.

Keywords: hypoglycemia, diabetes, insulin use, Ethiopia, Metu Karl referral hospital

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INTRODUCTION

Diabetes mellitus significantly contributes to the global health burden in the 21st century (1). Achieving good glycemic control is a valuable goal for people with diabetes (2); however, hypoglycemia is the greatest challenge to achieving tight glycemic control (3–7), which results in declined drug compliance, cardiovascular events, and even mortality (3). It is also related to a negative impact on health-related quality of life, healthcare resource use, and work productivity (5).

Hypoglycemic events are probably common in diabetic patients who use insulin (4, 8–12), and patients with type 1 diabetes (T1D) are more likely to develop hypoglycemia as compared with type 2 diabetic patients (4, 5, 13, 14). One of the most feared complications of diabetes treatment is hypoglycemia (15), which commonly occurs in clinical practice as approximately 90% of all patients who receive insulin have experienced hypoglycemic episodes (16).

Hypoglycemia, also known as an insulin reaction or insulin shock, is a clinical and biological syndrome caused by an abnormal decrease in plasma glucose levels to below 70 mg/dl and responsible for non-specific signs and symptoms, including shakiness, nervousness, sweating, chills and clamminess, dizziness, hunger and nausea, confusion, weakness, sleepiness, seizures, and loss of consciousness (17, 18). It is categorized as either mild or severe based on the seriousness of the event and whether it requires external support or is self-limiting (19, 20).

The factors associated with hypoglycemia are varied. It may include age, sex, occupational status, residence, body mass index, missed meals or inadequate caloric intake, alcohol consumption, concurrent use of an opioid, level of fasting blood sugar, blood glucose monitoring, glucose checkup period, insulin or diabetes medications, duration of diabetes, and presence of stroke (3, 5, 8, 21–24).

Individuals with T1D require a lifelong insulin supply for good treatment results. The most common problem among patients with T1D is lack of adherence to insulin therapy. The fear of hypoglycemia is the principal factor associated with their nonadherence to insulin therapy (25, 26). Like other diabetes complications, prevention is the best remedy for hypoglycemia, and recognizing the associated risk factors is the first step (27). Recognition of the problem, assessment of the risk factors, and application of the principles of intensive glycemic management are very important in reducing the possibility of hypoglycemia (28). The prevention and treatment of hypoglycemia in patients with T1D needs greater vigilance and education (29). Hypoglycemia in people living with diabetes mellitus is an unexplored area in literature in Africa (30), including Ethiopia. Any intervention aimed at preventing hypoglycemia in patients with T1D can only be possible when there is sufficient data on the issue. Therefore, this study aimed to assess the prevalence and the associated factors of hypoglycemia among T1D patients after insulin use at Metu Karl Referral Hospital in southwest Ethiopia.

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; COR, crude odds ratio; DM, diabetes mellitus; G-8, grade 8; MKRH, Metu Karl Referral Hospital; SPSS, Statistical Package for the Social Sciences; SD, standard deviation; T1D, type 1 diabetes.

MATERIALS AND METHODS

Study Setting and Period

The study was conducted at Metu Karl Heinz Referral Hospital (MKRH) from January 1 to 30, 2019. MKRH is located in the Oromia region, Ilu Abbabor zone, Metu town, 600 km southwest of Addis Ababa, the capital city of Ethiopia. The details of the study area were better described in a previous publication (31).

Study Design and Populations

A cross-sectional study was carried out. All insulin-dependent self-injecting T1D patients who had chronic follow-up visits at Metu Karl Referral Hospital during the study period were included in the source population. The study population was randomly selected among self-injecting T1D patients who fulfill the inclusion criteria during the study period. The details of the study population were better explained in a previous publication (31).

Sample Size Determination

The sample size was determined using a single-population proportion formula with an input of the expected proportion of hypoglycemia patients (50%), 5% margin of error, and 95% confidence level. The sample size computed was 384. However, the source population ($N = 535$ patients who were taking insulin therapy at the time of data collection) was less than 10,000; by applying the correction formula, it then became 223, but by adding 10% for non-response compensation, the final sample size was determined to be 245.

Sampling Method

Unless there is any disease-related emergency, every diabetes patient is set with a monthly appointment to have a checkup and for them to collect their monthly medication. A systematic random sampling method was used to select the study populations. The details of the sampling method were better explained in a previous publication (31).

Study Variables and Measurements

The dependent variable was hypoglycemia. The independent variables were sociodemographic factors (age, sex, marital status, and educational status), knowledge of diabetes, knowledge of insulin self-administration, and health profiles [family history of diabetes mellitus (DM), membership in a DM association, and duration of insulin use].

Hypoglycemia is a clinical and biological syndrome which is responsible for non-specific signs and symptoms, including shakiness, nervousness, sweating, chills and clamminess, dizziness, hunger and nausea, confusion, weakness, sleepiness, seizures, and loss of consciousness (18), and the symptoms are relieved upon consuming sugar/candy/honey (32).

Prevalence was defined as the frequency at which the study subjects experienced at least two symptoms of hypoglycemia in the last month.

Good knowledge of insulin self-administration refers to a person who scores greater than the mean value (≥5 or ≥62.5%) of knowledge-based questions; otherwise, knowledge of insulin self-administration is considered poor (31).

Awareness of hypoglycemia is demonstrated by participants who answered "always" to the question "Can you feel when your blood sugar is low?"; otherwise, they were classified as unaware of hypoglycemia (33).

Data Collection Instrument and Procedures

The data were collected through a face-to-face interview. The prevalence of hypoglycemia was assessed by a structured questionnaire in which all the possible symptoms of hypoglycemia were included (18). Two criteria were used, both of which suggest that the symptoms of a patient result from hypoglycemia. The criteria include the following:

1. symptoms of hypoglycemia happening after injecting insulin
2. relief of the symptoms using sugar/candy/honey

The common symptoms of hypoglycemia were listed. The patients were asked if they had ever experienced any one of the listed symptoms after injecting insulin in the past month. If the answer was "yes", then they were enquired about the frequency of the above-mentioned symptoms. If they answered that they had experienced the symptoms at least two times in a month and the symptoms were relieved upon consuming sugar/candy/honey, then they were considered to have had a hypoglycemic episode. Hence, if the abovementioned criteria were met, the patients were identified to have had a hypoglycemic episode. A hypoglycemic event that required the assistance of another person or which required medical assistance in a hospital for corrective measures was documented as a severe episode of hypoglycemia. The questionnaire was tested for reliability and validity. The face validity was performed by an internist who worked at the diabetes clinic of the hospital. The reliability of the analysis was determined using Cronbach's alpha test where the reliability coefficient was found to be significant (Cronbach's alpha: 0.77). To assess the quality, the questionnaire had been pre-tested in similar setups before the actual data collection was commenced. Training was given to data collectors and supervisors concerning the objective and the process of data collection.

Data Processing and Analysis

The data collected were entered into Epi-data, version 4.2.0.0, and analyzed using SPSS, version 20. Binary logistic regression analysis was done. Independent variables with a *P*-value of less than 0.25 in bivariate logistic regression were included in multivariable logistic regression. Multivariable logistic regression analysis was done to control for potential confounding factors and identify the most important determinate variables. The level of significance was declared at *P*-value <0.05 in the multivariable logistic regression analysis.

RESULTS

Socio-Demographic Characteristics

Of the sample size of 245, 242 T1D patients have participated in the study, yielding a response rate of 98.7%. The mean age of the respondents was 33.7 (± 12.6 SD) years, with a range of 19 to 70 years. The majority (45.4%) of the respondents were in the age group of 19–28 years. One hundred eight (44.6%) of the participants were Protestant followers. One hundred twelve (46.3%) and 58 (24%) of the respondents were out of marriage and cannot read and write, respectively (**Table 1**).

Health-Related Profiles

Ninety-nine (40.9%) of the respondents had a family history of diabetes. One hundred forty-one (58.3%) of the participants were members of Ethiopian diabetes associations. One hundred ninety-five (80.6%) of the respondents had good knowledge of diabetes. The majority (61.6%) of the respondents had poor knowledge of insulin self-administration. More than three-

TABLE 1 | Socio-demographic characteristics of type 1 diabetes patients at metu karl heinz referral hospital in ethiopia.

Variables	Categories	Frequency	Percent
Age group (years)	19–28	110	45.4
	29–38	57	23.6
	39–48	41	16.9
	49–58	14	5.8
	59–70	20	8.3
Sex	Male	98	40.5
	Female	144	59.5
Religion	Protestant	108	44.6
	Orthodox	75	31.0
	Muslim	59	24.4
Marital status	Out of marriage	112	46.3
	Within marriage	130	53.7
Educational status	Cannot read and write	58	24.0
	Can read and write up to G-8	85	35.1
	Secondary and above	99	40.9

fourth (88%) of the respondents were treated for five or more years (**Table 2**).

Prevalence of Self-Reported Hypoglycemia

Of the 242 respondents interviewed, 170 (70.3%) respondents were aware of hypoglycemia symptoms. One hundred fourteen (47.1%) respondents reported a history of hypoglycemia after injecting insulin. A total of 366 hypoglycemic events happened in the last month. The most reported symptom of hypoglycemia was sweating (91.7%), followed by dizziness and hunger and nausea with a prevalence of 24.8 and 14.5%, respectively (**Table 3**). Of those who developed hypoglycemia, 86 (75.4%), 20 (17.6%), and eight (7.0%) were managed by home treatment using sugar, candy, and honey, respectively. No respondent reported inpatient admission to the hospital due to hypoglycemia.

Factors Associated With Hypoglycemia

After adjusting for age group, educational status, and knowledge of insulin self-administration as confounding factors, educational status, knowledge of diabetes and insulin self-administration, and duration of insulin use were significantly associated with hypoglycemia at a *P*-value <0.05 (**Table 4**).

DISCUSSION

Indeed it is impossible to eliminate hypoglycemia from the lives of T1D patients (34), but recognition of the problem, evaluation of the risk factors, and application of the principles of intensive glycemic management are very important for minimizing hypoglycemia (28). Based on the abovementioned facts, this study aimed to assess the prevalence and the associated factors of hypoglycemia among T1D patients after insulin use at Metu Karl Referral Hospital in southwest Ethiopia. As a result, the proportion of self-reported hypoglycemia among type 1 diabetes was 114 (47.1%), at 95% confidence interval of 40.8–53.4%. This study was in line with 50% of the RECAP-DM study in Argentina (35). This finding was lower than 86.7% in Debre Markos Referral Hospital (5), 88% in Tikur Anbessa Specialized Hospital (23), and 94.3% in St. Paul's Hospital Millennium Medical College (22) studies in Ethiopia and 91.7% from HAT study in Brazil (36), 97.1% in Colombia (37), 97.4% from an international survey in nine countries (38), and 100% from the Southeast Asia cohort of IO HAT study (39). The variation observed between this and other studies may be due to the operational definition used. Unlike other studies, this study classified the individuals as having experienced hypoglycemia when they experience the hypoglycemia symptoms at least two times in 1 month. Besides that, unlike this study, the other studies with a reported high prevalence were done in more urban and vigilant societies, such that the subjects may be easily aware of a hypoglycemic episode, resulting in increased reports of hypoglycemia symptoms.

Respondents who can read and write and with up to primary and secondary and above education were 59 and 68%, respectively, less likely to develop hypoglycemia. Being educated was significantly associated with less occurrence of hypoglycemia. This could be because an individual who had better education may be associated with better knowledge of good insulin administration techniques to avoid the problem resulting from using an inappropriately high dose of insulin. This finding was supported by Wako et al., who revealed that low educational status was associated with the occurrence of hypoglycemia (23).

Respondents who had good knowledge of insulin self-administration were 46% less likely to develop hypoglycemia than those who had poor knowledge of insulin self-administration. Having good knowledge may be related to less chance of acquiring hypoglycemia. This could be explained by the good knowledge of insulin self-administration, which may prevent related hypoglycemia due to an inappropriately high dose of insulin.

Respondents who had poor knowledge of diabetes had 2.3 times increased odds of developing hypoglycemia than those who had good knowledge of diabetes. Having good knowledge of diabetes was significantly associated with less chance of developing hypoglycemia. This could be due to the good knowledge of diabetes being related to good knowledge of managing diabetes and the ways on how to prevent related complications. This finding was in line with a study by Gebrewahd and Teklewoini, which revealed that good knowledge of diabetes and hypoglycemia and a favorable attitude towards diabetes are positive predictors of good hypoglycemia prevention practice (40).

Respondents who were treated for 5 or more years had 3.9 times increased odds of developing hypoglycemia than those who were treated for less than 5 years. One of the reasons for recurrent hypoglycemia in patients with diabetes for more than

TABLE 2 | Health-related profiles of type 1 diabetes patients at metu karl heinz referral hospital in ethiopia.

Variables	Categories	Frequency	Percent
Family history of diabetes	Yes	99	40.9
	No	143	59.1
Member of the diabetes association	Yes	141	58.3
	No	101	41.7
Knowledge of diabetes	Good	195	80.6
	Poor	47	19.4
Knowledge of insulin self-administration	Good	93	38.4
	Poor	149	61.6
Duration of insulin therapy (years)	<3	11	4.5
	3–5	18	7.4
	≥5	213	88.0

TABLE 3 | Self-reported symptoms of hypoglycemia among type 1 diabetes patients at metu karl heinz referral hospital in ethiopia.

Symptoms of hypoglycemia	Categories	Frequency (*n*)	Percent (%)
Sweating	Yes	222	91.7
	No	20	8.3
Dizziness	Yes	60	24.8
	No	182	75.2
Hunger and nausea	Yes	35	14.5
	No	207	85.5
Sleepiness	Yes	22	9.1
	No	220	90.9
Chills and clamminess	Yes	18	7.4
	No	224	92.6
Shakiness	Yes	9	3.7
	No	233	96.3

TABLE 4 | Factors associated with hypoglycemia among type 1 diabetes patients at metu karl heinz referral hospital in ethiopia.

Variables	Categories	Hypoglycemia		COR (95% CI)	AOR (95% CI)
		Yes	No		
Age group (years)	19–28	50	60	1	1
	29–38	25	32	0.94 (0.49–1.79)	1.10 (0.53–2.28)
	39–48	23	18	1.53 (0.75–3.16)*	1.00 (0.42–2.35)
	49–58	5	9	0.67 (0.21–2.12)	0.36 (0.10–1.26)
	59–70	11	9	1.47 (0.56–3.82)	1.15 (0.40–3.32)
Educational status	Cannot read and write	35	23	1	1
	Can read and write up to G-8	37	48	0.51 (0.26–0.99)**	0.41 (0.19–0.88)**
	Secondary and above	42	57	0.48 (0.25–0.94)**	0.32 (0.14–0.70)**
Knowledge of diabetes	Yes	85	110	1	1
	No	29	18	2.09 (1.09–4.00)**	2.26 (1.06–4.84)**
Knowledge of insulin	Good	64	85	0.65 (0.39–1.09)*	0.54 (0.30–0.99)**
self-administration	Poor	50	43	1	1
Duration of insulin use	<5 years	6	23	1	1
	≥5 years	108	105	3.94 (1.54–10.1)**	3.93 (1.44–10.7)**

AOR, adjusted odds ratio; CI, confidence interval; COR, crude odds ratio.
**p-value < 0.25, **p-value < 0.05.*

5 years could be "unrecognized" chronic kidney disease, as the kidney is the site for the degradation of insulin. Besides this, it could be due to the fact that those who are treated for a long duration may have become fatigued and caught napping on the techniques to administer insulin by themselves. This finding was supported by studies done in Ethiopia (5, 22).

Strengths and Limitations of the Study

Despite that this study assesses the most understudied problem in Ethiopia, it has some limitations. First was the failure to use a finger stick blood glucose test since hypoglycemia was based on self-reported hypoglycemia symptoms. Secondly, the study was carried out in a single referral hospital; the findings from this study cannot be generalized to different populations and settings across Ethiopia. Lastly, there was failure to assess the daily activity of patients and their nutritional intake as factors associated with the outcome variable.

CONCLUSION

The prevalence of hypoglycemia among T1D patients in the study area was found remarkable. We can conclude that hypoglycemia is a public health problem among T1D patients. Since the study assesses hypoglycemia after insulin injection, this prevalence may be due to the poor practice of insulin injection. Therefore, imparting education on the proper technique of insulin administration should be considered at each follow-up visit.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical approval was obtained from Mizan-Tepi University Ethical Review Board. The ethical approval number was MTUERB/86/2019. Permission was obtained from Metu Karl Referral Hospital. All study participants were informed about the purpose of the study, their right to deny participation, anonymity, and confidentiality of the information. Written

informed consent was also obtained before participation in the study.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REFERENCES

1. Misra A, Gopalan H, Jayawardena R, Hills AP, Soares M, Reza-Albarran AA, et al. Diabetes in Developing Countries. *J Diabetes* (2019) 11:522–39. doi: 10.1111/1753-0407.12913
2. Cryer PE. The Barrier of Hypoglycemia in Diabetes. *Diabetes* (2008) 57 (10):3169–76. doi: 10.2337/db08-1084
3. Samya V, Shriraam V, Jasmine A, Akila GV, Rani MA, Durai V, et al. Prevalence of Hypoglycemia Among Patients With Type 2 Diabetes Mellitus in a Rural Health Center in South India. *J Prim Care Community Health* (2019) 10:1–7. doi: 10.1177/2150132719880638
4. Ilan G, Shamoon H. Hypoglycemia in Diabetes: Common, Often Unrecognized. *Cleve Clin J Med* (2004) 71(4):335–42. doi: 10.3949/ccjm.71.4.335
5. Tiruneh GG, Abebe N, Dessie G. Self-Reported Hypoglycemia in Adult Diabetic Patients in East Gojjam, Northwest Ethiopia: Institution Based Cross-Sectional Study. *BMC Endocr Disord* (2019) 19:17. doi: 10.1186/s12902-019-0341-z
6. Wilson DM, Calhoun PM, Maahs DM, Chase HP, Messer L, Buckingham BA, et al. Factors Associated With Nocturnal Hypoglycemia in At-Risk Adolescents and Young Adults With Type 1 Diabetes. *Diabetes Technol Ther* (2015) 17(6):385–91. doi: 10.1089/dia.2014.0342
7. Shriraam V, Mahadevan S, Anitharani M, Jagadeesh NS. Knowledge of Hypoglycemia and Its Associated Factors Among Type 2 Diabetes Mellitus Patients in a Tertiary Care Hospital in South India. *Indian J Endocrinol Metab* (2015) 19(3):378–82. doi: 10.4103/2230-8210.152779
8. Jeon JY, Kim SR, Kim HJ, Kim DJ, Lee K, Lee J, et al. Risk Factors of Severe Hypoglycemia Requiring Medical Assistance and Neurological Sequelae in Patients With Diabetes. *Med (Baltimore)* (2016) 95:47. doi: 10.1097/MD.0000000000005365
9. MacLeish SA, Wallace S, Madhun Z, Hostoffer RJ. Treatment of Hypoglycemia With Intramuscular Immunoglobulin in Insulin Dependent-Diabetics With Insulin Antibodies. *J Allergy Clin Immunol* (2007) 119(1):s18. doi: 10.1016/j.jaci.2006.11.087
10. Cox DJ, Kovatchev BP, Julian DM, Gonder-Frederick LA, Polonsky WH, Schlundt DG, et al. Frequency of Severe Hypoglycemia in Insulin-Dependent Diabetes Mellitus Can Be Predicted From Self-Monitoring Blood Glucose Data. *J Clin Endocrinol Metab* (1994) 79(6):1659–62. doi: 10.1210/jcem.79.6.7989471
11. Abraham MB, Jones TW, Naranjo D, Karges B, Oduwole A, Tauschmann M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Assessment and Management of Hypoglycemia in Children and Adolescents With Diabetes. *Pediatr Diabetes* (2018) 19(Supp.27):178–92. doi: 10.1111/pedi.12698
12. Haim B-A, Pradeep N, Ayelet M, Y E. Drug-Induced Hypoglycemic Coma in 102 Diabetic Patients. *Arch Intern Med* (1999) 159:281–4. doi: 10.1001/archinte.159.3.281
13. DuBose SN, Weinstock RS, Beck RW, Peters AL, Aleppo G, Bergenstal RM, et al. Hypoglycemia in Older Adults With Type 1 Diabetes. *Diabetes Technol Ther* (2016) 18(12):765–71. doi: 10.1089/dia.2016.0268
14. Yale J-F, Paty B, A P. Hypoglycemia. *Can J Diabetes* (2018) 42:S104–8. doi: 10.1016/j.jcjd.2017.10.010
15. Briscoe VJ, Davis SN. "Hypoglycemia in Type 1 and Type 2 Diabetes: Physiology, Pathophysiology, and Management. *Clin Diabetes* (2006) 24 (3):115–21. doi: 10.2337/diaclin.24.3.115
16. Cryer PE. *Hypoglycemia: Pathophysiology, Diagnosis, and Treatment*. New York, NY, USA: Oxford University Press (1997).
17. Virally ML, Guillausseau PJ. Hypoglycemia in Adults. *Diabetes&Metabolism* (1999) 25(6):477–90.
18. Dangi-Garimella S. The Persistent Complication of Hypoglycemia in Diabetics. *Am J Manag Care* (2014) 20(8 Spec no.):E17.
19. Workgroup on Hypoglycemia ADA. Defining and Reporting Hypoglycemia in Diabetes: A Re- Port From the American Diabetes Association Workgroup on Hypoglycemia. *Diabetes Care* (2005) 28:1245–9. doi: 10.2337/diacare.28.5.1245
20. Joint British Diabetes Societies. The Hospital Management of Hypoglycaemia in Adults With Diabetes Mellitus. 3rd edition (2018) p. 8–25.
21. Emons MF, Bae JP, Hoogwerf BJ, Kindermann SL, Taylor RJ. Risk Factors for 30-Day Readmission Following Hypoglycemia-Related Emergency Room and Inpatient Admissions. *BMJ Open Diabetes Res Care* (2016) 4:e000160. doi: 10.1136/bmjdrc-2015-000160
22. Kahsay H, Fantahun B, Nedi T, Demoz GT. Evaluation of Hypoglycemia and Associated Factors Among Patients With Type 1 Diabetes on Follow-Up Care at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. *J Diabetes Res* (2019) 2019:9037374. doi: 10.1155/2019/9037374
23. Abdinasir W, Saba Belay YF, T K. Assessment of the Magnitude, Severity and Associated Factors of Hypoglycemia in Diabetic Patients Attending National Diabetes Referral Clinic at Tikur Anbessa Hospital, Addis Ababa, Ethiopia. *J Diabetes Metab* (2017) 8(5):1–8. doi: 10.4172/2155-6156.1000741
24. Huri HZ, Irfan M, Aziz A, Ratna S, Widodo RT. Factors Associated With Hypoglycemia Episodes in Hospitalized Type 2 Diabetes Mellitus Patients in a Tertiary Health Facility in Malaysia. *Trop J Pharm Res* (2016) 15(6):1313–20. doi: 10.4314/tjpr.v15i6.26
25. Almeda-valdes P, Riofrio P. Factors Associated With Insulin Nonadherence in Type 1 Diabetes Mellitus Patients in Mexico. *Int J Diabetes Metab* (2019) 25 (3-4):1–9. doi: 10.1159/000502903
26. Anderbro T. Behavior Change Intervention and Fear of Hypoglycemia in Type 1 Diabetes. *Karolinska Institutet* (2012), 68.
27. Yun J, Ko S. Risk Factors and Adverse Outcomes of Severe Hypoglycemia in Type 2 Diabetes Mellitus. *Diabetes Metab J* (2016) 40:423–32. doi: 10.4093/dmj.2016.40.6.423
28. American Diabetes Association. Minimizing Hypoglycemia in Diabetes. *Diabetes Care* (2015) 38:1583–91. doi: 10.2337/dc15-0279
29. Daneman D, Frank M, Perlman K, Tamm J, E R. Severe Hypoglycemia in Children With Insulin-Dependent Diabetes Mellitus: Frequency and Predisposing Factors. *J Pediatr* (1989) 115(5):681–5. doi: 10.1016/S0022-3476(89)80642-0
30. Ademolu AB. Analysis of Hypoglycemic Episodes in Diabetics in Africans Using Ademolus Classification of Hypoglycemia (ACH). *Acta Sci Med Sci* (2019) 3(3):138–45.
31. Yosef T. Knowledge and Attitude on Insulin Self-Administration Among Type 1 Diabetic Patients at Metu Karl Referral Hospital, Ethiopia. *J Diabetes Res* (2019) 2019:7801367. doi: 10.1155/2019/7801367
32. Seaquist ER, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L, et al. Hypoglycemia and Diabetes: A Report of a Workgroup of the American Diabetes Association and the Endocrine Society. *Diabetes Care* (2013) 36:1384–95. doi: 10.2337/dc12-2480
33. Pedersen-Bjergaard U, Pramming S, T B. Recall of Severe Hypoglycemia and Self-Estimated State of Awareness in Type 1 Diabetes. *Diabetes Metab Res Rev* (2003) 19:232–40. doi: 10.1002/dmrr.377
34. Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group.

Long-Term Effect of Diabetes and Its Treatment on Cognitive Function. *N Engl J Med* (2007) 356(18):1842–52. doi: 10.1056/NEJMoa066397

35. Gonzalez C, Monti C, Pinzon A, Monsanto H. Prevalence of Hypoglycemia Among a Sample of Sulfonylurea-Treated Patients With Type 2 Diabetes Mellitus in Argentina: The Real-Life Effectiveness and Care Patterns of Diabetes Management (RECAP-DM) Study. *Endocrinol Diabetes Nutr* (2018) 65(10):592–602. doi: 10.1016/j.endinu.2018.05.014
36. Lamounier RN, Geloneze B, Leite SO, Montenegro R Jr, Zajdenverg L, Fernandes M, et al. Hypoglycemia Incidence and Awareness Among Insulin – Treated Patients With Diabetes: The HAT Study in Brazil. *Diabetol Metab Syndr* (2018) 10:83. doi: 10.1186/s13098-018-0379-5
37. Gómez AM, Chica LG, Burbano ÁF, Vásquez EM, Jorge A, Arias PM, et al. Survey on Hypoglycemia Among Insulin-Treated Patients With Diabetes: The Colombian International Operations Hypoglycemia Assessment Tool Population. *Biomédica* (2019) 39:576–86. doi: 10.7705/biomedica.4365
38. Emral R, Pathan F, Augusto C, Corte Y, Murphy A, El-hefnawy MH, et al. Self-Reported Hypoglycemia in Insulin-Treated Patients With Diabetes: Results From an International Survey on 7289 Patients From Nine Countries. *Diabetes Res Clin Pract* (2017) 4:17–28. doi: 10.1016/j.diabres.2017.07.031
39. Pathan F, Goh S, Rudijanto A, Gadekar A, Jain A. Hypoglycaemia Among Insulin-Treated Patients With Diabetes: Southeast Asia Cohort of IO HAT Study. *J ASEAN Fed Endocr Soc* (2018) 33(1):28–36. doi: 10.15605/jafes.033.01.05
40. Gebremichael GB, Zemichael TM. Hypoglycemia Prevention Practice and Associated Factors Among Diabetic Patients on Follow-Up at Public Hospitals of Centeral Zone, Tigray, Ethiopia, 2018. *Int J Endocrinol* (2020) 2020.

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Metabolically Abnormal But Normal-Weight Individuals Had a Higher Risk of Type 2 Diabetes Mellitus in a Cohort Study of a Chinese Population

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Aims: Obesity is a heterogeneous disease in terms of body mass index (BMI) and metabolic status. The purpose of this study was to investigate the risk of type 2 diabetes mellitus (T2DM) in subjects with metabolically abnormal but normal weight (MANW) in China.

Materials and Methods: A prospective cohort with a total of 17,238 participants of the Zhejiang metabolic syndrome cohort was recruited. According to the standard of the Working Group on Obesity in China, general obesity is defined. Metabolic abnormality was defined as two or more abnormal components (elevated triglycerides (TG), low high-density lipoprotein cholesterol (HDL-C), elevated systolic blood pressure (SBP) or diastolic blood pressure (DBP) or use of antihypertensive therapy, and elevated fasting plasma glucose or antidiabetic treatment). The hazard ratio (HR) and its 95% CI were calculated using a multiple regression model, adjusted for the potential confounding factors.

Results: Compared with metabolically normal and normal weight (MNNW) subjects, the metabolically abnormal and obesity/overweight (MAO) subjects had the highest risk of T2DM disease, with an HR of 4.67 (95% CI: 3.23–6.76), followed by MANW subjects (HR = 2.61, 95% CI: 1.74–3.92) and metabolically normal but obesity/overweight (MNO) subjects (HR = 2.09, 95% CI: 1.29–3.38) after adjusting for age, sex, smoking, drinking, physical activity, and family history of diabetes. Compared with that in the MNNW

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subjects, the HR in MANW subjects was significantly higher than that in MNO subjects. In normal-weight subjects, the HR of T2DM was significantly positively correlated with the number of components with metabolic abnormalities.

Conclusions: MANW subjects had a higher risk of T2DM. MANW subjects should be given more attention in the prevention and control of common chronic diseases.

Keywords: obesity, heterogeneity, type 2 diabetes mellitus, metabolically abnormal but normal weight (MANW), metabolically normal but obesity/overweight (MNO)

INTRODUCTION

Over the past few decades, the prevalence of overweight and obesity has risen rapidly around the world and has become a serious public health concern (1). Obesity increases the risks of cardiovascular disease, type 2 diabetes mellitus (T2DM), and all-cause mortality (2). Obesity is a heterogeneous disease with different phenotypes. According to the metabolic status, obesity can be divided into metabolic normality and abnormality obesity (3). Subjects who are obese without metabolic abnormalities are called metabolically normal but obesity/overweight (MNO) and account for about 18%–44%. About 5%–45% of individuals with normal weight have abnormal metabolic profiles (4, 5), which are known as metabolically abnormal but normal weight (MANW) population, and this group is relatively easy to ignore. Currently, many studies have been conducted on the relationship between MNO and the risk of developing diabetes, although the findings are inconsistent (6–8). However, there are relatively few studies on the risk of diabetes in MANW.

Previous studies have reported that MANW subjects might have an increased risk of long-term effects (8–11), but there was not enough research on the risk of diabetes in MANW. There is little reliable evidence from prospective studies with large sample size, especially in Chinese groups. Unlike Westerners, the Chinese had a lower body mass index (BMI) and smaller body size but had a higher body and visceral fat and a lower fat-free mass (12). In comparison with Caucasians, Chinese have more severe adverse profiles of metabolic components at the same BMI (13, 14). Therefore, it is of far-reaching significance to determine the relationship between different metabolic phenotypes and the risk of developing diabetes in a Chinese population, especially to determine the relationship between this MANW population that is not easy to pay attention to and diabetes.

In this study, we investigated the association between the heterogeneous phenotype of obesity and the risk of T2DM using a prospective cohort study of a total of 17,238 participants in China, and we tested the hypothesis that MANW individuals are at increased risk of diabetes.

Abbreviations: MNNW, metabolically normal and normal weight; MANW, metabolically abnormal but normal weight; MNO, metabolically normal but obesity/overweight; MAO, metabolically abnormal and obesity/overweight; BMI, body mass index; WC, waist circumference; TG, triglycerides; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; WHR, waist–hip ratio; WHtR, waist–height ratio.

MATERIALS AND METHODS

Study Population

The subjects were recruited from the Zhejiang metabolic syndrome cohort in the Zhejiang Province, Southeastern China.

The cohort is a community-based prospective cohort that started from 2010 to 2014. This investigation was a study of 22,649 participants in five counties/communities in Zhejiang Province. The subjects were recruited from the residents of the cluster sample communities. This sample can represent the general population of the Zhejiang Province in terms of geographical location, demographic characteristics, and socioeconomic status. The epidemiological investigation, clinical health examination, and routine biochemical measurements were conducted at baseline. The protocols have been described in detail in the previous studies (1, 2, 15) and are briefly described below.

The participants were recruited for this study if they were ≥18 years old and had a BMI ≥18.5 kg/m^2. Participants were excluded from baseline if they had cancer, diabetes, severe cardiovascular disease, or cerebrovascular disease (angina, cerebral infarction, and renal insufficiency) or if they had missing data for physical examination, such as weight, height, blood pressure (BP), waist circumference (WC), or biochemical determination, including triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and fasting plasma glucose (FPG). The participants who failed follow-up or migrated to other places (out of the county or city) were also excluded from the study. Ultimately, a total of 17,238 participants were recruited in the cohort.

The protocol was approved by the Ethics Committees of both Zhejiang University School of Medicine. Written informed consent was obtained from each participant.

Epidemiological Investigation and Anthropometric Measurements

At the baseline, the participants were interviewed face to face with a structured questionnaire; the details were reported previously (2, 16). The information solicited in the questionnaire included demographic data, such as date of birth, sex, educational level, smoking and alcohol drinking behaviors, physical activity, dietary habits, and family history of diabetes. Original smoking behavior in the cohort was investigated as current, previous, and never. Current smoking was defined as a person's smoking at least one cigarette per day for (at least) 1 year. Previous smoking was defined as a person's having quit for at least 1 year. Both current smoking and previous smoking were defined as smoking in the multiple regression. The alcohol consumption in the cohort was measured as the frequency

of drinking and was categorized into two groups: ≥3 times/week and <3 times/week. The smoking rate and drinking rate were calculated as the percentage of smokers or drinkers in each group. Information on physical activity was collected with the International Physical Activity Questionnaire (IPAQ) (short vision) (3). According to the instructions, calculate the energy consumed by each activity in units of metabolic equivalent (MET) minutes per week (MET-m/week). The threshold of total physical activity ≥600 MET-m/week is considered to be a moderate or high physical activity level. Calculate the sedentary time per week, and divide the participants into light, moderate, and heavy according to the quartile of sedentary time.

The anthropometric index, which includes weight, height, WC, systolic BP (SBP), and diastolic BP (DBP), were measured by well-trained investigators or doctors with a standard protocol (2). Height and weight were measured when the subjects wore light clothing and without shoes. WC was measured at the midpoint between the iliac crest and lowest rib. The record retains a decimal place. BP was measured in a sitting position with a mercury sphygmomanometer. SBP and DBP were reported as the average of three repeat measurements at 30-s intervals.

The overnight fasting blood samples were collected for each subject. Biochemical variables, including TG, total cholesterol (TC), HDL-C, and low-density lipoprotein cholesterol (LDL-C), were determined by a biochemical autoanalyzer (Hitachi 7060, Tokyo, Japan). FPG was analyzed using the glucose oxidase method with the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA, USA).

Definitions of Obesity and Metabolic Abnormality

BMI was calculated as weight (kg) divided by the square of height (m^2). General obesity was defined by BMI with the criteria of the Working Group on Obesity in China (WGOC) (4). Obesity was operationalized as a BMI ≥28 kg/m^2, overweight was a BMI ≥24 and <28 kg/m^2, and normal weight was a BMI ≥18.5 and <24 kg/m^2. Metabolically abnormal components included 1) high TG (≥1.7 mmol/L), 2) low HDL-C (men <1.03 mmol/L and women <1.29 mmol/L), 3) high SBP (≥130 mmHg) or DBP (≥85 mmHg) or use of antihypertensive drug therapy, and (16) high FPG (≥5.6 mmol/L) or antidiabetic treatment (5). Metabolic abnormality was defined as ≥2 abnormal components, whereas metabolic normality was defined as ≤1 abnormal component.

Therefore, metabolically normal and normal weight (MNNW) represents metabolic normality and normal weight, and MANW represents metabolic abnormality but normal weight. Metabolically abnormal and obesity/overweight (MAO) represents metabolic abnormality and obesity/overweight, whereas MNO represents metabolic normality but obesity/overweight.

Follow-Up and Case Ascertainment

All the subjects were followed up after the baseline investigation and ended on the date of diagnosis of T2DM, lost to follow-up, or censoring date (December 31, 2017), whichever came first. After exclusion of type 1 diabetes mellitus, gestational diabetes mellitus, or diabetes due to other causes, T2DM was defined using the following criteria: 1) FPG ≥7.0 mmol/l, 2) any treatment for diabetes, and 3) self-reported history of diabetes, which were previously diagnosed by clinical physicians.

In the cohort, the incidence of T2DM was identified through the Zhejiang Chronic Disease Surveillance System or by field-epidemiological investigation. The details of the Zhejiang Chronic Disease Surveillance System were reported previously (15). The investigators also asked each participant if they had ever been diagnosed with T2DM by clinical physicians.

Statistical Analyses

Continuous variables of normal distribution were described as mean and SDs, while continuous variables of skewed distribution were expressed as medians and interquartile ranges (IQRs). Categorical variables were expressed as number (%).

The chi-square test was used to compare categorical variables such as metabolic components and behavioral factors. One-way ANOVA was used to compare the four metabolic phenotypes.

The person-year of follow-up was calculated from the date of recruitment to the end of the follow-up period. The incidence of T2DM was calculated as the number of new cases divided by the person-years of follow-up. We have tested assumptions of the Cox proportional-hazards model using the Kaplan–Meier survival curves and a graphical approach based on a cumulative risk function, and the study was consistent with the Cox proportional-hazards model assumptions. The hazard ratio (HR) and its 95% CI of the obese phenotypes were calculated using a multiple Cox regression model, with the MNNW group as a reference group. There are three models used in the analysis to adjust for potential confounding factors: unadjusted model 1, model 2 with adjustments for age and sex, and model 3 with adjustments for age, sex, smoking, drinking, physical activity, and family history of diabetes.

In the sensitivity analysis, we first excluded subjects whose outcomes occurred in the first 2 years of follow-up to avoid reverse causation. The analysis was performed using the strict definition of metabolic normality as zero abnormal components, while metabolic abnormality was defined as ≥1 abnormal components. We also analyzed subjects who were restricted to non-smokers or stratified by sex.

A two-tailed $p < 0.05$ was considered statistically significant. All statistical analyses were conducted using PASW Statistics version 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline Characteristics of the Subjects

There were 17,238 participants recruited in this study. Their mean age is 54.1 years (SD: 13.9 years), and 42.6% of the subjects were male.

The basic characteristics of the subjects with the four obesity phenotypes are presented in **Table 1**. The prevalence of MAO, MNO, and MANW among the subjects was 25.8%, 13.4%, and 25.1% (in **Supplementary Table 1**). There were significant differences in anthropometric variables and metabolic components among the subjects with the four obesity phenotypes except for drinking rate (all p-values <0.05).

TABLE 1 | Baseline characteristics of subjects in the Zhejiang cohorts.

Variables	Zhejiang cohort				
	MNNW	MNO	MANW	MAO	*P*-Value
Number	6,159	2,315	4,321	4,443	
Men (%)	42.3	44.5	41.4	43.2	<0.001
Age (years)	49.3 (14.2)	49.2 (12.3)	60.6 (12.5)	56.8 (12.1)	<0.001
Weight (kg)	55.7 (6.7)	67.7 (8.4)	55.5 (7.0)	68.6 (9.0)	<0.001
BMI (kg/m^2)	21.4 (1.5)	26.1 (2.0)	21.8 (1.5)	26.7 (2.2)	<0.001
WC (cm)	75.1 (6.8)	85.8 (7.2)	77.7 (6.5)	88.7 (7.3)	<0.001
Smoking rate, n (%)	1,419 (24.5)	519 (23.7)	892 (21.9)	923 (21.9)	0.003
Drinking rate, n (%)	1,504 (27.3)	562 (27.0)	1,045 (27.0)	1,074 (26.5)	0.829
Physical activity					<0.001
Heavy, n (%)	1,094 (19.2)	395 (18.4)	653 (16.2)	706 (16.8)	
Moderate, n (%)	1,096 (19.3)	439 (20.5)	604 (15.0)	621 (14.8)	
Light, n (%)	3,495 (61.5)	1,310 (61.1)	2,780 (68.9)	2,869 (68.4)	
SBP (mmHg)	115.2 (11.6)	118.3 (10.4)	143.3 (20.2)	142.9 (19.7)	<0.001
DBP (mmHg)	70.7 (8.4)	73.6 (7.8)	83.0 (11.2)	85.0 (11.4)	<0.001
TG (mmol/l)	1.1 (0.6)	1.4 (0.8)	1.8 (1.4)	2.2 (1.6)	<0.001
FPG (mmol/l)	4.7 (0.6)	4.8 (0.6)	5.0 (0.7)	5.1 (0.7)	<0.001
HDL-C (mmol/l)	1.7 (6.6)	1.6 (6.6)	1.5 (5.0)	1.5 (8.8)	<0.001

MNNW, metabolically normal weight; MANW, metabolically abnormal but normal weight; MNO, metabolically normal obesity/overweight; MAO, metabolically abnormal obesity/overweight; n (%), count (percentage); BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol.

The Risk of Type 2 Diabetes Mellitus in Subjects With Different Metabolic Phenotypes of Obesity/Overweight

In the Zhejiang cohort, 327 new T2DM cases were identified during follow-up. The MNNW subjects had 41,233.0 person-years of follow-up, whereas MNO had 15,705.0 person-years, MANW had 27,945.0 person-years, and MAO had 292,74.0 person-years. The incidence rates of T2DM in the MNNW, MNO, MANW, and MAO were 1.02, 2.23, 3.08, and 5.60 per 1,000 person-years, respectively. The overall trends of T2DM incidence in the subjects with different phenotypes of obesity/overweight are presented in **Figure 1** and **Table 2**. The subjects with MAO had

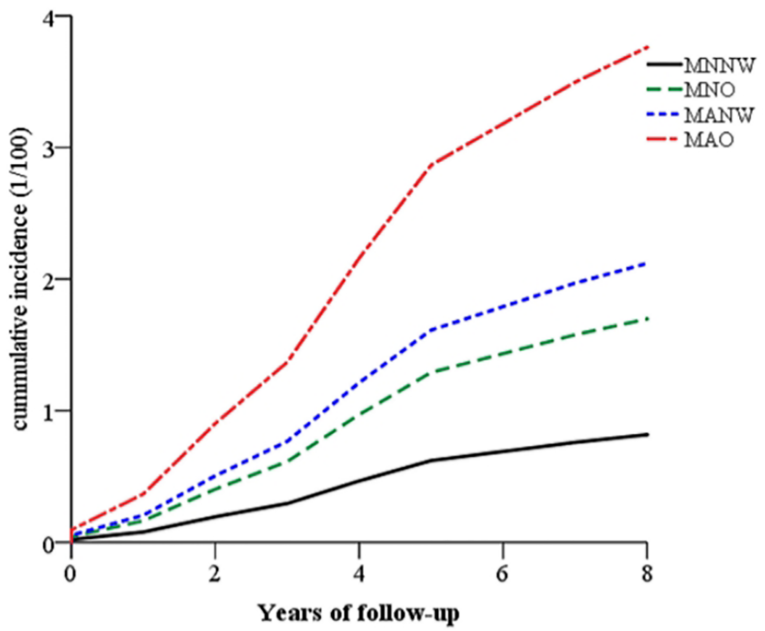

FIGURE 1 | The overall trends of the cumulative incidence of T2DM in the subjects with different phenotypes of obesity after follow-up in the Zhejiang cohort. The subjects with MAO (red and solid line) had the highest incidence of T2DM, followed by MANW (blue and dotted line) and MNO (green and dashed line). MNNW (black and dashed-dotted line) had a lower risk. T2DM, type 2 diabetes mellitus; MAO, metabolically abnormal and obesity/overweight; MANW, metabolically abnormal but normal weight; MNO, metabolically normal but obesity/overweight; MNNW, metabolically normal and normal weight.

TABLE 2 | Incidences and hazard ratios for type 2 diabetes mellitus in different obese/overweight phenotypes in the Zhejiang cohorts.

	MNNW	MNO	MANW	MAO
N	6,159	2,315	4,321	4,443
Person years of follow-up	41,233.0	15,705.0	27,945.0	29,274.0
Cases of type 2 diabetes	42	35	86	164
Incidence rate (per 1,000 person years)	1.02	2.23	3.08	5.60
Hazard ratio (95% CI)[§]				
Model 1	Ref	2.20 (1.41–3.45)	3.00 (2.07–4.34)	5.51 (3.92–7.73)
Model 2	Ref	2.21 (1.41–3.46)	2.68 (1.83–3.92)	5.12 (3.63–7.22)
Model 3	Ref	2.09 (1.29–3.38)	2.61 (1.74–3.92)	4.67 (3.23–6.76)

MNNW, metabolically normal and normal weight; MANW, metabolically abnormal but normal weight; MNO, metabolically normal but obesity/overweight; MAO, metabolically abnormal and obesity/overweight.

[§]Model 1: unadjusted Cox model. The overall model significance as calculated using Wald test is $p < 0.001$.

Model 2: adjusted for age and sex. The overall model significance as calculated using Wald test is $p < 0.001$.

Model 3: adjusted for age, sex, smoking, drinking, physical activity, and family history of diabetes. The overall model significance as calculated using Wald test is $p < 0.001$.

the highest incidence of T2DM, followed by MANW, MNO, and MNNW (**Figure 1**). After adjustment for age, sex, smoking, drinking, physical activity, and family history of diabetes in a multiple Cox regression model, MAO increased the risk for T2DM, with an HR of 4.67 (95% CI: 3.23–6.76) compared with that of the MNNW subjects. MANW and MNO had HRs of 2.61 (95% CI: 1.74–3.92) and 2.09 (95% CI: 1.29–3.38), respectively (**Table 2**). Consistent results were found in model 1 and model 2. In analyzing the subjects with overweight and obesity, subjects with metabolic abnormalities had a risk of T2DM, with HRs of 5.92 (3.77–9.31) for obesity, 4.29 (2.92–6.31) for overweight, and 2.60 (1.73–3.90) for normal weight. The subjects with metabolically normal obesity had risks of 3.08 (1.30–7.30) and 1.93 (1.16–3.23) in metabolically normal overweight subjects. However, no significant interaction was found ($p > 0.05$), and this result may be due to the limited sample size (**Supplementary Table 2**).

The Correlations Between the Number of Components With Metabolic Abnormalities and the Risk of Type 2 Diabetes Mellitus in Normal-Weight Subjects

Table 3 shows the correlations between the number of components with metabolic abnormalities and the HRs of T2DM with the normal-weight subjects. Taking subjects with no abnormal component as a reference, all other subjects had an increased risk of T2DM after adjusted for age, sex, smoking, drinking, physical activity, and family history of diabetes. Furthermore, the HRs of T2DM were 2.38 (95% CI: 1.42–4.01) in the group with two abnormalities and 3.92 (95% CI: 2.17–7.10) in the group with three and four abnormalities. The HRs of T2DM were significantly positively correlated with the number of components with metabolic abnormalities (all *p*-values for trends <0.05).

TABLE 3 | The correlations between the number of components with metabolic abnormalities and hazard ratios of T2DM in normal-weight subjects.

Number of abnormal metabolic components	HR (95% CI)
0	Ref
1	0.83 (0.48–1.46)
2	2.38 (1.42–4.01)
3+4	3.92 (2.17–7.10)
p for trend	<0.001

The HR (95% CI) adjusted for age, sex, smoking, drinking, physical activity, and family history of diabetes.

T2DM, type 2 diabetes mellitus; HR, hazard ratio.

Sensitivity Analysis

The results of the sensitivity analysis of the study are presented in **Table 4**. Consistent results were found after stratification by sex in males and when using WC as an index of obesity evaluation, and the risk of MNO compared with MNNW was higher than that of MANW. No significant difference was found if metabolically normality was defined as zero abnormalities in metabolic components or restricting the subjects to non-smokers.

Qualitatively consistent results were also found when the analysis was performed without adjustment, when adjusting for sex and age only or when making additional adjustments for smoking, drinking, physical activity, and family history of diabetes (**Tables 2**, **3**).

DISCUSSION

In this prospective cohort study, we found that MANW subjects had a higher T2DM risk than the MNNW subjects. The risk was also positively correlated with the number of abnormal components in normal-weight subjects.

There are different metabolic heterogeneities in obese and normal-weight subjects. Heterogeneous phenotypes may be associated with the risk of long-term effects. Many previous studies have investigated the effects of MNO, but the results have been inconsistent (6–13). These inconsistencies may be due to the different definitions of obesity and metabolic abnormalities, race, region, age, gender, etc. These inconsistencies raised the debate of MNO regarding whether Metabolic health but obesity (MHO) is a health status or a transitional status to MAO (7, 14). In this prospective study with a relatively large sample, we found that MNO increased the risk of T2DM compared with MNNW counterparts but had less risk than MAO participants. These results were consistent with many previous studies (6, 12, 13, 17).

TABLE 4 | Sensitivity analysis.

	MNNW	MNO	MANW	MAO
Metabolic normality that defined as zero abnormal component	Ref	0.97 (0.39–2.4)	1.98 (1.22–3.2)	4.18 (2.64–6.63)
Subjects without smoking	Ref	1.88 (1.09–3.22)	2.79 (1.79–4.34)	4.00 (2.65–6.05)
WC as index of obesity	Ref	3.95 (1.83–8.50)	2.81 (2.06–3.83)	6.57 (4.12–10.46)
after excluding the first 2 years of follow-up	Ref	1.89 (1.05–3.42)	2.93 (1.82–4.73)	5.06 (3.26–7.86)
Males	Ref	3.57 (1.58–8.05)	3.03 (1.45–6.36)	6.67 (3.39–13.11)
Females	Ref	1.62 (0.87–3.01)	2.70 (1.65–4.44)	4.39 (2.78–6.94)

Adjusted for age, sex, smoking, drinking, physical activity, and family history of disease.
MNNW, metabolically normal and normal weight; MANW, metabolically abnormal but normal weight; MNO, metabolically normal but obesity/overweight; MAO, metabolically abnormal and obesity/overweight; WC, waist circumference.

From this perspective, MNO was not a healthy status but had a lower risk than that associated with MAO.

Although previous studies have mainly focused on MNO, recently, researchers have started to pay attention to the biological effect of MANW. MANW is an individual with a normal BMI and metabolic abnormalities. This phenotype accounted for approximately 5% to 45% of the normal-weight individuals (18). This prevalence varied based on the definition of MANW, ethnicity, region, age, and gender distributions. In this study, we found that 25.1% of normal-weight individuals were MANW. Compared with their MNNW counterparts, MANW subjects usually have higher levels of visceral and ectopic fat as well as lower lean body mass and insulin sensitivity (19). MANW subjects might have higher risks of long-term effects (17, 20, 21). Reliable evidence is still lacking from prospective studies with large sample sizes, especially from Asian populations, including Chinese populations. Chinese people usually have a low BMI and fat-free mass and higher body fat and visceral fat (19), and they have more abnormal profiles of metabolic components with the same BMI (22, 23). Therefore, the effects of heterogeneous phenotypes might be different between Asian and Western populations. In this study, we found that MANW subjects had approximately three times the T2DM risk compared with MNNW subjects (HR = 2.61, 95% CI: 1.74–3.92) in model 3. Furthermore, MANW subjects also had a higher T2DM risk than their MNO counterparts when compared with MNNW.

In the cohort, the subjects with obesity and abnormalities also had the highest risks (HR = 5.92, 95% CI: 3.77–9.31) (in **Supplementary Table 2**); however, their interaction did not reach statistical significance. This negative association might be due to the limited sample size. In addition to adverse metabolic effects, obesity has multiple other effects that might increase T2DM risk. On the other hand, other risk factors induce metabolic abnormalities in addition to obesity. Therefore, a synergistic effect may be yielded once obesity and metabolic abnormalities are combined. In normal-weight subjects, the number of abnormal components significantly correlated with the HRs of T2DM. Integrating these results, metabolic status seems more important than obesity. This finding was consistent with the result of Liu's study (13).

These results indicated that MANW individuals were also the higher-risk population for T2DM. Compared with individuals with MNO and MAO, MANW more easily masks the need for screening, thereby delaying diagnosis and treatment and neglecting health management due to seemingly normal weight or normal BMI. Therefore, the public health implication in this study lies in the fact that phenotype-based management is important for obesity and that MANW individuals should be given more attention in clinical and public practice. Our results showed that the average age of the MANW group was higher than that of the other three groups and that in addition to weight control, attention should be paid to changes in biochemical indicators such as blood glucose, BP, HDL-C, and TG in the MANW (24), because in these people with normal BMI, it is easy to ignore the detection of these indicators. Annual health checkups and the modification of dietary habits are necessary. For example, improving the diet such as a diet rich in fruits and vegetables can reduce BP (25), and modifying macronutrient composition can affect lipid levels (26).

The Zhejiang cohort was recruited with a relatively large sample size of 17,238 participants and a long follow-up period of 10 years. The epidemiological data and the biochemical measurements were collected by trained health professionals following standard protocols. The incidences of T2DM were ascertained by record linkage with the data from the local chronic disease surveillance system or by field-epidemiological investigation. The potential confounding bias was controlled with a multiple Cox regression model, although similar results were found using different models. Sensitivity analysis also indicated consistent results under different conditions. These strengths increased the reliability of the evidence.

However, there were some limitations in the study. First, there were multiple definitions of obesity and metabolic abnormalities, and no consensus has been reached until now, so the results may restrict the extrapolation to some previous studies using different definitions. To increase extrapolation, the present study used the most common definition of metabolic abnormality of ≥2 abnormal metabolic components. Second, repeated baseline surveys are ongoing, and the dynamic information on metabolic and obesity status has not been completely collected.

CONCLUSION

Briefly, we found that MANW subjects had a higher T2DM risk, and this risk was higher than that in MNO subjects when compared with MNNW. The risk positively correlated with the number of abnormal components in normal-weight subjects. Phenotype-based management is important for obesity, and MANW individuals should be given more attention in clinical and public practice.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed in the current study are available from the corresponding authors and investigating coordinators upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committees of Zhejiang University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.724873/full#supplementary-material

REFERENCES

1. Zheng R, Liu C, Wang C, Zhou B, Liu Y, Pan F, et al. Natural Course of Metabolically Healthy Overweight/Obese Subjects and the Impact of Weight Change. *Nutrients* (2016) 8(7). doi: 10.3390/nu8070430
2. Zheng R, Yang M, Bao Y, Li H, Shan Z, Zhang B, et al. Prevalence and Determinants of Metabolic Health in Subjects With Obesity in Chinese Population. *Int J Environ Res Public Health* (2015) 12(11):13662–77. doi: 10.3390/ijerph121113662
3. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International Physical Activity Questionnaire: 12-Country Reliability and Validity. *Med Sci Sports Exerc* (2003) 35(8):1381–95. doi: 10.1249/01.MSS.0000078924.61453.FB
4. Zhou BFCooperative Meta-Analysis Group of the Working Group on Obesity in C. Predictive Values of Body Mass Index and Waist Circumference for Risk Factors of Certain Related Diseases in Chinese Adults–Study on Optimal Cut-Off Points of Body Mass Index and Waist Circumference in Chinese Adults. *BioMed Environ Sci* (2002) 15(1):83–96.
5. Alberti KGMM, Zimmet P, Shaw J. The Metabolic Syndrome - A New Worldwide Definition. *Lancet* (2005) 366(9491):1059–62. doi: 10.1016/S0140-6736(05)67402-8
6. Aune D, Sen A, Norat T, Janszky I, Romundstad P, Tonstad S, et al. Body Mass Index, Abdominal Fatness, and Heart Failure Incidence and Mortality: A Systematic Review and Dose-Response Meta-Analysis of Prospective Studies. *Circulation* (2016) 133(7):639–49. doi: 10.1161/CIRCULATIONAHA.115.016801
7. Boonchaya-Anant P, Apovian CM. Metabolically Healthy Obesity-Does It Exist? *Curr Atheroscl Rep* (2014) 16(10). doi: 10.1007/s11883-014-0441-1
8. Hamer M, Stamatakis E. Metabolically Healthy Obesity and Risk of All-Cause and Cardiovascular Disease Mortality. *J Clin Endocrinol Metab* (2012) 97(7):2482–8. doi: 10.1210/jc.2011-3475
9. Meigs JB, Wilson PW, Fox CS, Vasan RS, Nathan DM, Sullivan LM, et al. Body Mass Index, Metabolic Syndrome, and Risk of Type 2 Diabetes or Cardiovascular Disease. *J Clin Endocrinol Metab* (2006) 91(8):2906–12. doi: 10.1210/jc.2006-0594
10. Schulze MB. Metabolic Health in Normal-Weight and Obese Individuals. *Diabetologia* (2019) 62(4):558–66. doi: 10.1007/s00125-018-4787-8
11. Stefan N, Haring HU, Hu FB, Schulze MB. Metabolically Healthy Obesity: Epidemiology, Mechanisms, and Clinical Implications. *Lancet Diabetes Endocrinol* (2013) 1(2):152–62. doi: 10.1016/S2213-8587(13)70062-7
12. Janghorbani M, Salamat MR, Amini M, Aminorroaya A. Risk of Diabetes According to the Metabolic Health Status and Degree of Obesity. *Diabetes Metab Syndr* (2017) 11(Suppl 1):S439–44. doi: 10.1016/j.dsx.2017.03.032
13. Liu M, Tang R, Wang JH, He Y. Distribution of Metabolic/Obese Phenotypes and Association With Diabetes: 5 Years' Cohort Based on 22,276 Elderly. *Endocrine* (2018) 62(1):107–15. doi: 10.1007/s12020-018-1672-7
14. Bluher M. Are Metabolically Healthy Obese Individuals Really Healthy? *Eur J Endocrinol* (2014) 171(6):R209–19. doi: 10.1530/EJE-14-0540
15. Zhou Y, Zhang X, Zhang L, Li Z, Wu Q, Jin Z, et al. Increased Stroke Risk in Metabolically Abnormal Normal Weight: A 10-Year Follow-Up of 102,037 Participants in China. *Transl Stroke Res* (2020) 12(72):725–34. doi: 10.1007/s12975-020-00866-1
16. Wu S, Jin C, Li S, Zheng X, Zhang X, Cui L, et al. Aging, Arterial Stiffness, and Blood Pressure Association in Chinese Adults. *Hypertension* (2019) 73(4):893–9. doi: 10.1161/HYPERTENSIONAHA.118.12396
17. Aung K, Lorenzo C, Hinojosa MA, Haffner SM. Risk of Developing Diabetes and Cardiovascular Disease in Metabolically Unhealthy Normal-Weight and Metabolically Healthy Obese Individuals. *J Clin Endocrinol Metab* (2014) 99(2):462–8. doi: 10.1210/jc.2013-2832
18. Ding C, Chan Z, Magkos F. Lean, But Not Healthy: The 'Metabolically Obese, Normal-Weight' Phenotype. *Curr Opin Clin Nutr Metab Care* (2016) 19(6):408–17. doi: 10.1097/MCO.0000000000000317
19. Haldar S, Chia SC, Henry CJ. Body Composition in Asians and Caucasians: Comparative Analyses and Influences on Cardiometabolic Outcomes. *Adv Food Nutr Res* (2015) 75:97–154. doi: 10.1016/bs.afnr.2015.07.001
20. Lassale C, Tzoulaki I, Moons KGM, Sweeting M, Boer J, Johnson L, et al. Separate and Combined Associations of Obesity and Metabolic Health With Coronary Heart Disease: A Pan-European Case-Cohort Analysis. *Eur Heart J* (2018) 39(5):397–406. doi: 10.1093/eurheartj/ehx448
21. Seo YG, Choi HC, Cho B. The Relationship Between Metabolically Obese Non-Obese Weight and Stroke: The Korea National Health and Nutrition Examination Survey. *PloS One* (2016) 11(8). doi: 10.1371/journal.pone.0160846
22. Consultation. WE. Appropriate Body-Mass Index in Asian Populations and Its Implications for Policy and Intervention Strategies. *Lancet* (2004) 363(9412):902. doi: 10.1016/S0140-6736(03)15268-3
23. He W, Li Q, Yang M, Jiao J, Ma X, Zhou Y, et al. Lower BMI Cutoffs to Define Overweight and Obesity in China. *Obes (Silver Spring)* (2015) 23(3):684–91. doi: 10.1002/oby.20995
24. Eckel N, Muhlenbruch K, Meidtner K, Boeing H, Stefan N, Schulze MB. Characterization of Metabolically Unhealthy Normal-Weight Individuals: Risk Factors and Their Associations With Type 2 Diabetes. *Metabolism* (2015) 64(8):862–71. doi: 10.1016/j.metabol.2015.03.009

25. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A Clinical Trial of the Effects of Dietary Patterns on Blood Pressure. DASH Collaborative Research Group. *N Engl J Med* (1997) 336(16):1117–24. doi: 10.1056/NEJM199704173361601
26. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of Dietary Fatty Acids and Carbohydrates on the Ratio of Serum Total to HDL Cholesterol and on Serum Lipids and Apolipoproteins: A Meta-Analysis of 60 Controlled Trials. *Am J Clin Nutr* (2003) 77(5):1146–55. doi: 10.1093/ajcn/77.5.1146

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Syndrome Differentiation and Treatment Regularity in Traditional Chinese Medicine for Type 2 Diabetes: A Text Mining Analysis

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Objective: The goal of this study was to systematically summarize and categorize the syndrome differentiation, medication rules, and acupoint therapy in the domestic traditional Chinese medicine (TCM) literature on type 2 diabetes mellitus (T2DM), such that guidelines and new insights can be provided for future practitioners and researchers.

Methods: Taking randomized controlled trials (RCTs) on the treatment of T2DM in TCM as the research theme, we searched for full-text literature in three major clinical databases, including CNKI, Wan Fang, and VIP, published between 1990 and 2020. We then conducted frequency statistics, cluster analysis, association rules extraction, and topic modeling based on a corpus of medical academic words extracted from 3,654 research articles.

Results: The TCM syndrome types, subjective symptoms, objective indicators, Chinese herbal medicine, acupuncture points, and TCM prescriptions for T2DM were compiled based on invigorating the kidney and *Qi*, nourishing *Yin*, and strengthening the spleen. Most TCM syndrome differentiation for T2DM was identified as "*Zhongxiao*" (the lesion in the spleen and stomach) and "*Xiaxiao*" (the lesion in the kidney) deficiency syndromes, and most medications and acupoint therapies were focused on the "Spleen Channel" and "Kidney Channel." However, stagnation of liver *Qi* was mentioned less when compared with other syndromes, which did not have symptomatic medicines.

Conclusion: This study provides an in-depth perspective for the TCM syndrome differentiation, medication rules, and acupoint therapy for T2DM and provides practitioners and researchers with valuable information about the current status and frontier trends of TCM research on T2DM in terms of both diagnosis and treatment.

Keywords: type 2 diabetes mellitus, traditional Chinese medicine syndromes, acupoint therapy, Chinese herbal medicine, text mining

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic endocrine and metabolic disorder characterized by either reduced insulin production or insulin resistance resulting in drastically increased blood glucose levels (1). T2DM has been contributing to the burden of mortality and disability worldwide (2). Globally, at least 1 in 11 adults has diabetes mellitus (nearly 90% of them having T2DM pathology), while the T2DM cases in Southeast Asia are projected to reach the 151 million mark by the end of 2045 (3–6). The latest epidemiological survey data indicate that the prevalence of T2DM in mainland China is about 12.8%, according to the American Diabetes Association (7). Since the initiation and progression of diabetes associated with multifaceted comorbid conditions, it has been urgently required to re-optimize the diagnostic and treatment procedures, keeping pace with the advancement of medical sciences and counting in any novel pathological conditions related to diabetes.

For centuries traditional Chinese medicine (TCM) has been proven to be highly effective in treating numerous chronic and critical illnesses, including diabetes, which can be traced back to the theories illustrated in the Inner Canon of Yellow Emperor. Notably, the research has found that the clinical benefits of kai-yu-jiang-zhuo decoction are similar to that of metformin, the most used diabetes medicine (PMID: 25132859). Other studies have shown that Tianqi notoginseng root extract can dramatically reduce the risk of transforming impaired glucose tolerance into chronic T2DM by nearly 32% compared to a placebo treatment (PMID: 24432995). Furthermore, routine treatment with tang-min-ling pills, a combination of ten Chinese herbal medicines, can significantly improve the insulin secretion by pancreatic β cells and reduce the level of fasting blood glucose and glycosylated hemoglobin in patients with diabetes (PMID: 23231379). Since TCM treatments rely greatly on the syndrome differentiation, they, can formulate the most effective personalized herbal medicine regimen. Thus, this strategy has shown potential efficacy in treating T2DM and related disorders in several studies (PMID: 16466178, PMID: 7841750, PMID: 11783182).

In TCM, "*xiaoke*" is the name for diabetes (PMID: 20923535). Classically, diabetes syndromes can be divided into four deficiency syndromes: *Qi* (i.e., life force or vital energy, which belongs to *Yang*) deficiency; *Yin* (i.e., the opposite of *Yang*; e.g., liquid, blood) deficiency and excessive heat syndrome; *Qi* and *Yin* deficiencies and kidney *Yin* deficiency; and two excess syndromes that include blood stasis and phlegm-dampness syndromes (8). In TCM, early-phase diabetes-associated lesions are also classified distinctly as "*shangxiao*" for lung lesions, "*xiaxiao*" for kidney lesions, and "*tripteryg*" for spleen and stomach lesions (PMID: 31704615).

Studies have found that microvascular complications in diabetes influence chronic comorbid complications (PMID: 27230641). Therefore, in practice, TCM clinicians primarily focus on removing blood stasis to improve blood circulation, similar to practices in Western medical therapies (PMID: 23238996). Blood stasis occurs due to *Qi* deficiency, stagnation, and cold retention (PMID: 23163160). Hence, invigorating the spleen and promoting *Qi* using TCM herbs can significantly reduce the risk of diabetic nephropathy (PMID: 28641649).

The treatment for T2DM in TCM includes Chinese herbal medicine (9–12), acupuncture (13, 14), exercise therapy (15–17), and diet (18, 19), with their therapeutic effects supported by previous clinical reports (20). Unlike Western therapies, which mainly focus on blood glucose regulation in patients with diabetes, TCM emphasizes symptom improvement, which can provide long-term benefits and prevent secondary complications (20).

Despite the potential efficacy and importance of TCM applications in the treatment of T2DM, no studies have systematically summarized the research hotspots and fundamental mechanisms underlying this procedure. Randomized controlled trials (RCTs) are the gold standard for evaluating therapeutic effects, and increasingly well-designed RCTs have been conducted worldwide to evaluate the efficacy of TCM-based therapies (21–24). This study intends to systematically review the literature on RCTs for the treatment of T2DM based on TCM and analyze the frequency of medical/academic words utilized in these RCTs by extracting them using text mining with natural language process (NLP) tools.

Natural language processing (NLP) is a field of artificial intelligence that utilizes computers to analyze, understand, and interpret human language. It has received increasing attention in medical research in recent years. For a large variety of unstructured texts such as diagnostic tests, surgical records, test reports, medical orders, progress note, and nursing records, NLP has become an important method and tool for mining clinically useful information in medical texts. For example, some researchers used deep learning Word2vec and TF-IDF to extract text features before, applying association rule algorithm and complex network analysis methods to analyze the compatibility between pairs of drugs and correlations of drugs with symptoms (25–29). In addition, NLP tools have been applied in the field of TCM in recent years. For example, Wei et al. extracted Chinese herbal medicines with high frequencies through data mining of prescriptions in classic TCM literature (30). They applied NLP to extract diagnostic information obtained from tongue images of patients to generate prescriptions by a convolutional neural network (31).

At present, the NLP applications in the field of TCM primarily focus on the unstructured data of medical records, including TCM syndrome, tongue image, pulse condition, prescription drug rule (32–34), whereas there are few researchers who have applied NLP for extracting information in TCM research articles. In TCM research, manual extraction of a large amount of information is still applied to transform unstructured data into structured data, a time-consuming and laborious process, and mistakes can easily be made. In this study, with text mining using NLP tools, the current research status of the focused medical academic words and inter-relationship among them can be discovered, providing novel knowledge and in-depth guidelines for future research on TCM-based treatments for T2DM.

METHODS

Data Collection

Research articles for the treatment of T2DM in TCM published from 1990 to 2020 were retrieved from the three major Chinese literature databases (Wan Fang, VIP, and CNKI). The following queries were used to search for articles by title, summary, or keywords: ("type 2 diabetic mellitus" or "type 2 diabetes" or "T2DM" or "type II diabetic mellitus" or " DM II "or "type II diabetes") and ("Chinese medicine" or "TCM" or "Chinese herbal medicine" or "traditional medicine" or "alternative medicine" or "complementary medicine" or "herbal" or "prescription" or "recipe" or "formula" or "Chinese patent medicine" or "Chinese traditional patent medicine" or "han prescription" or "TCMWM" or "acupuncture" or "needle" or "moxibustion" or " points" or "syndromes" or "syndrome" or "syndrome elements" or "integrated traditional Chinese and western medicine" or "integrated Chinese and western medicine" or "integrated traditional and western medicine" or "TCMWM" or "TCM-WM" or "integrated TCM WM").

Inclusion Criteria

For simplicity and objectivity, only RCTs of T2DM research in TCM were selected to establish the corpus of medical academic words and future analysis in this study.

Exclusion Criteria

Articles were excluded if they focused on (1) Type 1 diabetes; (2) only Western medicine treatment; (3) diagnosis; (4) policy; (5) detection; (6) nursing therapy; (7) risk prediction; (8) massage; (9) extraction process of botanical ingredients; (10) patient compliance. Finally, 3,654 published full-text RCTs were selected for the subsequent text mining. The selection process of this study is depicted in **Figure 1**.

Corpus Establishment

Based on the chosen RCTs, we established a corpus of medical academic words with Python 3.7. First, the PDF documents were recognized using the optical character recognition (OCR) technology; then, the focused medical academic words were extracted by segmenting words, removing stop words, and applying a custom dictionary (specific details are shown in **Figure 2**). Furthermore, a total of 41 valid fields were extracted by our automated extraction system, including patient age, group, prescription, herbal medicine, dosage, blind method. The primary rule-based information extraction used in this work is regular expression—a string of texts that allows users to create patterns to match, locate, and manage text—with extracted fields determined by clinical experts of TCM (specific results are shown in **Supplementary 1**, and **Supplementary Figures 1**, **2**). To illustrate the extraction procedure, we took the extraction of TCM prescriptions of T2DM as an example and constructed a binary matrix in which columns are herbal materials, rows represent prescriptions, and each cell has a value of either 0 or 1 (shown in **Table 1**).

Data Analysis

Frequency Statistics

We used Python 3.7 for word frequency analysis for each word list categorized as subjective symptoms, TCM syndrome types, TCM prescriptions and herbal medicine, and acupuncture points.

Topic Modeling

We used Python 3.7 to conduct topic modeling. The latent Dirichlet allocation (LDA) algorithm was applied for topic modeling (**Figure 3**) to allocate topics to the focused RCTs and words of each RCT to topics based on the Dirichlet distribution. This study used feature weight (TF – IDF = TF × IDF) to evaluate the importance of a word to a text as important evidence for classifying text on the same topic. The TF represents the frequency of a word t_i appearing in the text d_j, expressed by the formula:

$$TF_{i,j} = \frac{n_{i,j}}{\sum_k n_{k,j}}$$

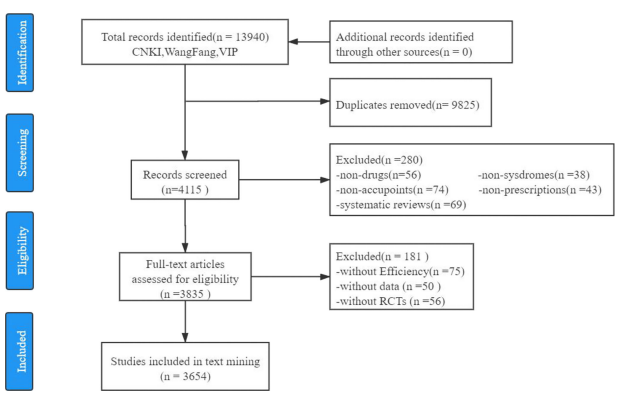

FIGURE 1 | Flowchart for the selection of RCTs.

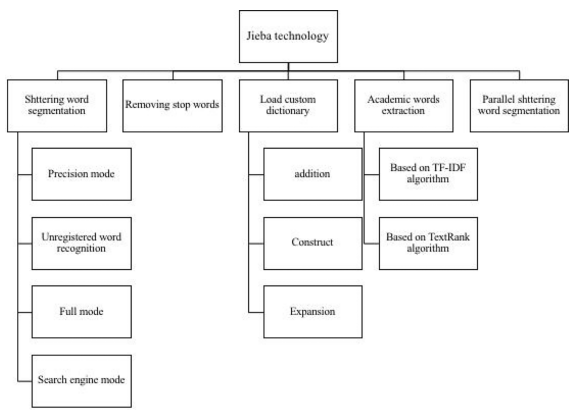

FIGURE 2 | Schematic diagram of stuttering word segmentation. Stuttering word segmentation technology roadmap, from word segmentation, removing stop words, loading custom dictionaries, keyword extraction, part-of-speech tagging to drawing keyword word cloud maps.

TABLE 1 | An example of a database constructed from literature.

	Astragalus	Rehammania	Yam	Puerarin	Coptidis rhizoma
Prescription1	1	0	1	0	0
Prescription2	1	1	0	1	0
Prescription3	0	0	1	0	1
Prescription4	0	0	1	0	1
Prescription5	1	1	0	0	1
Prescription6	0	0	1	0	0
…					
Prescription323	1	0	0	1	0

The IDF refers to the reverse file frequency, and the calculation formula is:

$$idf_i = \log \frac{|D|}{|\{j : t_i \in d_j\}|}$$

where $|D|$ is the total number of files in the corpus, $|\{j: t_i \in d_j\}|$ indicates the number of files containing the word t_i. As indicated by the formula of the feature weight, the more frequently a word appears in one document and the less frequently it appears in another document, the higher the weight and TF – IDF would be.

Association Rule Extraction

SPSS Modeler 14.1 was applied to extract association rules for the high-frequency words (i.e., greater than 2%) in subjective symptoms, TCM syndrome types, Chinese herbal medicines, acupuncture points, and TCM prescriptions in each focused RCT. An *a priori* algorithm was applied; the minimum support was set at 10%, minimum confidence at 80%, and the lift at >1.

Cluster Analysis

In order to cluster the focused RCTs into groups with similar characteristics, SPSS 22.0 was employed to conduct cluster analysis for the occurrence of high-frequency words (i.e., greater than 2%) in subjective symptoms, TCM syndrome types, Chinese herbal medicines, acupuncture points, and TCM prescriptions in each of the focused RCTs. In this study, a hierarchical clustering algorithm with between-groups linkage was applied based on correlation-based distance.

RESULTS

The Distribution of Subjective Symptoms

First, we analyzed the distribution of subjective symptoms based on the frequency of the occurrence, applying an arbitrary cutoff threshold of 2% (**Figure 4**). Among them, obesity was the most frequent symptom, followed by polyphagia, polydipsia, polyuria, tiredness and fatigue, thirst and polyphagia, dysphoria in chest, palms, and soles, and soreness and weakness of the waist and knees. From the close observation of frequencies of various symptoms, we found that the nature of the lesion had been focused on deficiencies in multiple energy metabolism systems, and the lesion sites were concentrated in the "spleen" and "kidney."

The Distribution of Syndromes

The frequency-based distribution of TCM syndromes is shown in **Figure 5**. Syndromes with the occurrence of higher than 2% were dampness stagnancy and spleen deficiency, *Yin* and *Yang* deficiency, *Yin* deficiency, liver-*Qi* stagnation, liver and kidney *Yin* deficiency, spleen and kidney *Yang* deficiency, kidney *Yin* deficiency, stagnation of liver *Qi* and spleen deficiency, stomach heat flaming syndrome, spleen deficiency syndrome, and spleen and kidney deficiency syndrome. The pathogenic factors mainly were related to "dampness, heat", which obstructed the movement of *Qi* and blood, leading to "fat".

The Distribution of Prescriptions and Herbal Medicine

This research contains 323 prescriptions and 273 herbal materials. The frequency of TCM prescribed medicines for

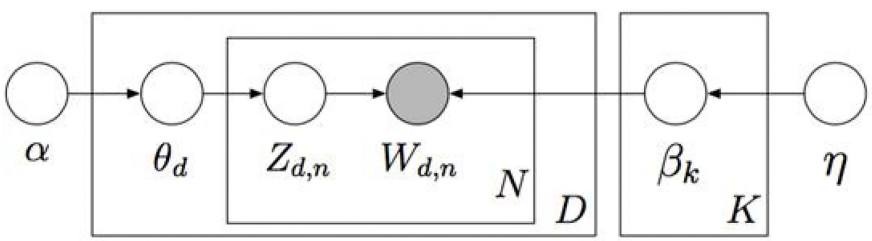

FIGURE 3 | Graphic model for Latent Dirichlet allocation. K, total number of topics; β_k, topic, a distribution over the vocabulary; D, total number of documents; θ_d, per-document topic proportions; N, total number of words in a document (in fact, it should be N), $Z_{d,n}$, per-word topic assignment; $W_{d,n}$, observed word; α, ?, Dirichlet parameters.

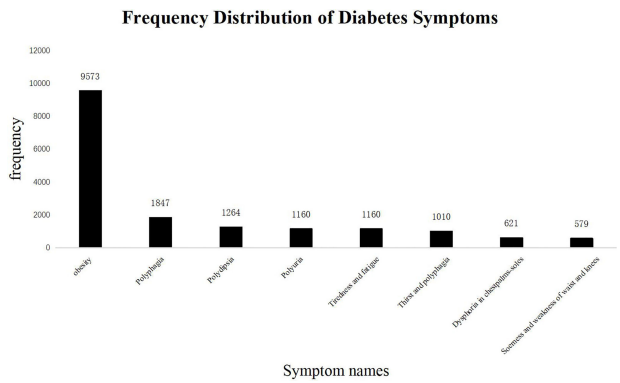

FIGURE 4 | Frequency Distribution of Diabetes Symptoms. The frequency of TCM subjective symptoms in the diabetes literature. The abscissa represents the name of the subjective symptoms, and the ordinate shows the frequency of each symptom. The total number of subjective symptoms of TCM was 27,448, of which more than 2% are as follows: Obesity: 9,573 (34.8%); Polyphagia: 1,847 (6.7%); Polydipsia: 1,264 (4.6%); Polyuria: 1,160 (4.2%); Tiredness and fatigue: 1,160 (4.2%); Thirst and polyphagia: 1,010 (3.6%); Dysphoria in chest, palms, and soles: 621 (2.2%); Soreness and weakness of waist and knees: 579 (2.1%). The most frequent occurrence is "Obesity", a common symptom of "spleen deficiency and dampness".

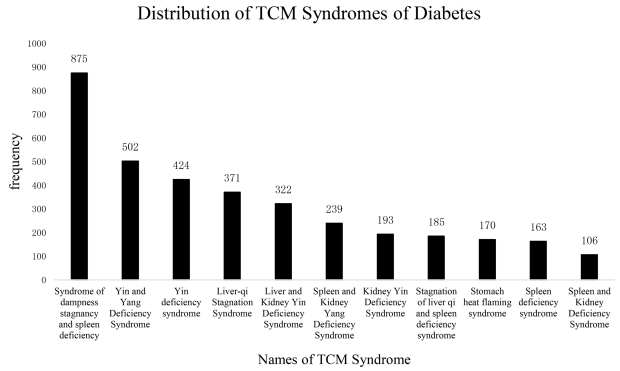

FIGURE 5 | Distribution of TCM Syndromes of Diabetes. The frequency of TCM syndromes in the diabetes literature. The abscissa represents the name of the syndrome; the ordinate shows the frequency of each name. The total number of TCM syndromes was 4,801, among which more than 2% are as follows: Syndrome of dampness stagnancy and spleen deficiency: 875 (18.2%); Yin and Yang deficiency syndrome: 502 (10.4%); Yin deficiency syndrome: 424 (8.8%); Liver-qi stagnation syndrome: 371 (7.7%); Liver and kidney Yin deficiency syndrome: 322 (6.7%); Spleen and kidney Yang deficiency: 239 (4.9%); Kidney Yin deficiency: 193 (4.0%); Stagnation of liver qi and spleen deficiency syndrome: 185 (3.8%); Stomach heat flaming syndrome: 170 (3.5%); Spleen deficiency syndrome: 163 (3.3%); Spleen and Kidney Deficiency Syndrome: 106 (2.2%). From the distribution of syndromes, it can be seen that the "spleen deficiency and dampness syndrome" appears most frequently, which may be related to diabetes the patient's "polydipsia, polyphagia" is related, the spleen deficiency, and the movement and transformation are unfavorable, which leads to the body dampness, evil, and blood stasis group.

treating diabetic symptoms is shown in **Figure 6**. Medicines prescribed in more than 2% of all literature were (in descending order) *Pill of ingredients with Rehmannia*, *Xiaoke Pills*, *Yiqiyangyinhuoxue Decoction*, *Jinlida Granules*, *Yiqihuoxue Decoction*, *Berberine*, and *Shenqi Jiangtang Granules*. As before, the prescription medicines were mainly composed of Chinese herbal drugs that could invigorate the spleen, replenish *Qi*, and nourish *Yin*.

Most prescriptions (formulas and recipes) comprise several herbal ingredients. The frequency distribution of Chinese herbal medicines based on their application popularity in T2DM treatment is shown in **Table 2**. Astragalus membranaceus (Huang Qi) remains the most frequently used medicinal herb in TCM practice, followed by Rehmannia (Di Huang), Chinese yam, Puerariae (Ge Gen), Coptis rhizome (Huang Lian), Radix Ophiopogonis (Mai Men Dong), Salvia miltiorrhiza (red sage or Dan Shen), Poria cocos (Fi Ling), Radix Trichosanthis (Tian Hua Fen), and Anemarrhena (Zhi Mu).

The Distribution of Acupuncture Points

Commonly used acupuncture points in TCM for T2DM treatments are shown in **Figure 7** in descending order based on their frequency of application in clinical practice, as *Zusanli* (*ST36*), *Sanyinjiao* (*SP36*), *Zhongwan* (*RN12*), *Pishu* (*BL20*), *Guanyuan* (*RN4*), *Tianshu* (*ST25*), *Fenglong* (*ST40*), *Shenshu* (*BL23*), *Qihai* (*RN6*), *Quchi* (*LI11*), *Taixi* (*KI3*), *Yinlingquan* (*SP9*), and *Guangming* (*GB37*). The above acupuncture points are primarily distributed on the Stomach Meridian of Foot-Yangming, Spleen Meridian of Foot-Taiyin, Kidney Meridian of Foot-Shaoyin, and Bladder Meridian of Foot-Taiyang. The principle treatment is directed towards replenishing *Qi*, invigorating the spleen, nourishing Yin, and clearing up the heat.

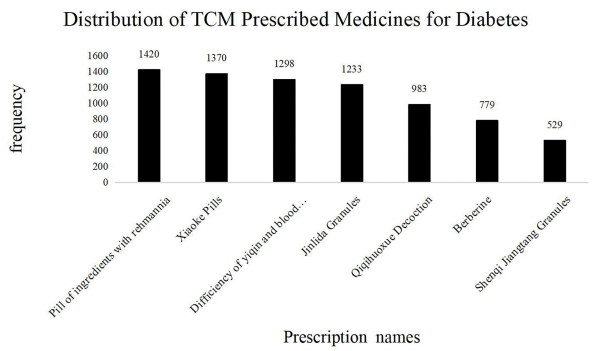

FIGURE 6 | Distribution of TCM Prescribed Medicines for Diabetes. The frequency of TCM prescriptions in the diabetes literature. The abscissa represents the name of the TCM prescription; the ordinate shows the frequency of each prescription name. The total number of TCM prescriptions was 24,748, of which more than 2% are as follows: Pill of ingredients with Rehmannia: 1,420 (5.7%); Xiaoke Pills: 1,370 (5.5%); Deficiency of yiqin and blood unsmooth: 1,298 (5.2%); Jinlida Granules: 1,233 (4.9%); Qiqihuoxue Decoction: 983 (3.9%); Berberine: 779 (3.1%); Shenqi Jiangtang Granules: 529 (2.1%). The most frequently used ones are "pill of ingredients with Rehmannia". The efficacy of the prescription is consistent with the above symptoms and syndromes.

TABLE 2 | Table of classification and frequency of commonly used Chinese medicines for treating diabetes.

ID	Names	Classification	Frequency	Percentage
1	Astragalus	Tonifying and replenishing medicinal	400	5.43%
2	Rehammania	Heat-clearing medicinal	310	4.21%
3	Yam	Tonifying and replenishing	298	4.05%
4	Pueraria	Exterior-releasing medicinal	269	3.65%
5	Coptis	Heat-clearing medicinal	260	3.53%
6	Ophiopogon	Tonifying and replenishing medicinal	250	3.39%
7	Salvia	Blood-activating and stasis-dispelling Medicinal	240	3.26%
8	Poria	Dampness-draining diuretic medicinal	232	3.15%
9	Radix Trichosanthis	Heat-clearing medicinal	224	3.04%
10	Anemarrhenae	Heat-clearing medicinal	222	3.01%

Astragalus (400, 5.43%), Rehmannia (310, 4.21%), Chinese yam (298, 4.05%), Puerariae (269, 3.65%), Coptis rhizome (260, 3.53%), Radix Ophiopogonis (250, 3.39%), Salvia miltiorrhiza (240, 3.26%), Poria cocos (232, 3.15%), Radix Trichosanthis (224, 3.04%), and Anemarrhena (222, 3.01%).

Text Topic Extraction

In this study, all included documents were divided into four topic-based categories, and each topic extraction selects the top 10 medical academic keywords with the highest frequency. The results of topic-keywords extraction are shown in **Supplementary Figures 3**, **4** and **Table 1**. We determine the number of topic categories by calculating Perplexity and MPI-score, as shown in our results. When the topic number K was set to 4, there was no intersection between each topic, indicating that 3,654 published RCTs in the literature could be divided into four exclusive topics. Topic 1 was diabetes complicated with cardiovascular and renal dysfunctions, such as urinary protein, glomerulus, proteinuria, diabetic nephropathy, tripterygium wilfordii, urea nitrogen, and chronic nephritis, indicating that these studies were mainly focused on diabetic nephropathy. Topic 2 included non-drug therapy as the theme. In Topic 3, we found diabetic complications related to aging. Moreover, in Topic 4, we observed that obesity and hypertension were the leading symptoms of T2DM.

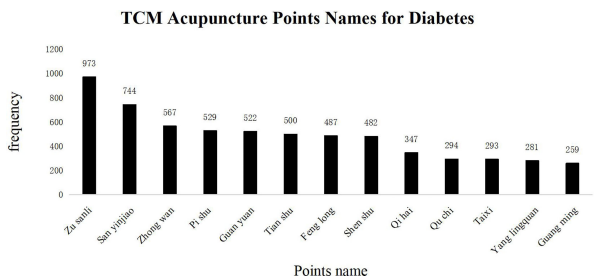

FIGURE 7 | TCM Acupuncture Points Names for Diabetes. The frequency of occurrence of acupuncture points in the diabetes literature. The abscissa represents the name of acupuncture points. The total number of acupuncture points was 12,458, of which more than 2% are the following: ST36: 973 (7.8%), SP36: 744 (5.9%), RN12: 567 (4.5%), BL20: 529 (4.2%), RN4: 522 (4.1%), ST25: 500 (4.0%), ST40: 487 (3.9%), BL23: 482 (3.8%), RN6: 347 (2.7%), LI11: 294 (2.3%), KI3: 293 (2.3%), SP9: 281 (2.2%), GB37: 259 (2.0%). The ordinate shows the frequency of appearance of each acupoint name; ST36 and SP36 are located on the Stomach Meridian of Foot-Yangming and Spleen Meridian of Foot-Taiyin. The abovementioned conforms to the TCM treatment principle.

Association Rule Analysis

The correlation between diagnosis and treatment information was mined based on the *a priori* algorithm (PMID: 25926855). The minimum support was 10%, the minimum confidence was 80%, and the maximum preceding item was 2. In this study, we used association rule analysis (ARA) to explore the information on the diagnosis and treatment of T2DM. The most common combinations were syndrome of excessive dampness and spleen deficiency with Astragalus membranaceus; obesity and *ST36*; pill of ingredients with Rehmannia extract and *Yin* deficiency; obesity with syndromes of excessive dampness and spleen deficiency. **Tables 3**, **4**, respectively, list the association rules of combining two kinds of diagnosis and three kinds of diagnosis.

Cluster Analysis

The clustering of information in more than 2% of the literature in this study formed four clusters. Cluster-1 mainly treated the manifestations of spleen-kidney *Yang* deficiency syndrome, such as low spirit due to *Qi* deficiency, polyuria, *BL23*, and *GB37*; Cluster-2 primarily focused on the stagnation of liver *Qi* and spleen deficiency syndrome; Cluster-3 mainly addressed the liver and kidney *Yin* deficiency, such as dysphoria in the chest, palms, and soles, thirst and drink, soreness and weakness of waist and knees, thirsty and drinking, pill of ingredients with Rehmannia extract; and Cluster-4 included spleen deficiency and treatment with Astragalus membranaceus. The results are shown in **Figure 8**.

DISCUSSION

Subjective Symptoms and Syndrome Differentiation for T2DM in TCM

This study found that "obesity" was the most common symptom for T2DM closely related to "spleen deficiency dampness syndrome," which is consistent with T2DM pathology in TCM. According to TCM theory, the lack of spleen *Qi* might lead to malfunctioning transport, affecting nutrient transport and water metabolism and leading to obesity. Also, obesity symptoms were related to dampness-heat stasis, and swift digestion resulted from gastric heat.

TABLE 3 | Association rules of two-diagnosis and treatment information.

Consequent	Antecedent	Support (%)	Confidence (%)	Lift (%)
Syndrome of dampness stagnancy and spleen deficiency	Astragalus membranaceus	85.91	96.09	1.03
Spleen deficiency syndrome	Astragalus membranaceus	85.90	90.62	1.02
Qiqihuoxue decoction	Astragalus membranaceus	85.90	90.62	1.16
Obesity	ST36	34.22	100	1.73
Spleen deficiency syndrome	ST36	34.22	92.15	1.04
Stomach heat flaming syndrome	Pill of ingredients with rehmannia	28.92	88.37	1.10
Radix Ophiopogonis	Pill of ingredients with rehmannia	28.86	97.67	1.25
Yin deficiency syndrome	Pill of ingredients with rehmannia	28.86	93.02	1.27
Chinese yam	Pill of ingredients with rehmannia	28.86	100	1.40
Obesity	Pill of ingredients with rehmannia	28.85	86.04	1.49
Poria cocos	Pill of ingredients with rehmannia	28.86	100	3.23
Spleen deficiency syndrome	Syndrome of dampness stagnancy and spleen deficiency	93.28	90.64	1.02
Obesity	Syndrome of dampness stagnancy and spleen deficiency	93.28	82.73	1.03

The results showed that the most common combinations were: syndrome of dampness stagnancy and spleen deficiency with Astragalus membranaceus, obesity and ST36, Pill of ingredients with rehmannia and Yin deficiency, Obesity and syndrome of dampness stagnancy and spleen deficiency.

Our results indicate that the primary phenotypic manifestation of T2DM patients was not categorized exclusively based on the typical "polydipsia, polyphagia, polyuria, and weight loss" symptoms. Interestingly, in addition to obesity, we found the following symptoms in the selected RCTs: (1) tiredness, weakness, shortness of breath, and low spirit due to *Qi* deficiency; (2) thirst, dry throat, dry mouth, and dysphoria with feverish sensation in chest, palms, and soles resulting from kidney *Yin* deficiency; (3) numbness and stasis of the limbs caused by *Qi* deficiency and blood stasis.

According to The Guidelines for the Diagnosis and Treatment of Common Diseases in Internal Medicine of TCM (35) and International Guidelines for the Diagnosis and Treatment of Diabetes in TCM (36), the syndrome differentiation and treatment of T2DM are created according to the deficiency of Yin and Jing (i.e., Qi in concentrated form, which belongs to Yin), and excessive dryness-heat. In addition, there are other categories of TCM syndrome differentiation for T2DM. For example, Internal Medicine of Traditional Chinese Medicine (2nd edition) classifies different syndromes of thirst elimination as "Shang Xiao," "Zhong Xiao," and "Xia Xiao." "Shang Xiao" refers to lung thermo in injury syndrome; "Zhong Xiao" indicates common stomach heat burning syndrome and Qi Yin deficiency syndrome; while "Xia Xiao" is divided into the deficiency syndrome of kidney Yin and the deficiency syndrome of Yin and Yang (37). Furthermore, in Guiding Principles for Clinical Research of Chinese Medicine New Drugs (Trial) (38) and relevant discussion of TCM diagnostics in various versions of TCM internal medicine textbooks (39–41), TCM syndromes of diabetes can be divided wholly into four syndromes: lung heat and fluid injury syndrome, stomach heat and excessive heat syndrome, deficiency of Qi and Yin syndrome, and deficiency of Yin and Yang syndrome. Thus, the pathogenesis of diabetes involves deficiency syndromes and excess syndromes, and

TABLE 4 | Association rules of three-diagnosis and treatment information.

Consequent	Antecedent	Support (%)	Confidence (%)	Lift (%)
Syndrome of dampness stagnancy and spleen deficiency	K13 and Astragalus membranaceus	40.939	93.442	1.001
Spleen deficiency syndrome	Astragalus membranaceus and Chinese yam	60.402	88.888	1.003
Syndrome of dampness stagnancy and spleen deficiency	Puerariae and Astragalus membranaceus	44.966	94.029	1.007
Obesity	ST36 and Astragalus membranaceus	26.845	100	1.732
Obesity	ST36 and syndrome of dampness stagnancy and spleen deficiency	30.872	100	1.733
Syndrome of dampness stagnancy and spleen deficiency	ST36 and Chinese yam	31.543	93.617	1.003
Astragalus membranaceus	Syndrome of dampness stagnancy and spleen deficiency	93.288	88.489	1.030
Astragalus membranaceus	Spleen deficiency syndrome and syndrome of dampness stagnancy and spleen deficiency	84.563	89.682	1.043
Astragalus membranaceus	Puerariae and syndrome of dampness stagnancy and spleen deficiency	44.295	95.454	1.111
Astragalus membranaceus	Radix Ophiopogonis and syndrome of dampness stagnancy and spleen deficiency	44.966	95.522	1.112
Astragalus membranaceus	BL20 and syndrome of dampness stagnancy and spleen deficiency	17.449	100	1.164
Astragalus membranaceus	Spleen and kidney Yang deficiency and syndrome of dampness stagnancy and spleen deficiency	17.449	100	1.164
Astragalus membranaceus	Low spirit and syndrome of dampness stagnancy and spleen deficiency	22.818	100	1.164

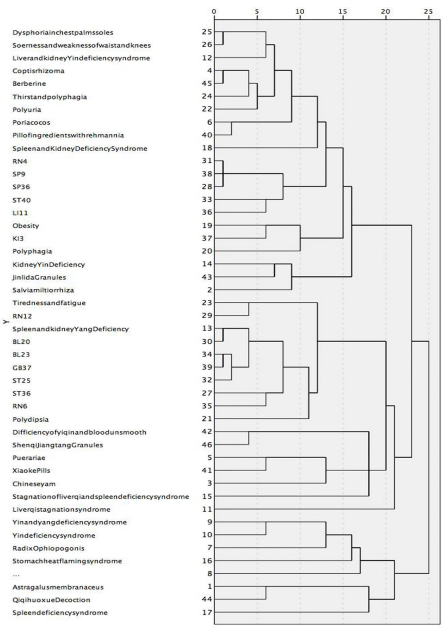

FIGURE 8 | A tree graph using an average join (between groups). Category 1 mainly treated the manifestations of spleen-kidney Yang deficiency syndrome, such as low spirit due to Qi deficiency, polyuria, *BL23* and *GB37*; Category 2 was mainly Stagnation of liver qi and spleen deficiency syndrome; Category 3 was mainly liver and kidney Yin deficiency, such as dysphoria in chest, palms, and soles, thirst and drink, soreness and weakness of waist and knees, thirsty and drinking, *Pill of ingredients with Rehmannia*; Category 4 was mainly spleen deficiency, treatment with Astragalus membranaceus.

deficiency syndromes are mainly manifested in the form of Qi, Yin, and Yang deficiencies.

Like the expansion of symptom corpus, we also expanded the corpus for TCM syndromes of T2DM, which might help TCM practitioners give greater attention to additional subjective symptoms of T2DM patients and interpret their causes from more perspectives. In this study, the top 10 syndromes of TM2D included (1) syndrome of dampness stagnancy and spleen deficiency; (2) *Yin* and *Yang* deficiency syndrome; (3) *Yin* deficiency syndrome; (4) liver *Qi* stagnation syndrome; (5) liver and kidney *Yin* deficiency syndrome; (6) spleen and kidney *Yang* deficiency; (7) kidney *Yin* deficiency; (8) stagnation of liver *Qi* and spleen deficiency syndrome; (9) stomach heat flaming syndrome; and (10) spleen deficiency syndrome. According to the results, the TCM syndrome types of diabetes are complex and dynamic, increasing the difficulty in interpreting them for TCM clinicians. To help clinicians better understand these syndromes, we proposed the following summary: (1) the course of T2DM is considered to be a mixture and alternation of deficiency and excess; (2) the disease lesions are mainly spleen, liver, and kidney; (3) the disease elements are dampness, stasis, and heat. So the primary treatment is for tonifying, nourishing *Yin*, clearing heat, and relieving stasis.

Prescriptions, Herbal Medicines, and Acupoint Treatment Patterns

T2DM symptoms are mainly manifested as deficiency syndrome, and its fundamental causes are *Yin* deficiency and excessive heat, according to the TCM theory. TCM physicians believe that diabetes pathogenesis is due to the loss of body fluid due to excessive body heat, and patients need treatment to nourish *Yin* and reduce heat. For example, pills of ingredients with Rehmannia extract, which contains Radix Rehmanniae Praeparata, is the appropriate choice to nourish *Yin* and kidney, help produce saliva and quench thirst, clear heat, moisten the lung, and improve both liver and kidney functions (42).

Astragalus was the most frequently prescribed natural drug in our analysis. It has the effect of replenishing *Qi* and raising *Yang* and can generate blood, promote body fluid circulation, cure spleen deficiency, and eliminate thirst. Also, Astragalus and Danshen can be applied together to replenish *Qi*, enhancing the effect of Danshen to promote blood circulation, strengthen the spleen, and reduce dampness and blood stasis. More importantly, modern studies have shown that Astragalus polysaccharide can effectively reduce blood glucose levels, improve insulin sensitivity, inhibit the apoptosis of pancreatic β cells, and play a key role in treating diabetes complications (43). Yam can nourish the *Qi* of the spleen, lung, and kidney and nourish the *Yin* of these organs. Therefore, it serves as an essential medicine for the treatment of T2DM (44). Atractylodes and Poria can invigorate the spleen and reduce dampness, and they are often used to treat T2DM of damp-heat accumulating spleen syndrome (45). Ophiopogon japonicus can nourish the lung and stomach *Yin*; when prescribed together with dogwood, it can nourish liver and kidney *Yin (46)*.

In addition to these drugs prescribed for replenishing *Qi* and invigorating *Jin* (i.e., liquid in the body), the second most prescribed type for T2DM was antipyretic and dehumidifying drugs, whose mechanism has been well studied. Internal fever is believed to be an important patho-mechanism of spleen impairment, and primary attention should be given to eliminating body heat (47). Among antipyretic drugs, Coptis is the most frequently used medicine. Since ancient times, Rhizoma Coptis has been an important anti-thirst drug (48), also known as the "hypoglycemic holy drug." Modern pharmacological studies have also shown that Rhizoma Coptis can regulate glucose and lipid metabolism, improve insulin resistance, and protect islet β cells (49). Also, Pueraria can clear heat, promote fluid circulation, and quench thirst (50).

Acupuncture and moxibustion—the essential external treatments in TCM—can regulate *Qi*, blood, *Yin*, and *Yang* of the human body by activating acupoints related to respective diseases. Studies have shown that acupuncture at *ST36* can improve insulin sensitivity and the morphology of pancreatic β cells (51). Likewise, activation of *ST40* can improve stomach function. T2DM-associated obesity and hyperlipidemia, related to spleen dysfunction, prevent nutrients from transporting to the whole body, leading to phlegm and dampness accumulation in the waist and abdomen. The ancient TCM book *Song Yu Long*

has suggested that acupuncture at *ST40* can help remove phlegm (52).

Moreover, rodent studies have shown that acupuncture at *ST40* can prevent rats from obesity and help them improve fat metabolism and insulin resistance (53). The acupuncture points *BL20* and *BL23* are located on the bladder meridian. Studies have shown that acupuncture at Back-shu acupoint can form a direct neural pathway through spinal ganglia to adjust the functions of internal organs (54). The spleen and stomach belong to "Zhong *xiao*," interacting with each other, where their chief function is to transport nutrients and water to different parts of the body. The liver and kidney belong to "X*ia xiao*," whose main function is to channelize gas and regulate mood. It also helps the spleen and stomach transport nutrients, and it improves the normal metabolism of sugar and fat. Animal experiments have shown that acupuncture at *RN12* can increase the expression levels of corticotropin-releasing hormone (CRH) and c-fos protein in the hypothalamus as well as the levels of peripheral insulin and β-endorphin, and it plays a key role in regulating blood sugar (55). *RN4* is the small intestine Mu-front acupoint at Ren meridian and Foot-three Yin meridian intersection point, related to vital energy. *SP36* is the acupoint at foot three Yin meridian intersection points. Chronic T2DM is usually related to deficiency of both *Qi* and *Yin*, along with dryness-heat, which is usually treated by activation of *RN4* and *SP36* acupoints to improve *Qi* and *Yin* for liver, spleen, and kidney jointly.

Text Topic Extraction Analysis

The LDA extracted the text medical academic words by calculating the TF – IDF value between keywords. When we set K to be 4, we get four separate themes with no intersection.

As shown in our results, diabetes complications are one of the most popular research themes. The T2DM is initially manifested as microalbuminuria and albuminuria and then gradually develops to nephrotic syndrome, and even may lead to renal failure, presenting a progressive aggravation (56). When diabetes induces edema and abnormal urination, diabetic nephropathy (DN) initiates symptoms related to the TCM concept of kidney *Qi* deficiency. Other studies have shown that spleen and kidney deficiency is the key to T2DM, leading to spleen damage and proteinuria (57). Several studies on TCM therapies have shown that patients' blood glucose indexes can be gradually controlled under comprehensive conditions. At the same time, their blood lipid and insulin resistance will be improved accordingly (58), potentially beneficial to the recovery of patients' islet cells' function.

Evaluation of the Treatment Effects for RCT Articles

Since the efficacy information in the RCT literature is described in various forms, lacking standardization, further breakthroughs are needed to extract them from RCT articles as other core information systematically. Therefore, a random sample of 200 RCTs was selected from the 3,654 chosen RCTs to evaluate their treatment effects to illustrate the overall treatment effects. We found that 94.5% of these articles in the intervention group had an effective rate greater than 70%. Thus, in these research articles, the effective rate in the intervention group was significantly higher than that in the control group, with p values less than 0.05, indicating that the diagnosis and treatment information extracted in this study has effective implications for clinical practitioners.

CONCLUSION

In this study, NLP technology and data mining methodologies were used to sort out the subject symptoms, classification of TCM syndromes, and treatment methods for T2DM based on unstructured data of RCTs, to make innovative attempts in technical methods and provide a basis for future clinical data research and methodological research. Unlike the theoretical knowledge of syndrome types and treatment methods for T2DM in TCM textbooks or guidelines, we have illustrated the most recent research findings for clinical practitioners. Therefore, syndrome differentiation should be considered comprehensively and flexibly to provide the basis for the diagnosis and treatment for clinicians, rather than only following the theoretical knowledge of textbooks. Furthermore, we have expanded the corpus of common symptoms and syndromes for T2DM, providing researchers and doctors focusing on TCM treatment of T2DM with additional perspectives.

As suggested in this study, the main treatment theories for T2DM in TCM are to invigorate the kidney and promote blood circulation, invigorate *Qi*, and dissipate blood stasis, which is achieved by taking TCM medicine or external treatments (e.g., acupuncture and moxibustion). Different from these TCM treatment methods for T2DM, modern therapies mainly concentrate on proper diet, routine exercise (59), precise medication (60–63), adjustment of lifestyle (64), and other relevant methods (65, 66). Our research findings could also provide insights for researchers and practitioners of Western medicine in the field of T2DM.

Different from another study that analyzed the prescription rules of TCM in the treatment of T2DM based on data mining (67), we found a high frequency of stagnation of liver *Qi* syndrome, and we showed that a pill of ingredients with Rehmannia mainly was used to treat liver dysfunction. Therefore, in clinical treatment, we should pay attention to nourishing *Yin* and invigorating *Jin*, nourishing *Qi* to invigorate the spleen, soothing the liver, relieving depression, clearing away heat, and removing dampness.

In the future, the relationship among symptoms, syndrome, drugs, and efficacy based on the unstructured information extracted in this study can be further explored by using the machine learning algorithm. However, since this study can only be used as a macro-scale study on the distribution of T2DM diagnosis and treatment information, it is necessary to increase the size of the sample of research articles and fine-tune the analytical methods to obtain a more precise pattern for T2DM diagnosis and treatment in TCM.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

YXL, YX, JZ, and DH: Essay writing guidance. YZL, YZ, LZ, ZH, and HS: Data collation. LW: Technical support. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Xu JY, Wu YL. *National Famous Old Traditional Chinese Medicine Doctor's Prescription for Diabetes Mellitus*. Zhengzhou: Zhongyuan Peasant Publishing House of Zhongyuan Publishing and Media Group (2010). p. 1.
2. GBD 2013 Mortality and Causes of Death Collaborators. Global, Regional, and National Age-Sex Specific All-Cause and Cause-Specific Mortality for 240 Causes of Death, 1990–2013: A Systematic Analysis for the Global Burden of Disease Study 2013. *Lancet* (2015) 385:117–71. doi: 10.1016/S0140-6736(14)61682-2
3. Zheng Y, Ley SH, Hu FB. Global Aetiology and Epidemiology of Type 2 Diabetes Mellitus and Its Complications. *Nat Rev Endocrinol* (2018) 14(2):88–98. doi: 10.1038/nrendo.2017.151
4. International Diabetes Federation. *IDF Diabetes Atlas-7th Edition*. Diabetes Atlas (2015). Available at: http://www.diabetesatlas.org/.
5. Holman N, Young B, Gadsby R. Current Prevalence of Type 1 and Type 2 Diabetes in Adults and Children in the UK. *Diabetes Med* (2015) 32:1119–20. doi: 10.1111/dme.12791
6. Bruno G, Runzo C, Cavallo-Perin P, Merletti F, Rivetti M, Pinach S, et al. Incidence of Type 1 and Type 2 Diabetes in Adults Aged 30–49 Years: The Population-Based Registry in the Province of Turin, Italy. *Diabetes Care* (2005) 28:2613–9. doi: 10.2337/diacare.28.11.2613
7. Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, et al. Prevalence of Diabetes Recorded in Mainland China Using 2018 Diagnostic Criteria From the American Diabetes Association: National Cross-Sectional Study. *BMJ* (2020) 369:m997. doi: 10.1136/bmj.m997
8. A. *The Inner Canon of Emperor Huang*. Beijing: Chinese Medical Ancient Books Publishing House (2003).
9. Chen WC. Clinical Observation of Modified Liu Jun Zi Decoction in the Intervention of Prediabetes. *Chin J Pharmacol* (2012) 10(22):292–3. doi: 10.15912/j.cnki.gocm.2012.22.026
10. Zhang HS, Zhang ZJ, Wei W, et al. Effects of Jian Pi Tiao Tang Prescription on Glucagon-Insulin-Resistance in Pre-Diabetic Patients With Phlegm-Dampness. *Chin Traditional Chin Med Sci Technol* (2019) 26(1):11–3.
11. Zhang LM, Feng DY, Zou LX, Wu ZQ, Zhang XY, Tan Y. The Effect of Xiao Zheng Cheng Tang on Phlegm Dampness and Heat Accumulation in Pre-Diabetes. *J Hunan Univ Chin Med* (2018) 4):467–9.
12. Li NY. Effect of Da Chai Hu Decoction on the Treatment of Pre-Diabetic Patients. *J Contemp Med* (2018) 16(5):207–8.
13. Wang Y, Zhu YH, Zhang XT, Cheng F. A Review of Non-Drug Intervention in Prediabetes Mellitus. *Henan Traditional Chin Med* (2018) 38(3):479–83. doi: 10.16367/j.issn.1003-5028.2018.03.0127
14. Zhao ZH, Chen, Sun L, et al. A Review of the Treatment of Dry Preglycosuria in Chinese Medicine. *Shi Zhen Chin Med Chin Med* (2014) 25(2):439–40.
15. Mei CH, Wang DH. The Effect of Lifestyle Intervention on Pre-Diabetic Patients Based on TCM Physique Identification. *Jilin J Traditional Chin Med* (2017) 37(5):440–2. doi: 10.13463/j.cnki.jlzyy.2017.05.003
16. Wu YM, Lin KL, Chen RF. Clinical Analysis of Diabetes Mellitus. *J Guangzhou Univ Chin Med* (2011) 28(2):109–12.
17. Yang XY, Song ZL, Yang WQ. Huanglian Yueju Pill Combined Lifestyle Intervention. *Modern Chin Med* (2011) 31(2):25–27. doi: 10.13424/j.cnki.mtcm.2011.02.031
18. Liu MM, Fang ZH. Chinese Medicine Food Therapy for Diabetes and Pre-Diabetes Patients. *Clin J Chin Med* (2018) 30(12):2198–200. doi: 10.16448/j.cjtcm.2018.0673
19. Gong GM, Liu A, Zhang XY, Chen N. Clinical Observation of Prediabetes Mellitus Based on the Theory of "Curing Disease" With Medicated Diet Steamed Bread as the Main Comprehensive Intervention. *J Chengdu Univ Traditional Chin Med* (2018) 41(2):25–8. doi: 10.13593/j.cnki.51-1501/r.2018.02.025
20. Wang CY, Zhang HY. Research Progress in the Treatment of Prediabetes Mellitus With Traditional Chinese Medicine. *Chin Med Guide* (2017) 15(15):174.
21. Available at: https://scholar.google.com/scholar?cites=17756175773309118945&as_sdt=5,41&sciodt=0,41&hl=en.
22. Qi J. *Searching for Economic Effects of User Specified Event Based on Topic Modelling and Event Reference*. Jordery School of Computer Science, Acadia University (2015).
23. Blei DM. Probabilistic Topic Models. *Commun ACM Vol* (2012) 55(4):77–84. doi: 10.1145/2133806.2133826
24. Blei DM, Lafferty JD. A Correlated Topic Model of Science. *Ann Appl Stat* (2007) 1(1):17–35. doi: 10.1214/07-AOAS114
25. Xie J, Li Y, Wang N, et al. Feature Selection and Syndrome Classification for Rheumatoid Arthritis Patients With Traditional Chinese Medicine Treatment. *Eur J Integr Med* (2020) 34:101059.
26. Tang Y, Li Z, Yang D, Fang Y, Gao S, Liang S, et al. Research of Insomnia on Traditional Chinese Medicine Diagnosis and Treatment Based on Machine Learning. *Chin Med* (2021) 16(1):1–21. doi: 10.1186/s13020-020-00409-8
27. Pang H, Wei S, Zhao Y, He L, Wang J, Liu B, et al. Effective Attention-Based Network for Syndrome Differentiation of Aids. *BMC Med Inf Decis Making* (2020) 20(1):1–10. doi: 10.1186/s12911-020-01249-0
28. Kowsher M, Tithi FS, Rabeya T, Afrin F, Huda MN. Type 2 Diabetics Treatment and Medication Detection With Machine Learning Classifier Algorithm. In: Uddin M, Bansal J, editors. *Proceedings of International Joint Conference on Computational Intelligence*. Springer, Singapore: Algorithms for Intelligent Systems (2020). p. 519–31. doi: 10.1007/978-981-13-7564-4_44
29. Wen G, Wang K, Li H, Huang Y, Zhang S. Recommending Prescription via Tongue Image to Assist Clinician. *Multimedia Tools Appl* (2021) 80:1–22. doi: 10.1007/s11042-020-10441-3
30. Song Y, Zhao B, Jia J, Wang X, Xu S, Li Z, et al. A Review on Different Kinds of Artificial Intelligence Solutions in TCM Syndrome Differentiation Application. *Evidence-Based Complementary Altern Med* (2021) 2021(8):1–8. doi: 10.1155/2021/6654545
31. Reyes–Ortiz JA, González–Beltrán BA, Gallardo–López L. Clinical Decision Support Systems: A Survey of NLP–Based Approaches From Unstructured Data. In: *2015 26th International Workshop on Database and Expert Systems Applications (DEXA)*. Valencia (2015). p. 163–7. doi: 10.1109/DEXA.2015.47
32. Alemzadeh H, Devarakonda M. An NLP-Based Cognitive System for Disease Status Identification in Electronic Health Records. In: *2017 IEEE EMBS International Conference on Biomedical and Health Informatics, BHI 2017*. (2021). pp. 89–92. doi: 10.1109/BHI.2017.7897212
33. Zhong X, Jia Y, Li D, Zhang X. Classification of Acupuncture Points Based on the Bert Model*. *J Data Anal Inf Process* (2021) 9:123–35. doi: 10.4236/jdaip.2021.93008

34. Kang Y, Cai Z, Tan CW, Huang Q, Liu H. Natural Language Processing in Management Research: A Literature Review. *J Manage Anal* (2020) 7(2):139–72. doi: 10.1080/23270012.2020.1756939
35. Lan L. *Research on Chinses Word Segmentation Method Based on Word Embedding*. Harbin Engineering University (2017).
36. Peng KH, Liu LH, Chang CS, Lee CC. Predicting Personality Traits of Chinese Users Based on Facebook Wall Posts. In: *2015 24th Wireless and Optical Communication Conference (WOCC)*. (2015). pp. 9–14. doi: 10.1109/WOCC.2015.7346106
37. Xia T, Y Chai. An improvement to TF-IDF: Term Distribution Based Term Weight Algorithm. *JSW* (2011) 6(3):413–20.
38. Zheng XY. *Guiding Principles for Clinical Research of Chinese Medicine New Drugs: Trial Implementation*. Beijing: China Medical Science and Technology Press (2002).
39. Zhang BY. *Internal Medicine of Traditional Chinese Medicine*. Shanghai: Shanghai Science and Technology Press (2010) p. 204–8.
40. Wu MH, Wang XY. Internal Medicine of Traditional Chinese Medicine. Beijing: China Traditional Chin Med Press (2012). 60:340–8.
41. Ma WP. TCM Diagnostics. In: *3rd Edition*. Beijing: People's Medical Publishing House. (2014)
42. Xu X, Zhao K, Yang Z. Liuweidihuang Pill in Combination With Metformin on Hepatitis B Yin Asthenia and Internal Heat Effect and Vascular Endothelial Function in Patients With Diabetes. *Combine Traditional Chin western Med Mag World* (2020) 15(2):140–3. doi: 10.13935/j.cnki.sjzx.200229
43. Wang Y, Zhang L, Zhang Y, et al. Effect of Polysaccharides on Polysaccharides From Polysaccharides. *Chin J Pharmacol* (2012) 22(1):417.
44. Chinese Society of Traditional Chinese Medicine. *Guidelines for Diagnosis and Treatment of Common Diseases in Internal Medicine of Traditional Chinese Medicine. Part of Traditional Chinese Medicine Diseases*. Beijing: China Press of Traditional Chinese Medicine (2008).
45. Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, et al. Effects of Diet and Exercise in Preventing NIDDM in People With Impair Glucose Tolerance: The DA Qing IGT and Diabetes Study. *Diabetes Care* (1997) 20(4):537–44. doi: 10.2337/diacare.20.4.537
46. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, et al. Prevention of Type 2 Diabetes Mellitus by Changes in Lifestyle Among Subjects With Impaired Glucose Tolerance. *N Engl J Med* (2001) 344(18):1343–50. doi: 10.1056/NEJM200105033441801
47. Shi YJ, Zhang HY, Zhang D, Hu Q, Zhang Q, Zhang M. Dogwood Blood Glucose in Patients With Type 2 Diabetes Mellitus and the Impact of Body Mass. *Res Traditional Chin Med* (2014) 27(8):23–4.
48. Tong XL, Xu J, Lian FM, Yu XT, Zhao YF, Xu LP, et al. Structural Alteration of Gut Microbiota during the Amelioration of Human Type 2 Diabetes with Hyperlipidemia by Metformin and a Traditional Chinese Herbal Formula: a Multicenter, Randomized, Open Label Clinical Trial. *mBio* (2018) 9(3):e02392–17. doi: 10.1128/mBio.02392-17
49. Chen KJ, Chen MC. Clustering and Factor Analysis of Traditional Chinese Medicine for Diabetes in Ancient Literature. *Shizhen Traditional Chin Med Mater Med* (2013) 24(12):3076–7. doi: 10.3969/j.issn.1008-0805.2013.12.114
50. Wang J, Wang L, Lou GH, Zeng H, Hu J. Coptidis Rhizoma: A Comprehensive Review of Its Traditional USES Botany, Phytochemistry, Pharmacology and Toxicology. *Pharm Biol* (2019) 57(1):193–225. doi: 10.1080/13880209.2019.1577466
51. Chen GY, Fang JY, Zheng SS, Zhao HH. Study on the Effect of Pueraria Root on Diabetes Mellitus. *Chin J Pharmacol* (2017) 28(11):2716–8.
52. Shi XM, Xu GL, Li Y, Tu J. Research Progress in the Treatment of Diabetes With Traditional Chinese Medicine. *Jiangxi Traditional Chin Med* (2015) 46 (05):64–70.
53. Zhang Y, Chen DQ, Zhao FX, Su B, Lin CX. Effect of Low-Frequency Electroacupuncture at Zusanli on Glucose Metabolism in Type 2 Diabetic Rats. *Modern Chin Med Clinic* (2015) 22(1):49–52.
54. Gang WJ, Huang LX. Discrimination and Analysis of the Mistakes of Guanyuan Point in the Current "Jade Dragon Song". *Acupuncture Res* (2009) 34(1):70–1.
55. Wu JJ, Zhu WY, Sun YN, Zhu SP. Anti-Acupuncture at the "Fenglong" Point on the Prevention of Obesity in Rats and Its Effect on the Transcription Factor Peroxisome Proliferator-Activated Receptor γ. *China Gen Pract Med* (2019) 22(6):707–11.
56. Tong CG, Gu SZ, Yi HQ. Morphological Basis of Chest and Abdomen Qi Street. *Acupuncture Res* (2004) 29(4):270–3.
57. Yang L. *Low-Frequency Electroacupuncture at "Zhongwan" Point on the Immediate Hypoglycemic Effect and Its Mechanism of Diabetic Rats*. Wuhan: Hubei University of Traditional Chinese Medicine (2014).
58. Liu MC, Liu SJ, Wang P, Kang GR, Wang Y, Lv J, et al. Study on TCM Syndromes of Type 2 Diabetic Nephropathy Based on the Association of Disease, Syndromes and Symptoms. *Chin J Traditional Chin Med* (2014) 21 (12):2851–3. doi: 10.13193/j.issn.1673-7717.2014.12.010
59. Zheng SS, Xu YQ, Chen ZJ, Mou X, et al. Clinical Analysis of Diabetic Nephropathy. *Sci Technol Chin Traditional Med* (2019) 26(3):478–80.
60. Zhang WZ, Chen WH, Li SL. Study on the Distribution of TCM Syndromes in Patients With Early Renal Damage of Type 2 Diabetes Mellitus. *J Guangzhou Univ Traditional Chin Med* (2015) 13(3):406–9. doi: 10.13359/j.cnki.gzxbtcm.2015.03.005
61. Qiao L, Zheng H, Yang LX, Li XY, Li Q, Liu TH. Analysis of Literature Characteristics and Research Status of Astragalus Polysaccharides in the Treatment of Diabetes Based on CNKI Data Mining. *Western Chin Med* (2017) 30(3):56–9.
62. Liu X, Qu C, Wang ST, Shen XH, Yang YF, Shi Y. Analysis on the Rule of Drug Use in the Treatment of Type 2 Diabetes Mellitus Based on Data Mining. *Chin J Traditional Chin Med* (2020) 38(5):74–8. doi: 10.13193/j.issn.1673-7717.2020.05.017
63. Tong XL, Liu WK, Wang J, Ni Q, Wei JP. Differentiation and Application of Diabetes Mellitus in Different Stages. *Jilin J Traditional Chin Med* (2012) 32 (5):442–4. doi: 10.13463/j.cnki.jlzyy.2012.05.004
64. Wang J, Wu HR. Explanation of the Core Content of "International Guidelines for Diagnosis and Treatment of Diabetes in Traditional Chinese Medicine. *Beijing Traditional Chin Med* (2018) 37(08):730–2. doi: 10.16025/j.1674-1307.2018.08.010
65. Zhou ZY. *Internal Medicine of Traditional Chinese Medicine. (2nd Edition)*. Beijing: China Press of Traditional Chinese Medicine (2007) p. 407–15.
66. Wu TC, Yue RS, He ML, Wu SQ. Based on Data Mining to Explore the Prescription of Traditional Chinese Medicine in the Treatment of Prediabetes. *Pharmacol Clinics Chinese Materia Medica* (2021) 37(03):190–5. doi: 10.13412/j.cnki.zyyl.20201214.006
67. Bai WJ, Lu B. Modern Ziyinqingrefang Treatment of Diabetes Drug Law Analysis. *China Med News* (2021) 19(6):153–6.

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Potential Role of the Renal Arterial Resistance Index in the Differential Diagnosis of Diabetic Kidney Disease

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Aims: To investigate the potential role of renal arterial resistance index (RI) in the differential diagnosis between diabetic kidney disease (DKD) and non-diabetic kidney disease (NDKD) and establish a better-quantified differential diagnostic model.

Materials and Methods: We consecutively reviewed 469 type 2 diabetes patients who underwent renal biopsy in our center. According to the renal biopsy results, eligible patients were classified into the DKD group and the NDKD group. The diagnostic significance of RI was evaluated by receiver operating characteristic (ROC) curve analysis. Logistic regression analysis was used to search for independent risk factors associated with DKD. Then a novel diagnostic model was established using multivariate logistic regression analysis.

Results: A total of 332 DKD and 137 NDKD patients were enrolled for analysis. RI was significantly higher in the DKD group compared with those in the NDKD group (0.70 vs. 0.63, $p< 0.001$). The optimum cutoff value of RI for predicting DKD was 0.66 with sensitivity (69.2%) and specificity (80.9%). Diabetic retinopathy, diabetes duration ≥ 60 months, HbA1c ≥ 7.0(%), RI ≥ 0.66, and body mass index showed statistical significance in the multivariate logistic regression analysis. Then, we constructed a new diagnostic model based on these results. And the validation tests indicated that the new model had good sensitivity (81.5%) and specificity (78.6%).

Conclusions: RI has a potential role in discriminating DKD from NDKD. The RI-based predicting model can be helpful for differential diagnosis of DKD and NDKD.

Keywords: diabetic kidney disease, non-diabetic kidney disease, resistance index, differential diagnosis, type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is a global public health challenge affecting over 463 million adults, according to the report of the International Diabetes Federation in 2019 (1). In China, it is estimated that approximately 11.2% of the population has DM (129.8 million people) (2). Type 2 diabetes mellitus (T2DM) combined with renal impairment is correlated with increased cardiovascular

mortality and all-cause mortality (3). Diabetic kidney disease (DKD) is now one of the most frequent and severe complications of diabetes and continues to be the principal cause of end-stage kidney disease (ESKD) worldwide (4, 5). However, non-diabetic kidney disease (NDKD) occurs in T2DM patients as well (6). The prevalence of NDKD in T2DM varied widely from 36.8% to 82.9% (7–12). The therapy and prognosis of NDKD are pretty different from DKD (13, 14). It is believed that the renal outcomes of patients with DKD are relatively worse compared with their counterparts with biopsy-proven NDKD because the pathological changes of DKD are deemed difficult to reverse (15, 16). Therefore, it is critical to distinguish between DKD and NDKD in diabetes with renal impairment in clinical practice.

Currently, the renal pathological diagnosis is the gold standard to discriminate DKD from NDKD. However, the kidney biopsy is an invasive procedure that is impracticable in patients with contraindications, such as pyknotic kidney, bleeding tendency, solitary kidney, uncontrolled hypertension, or severe anemia. Moreover, a renal biopsy could not be routinely performed in some primary hospitals. Thus, the diagnosis and appropriate treatment were usually based on clinical indicators, such as diabetes duration, hematuria, diabetic retinopathy (DR), glycated hemoglobin (HbA1c), and other indices (17–22). However, those markers are not entirely accurate. For instance, lack of DR contributes to the diagnosis of NDKD but does not rule out DKD (23). In recent years, some studies have used some new markers and diagnostic models for the clinical differentiation between DKD and NDKD, such as dysmorphic erythrocytes and urinary neutrophil gelatinase-associated lipocalin (22, 24). However, these markers or risk model is not perfect and is still not good enough to meet clinical requirements. Therefore, it is necessary to find a new precise and sensitive non-invasive marker for clinical differentiation of DKD from NDKD.

The Renal atrial resistance index (RI), measured by doppler ultrasound, is a low-cost and non-invasive tool in detecting kidney diseases; it has been extensively used to evaluate renal blood flow as a semi-quantitative parameter. Previous studies suggested that RI is correlated with severe interstitial fibrosis and the progression of chronic kidney disease (CKD) (25–27). In addition, a few studies have noticed that RI in patients with DKD is significantly higher when compared with non-diabetic controls, which might be helpful for the identification and prediction of DKD (28, 29). However, the potential role of RI in the clinical differentiation of DKD from NDKD and the optimal cutoff value remains unclear. Thus, the present study was conducted to investigate the potential role of RI in the differential diagnosis between DKD and NDKD and establish a better-quantified differential diagnostic model.

MATERIALS AND METHODS

Study Subjects

A total of 469 T2DM patients with renal impairment from the department of nephrology at Xinqiao Hospital, Army Medical University, from January 2014 to September 2020 were retrospectively analyzed (**Figure 1**). All patients had received echo-color-Doppler examination of renal vessels, systematic screening for diabetic retinopathy, and renal biopsy. The diagnosis of T2DM met the criteria proposed by American Diabetes Association in 2019 (30). Eligible patients were divided into the DKD group and the NDKD group based on the kidney biopsy results. The inclusion criteria were: T2DM patients with renal impairment and received kidney biopsy; serum creatinine < 442 μmol/L. The exclusion criteria were: age above 75 years or below 18 years; lack of a fundus clinical information data and pathological data or clear medical history; severe complications, such as severe infection, heart failure, and hypertensive emergency; biopsy-proven DKD complicated by NDKD. All patients signed informed consent before kidney biopsy. The study was approved by the ethical committee of Xinqiao Hospital, and was in accordance with the principles of the Declaration of Helsinki.

Renal Biopsy and Pathological Examination

The kidney biopsies were performed by an experienced renal pathologist, and every kidney biopsy tissue was investigated by electron microscopy, light microscopy, and immunofluorescence. The kidney biopsy indications were in accordance with the KDOQI Guideline (31). The pathological diagnostic criteria for DKD was: diffuse mesangial proliferation, diffuse capillary glomerulosclerosis, presence of Kimmelstiel–Wilson nodular lesions, glomerular basement membrane thickening, hyaline exudative lesions (32). NDKD usually has some unique features based on the guidelines previously reported (12).

Clinical and Laboratory Information

The following data were collected at the time of kidney biopsy: age, sex, body mass index (BMI), systolic blood pressure, diastolic blood pressure, presence of hypertension and DR, medical history of DM, fasting blood glucose, HbA1c, hemoglobin, platelet, serum albumin, total cholesterol, triglyceride, serum creatinine, uric acid, blood urea nitrogen, 24-hour urine protein, hematuria, and RI value. Blood pressure was measured three times according to a standard protocol, and the average value was calculated. DR was confirmed by fundus photography. Ultrasonography was performed by a Philips IU22 Cart G Ultrasound System with C5-1(Made in United Kingdom). The estimated glomerular filtration rate (eGFR) was obtained using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation (33).

Statistical Analysis

Continuous variables were shown as average ± standard deviation or the median and interquartile depending on the data distribution, whereas enumeration data were described as percentages (%). The t-test was applied for normally distributed data, and the Mann-Whitney U test was applied for non-normally distributed data. The chi-squared test was applied for enumeration data. Receiver operating characteristic (ROC) curve analysis was used to explore the optimal cutoff point of the RI to predict DKD. Univariate and multivariate logistic regression analyses (stepwise forward) were performed to search the independent risk factors relating to the

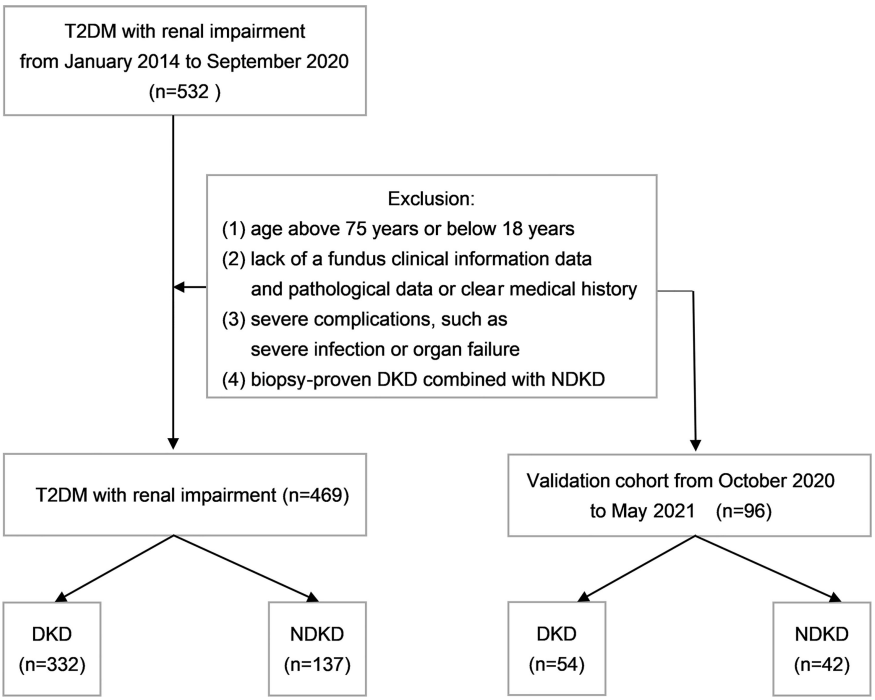


FIGURE 1 | Flowchart showing the procedure for the selection of study participants. T2DM, type 2 diabetes mellitus; DKD, diabetic kidney disease; NDKD, non-diabetic kidney disease.

DKD diagnosis, with results shown as the odds ratio (OR) and 95% confidence interval (CI). The final significant risk factors were included in two differential diagnostic models (with or without RI). The equation is as follows: $PDKD = exp\ (\alpha + \beta 1x1 + \beta 2x2 + \beta 3x3 + \ldots + \beta nxn)/[1 + exp(\alpha + \beta 1x1 + \beta 2x2 + \beta 3x3 + \ldots + \beta nxn)]$. PDKD is the probability of DKD diagnosis, α is a constant, β is the estimator, and x is the clinical predictor. If PDKD ≥ 0.5, the patient should be considered as DKD, while the diagnosis should be NDKD if PDKD < 0.5. The Delong test and the calculation of the net reclassification improvement and the integrated discrimination improvement were performed by R language to analyze two models. Then, a better model was selected. Finally, a back-substitution and a validation test (by a validation cohort of 96 cases) were conducted to evaluate the new model. Correlations between RI and clinical indices were analyzed by the Pearson test. Statistical analyses were performed by SPSS (IBM SPSS Statistics 23.0) and R language. $P < 0.05$ was considered statistically significant. The number of patients required for the validation cohort is computed using software PASS 15.0.5.

RESULTS

The Clinical Characteristics and Renal Pathological Features of the Included Patients

A total of 469 patients were divided into two groups based on kidney biopsy results, with 332 patients in the DKD group and 137 patients in the NDKD group. The general clinical information of the two groups was shown in **Table 1**. Compared with the NDKD group, patients in the DKD group had longer diabetes duration, higher incidence of DR, and higher levels of systolic blood pressure, fasting blood glucose, HbA1c, serum creatine, blood urea nitrogen and RI value, while lower levels of BMI, hemoglobin, triglyceride and eGFR. But no significant difference was noticed between the two groups regarding age, gender, diastolic blood pressure, platelets, serum albumin, total cholesterol, uric acid, urinary protein, or presence of hematuria, nephrotic syndrome, hypertension, cardiovascular disease. Moreover, renal pathological findings showed that membranous nephropathy (38 cases, 27.74%) and IgA nephropathy (38 cases, 27.74%) were the most common pathological type among the 137 NDKD patients, followed by mesangial proliferative glomerulonephritis (19 cases, 13.87%), hypertensive nephrosclerosis (16, 11.68%), and other types (**Supplementary Table 1**). The main pathological manifestations of the DKD group were advanced lesions, with 71.08% of the total were classified as class III (**Supplementary Table 2**).

Differential Diagnosis Performance of RI in Diabetic Patients With Renal Impairment

To explore the clinical value of RI in DKD and NDKD, we compared the RI value in the two groups. Results showed patients in the DKD group had a significantly higher RI value compared with those in the NDKD group (0.70 *vs.* 0.63, $p<0.001$, **Table 1**). Then, we performed a ROC curve to determine the cutoff point of RI for predicting DKD. The area under the curve (AUC) of RI was 0.785. The best cutoff point of RI was 0.66, with 69.2% sensitivity

TABLE 1 | The general clinical characteristics of the included patients.

Parameter	All cases (n = 469)	NDKD (n = 137)	DKD (n = 332)	P value
Age, (years)	51.53 ± 10.26	52.04 ± 11.05	51.32 ± 9.93	0.489
Gender, (male, %)	299 (63.75)	82 (59.85)	217 (65.36)	0.259
BMI, (kg/m^2)	25.24 ± 3.48	26.51 ± 4.01	24.80 ± 3.42	<0.001
SBP, (mm Hg)	142.73 ± 22.62	136.62 ± 21.42	145.26 ± 22.69	<0.001
DBP, (mm Hg)	84.43 ± 12.16	84.32 ± 12.58	84.47 ± 12.00	0.905
Duration of diabetes, (months)	76.03 ± 62.41	28.10 ± 30.59	94.66 ± 61.71	<0.001
Duration of diabetes ≥ 60 months (%)	240 (51.17)	23 (16.79)	217 (65.35)	<0.001
HbA1c	7.82 ± 2.14	7.32 ± 1.90	8.01 ± 2.19	0.004
HbA1c ≥ 7, (%)	216 (46.06)	45 (32.85)	171 (51.51)	<0.001
FBG, (mmol/L)	7.34 ± 3.33	6.76 ± 2.92	7.58 ± 3.46	0.015
Hemoglobin, (g/L)	117.51 ± 24.93	127.02 ± 22.02	113.59 ± 25.03	<0.001
PLT, (10^9/L)	205.76 ± 79.36	206.44 ± 80.91	205.48 ± 78.84	0.906
Serum albumin, (g/L)	35.05 ± 9.43	34.20 ± 10.81	35.40 ± 8.79	0.215
TC, (mmol/L)	5.47 ± 1.98	5.65 ± 2.45	5.40 ± 1.76	0.217
TG, (mmol/L)	2.21 ± 1.86	2.57 ± 2.19	2.07 ± 1.69	0.008
Uric acid, (μmol/L)	378.59 ± 96.49	372.42 ± 108.05	381.04 ± 91.57	0.388
Scr, (μmol/L)	127.32 ± 76.47	102.41 ± 60.89	137.52 ± 79.87	<0.001
BUN, (mmol/L)	7.86 ± 3.67	6.89 ± 3.39	8.26 ± 3.71	<0.001
eGFR, (ml/min/1.73m^2)	68.08 ± 35.44	82.67 ± 34.37	61.32 ± 34.23	<0.001
Urinary protein, (g/24 h)	3.41 ± 3.71	3.49 ± 4.39	3.38 ± 3.41	0.778
Hematuria (%)	320 (68.23)	96 (70.07)	224 (67.47)	0.372
RI	0.68 ± 0.07	0.63 ± 0.05	0.70 ± 0.07	<0.001
Clinical comorbidities				
Nephrotic syndrome (%)	109 (23.24)	32 (23.36)	77 (23.19)	0.969
Hypertension (%)	325 (69.29)	90 (65.69)	235 (70.78)	0.282
Cardiovascular disease (%)	180 (38.38)	52 (37.96)	128 (38.55)	0.799
DR (%)	227 (48.40)	12 (8.76)	215 (64.76)	<0.001

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; FBG fasting blood glucose; PLT, platelet; TC total cholesterol; TG triglyceride; Scr serum creatine; BUN blood urea nitrogen; RI, resistance index; DR, diabetic retinopathy. Data were presented as the mean ± standard, the median with range or counts and percentages. A two-tailed P < 0.05 was considered statistically significant.

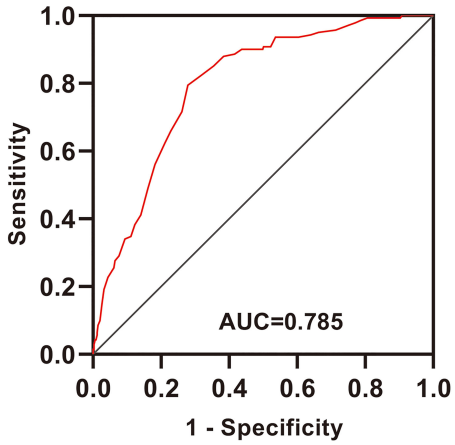
and 80.9% specificity, as calculated by obtaining the best Youden index (**Figure 2**). Then, we assigned a value of 1 to RI ≥ 0.66 and converted it into a binary variable. Considering the value of RI in the differential diagnosis of two groups, the correlation analysis between RI and other clinical information was performed. There were 256 (54.6%) patients with RI ≥ 0.66 and 213 (45.4%) patients with RI < 0.66, respectively. Compared with patients with RI < 0.66, patients with RI ≥ 0.66 had higher age, systolic blood pressure, duration of diabetes, serum creatinine, blood urea nitrogen, the proportion of hypertension, diabetic retinopathy, and nephrotic syndrome, while a lower level of BMI, serum albumin, hemoglobin, TG, and eGFR (**Table 2**). Further analyses was performed by linear regression, RI levels were positively correlated with age (r=0.245; p<0.001), duration of diabetes (r=0.341; p<0.001), systolic blood pressure (r=0.274; p<0.001); serum creatinine (r =0. 335; p<0.001); blood urea nitrogen (r=0.037; p<0.001) and total urinary protein, g/24 h (r=0.141; p=0.003), whereas it was inversely correlated with BMI (r= −0.143; p=0.002), hemoglobin (r=−0.424; p<0.001), triglyceride (r= −0.145; p=0.002), and serum albumin (r=−0.163; p<0.001) in diabetic patients with renal impairment (**Supplementary Table 3**).

Screening for DKD Diagnosis-Related Factors

Previous studies found that the duration of diabetes ≤ 60 months was an independent risk factor for NDKD (11). Many researches indicated that intensive blood glucose control (HbA1c 6.5–7.0%) could reduce the risk of DKD. HbA1c <7.0 was considered as a common indicator of clinical blood glucose control (34). Therefore, we assigned a value of 1 to the duration of diabetes ≥ 60 months and HbA1c ≥ 7% respectively. Then those indicators were converted into binary variables. Univariate regression analysis indicated that duration of diabetes ≥ 60 months, BMI, systolic blood pressure, DR, fasting blood glucose, HbA1c ≥ 7%, hemoglobin, triglyceride, serum creatinine, blood urea nitrogen, and RI ≥ 0.66 were related to the diagnosis of DKD. After adjusting for the factors mentioned above using multivariate logistic regression analysis, RI ≥ 0.66 was still an independent risk factor for the DKD diagnosis, as well as the duration of diabetes ≥ 60 months, BMI, DR, and HbA1c ≥ 7% (**Table 3**).

Establishment and Validation of the New Differential Diagnostic Model

Then, we used two multivariate logistic regression analyses to establish two differential diagnostic models (with or without RI value) to explore the RI value for the clinical differentiation between DKD and NDKD (**Table 4**). The traditional model was built by four independent risk factors other than RI, and the RI-based model was built by five independent risk factors, including RI≥ 0.66. The detailed equation of the two models is shown in **Supplementary Table 4**. The area under ROC curve of the traditional model was 0.889. After adding RI, the area under the ROC curve of the RI-based model increased to 0.912



	Cutoff	Sensitivity	Specificity	AUC(95%CI)	Youden index	P value
RI	0.660	69.2%	80.9%	0.785(0.745,0.829)	0.501	P<0.001

FIGURE 2 | Differential diagnosis performance of RI in type 2 diabetic patients with renal impairment evaluated by ROC curve. RI, resistance index; AUC, the area under ROC curve; CI, confidence interval; ROC, receiver operating characteristic.

(**Figure 3A**). The statistical significance of two ROC AUC by DeLong's test (Z = 2.5964, P value = 0.00942), the net reclassification improvement (NRI = 0.1837, P value = 0.00278), and the integrated discrimination improvement (IDI = 0.0572, P value = 0.00013) implied that the RI-based model has improved the efficiency of differential diagnosis. The sample size of the validation cohort was computed by software PASS based on the AUC of the traditional model (AUC=0.912). The results showed that a random sample of 51 subjects from the positive population and 40 from the negative population produced a two-sided 95.0% confidence interval with a width of 0.120. Then, we recruited another 96 patients (from October 2020 to May 2021)

TABLE 2 | Comparison of clinical findings in T2DM with renal impairment according to renal resistance index.

Parameters	R I< 0.66 (n = 213)	RI ≥ 0.66 (n = 256)	P value
Age, (years)	49.20 ± 10.47	53.37 ± 9.64	<0.001
Gender, (male, %)	132(61.97)	166 (64.84)	0.526
BMI, (kg/m^2)	25.67 ± 3.64	24.87 ± 3.31	0.013
SBP, (mm Hg)	136.73 ± 20.47	147.62 ± 23.17	<0.001
DBP, (mm Hg)	84.92 ± 11.53	83.96± 12.69	0.398
Duration of diabetes, (months)	51.46 ± 55.12	95.69 ± 61.01	<0.001
Duration of diabetes ≥ 60, months (%)	70 (32.86)	169(66.01)	<0.001
HbA1c, (%)	7.70 ± 1.88	7.94 ± 2.33	0.284
HbA1c ≥ 7, (%)	97(45.54)	118 (46.09)	0.832
FBG, (mmol/L)	7.17 ± 3.08	7.48 ± 3.54	0.313
Hemoglobin, (g/L)	126.00 ± 23.53	110.60 ± 23.95	<0.001
PLT, (10^9/L)	211.45± 82.92	200.98 ± 76.00	0.157
Serum albumin, (g/L)	36.13 ± 10.45	34.26 ± 8.37	0.034
TG, (mmol/L)	2.44 ± 1.97	2.03 ± 1.75	0.018
TC, (mmol/L)	5.47 ± 2.24	5.48 ± 1.75	0.920`
Scr, (μmol/L)	106.47± 60.54	144.76 ± 83.96	<0.001
eGFR, (ml/min/1.73m^2)	79.03 ± 35.72	59.22 ± 32.71	<0.001
BUN, (mmol/L)	7.01 ± 3.21	8.56 ± 3.90	<0.001
Uric acid, (μmol/L)	375.98 ± 103.04	381.33 ± 91.05	0.555
Urinary protein, (g/24 h)	3.03 ± 3.96	3.73 ± 3.47	0.054
Hematuria (%)	134 (62.91)	184 (71.88)	0.056
Cardiovascular disease (%)	78 (36.61)	101 (39.45)	0.479
Hypertension (%)	121 (56.81)	191 (74.61)	0.003
DR (%)	63 (29.58)	163 (63.67)	<0.001
Nephrotic syndrome (%)	40 (18.78)	69 (26.95)	0.037

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; FBG fasting blood glucose; PLT, platelet; TG triglyceride; TC total cholesterol; Scr serum creatine; BUN blood urea nitrogen; DR, diabetic retinopathy. Data were presented as the mean ± standard, the median with range or counts and percentages. A two-tailed p < .0.05 was considered statistically significant.

TABLE 3 | Predictors of DKD in T2DM with renal impairment.

Parameters	Univariate		Multivariate	
	OR (95%CI)	P value	OR (95%CI)	P value
Duration of diabetes ≥ 60 months (yes/no)	9.34 (5.61,15.56)	0.000	5.29 (2.63,10.59)	<0.001
BMI, (kg/m^2)	0.89 (0.84,0.94)	0.000	0.90 (0.82,1.00)	0.045
SBP, (mmHg)	1.02 (1.01,1.03)	0.001		
DR (yes/no)	19.14 (10.16,36.07)	0.000	14.80 (6.40,34.26)	<0.001
HbA1c ≥ 7% (yes/no)	2.20 (1.40,3.45)	0.001	2.19 (1.14,4.21)	0.019
FBG, (mmol/L)	1.09 (1.02,1.17)	0.016		
Hemoglobin, (g/L)	0.98 (0.97,0.99)	0.000		
TG, (mmol/L)	0.87 (0.78,0.97)	0.014		
Scr, (μmol/L	1.01 (1.01,1.01)	0.000		
BUN, (mmol/L)	1.13 (1.06,1.21)	0.000		
RI ≥ 0.66 (yes/no)	9.50 (5.84,15.46)	0.000	5.19 (2.59,10.38)	<0.001

BMI, body mass index; SBP, systolic blood pressure; DR, Diabetic retinopathy; HbA1c, glycosylated hemoglobin; FBG, fasting blood glucose; TG, triglyceride; Scr, serum creatine; BUN, blood urea nitrogen; RI, resistance index; CI, confidence interval.

that met the same inclusion criteria to validate the RI-based model's efficacy further. The Validation cohort test demonstrated that the RI-based model had a sensitivity of 81.5% and a specificity of 78.6% (**Table 5**). The ROC curve (AUC=0.857) based on the validation cohort indicated that the RI-based model had good diagnostic efficiency (**Figure 3B**).

DISCUSSION

For T2DM patients with renal impairment, it is of great significance to differentiate between DKD and NDKD to guide clinical diagnosis and treatment. Kidney biopsy is the ultimate method of differential diagnosis, but its clinical application is restricted by many contraindications (35, 36). Therefore, it is necessary to find a new precise and sensitive non-invasive indicator for clinical differentiation of DKD from NDKD. The present study found that the RI value, which reflects vascular disease (37), was remarkably higher in the DKD group in contrast to the NDKD group. Furthermore, the RI-based differential model has good sensitivity and specificity.

Doppler ultrasound as a non-invasive, low-cost method has been extensively used in the detection of reno-vascular diseases. RI is calculated by the ratio of the difference between peak systolic velocity (PSV) and end-diastolic velocity (EDV) divided by peak systolic velocity (PSV), obtained by the Doppler spectrum analysis from segmental or interlobar arteries. PSV mainly reflects the degree of renal vascular filling and blood supply, while the EDV reflects renal blood perfusion, and RI mainly reflects vascular bed resistance (38). There is no uniform standard for RI average value. The normal mean renal RI value reported in previous literature are listed in **Supplementary Table 5**. Generally, a value of 0.60 is considered a normal value for renal RI (39, 40). Current studies have found that RI can effectively assess the renal blood perfusion status, whether for renal damage caused by hypertension and diabetes (41–43) or for risk prediction and disease assessment of early acute renal injury induced by various diseases (44–47). In patients with CKD, RI ≥ 0.70 is an independent risk factor for the progression of renal failure (25, 48). And RI ≥ 0.80 was associated with a lower survival rate (49). Moreover, studies have also confirmed that the RI is higher in the newly diagnosed and untreated DKD patients than healthy controls (28, 29, 50), even before the onset of microalbuminuria, supporting the dynamic evaluation of renal RI as an early detector of renal vascular alterations in the presence of T2DM (51). A previous study has also demonstrated a higher value of RI in DKD compared with diabetic patients without kidney disease (52). Different from previous studies, our study focused on comparing the RI level between the DKD group and the NDKD group. We found a significantly higher RI value in the DKD group. RI ≥ 0.66 was proved to be an independent risk factor for the diagnoses of DKD in T2DM patients with renal impairment, which could improve the efficiency of differential diagnosis in identifying cases with a higher clinical suspicion for DKD. It is worth noting that the RI value is affected by age, race, region and many other factors so

TABLE 4 | Development of two differential diagnostic mode.

Parameters	The RI-Based Model				The Traditional Model			
	OR (95%CI)	B	SE	P value	OR (95%CI)	β	SE	P value
Dm (0/1)	5.320 (2.653,10.66)	1.671	0.35	0.000	6.984 (3.617,13.483)	1.944	0.336	0.0000
BMI	0.904 (0.819,0.998)	-0.101	0.050	0.046	0.887 (0.809,0.973)	-0.120	0.047	0.011
DR (0/1)	14.921 (6.445,34.546)	2.703	0.428	0.000	15.790 (7.077,35.227)	2.759	0.409	0.000
Gh (0/1)	2.214 (1.151,4.258)	0.795	0.334	0.017	1.799 (0.978,3.311)	0.587	0.311	0.009
RI (0/1)	5.239 (2.618,10.483)	1.656	0.000	0.000				
Constant	2.655	0.976	1.318	0.459	8.137	2.096	1.204	0.082

OR, odds ratio; SE, standard error; Dm, diabetes duration ≥ 60 months (1 yes, 0 no); BMI, body mass index; DR, diabetic retinopathy (1 yes, 0 no); Gh, HbA1c ≥ 7.0% (1 yes, 0 no); RI, Resistance index ≥ 0.66 (1 yes, 0 no).

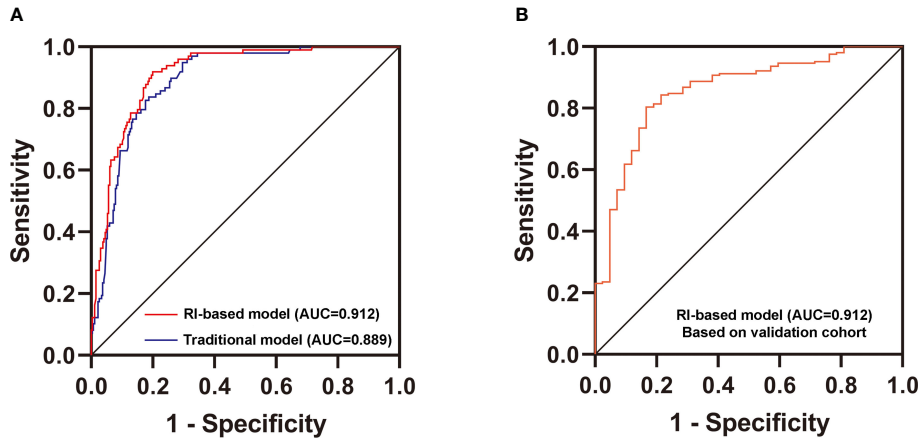


FIGURE 3 | Validation of the new differential diagnostic model. **(A)** Comparison of the area under the curve (AUC) of the RI-based model and the traditional model. DeLong's test was applied by R language to compare the AUC of two models (with or without RI). Z = 2.5964, P value = 0.00942. **(B)** Receiver operating characteristics (ROC) curve for the discriminative effect of the RI-based model in the Validation cohort. AUC, the area under the curve =0.857.

that the best cutoff value may be different in different studies. The cutoff value of RI in this study was derived from a small group of people in this specific region. A more appropriate cutoff value of RI needs further clinical research with a larger sample size in the future.

Glomerular hemodynamic changes (including hyperfiltration and hypoperfusion) are key pathological processes in the development of DKD, which could explain why RI value as an indicator of renal hemodynamics has a potential value in the differential diagnosis of DKD (53). The cause of glomerular hyperfiltration is currently believed to be caused by an increase in the trans-glomerular pressure gradient. The high glucose leads to the glycation of the basement of small blood vessels, particularly the efferent arteriole, making it thicken and stiffer and increasing the pressure within the glomerulus. Simultaneously, the afferent arteriole dilates, letting more blood flow into the glomeruli and increasing the pressure even further (54). With the deterioration of DKD, renal perfusion pressure and glomerular filtration rate continue to rise, resulting in glomerular capillary wall thickening, permeability enhancement, vascular lumen stenosis, glomerular capillary pressure increases. Those changes eventually lead to increased blood flow forward resistance and other renal artery hemodynamic disorders. Ultrasound showed that PSV and EDV decreased, especially EDV decreased and RI increased (53). Then, glomerular capillary basement membrane (GBM) thickening and mesangial matrix increased gradually, leading to glomerulosclerosis (55). Patients with NDKD often have less vascular involvement, such as membranous nephropathy mainly characterized by podocyte changes, and IgA nephropathy is primarily characterized by mesangial proliferation (56, 57). Although both DKD and NDKD patients suffered from renal hemodynamics changes, more significant vascular lesions and a higher RI value were detected in DKD patients.

Our research also found that DR and diabetic duration are powerful predictors of DKD, which is consistent with previous studies (11, 18). Generally, the diabetes duration in DKD patients is longer than that in NDKD patients. Diabetes history ≥ 5 years is an independent risk factor for DKD (58). In the present study, the diabetes duration is defined as the time from the diagnosis of diabetes to the time of renal biopsy. The diagnosis is usually delayed in patients with T2DM. It is hard to clarify their actual course of diabetes before diagnosis, so DKD cannot be ignored due to a short history of diabetes. Similarly, DR is related to DKD (59). CKD patients with DR are conducive to the diagnosis of DKD, in which

TABLE 5 | Predictive value of the Back-substitution and Validation cohort test.

	Back-Substitution Test			Validation Cohort Test		
	DKD	**NDKD**	**Total**	**DKD**	**NDKD**	**Total**
Diagnosed as DKD	300	45	345	44	9	53
Diagnosed as NDKD	32	92	124	10	33	43
Total	332	137	469	54	42	96
Sensitivity		90.4%			81.5%	
Specificity		67.2%			78.6%	
Positive predictive value		87.0%			83.0%	
Negative predictive value		74.2%			76.7%	
Total consistency		83.6%			80.2%	

DKD, diabetic kidney disease; NDKD, non-diabetic kidney disease.

proliferative retinopathy has higher specificity. DR often accompanies kidney damage in T1DM, but the incidence of DR in T2DM is only 40%-60%. DR cannot completely distinguish DKD from NDKD because some diabetic patients without DR also have biopsy-proven DKD. Therefore, we choose to combine previous classic predictive indicators and RI that reflect vascular disease to construct a diagnostic model, significantly improving diagnostic efficiency. In addition, RI obtained by ultrasound examinations can be easily performed in most medical institutions, making our model more practical and economical in clinical practice. Therefore, we propose to incorporate the RI measure in the examination of DM combined with renal impairment. Interestingly, our study found for the first time that lower BMI was an independent risk factor for DKD. BMI was significantly lower in the DKD group than the NDKD group, which may be due to a longer duration of diabetes in the DKD group. However, compared with other independent risk factors, the OR value of BMI was only 0.9. Further studies are needed to power it as an independent risk factor for DKD.

There are several limitations in the present research. First of all, this research was a retrospective study performed in a single center with limited data. Secondly, all validation cohort patients came from the department of nephrology of Xinqiao Hospital, lacking external validation. Thirdly, we only measure RI value at the time of kidney biopsy, not continuous testing; certain errors are unavoidable.

In conclusion, the RI value might serve as a novel potential indicator in the differential diagnosis of DKD. The RI-based differential diagnostic model has improved the accuracy and could be commonly used in clinical practice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The ethical committee of Xinqiao Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HL and YS carried out the studies, participated in data collection, statistical analysis, and the writing of the manuscript. ZY and YH contributed to the data analysis. TH, TX, and YL contributed to the data collection. JZ and JX reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.731187/full#supplementary-material

REFERENCES

1. Zheng Y, Ley SH, Hu FB. Global Aetiology and Epidemiology of Type 2 Diabetes Mellitus and its Complications. *Nat Rev Endocrinol* (2018) 14(2):88–98. doi: 10.1038/nrendo.2017.151
2. Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, et al. Prevalence of Diabetes Recorded in Mainland China Using 2018 Diagnostic Criteria From the American Diabetes Association: National Cross Sectional Study. *BMJ (Clinical Res ed)* (2020) 369:m997. doi: 10.1136/bmj.m997
3. Afkarian M, Sachs MC, Kestenbaum B, Hirsch IB, Tuttle KR, Himmelfarb J, et al. Kidney Disease and Increased Mortality Risk in Type 2 Diabetes. *J Am Soc Nephrol* (2013) 24(2):302–8. doi: 10.1681/asn.2012070718
4. Umanath K, Lewis JB. Update on Diabetic Nephropathy: Core Curriculum 2018. *Am J Kidney Dis* (2018) 71(6):884–95. doi: 10.1053/j.ajkd.2017.10.026
5. Ma RCW. Epidemiology of Diabetes and Diabetic Complications in China. *Diabetologia* (2018) 61(6):1249–60. doi: 10.1007/s00125-018-4557-7
6. Yan S-T, Liu J-Y, Tian H, Li C-L, Li J, Shao Y-H, et al. Clinical and Pathological Analysis of Renal Damage in Elderly Patients With Type 2 Diabetes Mellitus. *Clin Exp Med* (2016) 16(3):437–42. doi: 10.1007/s10238-015-0362-5
7. Pham TT, Sim JJ, Kujubu DA, Liu IL, Kumar VA. Prevalence of Nondiabetic Renal Disease in Diabetic Patients. *Am J Nephrol* (2007) 27(3):322–8. doi: 10.1159/000102598
8. Mou S, Wang Q, Liu J, Che X, Zhang M, Cao L, et al. Prevalence of Non-Diabetic Renal Disease in Patients With Type 2 Diabetes. *Diabetes Res Clin Pract* (2010) 87(3):354–9. doi: 10.1016/j.diabres.2009.11.012
9. Zhuo L, Ren W, Li W, Zou G, Lu J. Evaluation of Renal Biopsies in Type 2 Diabetic Patients With Kidney Disease: A Clinicopathological Study of 216 Cases. *Int Urol Nephrol* (2013) 45(1):173–9. doi: 10.1007/s11255-012-0164-6
10. Li L, Zhang X, Li Z, Zhang R, Guo R, Yin Q, et al. Renal Pathological Implications in Type 2 Diabetes Mellitus Patients With Renal Involvement. *J Diabetes Complications* (2017) 31(1):114–21. doi: 10.1016/j.jdiacomp.2016.10.024
11. Dong Z, Wang Y, Qiu Q, Zhang X, Zhang L, Wu J, et al. Clinical Predictors Differentiating Non-Diabetic Renal Diseases From Diabetic Nephropathy in a Large Population of Type 2 Diabetes Patients. *Diabetes Res Clin Pract* (2016) 121:112–8. doi: 10.1016/j.diabres.2016.09.005
12. Sharma SG, Bomback AS, Radhakrishnan J, Herlitz LC, Stokes MB, Markowitz GS, et al. The Modern Spectrum of Renal Biopsy Findings in Patients With Diabetes. *Clin J Am Soc Nephrol* (2013) 8(10):1718–24. doi: 10.2215/cjn.02510213
13. Anders HJ, Huber TB, Isermann B, Schiffer M. CKD in Diabetes: Diabetic Kidney Disease Versus Nondiabetic Kidney Disease. *Nat Rev Nephrol* (2018) 14(6):361–77. doi: 10.1038/s41581-018-0001-y
14. Chen Y, Lee K, Ni Z, He JC. Diabetic Kidney Disease: Challenges, Advances, and Opportunities. *Kidney Dis (Basel Switzerland)* (2020) 6(4):215–25. doi: 10.1159/000506634

15. Bermejo S, González E, López-Revuelta K, Ibernon M, López D, Martín-Gómez A, et al. Risk Factors for non-Diabetic Renal Disease in Diabetic Patients. *Clin Kidney J* (2020) 13(3):380–8. doi: 10.1093/ckj/sfz177
16. Chang TI, Park JT, Kim JK, Kim SJ, Oh HJ, Yoo DE, et al. Renal Outcomes in Patients With Type 2 Diabetes With or Without Coexisting non-Diabetic Renal Disease. *Diabetes Res Clin Pract* (2011) 92(2):198–204. doi: 10.1016/j.diabres.2011.01.017
17. Xu J, Hu XF, Huang W, Shen PY, Zhang W, Ren H, et al. [The Clinicopathological Characteristics of Diabetic Nephropathy and Non-Diabetic Renal Diseases in Diabetic Patients]. *Zhonghua Nei Ke Za Zhi* (2017) 56(12):924–9. doi: 10.3760/cma.j.issn.0578-1426.2017.12.007
18. Zhou J, Chen X, Xie Y, Li J, Yamanaka N, Tong X. A Differential Diagnostic Model of Diabetic Nephropathy and Non-Diabetic Renal Diseases. *Nephrol Dial Transplant* (2007) 23(6):1940–5. doi: 10.1093/ndt/gfm897
19. Liu MY, Chen XM, Sun XF, Zhou JH, Zhang XG, Zhu HY, et al. Validation of a Differential Diagnostic Model of Diabetic Nephropathy and Non-Diabetic Renal Diseases and the Establishment of a New Diagnostic Model. *J Diabetes* (2014) 6(6):519–26. doi: 10.1111/1753-0407.12150
20. Liang S, Zhang XG, Cai GY, Zhu HY, Zhou JH, Wu J, et al. Identifying Parameters to Distinguish Non-Diabetic Renal Diseases From Diabetic Nephropathy in Patients With Type 2 Diabetes Mellitus: A Meta-Analysis. *PloS One* (2013) 8(5):e64184. doi: 10.1371/journal.pone.0064184
21. Mak S, Gwi E, Chan K, Wong P, Lo K, Lee K, et al. Clinical Predictors of Non-Diabetic Renal Disease in Patients With non-Insulin Dependent Diabetes Mellitus. *Nephrol Dialysis Transplant* (1997) 12(12):2588–91. doi: 10.1093/ndt/12.12.2588
22. Duan S, Chen J, Wu L, Nie G, Sun L, Zhang C, et al. Assessment of Urinary NGAL for Differential Diagnosis and Progression of Diabetic Kidney Disease. *J Diabetes Complications* (2020) 34(10):107665. doi: 10.1016/j.jdiacomp.2020.107665
23. Prakash J, Gupta T, Prakash S, Bhushan P, Usha, Sivasankar M, et al. Non-Diabetic Renal Disease in Type 2 Diabetes Mellitus: Study of Renal - Retinal Relationship. *Indian J Nephrol* (2015) 25(4):222–8. doi: 10.4103/0971-4065.144420
24. Dong ZY, Wang YD, Qiu Q, Hou K, Zhang L, Wu J, et al. Dysmorphic Erythrocytes Are Superior to Hematuria for Indicating Non-Diabetic Renal Disease in Type 2 Diabetics. *J Diabetes Investig* (2016) 7(1):115–20. doi: 10.1111/jdi.12371
25. Sugiura T, Wada A. Resistive Index Predicts Renal Prognosis in Chronic Kidney Disease: Results of a 4-Year Follow-Up. *Clin Exp Nephrol* (2011) 15(1):114–20. doi: 10.1007/s10157-010-0371-3
26. Sugiura T, Wada A. Resistive Index Predicts Renal Prognosis in Chronic Kidney Disease. *Nephrol Dialysis Transplant* (2009) 24(9):2780–5. doi: 10.1093/ndt/gfp121
27. Bigé N, Lévy PP, Callard P, Faintuch JM, Chigot V, Jousselin V, et al. Renal Arterial Resistive Index Is Associated With Severe Histological Changes and Poor Renal Outcome During Chronic Kidney Disease. *BMC Nephrol* (2012) 13:139. doi: 10.1186/1471-2369-13-139
28. Ishimura E, Nishizawa Y, Kawagishi T, Okuno Y, Kogawa K, Fukumoto S, et al. Intrarenal Hemodynamic Abnormalities in Diabetic Nephropathy Measured by Duplex Doppler Sonography. *Kidney Int* (1997) 51(6):1920–7. doi: 10.1038/ki.1997.261
29. Masulli M, Mancini M, Liuzzi R, Daniele S, Mainenti PP, Vergara E, et al. Measurement of the Intrarenal Arterial Resistance Index for the Identification and Prediction of Diabetic Nephropathy. *Nutr Metab Cardiovasc Dis* (2009) 19(5):358–64. doi: 10.1016/j.numecd.2008.07.003
30. American Diabetes Association. Diabetes Advocacy: Standards of Medical Care in Diabetes-2019. *Diabetes Care*. (2019) 42(Suppl1):S182–s3. doi: 10.2337/dc19-S016
31. National Kidney Foundation. KDOQI Clinical Practice Guideline for Diabetes and CKD: 2012 Update. *Am J Kidney Dis* (2012) 60(5):850–86. doi: 10.1053/j.ajkd.2012.07.005
32. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic Classification of Diabetic Nephropathy. *J Am Soc Nephrol* (2010) 21(4):556–63. doi: 10.1681/asn.2010010010
33. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A More Accurate Method to Estimate Glomerular Filtration Rate From Serum Creatinine: A New Prediction Equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* (1999) 130(6):461–70. doi: 10.7326/0003-4819-130-6-199903160-00002
34. Jiang W, Wang J, Shen X, Lu W, Wang Y, Li W, et al. Establishment and Validation of a Risk Prediction Model for Early Diabetic Kidney Disease Based on a Systematic Review and Meta-Analysis of 20 Cohorts. *Diabetes Care* (2020) 43(4):925–33. doi: 10.2337/dc19-1897
35. Kumar S, Lim E, Covic A, Verhamme P, Gale CP, Camm AJ, et al. Anticoagulation in Concomitant Chronic Kidney Disease and Atrial Fibrillation: JACC Review Topic of the Week. *J Am Coll Cardiol* (2019) 74(17):2204–15. doi: 10.1016/j.jacc.2019.08.1031
36. Carey IM, Critchley JA, DeWilde S, Harris T, Hosking FJ, Cook DG. Risk of Infection in Type 1 and Type 2 Diabetes Compared With the General Population: A Matched Cohort Study. *Diabetes Care* (2018) 41(3):513–21. doi: 10.2337/dc17-2131
37. Di Nicolò P, Granata A. Renal Intraparenchymal Resistive Index: The Ultrasonographic Answer to Many Clinical Questions. *J Nephrol* (2019) 32(4):527–38. doi: 10.1007/s40620-018-00567-x
38. Spatola L, Andrulli S. Doppler Ultrasound in Kidney Diseases: A Key Parameter in Clinical Long-Term Follow-Up. *J Ultrasound* (2016) 19(4):243–50. doi: 10.1007/s40477-016-0201-x
39. Le Dorze M, Bouglé A, Deruddre S, Duranteau J. Renal Doppler Ultrasound: A New Tool to Assess Renal Perfusion in Critical Illness. *Shock* (2012) 37(4):360–5. doi: 10.1097/SHK.0b013e3182467156
40. Keogan MT, Kliewer MA, Hertzberg BS, DeLong DM, Tupler RH, Carroll BA. Renal Resistive Indexes: Variability in Doppler US Measurement in a Healthy Population. *Radiology* (1996) 199(1):165–9. doi: 10.1148/radiology.199.1.8633141
41. Doi Y, Iwashima Y, Yoshihara F, Kamide K, Hayashi S, Kubota Y, et al. Response to Renal Resistive Index and Cardiovascular and Renal Outcomes in Essential Hypertension. *Hypertension* (2013) 61(2):e23. doi: 10.1161/hypertensionaha.111.00664
42. Iwakura Y, Ito S, Morimoto R, Kudo M, Ono Y, Nezu M, et al. Renal Resistive Index Predicts Postoperative Blood Pressure Outcome in Primary Aldosteronism. *Hypertension* (2016) 67(3):654–60. doi: 10.1161/hypertensionaha.115.05924
43. Ikee R, Kobayashi S, Hemmi N, Imakiire T, Kikuchi Y, Moriya H, et al. Correlation Between the Resistive Index by Doppler Ultrasound and Kidney Function and Histology. *Am J Kidney Dis* (2005) 46(4):603–9. doi: 10.1053/j.ajkd.2005.06.006
44. Darmon M, Schortgen F, Vargas F, Liazydi A, Schlemmer B, Brun-Buisson C, et al. Diagnostic Accuracy of Doppler Renal Resistive Index for Reversibility of Acute Kidney Injury in Critically Ill Patients. *Intensive Care Med* (2011) 37(1):68–76. doi: 10.1007/s00134-010-2050-y
45. Giustiniano E, Meco M, Morenghi E, Ruggieri N, Cosseta D, Cirri S, et al. May Renal Resistive Index be an Early Predictive Tool of Postoperative Complications in Major Surgery? Preliminary Results. *BioMed Res Int* (2014) 2014:917985. doi: 10.1155/2014/917985
46. Song J, Wu W, He Y, Lin S, Zhu D, Zhong M. Value of the Combination of Renal Resistance Index and Central Venous Pressure in the Early Prediction of Sepsis-Induced Acute Kidney Injury. *J Crit Care* (2018) 45:204–8. doi: 10.1016/j.jcrc.2018.03.016
47. Lerolle N, Guérot E, Faisy C, Bornstain C, Diehl JL, Fagon JY. Renal Failure in Septic Shock: Predictive Value of Doppler-Based Renal Arterial Resistive Index. *Intensive Care Med* (2006) 32(10):1553–9. doi: 10.1007/s00134-006-0360-x
48. Parolini C, Noce A, Staffolani E, Giarrizzo GF, Costanzi S, Splendiani G. Renal Resistive Index and Long-Term Outcome in Chronic Nephropathies. *Radiology* (2009) 252(3):888–96. doi: 10.1148/radiol.2523080351
49. Radermacher J, Ellis S, Haller H. Renal Resistance Index and Progression of Renal Disease. *Hypertension* (2002) 39(2 Pt 2):699–703. doi: 10.1161/hy0202.103782
50. Brkljacic B, Mrzljak V, Drinkovic I, Soldo D, Sabljar-Matovinovic M, Hebrang A. Renal Vascular Resistance in Diabetic Nephropathy: Duplex Doppler US Evaluation. *Radiology* (1994) 192(2):549–54. doi: 10.1148/radiology.192.2.8029430
51. Bruno RM, Daghini E, Landini L, Versari D, Salvati A, Santini E, et al. Dynamic Evaluation of Renal Resistive Index in Normoalbuminuric Patients With Newly Diagnosed Hypertension or Type 2 Diabetes. *Diabetologia* (2011) 54(9):2430–9. doi: 10.1007/s00125-011-2148-y
52. Platt JF, Rubin JM, Ellis JH. Diabetic Nephropathy: Evaluation With Renal Duplex Doppler US. *Radiology* (1994) 190(2):343–6. doi: 10.1148/radiology.190.2.8284379

53. Rossing K, Christensen PK, Hovind P, Tarnow L, Rossing P, Parving HH. Progression of Nephropathy in Type 2 Diabetic Patients. *Kidney Int* (2004) 66 (4):1596–605. doi: 10.1111/j.1523-1755.2004.00925.x
54. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular Hyperfiltration in Diabetes: Mechanisms, Clinical Significance, and Treatment. *J Am Soc Nephrol* (2017) 28(4):1023–39. doi: 10.1681/asn.2016060666
55. Wolf G, Butzmann U, Wenzel UO. The Renin-Angiotensin System and Progression of Renal Disease: From Hemodynamics to Cell Biology. *Nephron Physiol* (2003) 93(1):P3–13. doi: 10.1159/000066656
56. Cattran DC, Brenchley PE. Membranous Nephropathy: Integrating Basic Science Into Improved Clinical Management. *Kidney Int* (2017) 91(3):566–74. doi: 10.1016/j.kint.2016.09.048
57. Suzuki H, Kiryluk K, Novak J, Moldoveanu Z, Herr AB, Renfrow MB, et al. The Pathophysiology of IgA Nephropathy. *J Am Soc Nephrol* (2011) 22 (10):1795–803. doi: 10.1681/asn.2011050464
58. Yenigun EC, Dede F, Ozturk R, Turgut D, Koc E, Piskinpasa SV, et al. Non-Diabetic Renal Disease in Diabetes Mellitus: Clinical Features and Renal Biopsy Findings. *Hippokratia* (2015) 19(2):148–52.
59. Fiorentino M, Bolignano D, Tesar V, Pisano A, Biesen WV, Tripepi G, et al. Renal Biopsy in Patients With Diabetes: A Pooled Meta-Analysis of 48 Studies. *Nephrol Dial Transplant* (2017) 32(1):97–110. doi: 10.1093/ndt/gfw070

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Validation of Controlled Attenuation Parameter Measured by FibroScan as a Novel Surrogate Marker for the Evaluation of Metabolic Derangement

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Background/Objectives: Renaming non-alcoholic fatty liver disease (NAFLD) to metabolic dysfunction-associated fatty liver disease (MAFLD) suggests a shift of emphasis to the accompanying metabolic disturbance. Controlled attenuation parameter (CAP) measured by FibroScan has been shown to be correlated with hepatic steatosis. We aim to validate its usefulness as a novel surrogate marker for evaluating metabolic derangement.

Subjects/Methods: Volunteers were recruited from medical staff at our hospital to undergo CAP measurements. Anthropometrics, CAP, and laboratory assessments for metabolic profiles and insulin resistance were collected. CAP < 238 dB/m denoted no hepatic steatosis, 238 ≤ CAP ≤ 259 dB/m denoted mild, 260 ≤ CAP ≤ 291 dB/m denoted moderate, and CAP > 291 dB/m denoted severe hepatic steatosis according to previous reports.

Results: Data of 824 participants were included for analysis. The age was 53.2 ± 15.4 years, body mass index (BMI) was 23.6 ± 3.1 kg/m^2, 24.4% were male subjects, and 22.0% met the criteria for metabolic syndrome (MetS). Taking the group with CAP < 238 dB/m as control, subjects with mild, moderate, and severe hepatic steatosis had increased odds of MetS by 3.51-, 3.32-, and 5.12-fold, respectively, after adjusting for multiple confounders (p = 0.020). Metabolic profiles, insulin resistance, and presence of MetS were similar between normal-weight subjects with CAP ≥ 238 dB/m and overweight subjects with CAP < 238 dB/m. Even in subjects with no MetS components, those with CAP ≥ 238 dB/m had higher BMI, waist circumferences, uric acid, triglyceride, white blood cell count, and insulin resistance, whereas lower adiponectin and estimated glomerular filtration rate. Waist circumference [OR 1.11 (1.04, 1.18), p = 0.001] and homeostatic model assessment of insulin resistance (HOMA-IR) [OR 2.39 (1.18, 4.83), p = 0.016] were predictive of hepatic steatosis according to CAP ≥ 238 dB/m.

Conclusions: CAP is a convenient, sensitive, and non-invasive indicator for metabolic derangement. Prospective studies are needed to further validate its usefulness as a surrogate marker for the transition of metabolic status over time.

Keywords: non-alcoholic fatty liver disease (NAFLD), controlled attenuation parameter (CAP), metabolic syndrome (MetS), diabetes mellitus, insulin resistance, FibroScan

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) affects nearly one billion people worldwide and is expected to be the leading cause of end-stage liver disease in the coming decades (1). Increasing evidence has indicated a link between NAFLD and metabolic dysfunction and subsequent cardiovascular and renal complications. Due to accumulated understanding of the pathogenesis and prevalence of NAFLD and its comorbidities, including obesity and diabetes mellitus, the nomenclature and diagnostic criteria have recently been revised by a panel of international experts from 22 countries (2). Metabolic dysfunction-associated fatty liver disease (MAFLD) has been proposed to replace NAFLD. Rather than an "exclusion criteria", "positive criteria" have been adopted to diagnose MAFLD, which are based on evidence of hepatic steatosis by imaging techniques, blood biomarkers, or liver histology, in addition to one of the following 3 criteria: 1) overweight/obesity, 2) type 2 diabetes mellitus, or 3) two of the seven cardiometabolic risk factors, which largely represent the criteria for metabolic syndrome (MetS) (3). The prevalence of MAFLD is expected to be more prominent because a less rigorous criterion is employed, where alcohol consumption and other concomitant liver diseases are no longer exclusion conditions, and more sensitive and inexpensive imaging techniques have come into clinical practice. The goal of the revised nomenclature is to draw public attention to the prevention and intervention of treatable metabolic dysfunction. Since specific pharmacotherapy for fatty liver disease is lacking, detection of metabolic derangement at an earlier stage to justify immediate intervention through lifestyle change should be both efficient and economical.

FibroScan is a non-invasive medical device originally designed to perform liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE). Since 2010, controlled attenuation parameter (CAP), which is a measurement of the ultrasound attenuation coefficient, can also be measured with FibroScan devices concomitantly to LSM and has been shown to be correlated with hepatic steatosis. When taking liver biopsy as the reference, an area under the receiver operating characteristic curve (AUROC) of CAP was equal to 0.79–0.91 and 0.77–0.95 for detection of more than 5%–10% and 33% of hepatic steatosis, respectively (4–6), the performance of which is superior to that of routine ultrasonography (4, 7). Recent studies have shown a close relationship between CAP and MetS components (8–10). However, current guidance still resists routine screening for NAFLD even in high-risk subjects attending primary care, diabetes, or obesity clinics due to uncertainties related to long-term benefits and cost-effectiveness (11). Thus, further evidence is needed to justify the early screening for fatty liver disease out of consideration for detecting metabolic derangement, rather than for liver lesion sequelae alone, such as steatohepatitis, hepatic cirrhosis, or liver cancer.

In this study, a total of 922 medical staff from our hospital were recruited to investigate the association between CAP and MetS components. We aim to validate the usefulness of CAP as a surrogate marker for evaluating metabolic derangement in subjects with fewer risks, or even with no MetS components.

SUBJECTS/MATERIALS AND METHODS

Subjects

The medical staff in our hospital who volunteered for the NAFLD study using FibroScan (502 Touch, Echosens, France) were recruited at the time of the annual physical checkup between January and March 2018. Information about the study participants was collected including previous medical history, drinking, and smoking status. Those with chronic liver diseases, including viral hepatitis B or C, autoimmune liver disease, recent infections, prolonged use of steroids, or estrogens were excluded. Individuals with daily alcohol consumption >20 g (for males) or >10 g (for females) were not included. Subjects with alanine transaminase (ALT) or aspartate transaminase (AST) higher than 5 times or plasma creatinine level higher than 2 times the upper normal limit were also excluded from the analysis. The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the First Affiliated Hospital of Sun Yat-sen University [Ethics Committee Review (2016) No. 146]. Informed consent was obtained from each participant.

Procedures

All subjects received clinical and laboratory assessments on the same day when the FibroScan examination was done. Anthropometric data including body weight and height, blood pressure, circumferences of the neck, waist, and hip were collected. Neck circumference was measured in the midway of the neck, between the mid-cervical spine and mid-anterior neck. Waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the ileal crest. Hip circumference measurement was taken around the widest portion of the buttock.

After a 10-h overnight fast, blood samples were drawn for laboratory testing, including fasting plasma glucose, lipid profiles, liver and renal function, uric acid, serum insulin, adiponectin, and whole blood cell counts. The estimated

glomerular filtration rate (eGFR) was estimated using a modification of diet in renal disease (MDRD) equation for the evaluation of renal function. Indices for insulin resistance assessment included homeostatic model assessment of insulin resistance (HOMA-IR), the product of fasting triglycerides and glucose (TyG), and metabolic score for insulin resistance (METS-IR). HOMA-IR was estimated by the formula as fasting insulin (U/ml) × fasting glucose (mmol/L)/22.5. TyG was calculated as Ln [fasting triglycerides (mmol/L) × 88.6 × fasting glucose (mmol/L) × 18]/2 (12). METS-IR was defined as Ln [2 × fasting glucose (mmol/L) × 18 + fasting triglycerides (mmol/L) × 88.6) × BMI]/Ln [fasting HDL-c (mmol/L) × 38.66] (13).

All FibroScan examinations were performed by the same trained operator following the manufacturer's instruction. CAP and LSM were assessed with the tip of the M probe placed on the skin between the ribs over the right lobe of the liver through the intercostal space. CAP was calculated only when LSM was valid to ensure an accurate attenuation value of the liver, and an attempt was made to collect ≥10 valid LSMs. The ratio of the interquartile range (IQR) to the median of the liver stiffness (IQR/Median, LSM) ≤30% was considered a reliable measurement. In our preliminary study, ROC curve analysis was performed to determine the cutoff value of CAP for diagnosing MetS. According to the maximum Youden's index, CAP ≥ 245 dB/m was used as a cutoff point in our group, with 79.6% sensitivity and 61.6% specificity. The AUROC was 0.706 (0.665, 0.747), which indicated a moderate efficiency. In contrast, when using CAP ≥ 238 dB/m, which was used as the cutoff point for hepatic steatosis previously, the sensitivity was 82.3%, the specificity was 55.7%, and the AUROC was 0.690 (0.649, 0.731). The diagnostic power of both was similar, while the latter had slightly higher sensitivity in diagnosing MetS in our group (**Supplementary Figure 1**). Thus, hepatic steatosis grade was decided by the cutoff values of CAP according to previous reports, where CAP < 238 dB/m denoted no steatosis (S0), 238 ≤ CAP ≤ 259 dB/m denoted mild (S1), 260 ≤ CAP ≤ 291 dB/m denoted moderate (S2), and CAP > 291 dB/m denoted severe steatosis (S3) (4, 10). The hepatic fibrosis cutoff value was LSM ≥ 7.0 kPa for significant fibrosis (10, 14) (**Supplementary Figure 2**).

According to the guideline developed by the Chinese Diabetes Society in 2017, MetS was diagnosed in subjects with more than any 3 of the following 5 components: 1) waist circumference ≥90 cm in men and ≥85 cm in women; 2) blood pressure ≥130/85 mmHg or treated with anti-hypertensive drugs; 3) fasting plasma glucose ≥6.1 mmol/L, 2-h post-load glucose ≥7.8 mmol/L, or treated with anti-hyperglycemic drugs; 4) fasting triglyceride ≥1.70 mmol/L; and 5) fasting high-density lipoprotein cholesterol (HDL-c) <1.04 mmol/L. Overweight was defined as body mass index (BMI) ≥ 24 kg/m^2, whereas obesity was BMI ≥ 28 kg/m^2 for the Chinese population (15).

Statistical Analysis

The Kolmogorov–Smirnov test was used to examine the distribution of all the continuous quantitative variables before comparison. Continuous variables with normal distribution were expressed as mean ± SD, or median (Q1, Q3) when normal distribution was not achieved. Categorical variables were expressed as counts and percentages. Continuous variables were compared using Student's *t*-test or Mann–Whitney test between 2 groups, or one-way ANOVA or Kruskal–Wallis test for more than two groups' comparison as appropriate. Bonferroni correction test was applied for *post-hoc* pairwise comparisons. Categorical variables were compared using the chi-squared test, and the 2×C cross-tabulation test was used for multiple comparisons, with Bonferroni-adjusted Z-tests for column proportions. Binary logistic regression analysis was used to calculate the odds ratios for the presence of MetS in different CAP-based hepatic steatosis groups, or in BMI and CAP combination categories, and for hepatic steatosis (CAP ≥ 238 dB/m) in subjects with no MetS components. All statistical analyses were performed using SPSS version 23.0. All statistical tests were two-sided and were evaluated at the 0.05 level of significance.

RESULTS

A total of 922 medical staff volunteered for the FibroScan examination, while 98 subjects were excluded (**Figure 1**). Data of anthropometries and laboratory tests of 824 participants were included for analysis. The average age of the included subjects was 53.2 years, and 24.4% were male subjects. The mean BMI was 23.6 kg/m^2, while 42.0% of them were overweight, and 7.9% were obese. One hundred forty-one (17.1%) subjects were diagnosed with diabetes. Three hundred forty (41.3%) subjects had a previous history of dyslipidemia, 120 of them were treated with statins, and 15 subjects were treated with fibrates. One hundred eighty-one (22.0%) subjects met the criteria for MetS. Subjects in the MetS group were approximately 13 years older, had a slightly more male subject composition, and had significantly higher BMI, waist and neck circumferences, and

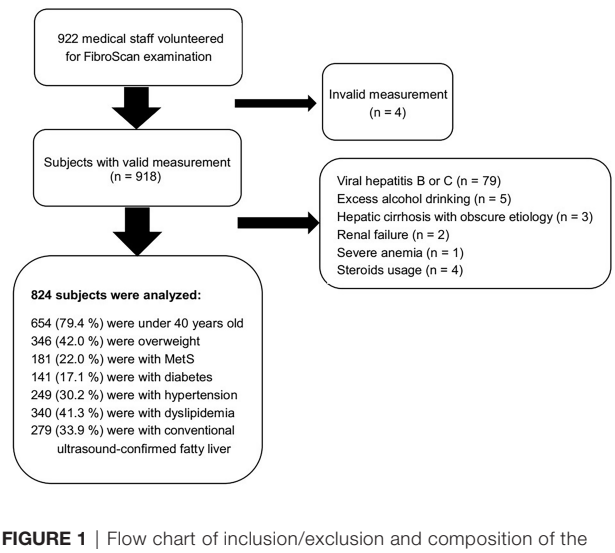

FIGURE 1 | Flow chart of inclusion/exclusion and composition of the included subjects.

waist-to-hip ratio. The median systolic and diastolic pressure was more than 10 mmHg higher in the MetS group. Around one-third of the subjects were diagnosed with fatty liver by using conventional ultrasound imaging, the percentage of which was significantly lower than that identified by FibroScan (52.7%) using CAP ≥ 238 dB/m as a cutoff value. The major difference was attributable to the fact that FibroScan detected more fatty liver in the non-MetS group than conventional ultrasonography, while the performances of both in the MetS group were similar (285/149 vs. 146/133, $p = 0.000$). The median LSM of all subjects was 4.0 kPa, only 18 subjects (2.2%) were found to have significant fibrosis (LSM ≥ 7.0 kPa), and 13 of them were in the MetS group (13/181 vs. 5/643, $p = 0.000$). The subjects in the MetS group had significantly higher fasting plasma glucose, serum insulin, triglyceride, uric acid, creatinine, ALT, AST level, and white blood cell count; their HDL-c, adiponectin, and eGFR were lower; and their total cholesterol and low-density lipoprotein cholesterol (LDL-c) were comparable with those of the non-MetS group. The CAP and LSM values, as well as insulin resistance indices including TyG, METS-IR, and HOMA-IR, were all significantly higher in the MetS group (**Table 1**).

The number of subjects with 5 MetS components was small (n = 9), so we combined them with subjects with 4 MetS components (n = 51) for further analysis. With accumulating number of MetS components, there was an upward trend in the percentage of subjects with CAP ≥ 238 dB/m in each group, from 25.1% in those with no MetS components to 81.7% in subjects with 4 or 5 MetS components. The number and percentage of subjects with CAP < 238 dB/m and 238 ≤ CAP ≤ 259 dB/m decreased, while those with CAP > 291 dB/m increased with accumulating MetS components. Likewise, the median and quartiles of CAP values increased with accumulating MetS components. When the subjects were categorized into 4 different groups according to hepatic steatosis severity as assessed by CAP measurement, the number and percentage of subjects with more MetS components (with 3, 4, or 5) increased, while those with no MetS components decreased with the

TABLE 1 | Clinical characteristics of the study population and comparisons between subjects with and without MetS.

Characteristics	Total	Non-MetS	MetS	*p*
	(n = 824)	(n = 643, 78%)	(n = 181, 22%)	
Age (years)	53.2 ± 15.4	50.4 ± 15.0	63.3 ± 12.6	0.000
Gender, male, n (%)	201 (24.4%)	146 (22.7%)	55 (30.4%)	0.034
Body mass index (kg/m^2)	23.6 ± 3.1	23.0 ± 2.7	25.9 ± 3.1	0.000
Waist circumference (cm)	81.7 ± 9.0	79.4 ± 8.1	89.5 ± 7.2	0.000
Waist-to-hip ratio	0.87 ± 0.06	0.86 ± 0.06	0.92 ± 0.05	0.000
Neck circumference (cm)	34.0 (32.4, 37.0)	33.5 (32.0, 36.0)	36.5 (34.5, 40.1)	0.000
Systolic pressure (mmHg)	121 (111, 135)	120 (110, 130)	136 (126, 147)	0.000
Diastolic pressure (mmHg)	72 (67, 80)	70 (65, 78)	80 (74, 85)	0.000
Drinking status, n (%)	139 (16.9%)	116 (18.0%)	23 (12.7%)	0.091
Smoking status, n (%)	49 (5.9%)	32 (5.0%)	17 (9.4%)	0.026
Diabetes, n (%)	141 (17.1%)	46 (7.2%)	95 (52.5%)	0.000
Hypertension, n (%)	249 (30.2%)	123 (19.1%)	126 (69.6%)	0.000
Dyslipidemia, n (%)	340 (41.3%)	206 (32.0%)	134 (74.0%)	0.000
Stroke, n (%)	15 (1.8%)	8 (1.2%)	7 (3.9%)	0.020
Coronary arterial disease, n (%)	68 (8.3%)	38 (5.9%)	30 (16.6%)	0.000
Fatty liver by ultrasound, n (%)	279 (33.9%)	146 (22.7%)	133 (73.5%)	0.000
Controlled attenuation parameter (dB/m)	241 (216, 278)	232 (207, 261)	285 (249, 318)	0.000
CAP ≥ 238 dB/m, n (%)	434 (52.7%)	285 (44.3%)	149 (82.3%)	0.000
Liver stiffness measurement (kPa)	4.0 (3.5, 4.8)	3.9 (3.4, 4.5)	4.5 (3.8, 5.9)	0.000
Fasting glucose (mmol/L)	5.2 (4.8, 5.9)	5.1 (4.8, 5.6)	6.4 (5.7, 7.8)	0.000
Cholesterol (mmol/L)	5.2 (4.6, 5.9)	5.1 (4.6, 5.8)	5.4 (4.5, 6.2)	0.259
Triglyceride (mmol/L)	1.14 (0.85, 1.67)	1.06 (0.77, 1.49)	1.85 (1.19, 2.66)	0.000
LDL-c (mmol/L)	3.40 ± 0.81	3.41 ± 0.75	3.37 ± 0.95	0.621
HDL-c (mmol/L)	1.44 ± 0.35	1.50 ± 0.35	1.27 ± 0.31	0.000
Alanine transaminase (U/L)	18 (14, 26)	17 (13, 24)	22 (17, 30)	0.000
Aspartate transaminase (U/L)	22 (18, 26)	22 (18, 25)	23 (19, 27)	0.008
Creatinine (μmol/L)	62 (55, 73)	62 (55, 72)	67 (56, 79)	0.005
Estimated glomerular filtration rate (ml/min/1.73 m^2)	93.5 ± 21.3	95.1 ± 20.0	88.0 ± 24.5	0.001
Uric acid (μmol/L)	335 (280, 401)	325 (274, 384)	382 (324, 448)	0.000
Adiponectin (μg/ml)	12.7 (7.7, 22.6)	13.7 (8.7, 23.5)	7.9 (4.7, 13.5)	0.000
Fasting serum insulin (μU/ml)	5.7 (3.9, 8.0)	5.2 (3.6, 7.3)	8.7 (6.7, 11.8)	0.000
White blood cell count (×10^9/L)	6.3 (5.3, 7.4)	6.2 (5.2, 7.3)	6.8 (5.9, 7.9)	0.000
TyG	4.61 (4.41, 4.82)	4.55 (4.37, 4.73)	4.92 (4.72, 5.14)	0.000
METS-IR	31.9 (27.8, 36.8)	30.2 (26.8, 33.7)	37.7 (34.2, 42.9)	0.000
HOMA-IR	1.33 (0.87, 1.97)	1.14 (0.81, 1.71)	2.51 (1.71, 3.47)	0.000

MetS, metabolic syndrome; HOMA-IR, homeostatic model assessment of insulin resistance; TyG, the product of fasting triglycerides and glucose; METS-IR, metabolic score for insulin resistance; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

increment of CAP-based groups. Similarly, the median and quartiles of the number of MetS components were also increased with increasing CAP-based groups (**Figure 2**).

There were 390 (47.3%) subjects who were diagnosed with no hepatic steatosis according to CAP, while the rest were evenly distributed among the mild, moderate, and severe hepatic steatosis groups (17.0%, 16.5%, and 19.2%, respectively) (**Table 2**). As compared with subjects with CAP < 238 dB/m, there was a notable escalating trend in age, BMI, waist and neck circumferences, waist-to-hip ratio, and blood pressure across groups with increasing CAP values. Fasting plasma glucose, serum insulin, triglyceride, ALT, AST, uric acid, white blood cell count, and indices of insulin resistance (TyG, METS-IR, and HOMA-IR) also increased, while adiponectin, HDL-c, and eGFR decreased accordingly. The differences between adjacent groups in cholesterol, LDL-c, AST, eGFR, white blood cell count, and LSM were not significant. Bonferroni *post-hoc* analysis showed that most of the clinical features in subjects with CAP < 238 dB/m were significantly different from those with CAP ≥ 238 dB/m, while differences between mild and moderate, or moderate and

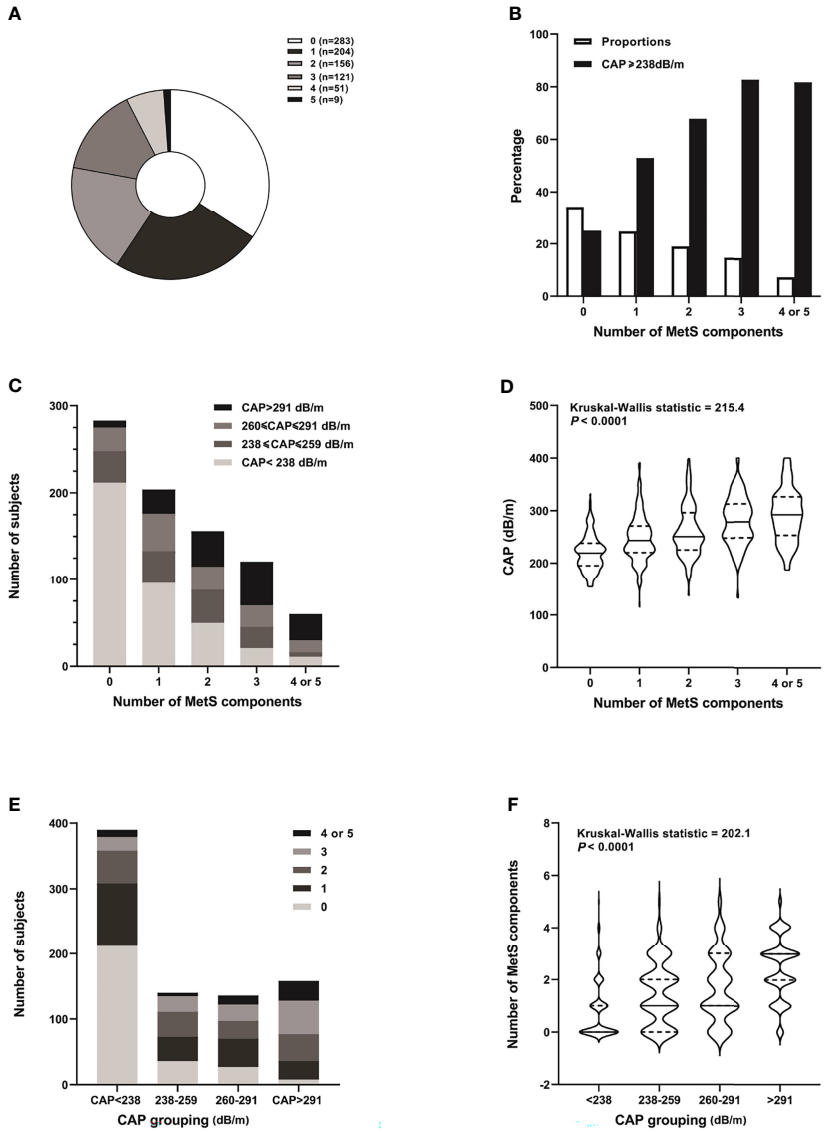

FIGURE 2 | Distribution of MetS components and CAP grades of the study subjects. **(A)** Pie chart distribution of the number of MetS components. **(B)** Proportion of subjects with different number of MetS components and percentage of those with CAP ≥ 238 db/m in each group. **(C)** Number of subjects with different grades of hepatic steatosis according to CAP in each MetS component group. **(D)** Violin chart distribution of the median and quartiles of CAP in each MetS component group. **(E)** Number of subjects with 0-5 MetS components in different hepatic steatosis groups according to CAP. **(F)** Violin chart distribution of the median and quartiles of number of MetS components in different CAP-based groups.

TABLE 2 | Comparison of clinical characteristics among subjects in different CAP categories.

Characteristics	Controlled attenuation parameter (dB/m)				*p*	*p*	*p*
	<238 (Group 1)	238–259 (Group 2)	260–291 (Group 3)	>291 (Group 4)	Group 1 vs. 2	Group 2 vs. 3	Group 3 vs. 4
n (%)	390 (47.3%)	140 (17.0%)	136 (16.5%)	158 (19.2%)	—	—	—
Age (years)	49.6 ± 15.5	54.0 ± 14.7	58.2 ± 14.7	57.4 ± 14.0	0.018	0.115	1.000
Gender, male, n (%)	74 (19.0%)	43 (30.7%)	38 (23.3%)	46 (29.1%)	0.004	0.613	0.824
Body mass index (kg/m^2)	22.3 ± 2.7	23.8 ± 2.8	24.7 ± 2.8	25.7 ± 2.8	0.000	0.037	0.009
Waist circumference (cm)	77.4 ± 7.9	82.5 ± 8.0	84.8 ± 7.5	88.6 ± 7.8	0.000	0.098	0.000
Waist-to-hip ratio	0.85 ± 0.06	0.88 ± 0.06	0.89 ± 0.05	0.91 ± 0.05	0.000	0.602	0.015
Neck circumference (cm)	33.0 (31.5, 34.8)	34.5 (33.0, 37.9)	35.0 (33.4, 37.9)	36.4 (34.5, 39.3)	0.000	1.000	0.061
Systolic pressure (mmHg)	118 (110, 130)	125 (112, 140)	122 (113, 136)	130 (120, 142)	0.000	1.000	0.024
Diastolic pressure (mmHg)	70 (65, 78)	75 (68, 80)	75 (70, 80)	78 (70, 85)	0.002	1.000	0.047
Controlled attenuation parameter (dB/m)	214 (195, 225)	248 (244, 253)	272 (266, 281)	318 (302, 338)	0.000	0.000	0.000
Liver stiffness measurement (kPa)	3.9 (3.4, 4.5)	4.0 (3.5, 4.7)	4.2 (3.5, 5.0)	4.4 (3.9, 5.3)	0.746	1.000	0.112
Fasting glucose (mmol/L)	5.0 (4.7, 5.5)	5.2 (4.8, 5.8)	5.4 (5.0, 5.9)	5.8 (5.2, 6.8)	0.136	0.191	0.021
Cholesterol (mmol/L)	5.00 (4.50, 5.70)	5.10 (4.50, 6.10)	5.30 (4.60, 6.10)	5.45 (4.65, 6.10)	0.319	1.000	1.000
Triglyceride (mmol/L)	0.96 (0.72, 1.29)	1.21 (0.94, 1.75)	1.48 (0.98, 1.92)	1.69 (1.14, 2.52)	0.000	0.471	0.026
LDL-c (mmol/L)	3.30 ± 0.76	3.47 ± 0.87	3.45 ± 0.75	3.51 ± 0.88	0.355	1.000	1.000
HDL-c (mmol/L)	1.56 ± 0.37	1.41 ± 0.34	1.41 ± 0.33	1.27 ± 0.27	0.001	1.000	0.006
Alanine transaminase (U/L)	16 (12, 22)	18 (13, 23)	21 (16, 29)	24 (18, 34)	0.181	0.006	0.263
Aspartate transaminase (U/L)	20 (18, 24)	22 (18, 26)	23 (20, 27)	24 (20, 28)	0.927	0.243	1.000
Creatinine (μmol/L)	60 (54, 71)	64 (56, 77)	67 (58, 78)	62 (54, 71)	0.029	1.000	0.152
Estimated glomerular filtration rate (ml/min/1.73 m^2)	96.2 ± 21.5	91.4 ± 19.1	87.5 ± 19.3	94.0 ± 23.1	0.146	0.765	0.056
Uric acid (μmol/L)	303 (261, 366)	341 (296, 399)	363 (308, 422)	376 (321, 446)	0.001	0.854	1.000
Adiponectin (μg/ml)	16.5 (10.7, 26.2)	11.3 (7.7, 19.4)	9.4 (5.8, 16.7)	7.8 (4.7, 13.7)	0.004	0.792	0.531
Fasting serum insulin (μU/ml)	4.45 (3.24, 6.21)	6.09 (3.95, 7.24)	6.89 (4.88, 8.88)	8.47 (6.32, 11.1)	0.008	0.082	0.021
White blood cell count (×10^9/L)	6.0 (5.1, 7.0)	6.3 (5.6, 7.5)	6.4 (5.5, 7.6)	6.9 (6.0, 7.9)	0.063	1.000	0.146
TyG	4.48 (4.32, 4.67)	4.68 (4.43, 4.82)	4.73 (4.52, 4.93)	4.83 (4.65, 5.04)	0.000	0.224	0.010
METS-IR	28.8 (25.8, 32.4)	32.3 (29.3, 36.8)	34.1 (30.1, 38.2)	36.9 (32.9, 41.1)	0.000	0.428	0.002
HOMA-IR	1.00 (0.69, 1.46)	1.34 (0.89, 1.88)	1.72 (1.13, 2.20)	2.29 (1.56, 3.17)	0.025	0.025	0.009

CAP, controlled attenuation parameter; LSM, liver stiffness measurement; eGFR, estimated glomerular filtration rate; WBC, whit blood cell; HOMA-IR, homeostatic model assessment for insulin resistance; TyG, the product of fasting triglycerides and glucose; METS-IR, metabolic score for insulin resistance.

severe hepatic steatosis were less prominent (**Supplementary Table 1**). Among the 390 subjects with CAP < 238 dB/m, 32 (8.2%) of them met the criteria for MetS, while more than half of the subjects (51.3%) with CAP > 291 dB/m were categorized as having 3 or more MetS components. Taking the group with no hepatic steatosis (CAP < 238 dB/m) as control, subjects with mild, moderate, and severe hepatic steatosis had increased odds of having MetS by 2.92-, 4.50-, and 11.8-fold, respectively (p = 0.000). The odds were still significant after adjusting for multiple confounders (p = 0.020) (**Table 3**).

The subjects were divided into 4 groups based on whether they were overweight or not using BMI ≥ 24 kg/m^2 as the cutoff point, and whether they were with (CAP ≥ 238 dB/m) or without (CAP < 238 dB/m) hepatic steatosis (**Table 4**). In the same BMI category of either normal weight or overweight, subjects with CAP ≥ 238 dB/m had significantly higher BMI, waist and neck circumferences, waist-to-hip ratio, blood pressure, fasting glucose and lipid profiles, liver enzymes, white blood cell count, and insulin resistance indices, but lower adiponectin and HDL-c levels as compared with those of their counterparts. Overweight subjects with CAP ≥ 238 dB/m had the worst metabolic profiles as compared with those of the other 3 groups. Forty-six percent of the subjects in this group met the criteria for MetS, nearly 10 times that of subjects in the normal-weight and CAP < 238 dB/m group (4.9%). Interestingly, other than somatotype indices (BMI, waist circumference, and neck circumference), metabolic profiles in subjects who were normal weight but with CAP ≥ 238 dB/m were comparable with those of subjects who were overweight but with CAP < 238 dB/m, including indices of insulin resistance, such as TyG and HOMA-IR. The difference in METS-IR between these 2 groups might be due to different BMIs used in the formula. The proportions of MetS in both groups were also similar (16.8% vs. 19.8%, p = 0.551) (**Supplementary Table 2**). Taking the normal-weight and CAP < 238 dB/m group as control, subjects who were normal weight but with CAP ≥ 238 dB/m had 3.88 times higher odds of having MetS, while the odds in subjects who were overweight with CAP < 238 dB/m was 4.75 times higher. The odds ratio increased to 16.40 in overweight subjects with CAP ≥ 238 dB/m. After age, BMI, waist, and multiple metabolic indices were adjusted, the odds ratios in normal weight with CAP ≥ 238 dB/m and overweight with CAP < 238 dB/m were similar to those of the control. However, overweight subjects with CAP ≥ 238 dB/m still had 3.24 times higher odds of having MetS. (p = 0.018) (**Table 5**).

There were 283 subjects who were with no MetS components in our study. Clinical characteristics were compared in these subjects

TABLE 3 | Proportion and odds ratio of having MetS according to CAP-based categories.

CAP categories	n (%)	Model 1* OR (95% CI)	Model 2** OR (95% CI)	Model 3*** OR (95% CI)
<238 dB/m	32/390 (8.2%)	1	1	1
238–259 dB/m	29/140 (20.7%)	2.92 (1.69, 5.05)	1.96 (1.05, 3.67)	3.51 (1.17, 10.6)
260–291 dB/m	39/136 (28.7%)	4.50 (2.68, 7.6)	2.00 (1.10, 3.64)	3.32 (1.17, 9.46)
>291 dB/m	81/158 (51.3%)	11.8 (7.30, 19.0)	4.81 (2.75, 8.41)	5.12 (1.83, 14.3)
p for trend	0.000	0.000	0.000	0.020

MetS, metabolic syndrome; CAP, controlled attenuation parameter; BMI, body mass index; UA, uric acid; WBC, white blood cell; TyG, the product of fasting triglycerides and glucose.
**Model 1: unadjusted.*
***Model 2: adjusted for age, BMI, and waist.*
****Model 3: adjusted for age, BMI, adiponectin, ADP, UA, WBC, and TyG.*

TABLE 4 | Clinical characteristics in normal-weight versus overweight subjects with or without hepatic steatosis.

Characteristics	Normal-weight and CAP < 238 dB/m (Group 1)	Normal-weight and CAP ≥ 238 dB/m (Group 2)	Overweight and CAP < 238 dB/m (Group 3)	Overweight and CAP ≥ 238 dB/m (Group 4)	*p* Group 1 vs. 2	*p* Group 2 vs. 3	*p* Group 3 vs. 4
n (%)	304 (36.9%)	173 (21.0%)	86 (10.0%)	261 (31.7%)	—	—	—
Age (years)	48.1 ± 15.1	56.9 ± 14.4	54.9 ± 15.6	56.4 ± 14.7	0.000	1.000	1.000
Gender, male, n (%)	47 (15.5%)	32 (18.5%)	27 (31.4%)	95 (36.4%)	0.391	0.020	0.399
Body mass index (kg/m^2)	21.3 ± 1.7	22.1 ± 1.5	25.9 ± 2.5	26.6 ± 2.1	0.000	0.000	0.024
Waist circumference (cm)	75.1 ± 6.4	79.1 ± 5.7	85.9 ± 6.7	89.7 ± 6.7	0.000	0.000	0.000
Waist-to-hip ratio	0.83 ± 0.05	0.87 ± 0.05	0.88 ± 0.05	0.91 ± 0.06	0.000	0.391	0.001
Neck circumference (cm)	32.5 (31.2, 34.0)	33.5 (32.0, 34.8)	35.5 (34.0, 38.0)	37.0 (34.9, 40.0)	0.008	0.000	0.107
Systolic pressure (mmHg)	115 (109, 126)	122 (110, 139)	125 (118, 135)	128 (118, 140)	0.000	1.000	0.951
Diastolic pressure (mmHg)	70 (64, 77)	72 (67, 80)	72 (66, 80)	76 (70, 83)	0.005	1.000	0.011
Controlled attenuation parameter (dB/m)	208 (192, 224)	261 (248, 285)	221 (206, 229)	288 (261, 315)	0.000	0.000	0.000
Liver stiffness measurement (kPa)	3.9 (3.4, 4.5)	4.0 (3.4, 4.6)	3.8 (3.4, 4.5)	4.3 (3.6, 5.2)	1.000	0.771	0.000
Fasting glucose (mmol/L)	5.0 (4.7, 5.5)	5.4 (4.9, 5.9)	5.1 (4.8, 5.8)	5.6 (5.1, 6.4)	0.000	0.948	0.002
Cholesterol (mmol/L)	5.00 (4.40, 5.70)	5.40 (4.70, 6.10)	5.10 (4.70, 5.60)	5.30 (4.50, 6.05)	0.005	0.282	1.000
Triglyceride (mmol/L)	0.91 (0.68, 1.14)	1.27 (0.96, 1.82)	1.21 (0.97, 1.51)	1.55 (1.08, 2.18)	0.000	1.000	0.005
HDL-c (mmol/L)	1.60 ± 0.36	1.47 ± 0.34	1.43 ± 0.35	1.28 ± 0.27	0.003	1.000	0.008
LDL-c (mmol/L)	3.31 ± 0.80	3.47 ± 0.74	3.27 ± 0.62	3.49 ± 0.90	0.383	0.517	0.282
Alanine transaminase (U/L)	15 (12, 20)	19 (16, 27)	19 (13, 26)	21 (16, 26)	0.000	1.000	0.066
Aspartate transaminase (U/L)	20 (18, 24)	23 (20, 27)	22 (19, 26)	23 (19, 26)	0.000	0.340	1.000
Creatinine (μmol/L)	59 (54, 68)	62 (55, 70)	65 (55, 76)	67 (57, 81)	0.882	0.914	1.000
Estimated glomerular filtration rate (ml/min/1.73 m^2)	97.5 ± 21.1	92.6 ± 20.0	91.4 ± 22.5	90.2 ± 21.3	0.097	1.000	1.000
Uric acid (μmol/L)	298 (254, 354)	339 (288, 386)	337 (288, 400)	376 (328, 445)	0.000	1.000	0.015
WBC (×10^9/L)	5.8 (5.0, 6.9)	6.4 (5.6, 7.5)	6.4 (5.7, 7.6)	6.7 (5.8, 7.9)	0.001	1.000	1.000
Adiponectin (μg/ml)	18.7 (12.0, 27.1)	13.0 (7.9, 24.2)	10.8 (6.2, 16.0)	8.1 (5.7, 12.6)	0.006	0.584	0.624
Fasting insulin (μU/ml)	4.1 (3.0, 5.7)	6.0 (4.2, 7.8)	6.4 (4.5, 8.7)	7.8 (5.8, 10.0)	0.000	1.000	0.104
TyG	4.44 (4.30, 4.61)	4.67 (4.48, 4.89)	4.63 (4.46, 4.72)	4.77 (4.60, 4.99)	0.000	0.716	0.000
METS-IR	27.4 (25.0, 29.8)	30.2 (27.5, 32.8)	34.1 (31.5, 36.7)	38.0 (34.7, 41.4)	0.000	0.000	0.007
HOMA-IR	0.95 (0.66, 1.28)	1.37 (0.95, 2.02)	1.39 (0.98, 2.07)	1.90 (1.31, 2.80)	0.000	1.000	0.047
Metabolic syndrome, n (%)	15 (4.9%)	29 (16.8%)	17 (19.8%)	120 (46.0%)	0.000	0.551	0.000

CAP, controlled attenuation parameter; LSM, liver stiffness measurement; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; WBC, white blood cell; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostatic model assessment for insulin resistance; TyG the product of fasting triglycerides and glucose; METS-IR, metabolic score for insulin resistance.

based on whether they were with or without CAP ≥ 238 dB/m. Both groups were similar in gender composition, blood pressure, drinking and smoking status, LSM, cholesterol, LDL-c, HDL-c, liver enzymes, and fasting plasma glucose. Subjects with CAP ≥ 238 dB/m were approximately 4 years older. Even though they were with normal body build, adipose tissue distribution, and metabolic profiles, subjects with CAP ≥ 238 dB/m had higher BMI, waist and neck circumferences, waist-to-hip ratio, fasting serum insulin, uric acid, triglyceride, white blood cell count, and insulin resistance indices, whereas their adiponectin and eGFR were significantly lower (**Table 6**). Binary logistic regression analysis was used to calculate the odds of hepatic steatosis as determined by CAP ≥ 238 dB/m in these healthy subjects. After all the correlated factors identified by variance analysis were adjusted, including age, gender, BMI, neck circumference, waist-to-hip ratio, triglyceride, eGFR, uric acid, white blood cell count, adiponectin, and TyG, only waist circumference and HOMA-IR appeared to contribute to the increased odds of hepatic steatosis. For every 1-cm increase in

TABLE 5 | Odds ratio of having MetS in normal-weight versus overweight subjects with or without hepatic steatosis.

	Model 1* OR (95% CI)	Model 2** OR (95% CI)	Model 3*** OR (95% CI)	Model 4$ OR (95% CI)
Normal weight and CAP < 238 dB/m	1	1	1	1
Normal weight and CAP ≥ 238 dB/m	3.88 (2.02, 7.47)	2.18 (1.08, 4.42)	1.01 (0.27, 3.75)	1.01 (0.27, 3.77)
Overweight and CAP < 238 dB/m	4.75 (2.26, 9.97)	1.21 (0.51, 2.90)	0.42 (0.06, 2.72)	0.56 (0.09, 3.70)
Overweight and CAP ≥ 238 dB/m	16.40 (9.24, 29.09)	3.86 (1.93, 7.74)	4.80 (1.57, 14.67)	3.24 (1.03, 10.2)
p for trend	0.000	0.000	0.000	0.018

MetS, metabolic syndrome; CAP, controlled attenuation parameter; BMI, body mass index; UA, uric acid; TyG, the product of fasting triglycerides and glucose; LSM, liver stiffness measurement; ALT, alanine transaminase; AST, aspartate transaminase.

**Model 1 unadjusted.*

***Model 2: adjusted for age, BMI, and waist.*

****Model 3: adjusted for age, BMI, waist, adiponectin, UA, WBC, ALT, and TyG.*

$Model 4: adjusted for gender, BMI, waist, waist-to-hip ratio, LSM, fasting insulin, creatinine, cholesterol, LDL-c, AST, fasting plasma glucose, and TyG.

TABLE 6 | Comparison of clinical characteristics in subjects with no MetS components based on whether they were with or without hepatic steatosis.

Characteristics	Controlled attenuation parameter (dB/m)		*p*
	<238	≥238	
n	212	71	—
Age (years)	41.2 ± 11.3	45.4 ± 13.2	0.011
Gender, male, n (%)	27 (12.7%)	15 (21.1%)	0.085
Body mass index (kg/m^2)	21.5 ± 2.1	22.7 ± 2.2	0.000
Waist circumference (cm)	74.4 ± 6.0	78.3 ± 5.6	0.000
Waist-to-hip ratio	0.82 ± 0.05	0.85 ± 0.05	0.000
Neck circumference (cm)	32.5 (31.0, 33.8)	33.5 (32.0, 35.5)	0.001
Systolic pressure (mmHg)	110 (106, 120)	110 (107, 120)	0.484
Diastolic pressure (mmHg)	68 (62, 72)	69 (65, 72)	0.558
Drinking status, n (%)	39 (18.4%)	11 (15.5%)	0.720
Smoking status, n (%)	3 (1.4%)	1 (1.4%)	1.000
Dyslipidemia, n (%)	23 (10.8%)	14 (19.7%)	0.055
Controlled attenuation parameter (dB/m)	206 (189, 224)	259 (246, 280)	0.000
Liver stiffness measurement (kPa)	3.8 (3.3, 4.4)	3.7 (3.3, 4.4)	0.197
Fasting plasma glucose (mmol/L)	4.9 (4.6, 5.1)	4.9 (4.7, 5.3)	0.071
Cholesterol (mmol/L)	4.90 (4.40, 5.40)	5.00 (4.60, 5.60)	0.142
Triglyceride (mmol/L)	0.82 (0.65, 1.04)	0.94 (0.75, 1.22)	0.002
LDL-c (mmol/L)	3.26 ± 0.67	3.43 ± 0.64	0.122
HDL-c (mmol/L)	1.61 ± 0.29	1.56 ± 0.35	0.280
Alanine transaminase (U/L)	15 (12, 20)	17 (13, 22)	0.077
Aspartate transaminase (U/L)	20 (18, 23)	21 (18, 25)	0.328
Creatinine (μmol/L)	58 (53, 67)	60 (54, 72)	0.069
Estimated glomerular filtration rate (ml/min/1.73 m^2)	102.3 ± 19.0	96.7 ± 16.5	0.031
Uric acid (μmol/L)	295 (251, 346)	334 (273, 381)	0.005
Adiponectin (μg/ml)	20.6 (12.2, 29.7)	10.7 (6.8, 20.3)	0.000
Fasting serum insulin (μU/ml)	4.14 (2.96, 5.74)	5.32 (3.72, 6.61)	0.018
White blood cell count (×10^9/L)	5.8 (5.0, 6.7)	6.3 (4.8, 7.3)	0.041
TyG	4.38 (4.25, 4.51)	4.43 (4.31, 4.59)	0.001
METS-IR	27.1 (24.8, 29.4)	28.9 (25.5, 31.4)	0.011
HOMA-IR	0.87 (0.63, 1.26)	1.09 (0.79, 1.47)	0.023

MetS, metabolic syndrome; HOMA-IR, homeostatic model assessment of insulin resistance; TyG, the product of fasting triglycerides and glucose; METS-IR, metabolic score for insulin resistance; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.

waist circumference, the odds of having hepatic steatosis increased by 11%. And the odds increased by 1.39 times for every 1.0 increment in HOMA-IR (**Table 7**).

DISCUSSION

In this study, clinical data of 824 healthcare workers were analyzed for detection of hepatic steatosis using FibroScan and its association with metabolic derangement. We showed that CAP value increased in parallel with the number of MetS components, while the odds of having MetS increased significantly with increasing CAP grades. In the same category of either normal weight or overweight, metabolic profiles and insulin resistance differed significantly between subjects with and without hepatic steatosis, while clinical features and presence of MetS were similar between normal-weight subjects with CAP ≥ 238 dB/m and overweight subjects with CAP < 238 dB/m. Even

TABLE 7 | Predictive factors for increasing odds of hepatic steatosis in subjects with no MetS components.

	Wald's	Odds*	95% CI	*p*
Waist (cm)	10.5	1.11	(1.04, 1.18)	0.001
HOMA-IR	5.85	2.39	(1.18, 4.83)	0.016
Constant	15.82			0.000

*MetS, metabolic syndrome; HOMA-IR, homeostatic model assessment of insulin resistance; BMI, body mass index; TyG, the product of fasting triglycerides and glucose. *Adjusted for age, gender, BMI, neck, waist-to-hip ratio, triglyceride, glomerular filtration rate, uric acid, white blood cell count, adiponectin, and TyG.*

in subjects with no MetS components, those with CAP ≥ 238 dB/m had higher BMI, waist and neck circumferences, waist-to-hip ratio, fasting insulin, uric acid, triglyceride, white blood cell count, and indices of insulin resistance, whereas their adiponectin and eGFR were lower. Waist circumference and HOMA-IR were predictive factors for increasing odds of hepatic steatosis.

Non-Alcoholic Fatty Liver Disease Related to Metabolic Derangement

There is growing evidence showing that NAFLD is a multisystem disease, affecting multiple extra-hepatic organs and regulatory pathways (16, 17). Meta-analysis has shown that NAFLD doubled the incidence of type 2 diabetes as well as chronic kidney disease (18, 19). Several large prospective studies also showed that NAFLD increased the risk of fatal and non-fatal CVD events, independent of established CVD risk factors (20–22). NAFLD might be associated with such complications either as a consequence of shared cardio-metabolic risk or as an example of ectopic fat accumulation leading to insulin resistance. In fact, NAFLD is not only a simple marker of cardiac/vascular and kidney damage but may also directly contribute to the development of these complications through hepatic production of lipids, atherogenic lipoproteins, pro-inflammatory and pro-oxidant molecules, induction of hepatic/peripheral insulin resistance, and glycemia dysregulation (16, 23–25). Therefore, the significance of early detection of hepatic steatosis using reliable, non-invasive, and inexpensive techniques is essential to screen for the accompanying metabolic derangement and warrant the initiation of early intervention.

In our study, the subjects included are assumed to be at lower risk of having MetS as compared with the general population, because they have more knowledge of the healthcare consequences and disease prevention. They are engaged in more health behavioral counseling including physical activity, fruit and vegetable consumption, and ideal body weight maintenance. Indeed, the proportions of drinking (16.9%) and smoking (5.9%) in our study were relatively low, as were the percentages of obesity (7.9%), MetS (22%), and significant hepatic fibrosis (2.2%). We showed that there were clear-cut differences in metabolic derangement between subjects with and without hepatic steatosis by using CAP ≥ 238 dB/m as the cutoff value. The odds of having MetS significantly elevated with increased severity of hepatic steatosis. The metabolic profiles and odds of having MetS in normal-weight subjects with CAP ≥ 238 dB/m were very similar to those of overweight subjects with CAP < 238 dB/m, indicating that the metabolic impact of fat accumulation in the liver in a non-overweight subject is comparable with that of general fat accumulation other than the liver in an overweight subject. Even in apparently healthy subjects with no MetS components, those with CAP ≥ 238 dB/m had more anthropometric and hematological indices associated with MetS and insulin resistance. Thus, CAP is a convenient and sensitive non-invasive surrogate marker for early detection of metabolic derangement in a population with lower risk.

Controlled Attenuation Parameter Associated With Metabolic Syndrome Components

Large and prospective studies have demonstrated the accuracy of CAP in identifying hepatic steatosis (7, 26, 27). The usefulness of CAP in exploring the correlation between NAFLD and MetS components has also been validated previously. Mikolasevic et al. (10) showed that in 648 patients with one or more MetS components, in whom 67.3% met the criteria for MetS, the prevalence of NAFLD (CAP ≥ 238 dB/m, 88.3%) and advanced liver fibrosis (LSM ≥ 7.0 kPa, 16.5%) was high, and CAP and LSM correlated with the number of MetS components. Another study involved 1,983 community-based participants who underwent self-paid health examination, in which the proportion of MetS was relatively low (13.6%). It showed that CAP had a moderate prediction performance for MetS. The AUROC was 0.79 for CAP alone, and it rose to 0.85 when combined with gender, age, and BMI (8). Huh et al. (9) investigated the interplay of obesity and metabolic health on hepatic steatosis and fibrosis in 2,198 asymptomatic subjects who underwent health checkups using FibroScan. They showed that the presence of either metabolically unhealthy status or obesity was closely associated with the risk of hepatic steatosis, while significant liver fibrosis was more significantly associated with obesity rather than with unhealthy metabolic status. Our results agree with the above studies in that CAP increased in parallel with the number of MetS components. Risks of having MetS increased with the severity of hepatic steatosis as graded by CAP. Because the proportion of significant fibrosis was quite low (2.2%) in our study, we did not confirm the effect of LSM in association with obesity, metabolic disturbance, or insulin resistance. The increment in LSM appeared to be an accompanying change with CAP, and the differences in LSM were only significant in the CAP > 291 dB/m group or overweight subjects with CAP ≥ 238 dB/m as compared with other groups in our study.

There are two significant findings in our study. Firstly, we showed the distinct differences between subjects with and without hepatic steatosis using CAP ≥ 238 dB/m as a cutoff point in the same category of either normal weight or overweight. Thus, in subjects who are with normal body habitus, those with increased CAP value tend to have worse metabolic profiles and insulin sensitivity, and the biochemical indices and proportion of patients with MetS are similar to those who are overweight and with no hepatic steatosis. Subjects who are both overweight and with hepatic steatosis have the worst metabolic profiles and the greatest risk of having MetS. Therefore,

CAP ≥ 238 dB/m could serve as a sensitive surrogate marker for identifying metabolic derangement in both normal-weight and overweight populations. Subjects who are normal weight with CAP ≥ 238 dB/m might represent those who have normal total fat mass but impaired adipose tissue expandability and function. In contrast, subjects who are overweight but with CAP < 238 dB/m may represent those who have better adipose tissue function, less ectopic fat storage, and better insulin sensitivity, though with increased total fat mass. A consensus has been reached on the recognition of sub-phenotypes of body weight in association with metabolic health status, namely, "normal-weight metabolically healthy", "normal-weight metabolically unhealthy", "metabolically healthy obesity", and "metabolically unhealthy obesity", though the diagnostic criteria are not consistent, and the cardiovascular and metabolic consequences of the sub-phenotypes are disputable (28–32). Because CAP correlates well with MetS components, higher values of CAP may indicate increased metabolic derangement and insulin resistance. The role of CAP in identifying normal-weight subjects with hepatic steatosis is especially important for the Asian population since the recent guideline has highlighted the fact that Asian people are particularly susceptible to lean NAFLD, partly because of body composition differences in fat and muscle, as well as genetic susceptibility (33). More importantly, evidence showed that non-obese patients achieved equivalent remission of NAFLD by a modest (3%–5%) weight reduction through lifestyle intervention (34). Thus, prospective studies are needed to further validate the usefulness of CAP as a surrogate marker for the transition of metabolic health status over time and for the prediction of cardiovascular outcomes.

Additionally, we showed that in subjects with no MetS components, the differences between those with CAP < 238 dB/m and CAP ≥ 238 dB/m were apparent. Subjects with increased CAP value tend to be older and have higher BMI, waist and neck circumferences, waist-to-hip ratio, triglyceride, uric acid, fasting insulin, white blood cell count, and insulin resistance indices, whereas lower adiponectin and eGFR. These are routine indicators associated with metabolic derangement, insulin resistance, and chronic inflammation. Our findings may suggest that in metabolically healthy subjects with increased CAP, though within normal range, subtle adverse changes in body size, visceral adiposity, lipid profile, uric acid, inflammatory index, and insulin resistance have already clustered in the direction of MetS long before its occurrence. Because this is a cross-sectional study, we could not rule out the possibility that the differences between the two groups were due to an approximately 4 years' gap in age by chance, which resulted in aging-associated metabolic derangement and insulin resistance. However, in the subsequent binary logistic regression analysis, age was no longer a risk factor after adjusting for multiple correlated confounders. The odds of hepatic steatosis increase with elevated waist circumference and HOMA-IR, which are indicators of visceral adipose accumulation and hepatic insulin resistance. However, long-term follow-up of these subjects for their transition of metabolic health status is still needed to validate this point of view.

Limitations

Our study has several limitations. Firstly, we do not have our own liver biopsy data to support the cutoff point of CAP for diagnosing hepatic steatosis. As a matter of fact, age-, gender-, and ethnic-specific CAP cutoff values based on liver biopsy in the general population without known hepatic diseases are lacking. We chose the one most recognized in the literature. Nevertheless, we showed that CAP ≥ 238 dB/m is sensitive enough to distinguish between different metabolic statuses. Secondly, the proportion of male subjects is small; thus, we failed to confirm the gender discrepancy in association with MetS as described previously (35, 36). Likewise, the proportion of significant fibrosis is not enough to validate its contribution to assessing metabolic risk. Therefore, we may need a larger sample size, with a more sensible distribution across gender and liver stiffness in future studies. Finally, since this is a cross-sectional study, we cannot validate the causation between hepatic steatosis and MetS. However, the metabolic derangement between subjects with or without hepatic steatosis was evident even in those with no MetS components or in subjects with normal weight. Therefore, we believe that CAP ≥ 238 dB/m did make a significant impact on a subject, even though he/she may seem apparently healthy, with no known risk factors or metabolic derangement. Rather than label this subject with a disease name, it may be more appropriate to call for attention to the underlying or impending metabolic derangement and take action (through lifestyle modification) to avoid future adverse outcomes. We need a prospective and longitudinal study to verify our findings.

Summary

In summary, we showed that CAP is a convenient and sensitive non-invasive surrogate marker for early detection of metabolic derangement in a population with lower risk. The major benefit of detecting hepatic steatosis in apparently healthy individuals is to identify the underlying metabolic derangement, therefore allowing the initiation of early intervention through lifestyle modification. This is of particular importance for the Asian population with a substantial proportion of lean NAFLD. Prospective studies are needed to further evaluate the usefulness of CAP as a surrogate marker for the transition of metabolic health status and for the prediction of cardiovascular outcomes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the First Affiliated Hospital of Sun Yat-sen University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YL conceptualized the study, supported the execution with her fund, and revised and proofread the manuscript. ZH designed the study and wrote the manuscript with input from all the authors. ZH and KN analyzed the data. KN, HC, and WD executed the study and collected data from all the participants. All authors checked the final manuscript before submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.739875/full#supplementary-material

REFERENCES

1. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global Burden of NAFLD and NASH: Trends, Predictions, Risk Factors and Prevention. *Nat Rev Gastroenterol Hepatol* (2018) 15:11–20. doi: 10.1038/nrgastro.2017.109
2. Eslam M, Sanyal AJ, George J. International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* (2020) 158:1999–2014.e1. doi: 10.1053/j.gastro.2019.s11.312
3. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A New Definition for Metabolic Dysfunction-Associated Fatty Liver Disease: An International Expert Consensus Statement. *J Hepatol* (2020) 73:202–9. doi: 10.1016/j.jhep.2020.03.039
4. Sasso M, Beaugrand M, de Ledinghen V, Douvin C, Marcellin P, Poupon R, et al. Controlled Attenuation Parameter (CAP): A Novel VCTE™ Guided Ultrasonic Attenuation Measurement for the Evaluation of Hepatic Steatosis: Preliminary Study and Validation in a Cohort of Patients With Chronic Liver Disease From Various Causes. *Ultrasound Med Biol* (2010) 36:1825–35. doi: 10.1016/j.ultrasmedbio.2010.07.005
5. Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, et al. Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* (2019) 156:1717–30. doi: 10.1053/j.gastro.2019.01.042
6. Kim JK, Lee KS, Choi JR, Chung HJ, Jung DH, Lee KA, et al. Usefulness of the Controlled Attenuation Parameter for Detecting Liver Steatosis in Health Checkup Examinees. *Gut Liver* (2015) 9:405–10. doi: 10.5009/gnl14202
7. de Lédinghen V, Vergniol J, Capdepont M, Chermak F, Hiriart JB, Cassinotto C, et al. Controlled Attenuation Parameter (CAP) for the Diagnosis of Steatosis: A Prospective Study of 5323 Examinations. *J Hepatol* (2014) 60:1026–31. doi: 10.1016/j.jhep.2013.12.018
8. Lin YJ, Lin CH, Wang ST, Lin SY, Chang SS. Noninvasive and Convenient Screening of Metabolic Syndrome Using the Controlled Attenuation Parameter Technology: An Evaluation Based on Self-Paid Health Examination Participants. *J Clin Med* (2019) 8:1775. doi: 10.3390/jcm8111775
9. Huh JH, Kim KJ, Kim SU, Han SH, Han KH, Cha BS, et al. Obesity Is More Closely Related With Hepatic Steatosis and Fibrosis Measured by Transient Elastography Than Metabolic Health Status. *Metabolism* (2017) 66:23–31. doi: 10.1016/j.metabol.2016.10.003
10. Mikolasevic I, Milic S, Orlic L, Stimac D, Franjic N, Targher G. Factors Associated With Significant Liver Steatosis and Fibrosis as Assessed by Transient Elastography in Patients With One or More Components of the Metabolic Syndrome. *J Diabetes Complicat* (2016) 30:1347–53. doi: 10.1016/j.jdiacomp.2016.05.014
11. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance From the American Association for the Study of Liver Diseases. *Hepatology* (2018) 67:328–57. doi: 10.1002/hep.29367
12. Guerrero-Romero F, Simental-Mendía LE, González-Ortiz M, Martínez-Abundis E, Ramos-Zavala MG, Hernández-González SO, et al. The Product of Triglycerides and Glucose, a Simple Measure of Insulin Sensitivity. Comparison With the Euglycemic-Hyperinsulinemic Clamp. *J Clin Endocrinol Metab* (2010) 95:3347–51. doi: 10.1210/jc.2010-0288
13. Bello-Chavolla OY, Almeda-Valdes P, Gomez-Velasco D, Viveros-Ruiz T, Cruz-Bautista I, Romo-Romo A, et al. METS-IR, a Novel Score to Evaluate Insulin Sensitivity, is Predictive of Visceral Adiposity and Incident Type 2 Diabetes. *Eur J Endocrinol* (2018) 178:533–44. doi: 10.1530/EJE-17-0883
14. Wong VW, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, et al. Diagnosis of Fibrosis and Cirrhosis Using Liver Stiffness Measurement in Nonalcoholic Fatty Liver Disease. *Hepatology* (2010) 51:454–62. doi: 10.1002/hep.23312
15. Chinese Diabetes Society. Guidelines for the Prevention and Control of Type 2 Diabetes in China (2017 Edition). *Chin J Diabetes Mellitus* (2018) 10:4–67. doi: 10.3760/cma.j.issn.1674-5809.2018.01.003
16. Byrne CD, Targher G. NAFLD: A Multisystem Disease. *J Hepatol* (2015) 62:S47–64. doi: 10.1016/j.jhep.2014.12.012
17. Armstrong MJ, Adams LA, Canbay A, Syn WK. Extrahepatic Complications of Nonalcoholic Fatty Liver Disease. *Hepatology* (2014) 59:1174–97. doi: 10.1002/hep.26717
18. Musso G, Gambino R, Cassader M, Pagano G. Meta-Analysis: Natural History of non-Alcoholic Fatty Liver Disease (NAFLD) and Diagnostic Accuracy of non-Invasive Tests for Liver Disease Severity. *Ann Med* (2011) 43:617–49. doi: 10.3109/07853890.2010.518623
19. Musso G, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Hamaguchi M, et al. Association of non-Alcoholic Fatty Liver Disease With Chronic Kidney Disease: A Systematic Review and Meta-Analysis. *PloS Med* (2014) 11:e1001680. doi: 10.1371/journal.pmed.1001680
20. Stepanova M, Younossi ZM. Independent Association Between Nonalcoholic Fatty Liver Disease and Cardiovascular Disease in the US Population. *Clin Gastroenterol Hepatol* (2012) 10:646–50. doi: 10.1016/j.cgh.2011.12.039
21. Zhou YJ, Li YY, Nie YQ, Huang CM, Cao CY. Natural Course of Nonalcoholic Fatty Liver Disease in Southern China: A Prospective Cohort Study. *J Dig Dis* (2012) 13:153–60. doi: 10.1111/j.1751-2980.2011.00571.x
22. Lazo M, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, et al. Non-Alcoholic Fatty Liver Disease and Mortality Among US Adults: Prospective Cohort Study. *BMJ* (2011) 343:d6891. doi: 10.1136/bmj.d6891
23. Perry RJ, Samuel VT, Petersen KF, Shulman GI. The Role of Hepatic Lipids in Hepatic Insulin Resistance and Type 2 Diabetes. *Nature* (2014) 510:84–91. doi: 10.1038/nature13478
24. Targher G, Byrne CD. Clinical Review: Nonalcoholic Fatty Liver Disease: A Novel Cardiometabolic Risk Factor for Type 2 Diabetes and its Complications. *J Clin Endocrinol Metab* (2013) 98:483–95. doi: 10.1210/jc.2012-3093
25. Buzzetti E, Pinzani M, Tsochatzis EA. The Multiple-Hit Pathogenesis of Non-Alcoholic Fatty Liver Disease (NAFLD). *Metabolism* (2016) 65:1038–48. doi: 10.1016/j.metabol.2015.12.012

26. Kwok R, Choi KC, Wong GL, Zhang Y, Chan HL, Luk AO, et al. Screening Diabetic Patients for Non-Alcoholic Fatty Liver Disease With Controlled Attenuation Parameter and Liver Stiffness Measurements: A Prospective Cohort Study. *Gut* (2016) 65:1359–68. doi: 10.1136/gutjnl-2015-309265
27. Vuppalanchi R, Siddiqui MS, Van Natta ML, Hallinan E, Brandman D, Kowdley K, et al. NASH Clinical Research Network. Performance Characteristics of Vibration-Controlled Transient Elastography for Evaluation of Nonalcoholic Fatty Liver Disease. *Hepatology* (2018) 67:134–44. doi: 10.1002/hep.29489
28. Neeland IJ, Poirier P, Després JP. Cardiovascular and Metabolic Heterogeneity of Obesity: Clinical Challenges and Implications for Management. *Circulation* (2018) 137:1391–406. doi: 10.1161/CIRCULATIONAHA.117.029617
29. Goossens GH. The Metabolic Phenotype in Obesity: Fat Mass, Body Fat Distribution, and Adipose Tissue Function. *Obes Facts* (2017) 10:207–15. doi: 10.1159/000471488
30. Stefan N, Schick F, Häring HU. Causes, Characteristics, and Consequences of Metabolically Unhealthy Normal Weight in Humans. *Cell Metab* (2017) 26:292–300. doi: 10.1016/j.cmet.2017.07.008
31. Mongraw-Chaffin M, Foster MC, Anderson CAM, Burke GL, Haq N, Kalyani RR, et al. Metabolically Healthy Obesity, Transition to Metabolic Syndrome, and Cardiovascular Risk. *J Am Coll Cardiol* (2018) 71:1857–65. doi: 10.1016/j.jacc.2018.02.055
32. Eckel N, Li Y, Kuxhaus O, Stefan N, Hu FB, Schulze MB. Transition From Metabolic Healthy to Unhealthy Phenotypes and Association With Cardiovascular Disease Risk Across BMI Categories in 90257 Women (the Nurses' Health Study): 30 Year Follow-Up From a Prospective Cohort Study. *Lancet Diabetes Endocrinol* (2018) 6:714–24. doi: 10.1016/S2213-8587(18)30137-2
33. Wong VW, Chan WK, Chitturi S, Chawla Y, Dan YY, Duseja A, et al. Asia-Pacific Working Party on Non-Alcoholic Fatty Liver Disease Guidelines 2017-Part 1: Definition, Risk Factors and Assessment. *J Gastroenterol Hepatol* (2018) 33:70–85. doi: 10.1111/jgh.13857
34. Wong VW, Wong GL, Chan RS, Shu SS, Cheung BH, Li LS, et al. Beneficial Effects of Lifestyle Intervention in non-Obese Patients With Non-Alcoholic Fatty Liver Disease. *J Hepatol* (2018) 69:1349–56. doi: 10.1016/j.jhep.2018.08.011
35. Pucci G, Alcidi R, Tap L, Battista F, Mattace-Raso F, Schillaci G. Sex- and Gender-Related Prevalence, Cardiovascular Risk and Therapeutic Approach in Metabolic Syndrome: A Review of the Literature. *Pharmacol Res* (2017) 120:34–42. doi: 10.1016/j.phrs.2017.03.008
36. Gerdts E, Regitz-Zagrosek V. Sex Differences in Cardiometabolic Disorders. *Nat Med* (2019) 25:1657–66. doi: 10.1038/s41591-019-0643-8

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Comparison of the Finnish Diabetes Risk Score Model With the Metabolic Syndrome in a Shanghai Population

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Aims: This study aimed to compare the diagnostic accuracy of the metabolic syndrome with the Finnish Diabetes Risk Score (FINDRISC) to screen for type 2 diabetes mellitus (T2DM) in a Shanghai population.

Methods: Participants aged 25-64 years were recruited from a Shanghai population from July 2019 to March 2020. Each participant underwent a standard metabolic work-up, including clinical examination with anthropometry. Glucose status was tested using hemoglobin A1c (HbAlc), 2h-post-load glucose (2hPG), and fasting blood glucose (FBG). The FINDRISC questionnaire and the metabolic syndrome were examined. The performance of the FINDRISC was assessed using the area under the receiver operating characteristic curve (AUC-ROC).

Results: Of the 713 subjects, 9.1% were diagnosed with prediabetes, whereas 5.2% were diagnosed with T2DM. A total of 172 subjects had the metabolic syndrome. A higher FINDRISC score was positively associated with the prevalence of T2DM and the metabolic syndrome. Multivariable linear regression analysis demonstrated that the FINDRISC had a linear regression relationship with 2hPG levels (b'= 036, $p < 0.0001$). The AUC-ROC of the FINDRISC to identify subjects with T2DM among the total population was 0.708 (95% CI 0.639–0.776), the sensitivity was 44.6%, and the specificity was 90.1%, with 11 as the cut-off point. After adding FBG or 2hPG to the FINDRISC, the AUC-ROC among the total population significantly increased to 0.785 (95% CI 0.671–0.899) and 0.731 (95% CI 0.619–0.843), respectively, while the AUC-ROC among the female group increased to 0.858 (95% CI 0.753–0.964) and 0.823 (95% CI 0.730–0.916), respectively ($p < 0.001$). The AUC-ROC of the metabolic syndrome to identify subjects with T2DM among the total and female population was 0.805 (95% CI 0.767–0.844) and 0.830 (95% CI 0.788–0.872), respectively, with seven as the cut-off point.

Conclusions: The metabolic syndrome performed better than the FINDRISC model. The metabolic syndrome and the FINDRISC with FBG or 2hPG in a two-step screening model are both efficacious clinical practices for predicting T2DM in a Shanghai population.

Keywords: risk predictors, type 2 diabetes, prediabetes, metabolic sydrome, primary screening

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INTRODUCTION

The increasing prevalence of diabetes, particularly type 2 diabetes mellitus (T2DM), has reached epidemic proportions worldwide (1). In 2019, 463 million people was estimated to suffer from diabetes, and this number was projected to reach 578 million by 2030, and 700 million by 2045 (2). A recent study (3) indicated that the total number of patients with diabetes in mainland China was approximately 129.8 million (70.4 million men and 59.4 million women). Follow-up studies in China, Finland, and the United States (4) have found that early lifestyle and drug interventions can delay or reduce the risk of developing T2DM by 30–60%. Hence, the identification of these individuals is important and effective (5–8). The responsibility for the care of T2DM patients has been transferred from secondary to primary care in the last two decades, among which prediction tools are crucial for identifying individuals at high risk of T2DM (9).

Insulin resistance (IR), the main indicator used in the diagnosis of T2DM even at certain insulin concentrations, is defined as the decreased glucose uptake by cells. Several methods are available for diagnosing IR (10). The homeostasis model assessment of insulin resistance (HOMA-IR) is one of the most widely used methods for assessing IR. In addition, the homeostasis model for assessing insulin sensitivity (HOMA-IS) and β-cell function (HOMA-β) is always used to observe insulin secretion and pancreatic β-cell functions (11). However, it is necessary to measure serum insulin levels to calculate HOMA, and this measurement is not part of routine evaluation in health care services. In addition, subjects with prediabetes or T2DM are commonly accompanied by a set of risk factors, which is known as the metabolic syndrome. The metabolic syndrome is a cluster of IR and disturbed glucose metabolism, overweight and abdominal fat distribution, dyslipidemia, and high blood pressure (12). Subjects with the metabolic syndrome have a 5-fold higher risk of developing T2DM (13). The metabolic syndrome is present if at least three of the following five criteria are met (14): (1) waist circumference ≥ 102 cm in men or 88 cm in women; (2) blood pressure ≥ 130/85 mmHg; (3) triglyceride (TG) levels ≥ 1.7 mmol/L; (4) high-density lipoprotein cholesterol (HDL-C) levels ≤ 1.03 mg/dl in men or 1.3 mmol/L in women; (5) fasting blood glucose (FBG) ≥ 100 mg/dl. This makes it one of the most labor-intensive and expensive prediction tools for T2DM.

Simple prediction tools that can identify individuals at risk could reduce the cost and inconvenience of screening. With such tools, a two-step procedure could be used: first, subjects would be screened with a risk score, and then those individuals identified to have a high risk for T2DM would have their glycemic status assessed by FBG, 2-h post-load glucose (2hPG) using the oral glucose tolerance test (OGTT), or hemoglobin (Hb)A1c measurements (15). However, due to differences in diet, lifestyle, social environment, and genetic susceptibility of different populations, the applicability of the model is limited (16). Therefore, various regions need to continue to explore T2DM assessment models that are suitable for local populations. The ideal assessment model needs to accurately assess the disease risk of individuals and to identify high-risk groups that are more likely to develop T2DM (discernment), which is a prerequisite for mature risk assessment models (17).

The Finnish Diabetes Risk Score (FINDRISC) model is the most authoritative and widely used risk-scoring model in Europe and other populations (15). The European Society of Cardiology, the European Association for the Study of Diabetes, and the Canadian Preventive Health Care Working Group all recommend the use of the FINDRISC model in the screening of T2DM when formulating the prevention and treatment guidelines for diabetes (15). The original questions in the FINDRISC model included age, body mass index (BMI), waist circumference, physical activity, daily consumption of fruits and vegetables, use of antihypertensive medication, history of high blood glucose, and family history of diabetes. Population-wide screening for diabetes using a standard risk calculator is more acceptable than HbA1c, 2hPG, or FBG, which require invasive testing (17). Many countries, including Finland, Canada, and Thailand, routinely use standard risk calculators to determine who should undergo invasive testing for T2DM diagnosis (17). Receiver operating characteristic (ROC) curve analysis is the most frequently used method to evaluate the discernment of the FINDRISC model (18). The value of the area under the ROC curve (AUC-ROC) is a global summary statistic of the discriminative value of a model, which can be used to illustrate the possibility that scores are higher in individuals with T2DM than in those without T2DM.

Despite the widespread use of FINDRISC models, the discriminatory accuracy of the metabolic syndrome versus the FINDRISC model has not been tested in a Chinese population. Hence, in this study, the metabolic syndrome to the FINDRISC model as a screening tool for T2DM in a Shanghai population was compared.

METHODS

Source of Data

The study was a cross-sectional clinical study. Patients who underwent physical examination at two community hospitals in Sanlin and Zhangjiang (Shanghai), who were aged between 25 and 64 years, were recruited from July 2019 to March 2020. The target population was stratified and sampled according to age group (every 10 years was 1 segment) and gender to ensure the uniform distribution of all ages and genders. According to the annual incidence of IGT diabetes (8%-11%), the minimum sample size was 470 cases. In this study, 1,000 cases were participated, and finally a total of 713 participants have completed the study. The study protocol was approved by the Ethics Committee of Shuguang Hospital, affiliated with the Shanghai University of Traditional Chinese Medicine (Certificate number 2018-599-28-01). Written informed consent was obtained from all the participants before enrollment. Exclusion criteria included current pregnancy, inability to communicate or stand, previous diagnosis of T2DM, or refusal to participate in the study by not providing informed consent. The participants' diabetes risk was calculated based on their FINDRISCs.

Diabetes and Pre–Diabetes Definitions

T2DM was defined using the WHO criteria (19) based on one of the following: FBG ≥ 7.0 mmol/L, 2hPG ≥ 11.1 mmol/L, a random plasma glucose ≥ 11.1 mmol/L, HbA1c ≥ 6.5% (48 mmol/mol) (based on the American Diabetes Association (ADA) criteria); and the criteria for pre-diabetes diagnosis, which is based on impaired fasting glucose (IFG): 6.1 mmol/L ≤ FBG < 7.0 mmol/L or impaired glucose tolerance (IGT): 7.8 ≤ 2hPG < 11.1 mmol/L (20).

The Metabolic Syndrome Criteria

The metabolic syndrome was evaluated according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) (14). Subjects were classified as having the metabolic syndrome if more than three of the following criteria were met:

(1) Waist circumference ≥ 102 cm in men and ≥ 88 cm in women
(2) Hypertriglyceridemia: ≥ 150 mg/dl;
(3) Low-HDL cholesterol: < 40 mg/dl in men and < 50 mg/dl in women
(4) High blood pressure: ≥ 130/85 mmHg or on antihypertensive medication
(5) High FBG: ≥ 100 mg/dl.

Clinical Evaluation

Participants' name, age, sex, address, contact number, and history of hypertension, hyperlipidemia, hyperuricemia, and fatty liver disease were recorded in the questionnaire form. Each participant underwent a standard metabolic workup, including a clinical examination with anthropometry. All measurements were performed in the morning, with participants in fasting conditions and the lightest possible clothes. Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body weight was tested using a digital scale to the nearest 0.2 kg with the participants wearing the lightest possible clothes. Body mass index (BMI) was calculated as weight in kilograms over height in m^2 (21). Waist circumference was measured at the mid-level between the lower rib margin and the iliac crest with the participants in a standing position. Blood pressure was measured on the right arm with the participants in a sitting position. The questionnaire was completed without laboratory tests. The answer to each question was assigned different weighted scores, as shown in **Table 1**. The final score was the sum of the scores from the eight questions, which ranged from 0 to 26.

TABLE 1 | Finnish Diabetes Risk Score (FINDRISC) questionnaire.

Item	Standard	Score
Age		
	Under 45 years	0
	45–54 years	2
	55–64 years	3
	>64 years	4
BMI		
	<24 kg/m^2	0
	24–28 kg/m^2	1
	>28 kg/m^2	3
Waist circumference (male)		
	<90cm	0
	90–102 cm	3
	>102 cm	4
Waist circumference (female)		
	<80 cm	0
	80–88 cm	3
	>88 cm	4
Physical Activity ≥ 30 min/d		
	Yes	0
	No	2
Consume fruits and vegetables daily		
	Yes	0
	No	1
History of hypertension		
	Yes	2
	No	0
History of high glucose		
	Yes	5
	No	0
Family history of diabetes		
	No	0
	Yes (non-first-degree relatives)	3
	Yes (first-degree relatives)	5

Biochemical Variables

Laboratory inspection indicators included FBG, 2-h plasma glucose in the 75 g oral glucose tolerance (2hPG) test, fasting serum insulin level (FINS), HbA1c, triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and High-density lipoprotein cholesterol (HDL-C). Insulin resistance and β cell function were estimated using the homeostasis model assessment (HOMA), as described by Song, Manson (11). HOMA-IR was calculated as [FINS (mU/l) × FBG (mmol/L)]/22.5; β-cell function (HOMA%β) was calculated as 20 × (FINS [mU/l]/[FBG (mmol/L) - 3.5] × 100%; insulin sensitivity (HOMA%S) was calculated as 1000/(FINS (mU/l) × (FBG (mmol/L).

Procedures for blood sample collection were explained to the participants by trained laboratory staff. Participants were asked to provide venous blood samples after 8–12 h of fasting. The first blood sample was obtained at the first follow-up after verifying the fasting period. Subsequently, 300 mL of test solution containing 75 g of anhydrous glucose was used as recommended. A new blood sample was obtained after 2 h to measure glucose levels. Blood testing was performed at the Shuguang Hospital Testing Center.

Statistical Analysis

All categorical variables were summarized as numbers and percentages (%), and the chi-squared test was performed to detect differences between men and women. Continuous variables were expressed as means with standard deviations (SD), and between-group comparisons were conducted using independent sample 2-tailed *t*-tests or Mann-Whitney *U* tests. Pearson correlation analyses were used to measure the association between the variables and the FINDRISCs. Multivariable linear regression analyses were conducted using the FINDRISC as a dependent variable. ROC curves were constructed to show the relationship between the sensitivity and specificity of the

FINDRISC for identifying subjects with T2DM. The AUC-ROC was used to evaluate the discriminatory accuracy of the FINDRISC in identifying prediabetes and diabetes subjects in male, female, and overall populations. An AUC-ROC of 1.0 represents a perfect test, with no false positive rate and no false negative rate, while an AUC-ROC of 0.5 indicates that the test performed no better than chance. The cut-off points to identify prediabetes and diabetes were determined by the point with the shortest distance to the upper left corner in the ROC curve, which was calculated as the square root of [(1-sensitivity) + (1-specificity)]. The same statistical analysis was performed to evaluate the discriminatory accuracy of the metabolic syndrome to identify subjects with T2DM. Statistical analyses were performed using SPSS software (version 26.0; Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

RESULTS

Characteristics of the Study Participants

Table 2 summarizes the basic and clinical characteristics of the 713 participants involved in this study. The mean age of the participants was 45.2 ± 11.2 years (women: 45.1 ± 11.4 years; men: 45.4 ± 10.9 years), and women accounted for 60.6% of the total participants. There was no statistically significant difference between men and women in terms of age ($p = 0.655$). Men had higher height (168.5 *vs*. 157.7 cm; $p < 0.001$), waist circumference (83.2 *vs*. 77.9 cm; $p < 0.001$), weight (68.0 *vs*. 58.4 kg; $p < 0.001$), diastolic pressure (81.8 *vs*. 78.9 mmHg; $p < 0.001$), HOMA%S (287.7 *vs*. 286.6; $p = 0.016$), and TG (1.7 *vs*. 1.3 mmol/L; $p = 0.002$) values than women. Women had higher FBG (5.1 *vs*. 4.9 mmol/L; $p = 0.038$), fasting serum insulin (22.7 *vs*. 13.7 mmol/L; $p < 0.001$), HOMA%β (97.0 *vs*. 96.1%; $p = 0.016$), and HDL-C (1.4 *vs*. 1.2 mmol/L, $p < 0.001$) values than men. In addition, women had a stronger family history of high glucose (7.4 *vs*. 4.3%) and diabetes (23.4 *vs*. 18.1%) and were relatively more physically active (69.0 *vs*. 63.7%). With increasing values of basic characteristics in the original FINDRISC questionnaire and increasing levels of lipid metabolic and obesity-related indicators, including systolic and diastolic blood pressure, FBG, 2hPG, HbA1c, fasting serum insulin level, TC, and TG, the FINDRISC values increased significantly. In total, 91% of the participants had a very low to low risk (FINDRISC ≤ 11) of T2DM and 5% had a high or very high risk (FINDRISC ≥ 15) of developing T2DM, according to the FINDRISC scale (**Table 3**). There was no significant difference between men and women in terms of their risk of developing T2DM ($p = 0.067$).

According to the ADA diagnostic criteria, 37 participants were newly diagnosed with T2DM, 65 with prediabetes, and 611 participants were diagnosed with non-T2DM. In total, 172 participants met the diagnostic criteria for the metabolic syndrome. The FINDRISC score increased with worsening glucose status, including FBG, 2hPG, and HbA1c. Of the 172 participants who were diagnosed with the metabolic syndrome, 63 had a FINDRISC score ≥ 11.

Associations Between FINDRISCs and Patient Characteristics

According to the results of the Pearson's correlation analysis shown in **Table 4**, significant positive correlations between the FINDRISC and the metabolic syndrome were observed, including systolic and diastolic blood pressure ($r = 0.34$, and 0.300, respectively, $p < 0.001$), FBG, 2hPG, HbA1c, fasting serum insulin level ($r = 0.356$, 0.361, 0.358, and 0.219, respectively, $p < 0.001$), TC, TG, and LDL-C ($r = 0.276$, 0.281, and 0.238, respectively, $p < 0.001$). In contrast, there was an inverse association between HDL-C and the FINDRISC ($r = -0.211$, $p < 0.001$). Subsequently, a multivariable linear regression analysis was performed using the FINDRISC as an independent variable to reach the final regression model. The p values revealed that the FINDRISC had a significant linear regression relationship with 2hPG ($b' = 0.160$, $p < 0.0001$). According to the standardized coefficient, 2hPG showed the greatest impact on the FINDRISC among all characteristics ($R^2 = 0.527$; $F = 27.287$, $p < 0.0001$, **Table 5**).

Diagnostic Accuracy for Undiagnosed T2DM

Table 6 shows the cut-off points of the metabolic syndrome and FINDRISC for screening of undiagnosed T2DM using 2hPG, FBG, and HbA1c as the diagnostic criteria, separately for the overall, female, and male populations. The highest AUC-ROC value was observed for the FBG criteria in the FINDRISC (AUC-ROC = 0.858, 95% CI 0.753–0.964) among the female group, followed by the female group with the metabolic syndrome (AUC-ROC = 0.830, 95% CI 0.788–0.872), and using FINDRISC with 2hPG among the female group (AUC-ROC = 0.823, 95% CI 0.730–0.916) (**Figure 1**). FINDRISC and the metabolic syndrome appeared to have better performance in the female group than in the male group (**Figure 2**). The AUC-ROC of the FINDRISC to identify subjects with T2DM among the total population was 0.708 (95% CI 0.639–0.776); its sensitivity was 44.6% and its specificity was 90.1%, with 11 as the cut-off point. When adding FBG or 2hPG to the FINDRISC, the AUC-ROC among the total population significantly increased to 0.785 (95% CI 0.671–0.899) and 0.731 (95% CI 0.619–0.843), respectively, while the AUC-ROC among the female group increased to 0.858 (95% CI 0.753–0.964) and 0.823 (95% CI 0.730–0.916), respectively ($p < 0.001$) (**Figure 3**). The AUC-ROC of the metabolic syndrome to identify subjects with T2DM among the total and female population was 0.805 (95% CI 0.767–0.844) and 0.830 (95% CI 0.788–0.872), respectively, with seven as the cut-off point.

While using the FINDRISC model, adding FBG resulted in the highest AUC-ROC value (0.785) among the total population, compared to the addition of HbA1c (0.704) and 2hPG (0.731). Although the addition of FBG or 2hPG increased the AUC-ROC value of FINSDRISC for predicting T2DM, the discriminatory accuracy was lackluster for the metabolic syndrome. A sensitivity analysis of the influence of different threshold values was performed (**Tables S1–S15**). When adding FBG or HbA1c, the

TABLE 2 | Prevalence of components of the modified version of the Finnish Diabetes Risk Score (FINDRISC) by sex.

Characteristics		Female					Male						p
	Total (n = 432)	FINDRISC0–7 (n = 294)	FINDRISC8–10 (n = 70)	FINDRISC11–14 (n = 47)	FINDRISC15–20 (n = 21)	FINDRISC21–26 (n = 0)	Total (n = 281)	FINDRISC0–7 (n = 211)	FINDRISC8–10 (n = 42)	FINDRISC11–14 (n = 18)	FINDRISC15–20 (n = 9)	FINDRISC21–26 (n = 1)	
Blood tests (±)													
FBG (mmol/L)	5.1 (1.1)	4.9 (0.7)	5.2 (0.8)	5.9 (1.9)	6.6 (1.9)	0	4.9 (1.1)	4.8 (0.9)	4.9 (0.6)	6.3 (2.9)	5.2 (0.6)	5.0	0.038
2h OGTT (mmol/L)	5.9 (2.6)	5.3 (1.5)	6.3 (2.8)	7.2 (3.8)	9.3 (5.5)	0	5.8 (2.6)	5.5 (2.1)	5.7 (2.1)	8.1 (4.8)	8.1 (2.7)	13.4	0.921
HbA1c (%)	5.8 (0.7)	5.6 (0.5)	5.9 (0.4)	6.2 (1.0)	6.8 (1.3)	0	5.8 (0.7)	5.8 (0.6)	5.7 (0.4)	6.3 (1.4)	6.3 (0.5)	7.6	0.825
Fasting serum insulin, mmol/L	22.7 (26.8)	18.5 (22.4)	27.6 (29.5)	37.0 (33.9)	32.3 (36.5)	0	13.7 (20.9)	11.1 (18.3)	22.2 (22.2)	24.6 (35.6)	12.1 (12.5)	1.4	<0.001
HOMA%β	104.1 (80.9)	102.9 (75.0)	114.5 (98.1)	106.2 (99.4)	83.7 (68.4)	0	87.5 (71.4)	86.1 (71.1)	109.5 (86.1)	80.2 (41.6)	56.6 (24.6)	49.4	0.016
HOMA%S	260.2 (221.0)	262.3 (222.3)	245.3 (221.8)	268.9 (210.8)	259.5 (210.3)	0	320.9 (225.0)	330.0 (226.1)	261.7 (214.2)	270.0 (195.3)	444.2 (198.1)	51.7	0.002
HOMA-IR	1.2 (1.2)	1.1 (1.2)	1.3 (1.4)	1.2 (1.5)	0.9 (0.8)	0	0.9 (1.0)	0.8 (1.0)	1.1 (1.3)	0.8 (0.7)	0.5 (0.6)	1.9	0.003
TC (mmol/L)	4.7 (0.9)	4.5 (0.8)	5.0 (1.0)	4.9 (0.9)	5.2 (1.1)	0	4.6 (0.9)	4.5 (0.9)	5.0 (0.9)	4.8 (0.7)	4.8 (0.8)	6.0	0.491
TG (mmol/L)	1.3 (1.3)	1.1 (0.9)	1.5 (0.9)	1.7 (1.4)	2.9 (3.6)	0	1.7 (2.0)	1.5 (1.5)	2.0 (1.3)	3.1 (4.6)	3.7 (3.7)	2.0	0.002
HDL-C (mmol/L)	1.4 (0.3)	1.4 (0.3)	1.3 (0.3)	1.3 (0.2)	1.1 (0.3)	0	1.2 (0.3)	1.3 (0.3)	1.1 (0.2)	1.2 (0.3)	1.1 (0.2)	1.2	<0.001
LDL-C (mmol/L)	2.5 (0.7)	2.4 (0.6)	2.8 (0.7)	2.7 (0.7)	2.9 (0.7)	0	2.5 (0.7)	2.5 (0.7)	2.9 (0.8)	2.6 (0.6)	2.4 (0.5)	3.3	0.876
Anthropometrics (±)													
SBP (mmHg)	123.2 (19.0)	118.9 (17.2)	129.7 (17.2)	134.2 (20.6)	136.9 (22.1)	0	123.2 (15.4)	121.8 (14.7)	126.5 (14.9)	125.2 (19.2)	135.6 (17.3)	130	0.986
DBP (mmHg)	78.9 (10.3)	76.8 (9.7)	81.4 (10.5)	84.9 (8.1)	86.5 (11.2)	0	81.8 (9.7)	80.7 (9.6)	83.3 (9.0)	86.2 (10.9)	88.2 (7.3)	90	<0.001
Height (cm)	157.7 (5.3)	157.9 (5.3)	156.7 (4.9)	157.6 (5.7)	158.4 (5.7)	0	168.5 (5.9)	168.3 (6.1)	168.6 (5.8)	169.1 (3.7)	171.1 (3.5)	163.7	<0.001
Weight (kg)	58.4 (9.4)	55.2 (7.2)	62.3 (9.1)	66.9 (11.0)	70.3 (6.2)	0	68.0 (12.0)	65.1 (11.0)	75.9 (11.3)	75.4 (9.9)	81.6 (7.4)	83	<0.001
FINDRISC score (±)	5.8 (4.4)	3.3 (2.3)	8.8 (0.8)	11.9 (1.1)	16.2 (1.5)	0	5.0 (4.1)	3.1 (2.2)	8.6 (0.8)	11.7 (1.1)	16.3 (1.7)	24	0.017
Age, years	45.1 (11.4)	42.5 (11.2)	51.0 (9.8)	50.0 (10.5)	50.0 (7.8)	0	45.4 (10.9)	45.2 (11.1)	44.9 (10.5)	45.7 (9.6)	52.7 (8.2)	55	0.655
Waist circumference, cm	77.9 (10.4)	73.9 (7.8)	83.2 (10.3)	89.1 (9.7)	90.8 (5.9)	0	83.2 (10.0)	80.5 (9.2)	90.6 (7.1)	89.5 (8.1)	96.4 (6.8)	106	<0.001
BMI, kg/m^2	23.3 (4.0)	22.2 (2.6)	25.3 (3.2)	26.8 (3.4)	28.1 (2.7)	0	23.2 (5.5)	22.9 (3.4)	26.6 (2.9)	26.3 (3.3)	27.8 (1.8)	31.0	0.107
Physical activity ≥ 30 min/d, %	69.0	69.0	72.9	66.0	61.9	0	63.7	71.6	40.5	50.0	22.2	No	0.143
Consume fruits and vegetables daily, %	96.5	96.9	97.1	95.7	90.5	0	92.5	92.4	90.5	94.4	100.0	Yes	<0.001
History of hypertension, %	22.0	10.9	48.6	31.9	66.7	0	24.9	14.7	50.0	50.0	88.9	Yes	0.101
History of high glucose, %	7.4	0.01	0.07	23.4	57.1	0	4.3	0.005	0.024	0.33	0.33	Yes	0.846
Family history of diabetes, %	23.4	12.6	28.6	55.3	85.7	0	18.1	0.06	35.7	77.8	100.0	yes	0.439
Outcomes (%)													
Non-T2DM	372 (86)	273 (93)	59 (84)	28 (60)	12 (57)	0	239 (85)	187(89)	38(90)	11 (61)	3(33)	0	0.485
Prediabetes	36 (8)	17 (6)	6 (9)	11 (23)	2 (10)	0	29 (10)	17 (8)	3 (7)	4 (22)	5(56)	0	0.368
T2DM	24 (6)	4 (1)	5 (7)	8 (17)	7 (33)	0	13 (5)	7 (3)	1 (3)	3 (17)	1 (11)	1	0.585
The metabolic syndrome (MS)	110 (25)	33 (11)	32 (46)	29 (62)	16 (76)	0	62 (22)	29 (14)	14 (33)	10 (56)	8 (89)	1	0.300

Data were given by mean (SD). FBG, fasting blood glucose; 2h OGTT, 2-h plasma glucose in the 75 g oral glucose tolerance test; HbA1c, glycated hemoglobin; HOMA%β, homeostasis model assessment of β-cell function; HOMA%S, homeostasis model assessment of insulin sensitivity; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; H-CRP, hypersensitive C-reactive protein; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BMI= Body Mass Index, Weight (kg)/height (m)^2.

TABLE 3 | Risk levels of FINDRISC by sex.

		Female		Male		Total		P value
Risk	**Score**	**N**	**%**	**N**	**%**	**N**	**%**	**0.067**
Very low risk	**< 7**	260	60	197	70	457	64	
Low risk	**7–11**	126	29	68	24	194	27	
Moderate risk	**12–14**	25	6	6	2	31	4	
High risk	**15–20**	20	4.6	9	3	29	4	
Very high risk	**≥ 20**	1	0.4	1	1	2	1	

The bold P value (0.067) indicates that there was no significant difference between men and women in terms of their risk of developing T2DM.

optimal cut-off point according to the Youden's index was 11, while adding 2hPG to the FINDRISC model led its cut-off point to decrease to eight. In contrast, the optimal cut-off point for the metabolic syndrome was seven, with a sensitivity and specificity of 74.4% and 76.3%, respectively. For female and male subjects, the optimal cut-off was the same as that for the overall population.

DISCUSSION

The metabolic syndrome is a cluster of insulin resistance and disturbed glucose metabolism, overweight and abdominal fat distribution, dyslipidemia, and high blood pressure, and commonly occurs in the majority of patients with prediabetes or T2DM (12). Approximately 25% of adults worldwide are estimated to have the metabolic syndrome (22). In addition, patients with the metabolic syndromes have a 5-fold higher risk of developing T2DM than those with the normal metabolic syndrome (13). A study (23) reported that the FINDRISC assessment model exhibited a good ability to predict the metabolic syndrome in a cohort of first-degree relatives of T2DM patients, with an AUC value of 65%. In this study, the prevalence of the metabolic syndrome increased with increasing FINDRISCs. Several cross-sectional studies have assessed the FINDRISC model as a screening tool for the metabolic syndrome. The lipid metabolic indicators (blood pressure, FBG, TC, TG, HDL-C, and LDL-C) were found to be significantly related to the FINDRISC model, which are common risk factors for T2DM (24). This finding indicated that the FINDRISC model could be used for undiagnosed T2DM screening in this cohort. This in turn revealed that a non-invasive screening tool combined with a relatively inexpensive and feasible biochemical marker was a better tool to identify subjects with T2DM, as opposed to a cluster of clinical and biochemical markers for diagnosing the metabolic syndrome (24).

TABLE 4 | Pearson's correlation analysis between FINDRISC and variables.

Variable	Pearson's coefficient (r)	p value
Height	-0.041	0.281
Weight	0.446	**<0.001**
Systolic blood pressure	0.343	**<0.001**
Diastolic blood pressure	0.300	**<0.001**
FBG	0.356	**<0.001**
2hPG	0.361	**<0.001**
HbA1c	0.358	**<0.001**
Fasting serum insulin	0.219	**<0.001**
HOMA%β	0.040	0.372
HOMA%S	-0.018	0.677
HOMA-IR	0.017	0.701
TC	0.276	**<0.001**
TG	0.281	**<0.001**
HDL-C	-0.211	**<0.001**
LDL-C	0.238	**<0.001**

FBG, fasting blood glucose; 2hPG, 2-h plasma glucose; HOMA%β, homeostasis model assessment of β-cell function; HOMA%S, homeostasis model assessment of insulin sensitivity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; TG, triglycerides; TC, total cholesterol.
Bold P values indicate a significant difference.

In 2009, Chien (25) used a Cox regression analysis to establish a risk scoring model for T2DM, namely the Taiwan model of China. A total of 2960 subjects aged over 35 years with a 10-year follow-up were selected for this cohort study. Risk factors in this invasive model included TG, HDL-C, FBG, and white blood cell levels. The AUC-ROC value for this model was 0.70. In 2012, a Chinese population (26) was used to test several domestic and foreign T2DM evaluation models, including the FINDRISC and Framingham models. It was found that the Taiwan model of China had the highest predictive efficiency among all models, with AUC-ROC values of 0.75 among all models; these were similar to the AUC-ROC values (0.704 to 0.785) found using the FINDRISC model in this study.

Several diabetes risk-score models have been developed to predict the risk of T2DM. These models can be used in clinical practice to identify people at high risk of T2DM, and to guide clinical treatment (17). However, it is still not clear whether these models can be applied in local populations. The incidence and risk factors of T2DM in a population determine the suitability of a risk score. Some models developed in a particular population always do not perform well in other populations. There have been extensive studies on risk scores that have been developed specifically in Chinese settings. Mao (27) evaluated the performance of The New Chinese Diabetes Risk Score (NCDRS) model in detecting undiagnosed diabetes and prediabetes among 7,675 community residents in Jiangsu Province. The results showed that the participants with undiagnosed diabetes reported the highest NCDRS value, and the best cut-off points of NCDRS for detecting undiagnosed diabetes and prediabetes were 27. Their results indicated the excellent performance of NCDRS in screening undiagnosed diabetes in Jiangsu Province, and further provide evidence for using NCDRS in detecting prediabetes. Zhang (28) developed and validated a prediction model for T2DM (Chinese risk model) in a cohort of rural adult Chinese population. With the validation dataset, the performance of this model was superior to the FINDRISC and the other models of T2DM risk, which was widely applicable for predicting 6-year risk of T2DM in a rural adult Chinese population.

TABLE 5 | Multivariable linear regression of FINDRISC .

Variable	*b*	S_b	*b'*	*t*	*p*
Constants	-20.439	5.201	–	-3.930	<0.0001
Height	0.008	0.031	0.015	0.261	0.794
Weight	0.056	0.035	0.146	1.622	0.105
Systolic blood pressure	0.010	0.012	0.043	0.807	0.420
Diastolic blood pressure	0.010	0.020	0.025	0.488	0.626
FBG	0.423	0.273	0.074	1.549	0.122
2hPG	**0.302**	**0.083**	**0.160**	**3.620**	**<0.0001**
HbA1c	0.255	0.329	0.036	0.777	0.438
Fasting serum insulin	0.003	0.017	0.007	0.181	0.856
HOMA%β	0.000415	0.004	0.008	0.097	0.923
HOMA%S	0.000375	0.001	0.020	0.395	0.693
HOMA-IR	0.066	0.281	0.018	0.236	0.813
TC	0.440	0.462	0.093	0.954	0.341
TG	0.000480	0.157	0.000159	-0.003	0.998
HDL-C	-0.425	0.742	-0.026	-0.574	0.567
LDL-C	-0.362	0.548	-0.056	-0.660	0.510

Dependent variable: FINDRISC score; $R^2 = 0.527$; $F = 27.287$, $p < 0.0001$

FBG, fasting blood glucose; 2hPG, 2-h plasma glucose; HOMA%β, homeostasis model assessment of β-cell function; HOMA%S, homeostasis model assessment of insulin sensitivity; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; TG, triglycerides; TC, total cholesterol.
Bold text indicates a significant difference.

The FINDRISC was originally developed in a prospective cohort to identify individuals at high risk of developing T2DM (29). Previous studies have analyzed the performance of FINDRISCs for the detection of undiagnosed T2DM (30). The FINDRISC model has been verified in several Western populations for T2DM screening (31–34). These results showed that the optimal cut-off points ranged widely from 9 (29) to 15 (33). To date, only four studies have evaluated the use of FINDRISC in Asian populations, including populations in the Philippines, Malaysia, Mongolia, and India (35–38). All of these results suggest that the FINDRISC has a good performance for T2DM screening in the Asian population. However, there was no publication, if any, which focused on the validation of the FINDRISC for the screening of T2DM in a Chinese population.

It has been reported that the performance of a diabetes risk score can be improved by adding biochemical markers (39). The HbA1c test is a more convenient screening method than FBG and 2hPG, which does not require fasting, and may not be influenced by day-to-day variations (40). HbA1c was used to monitor glycemic control in diabetic patients as it can reflect the average blood glucose levels over 2–3 months (41). However, some studies have shown that there is poor concordance between HbA1c and FBG or 2hPG during an OGTT, which are the most widely accepted glucose-based methods for diagnosing T2DM. Several studies (40, 42) employed the WHO diabetes diagnostic criteria or only FBG values in the FINDRISC assessment model among other populations. With the introduction of HbA1c as a diagnostic criterion by the ADA guidelines in 2009, studies evaluating the performance of the FINDRISC model by using HbA1c as the gold standard showed good results, with AUC-ROC values ranging from 0.72 to 0.81 (43–45). A previous study (45) used FBG, 2hPG, or HbA1c as the diagnostic criteria in their FINDRISC assessment model. The results showed a higher sensitivity (75% in men *vs.* 72% in women) and AUC-ROC

TABLE 6 | FINDRISC area under ROC curve (AUC) for identifying T2DM.

	AUC (95% CI)	Sensitivity	Specificity	Youden's index	Cut-off	p
FINDRISC (both sexes)	0.708 (0.639–0.776)	44.6%	90.1%	0.347	11	<0.001
FINDRISC (male)	0.625 (0.508–0.742)	31.0%	92.5%	0.235	11	0.028
FINDRISC (female)	0.761 (0.683–0.840)	53.3%	88.6%	0.420	11	<0.001
FBG (both sexes)	0.785 (0.671–0.899)	65.2%	88.1%	0.533	11	<0.001
FBG (male)	0.482 (0.232–0.733)					0.881
FBG (female)	0.858 (0.753–0.964)	72.7%	88.1%	0.609	11	<0.001
HbA1c (both sexes)	0.704 (0.627–0.780)	45.9%	89.6%	0.355	11	<0.001
HbA1c (male)	0.653 (0.532–0.773)	34.6%	92.3%	0.270	11	0.01
HbA1c (female)	0.745 (0.649–0.841)	54.3%	87.9%	0.422	11	<0.001
2hPG (both sexes)	0.731 (0.619–0.843)	69.0%	72.7%	0.416	8	<0.001
2hPG (male)	0.597 (0.380–0.813)					0.258
2hPG (female)	0.823 (0.730–0.916)	82.4%	70.7%	0.531	8	<0.001
MS (both sexes)	0.805 (0.767–0.844)	74.4%	76.3%	0.508	7	<0.001
MS (male)	0.757 (0.683–0.830)	62.9%	79.5%	0.424	7	<0.001
MS (female)	0.830 (0.788–0.872)	80.9%	74.2%	0.551	7	<0.001

FBG, fasting blood glucose; 2hPG, 2-h plasma glucose; HbA1c, glycated hemoglobin; MS, the metabolic syndrome.

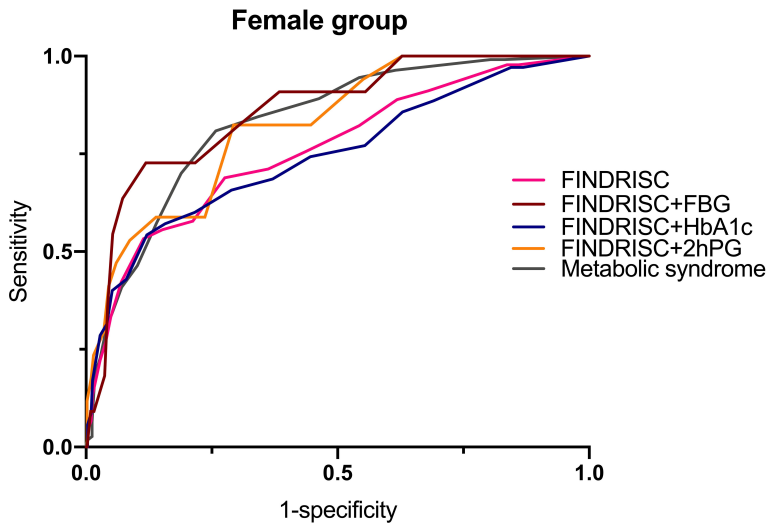

FIGURE 1 | FINDRISC area under ROC curve (AUC) for identifying T2DM in female populations.

value (0.75) than other studies that used 2hPG and/or FBG criteria. In contrast, two studies (15, 29) reported a higher AUC-ROC value than Zhang's study, which used 2hPG and/or FBG as the diagnostic criteria. Similarly, Costa (46) conducted a study to detect undiagnosed T2DM using FBG, 2hPG, and HbA1c alone instead of using combinations of these criteria. Their results suggested that 2hPG and FBG had better discriminatory power than HbA1c, which is in agreement with the observations in this study. In this study, FBG, 2hPG, and HbA1c levels were used as diagnostic criteria. Based on FBG or HbA1c criteria, 11 was the optimal cut-off point for identifying individuals with undiagnosed T2DM, while the optimal cut-off point was eight based on 2hPG criteria. Among these criteria, FBG displayed the best performance in screening for undiagnosed T2DM in the overall population, with an AUC-ROC value of 0.785, followed by 2hPG and HbA1c criteria, with AUC-ROC values of 0.731 and 0.704, respectively. The cut-off points using FBG or HbA1c were higher than those reported in European studies (15, 31, 34, 47). The studies used a value ≥ 9 as the best cut-off point without incorporating the peaks of the ROC curve, where the sum of the sensitivity and specificity is at maximum. In addition, all these diagnostic criteria performed better at screening for undiagnosed T2DM among the female population than for the overall population. However, FINDRISCs with 2hPG and FBG failed to screen for undiagnosed T2DM among the male population.

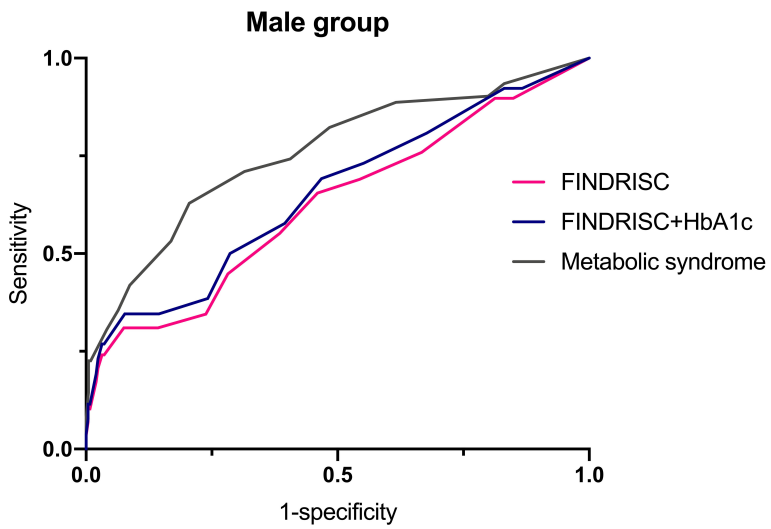

FIGURE 2 | FINDRISC area under ROC curve (AUC) for identifying T2DM in male populations.

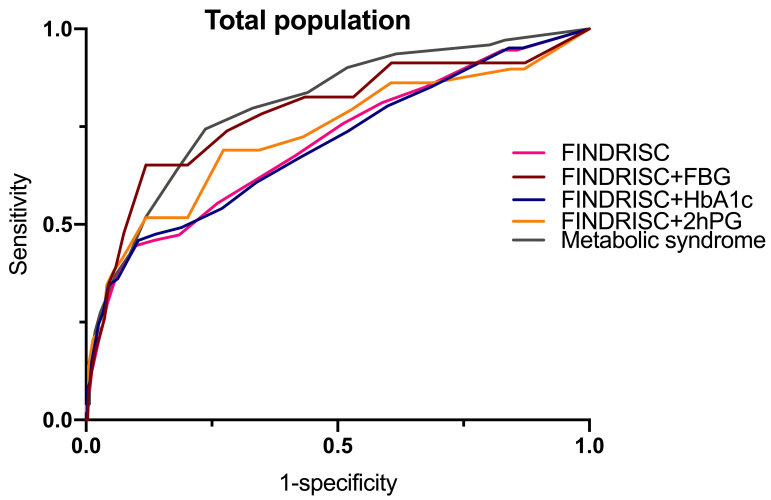

FIGURE 3 | FINDRISC area under ROC curve (AUC) for identifying T2DM in total populations.

The discriminatory accuracy in identifying subjects with diabetes of the original FINDRISC questionnaire was similar to that of the metabolic syndrome; however, the FINDRISC model is much easier to perform, as it did not require invasive testing. Despite the different performance when using different diagnostic criteria, the findings in this study indicated that the FINDRISC could be used as an initial screening tool to help clinicians identify patients at high risk of T2DM. In addition, FINDRISC with FBG and a cut-off point of 11 had a higher discriminative power for screening T2DM compared to 2hPG and HbA1c. To the best of our knowledge, this study is the first to assess the applicability of the FINDRISC model in a Chinese population. Even though the metabolic syndrome showed a better performance than using the FINDRISC screening model, adding FBG or 2hPG significantly increased the discriminative power of the FINDRISC. However, this study had several limitations. First, in this project, the sample size was relatively small, which was calculated based primarily on outpatients with risk factors who visited the hospital during the period from July 2019 to March 2020. The reason why the morbidity of this population is lower than that of Chinese population should be caused by insufficient sample size and population selection, which needs to be further proved by subsequent experiments. Further studies with larger sample sizes are needed to include conclusive data for clinical practice. Second, as participants were recruited from the districts in the metropolitan area of Shanghai, the results may not be applicable to the rest of the population of China.

CONCLUSION

In this study, we tried first to measure the FINDRISC model's performance in predicting current glucose disorders, and then to compare the results based on different sets of diagnostic criteria, which is one of the strengths of this study. According to our findings, the simple scoring model is less sensitive to the prediction of T2DM. Only when FBG or 2hPG was introduced, the prediction sensitivity of the model increased. It seems that the application of FINDRISC to predict T2DM might be less important. However, it is worth noting that including FPG, 2hPG, and HbA1c levels into the model introduces a bias, as these parameters are commonly used for the diagnosis of T2DM itself, which could falsely increase the discriminative power of this risk model. Nevertheless, a non-invasive screening tool with a relatively inexpensive and feasible biochemical marker is a better tool to identify subjects with diabetes than a cluster of clinical and biochemical markers. Hence, our findings may prompt the application of T2DM in a two-step model among the Chinese population.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZY and HL substantially contributed to the general idea and design of the study. SYJ completed the data analysis and the

manuscript. QGC, XH, YHL, and MJC assisted in case collection and clinical data recording. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.725314/full#supplementary-material

REFERENCES

1. Bommer C, Sagalova V, Heesemann E, Manne-Goehler J, Atun R, Bärnighausen T, et al. Global Economic Burden of Diabetes in Adults: Projections From 2015 to 2030. *Diabetes Care* (2018) 41(5):963–70. doi: 10.2337/dc17-1962
2. IDF. *IDF DIABETES ATLAS, Ninth edition* (2019). Available at: https://www.diabetesatlas.org/upload/resources/material/20200302_133351_IDFATLAS9e-final-web.pdf(2019).
3. Li Y, Teng D, Shi X, Qin G, Qin Y, Quan H, et al. Prevalence of Diabetes Recorded in Mainland China Using 2018 Diagnostic Criteria From the American Diabetes Association: National Cross Sectional Study. *BMJ* (2020) 369:m997. doi: 10.1136/bmj.m997
4. Galaviz KI, Narayan KMV, Lobelo F, Weber MB. Lifestyle and the Prevention of Type 2 Diabetes: A Status Report. *Am J Lifestyle Med* (2015) 12(1):4–20. doi: 10.1177/1559827615619159
5. Diabetes Prevention Program Research Group, Knowler WC, Fowler SE, Hamman RF, Christophi CA, Hoffman HJ, et al. 10-Year Follow-Up of Diabetes Incidence and Weight Loss in the Diabetes Prevention Program Outcomes Study. *Lancet* (2009) 374(9702):1677–86. doi: 10.1016/S0140-6736(09)61457-4
6. Haw JS, Galaviz KI, Straus AN, Kowalski AJ, Magee MJ, Weber MB, et al. Long-Term Sustainability of Diabetes Prevention Approaches: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. *JAMA Intern Med* (2017) 177(12):1808–17. doi: 10.1001/jamainternmed.2017.6040
7. Li G, Zhang P, Wang J, Gregg EW, Yang W, Gong Q, et al. The Long-Term Effect of Lifestyle Interventions to Prevent Diabetes in the China Da Qing Diabetes Prevention Study: A 20-Year Follow-Up Study. *Lancet* (2008) 371(9626):1783–9. doi: 10.1016/S0140-6736(08)60766-7
8. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, et al. Prevention of Type 2 Diabetes Mellitus by Changes in Lifestyle Among Subjects With Impaired Glucose Tolerance. *N Engl J Med* (2001) 344(18):1343–50. doi: 10.1056/nejm200105033441801
9. Ong SE, Koh JJK, Toh S-AES, Chia KS, Balabanova D, McKee M, et al. Assessing the Influence of Health Systems on Type 2 Diabetes Mellitus Awareness, Treatment, Adherence, and Control: A Systematic Review. *PloS One* (2018) 13(3):e0195086-e. doi: 10.1371/journal.pone.0195086
10. Ma M, Liu H, Yu J, He S, Li P, Ma C, et al. Triglyceride is Independently Correlated With Insulin Resistance and Islet Beta Cell Function: A Study in Population With Different Glucose and Lipid Metabolism States. *Lipids Health Dis* (2020) 19(1):121. doi: 10.1186/s12944-020-01303-w
11. Song Y, Manson JE, Tinker L, Howard BV, Kuller LH, Nathan L, et al. Insulin Sensitivity and Insulin Secretion Determined by Homeostasis Model Assessment and Risk of Diabetes in a Multiethnic Cohort of Women: The Women's Health Initiative Observational Study. *Diabetes Care* (2007) 30(7):1747–52. doi: 10.2337/dc07-0358
12. Bansal N. Prediabetes Diagnosis and Treatment: A Review. *World J Diabetes* (2015) 6(2):296–303. doi: 10.4239/wjd.v6.i2.296
13. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: A High-Risk State for Diabetes Development. *Lancet* (2012) 379(9833):2279–90. doi: 10.1016/S0140-6736(12)60283-9
14. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* (2001) 285(19):2486–97. doi: 10.1001/jama.285.19.2486
15. Li J, Bergmann A, Reimann M, Bornstein SR, Schwarz PE. A More Simplified Finnish Diabetes Risk Score for Opportunistic Screening of Undiagnosed Type 2 Diabetes in a German Population With a Family History of the Metabolic Syndrome. *Horm Metab Res* (2009) 41(2):98–103. doi: 10.1055/s-0028-1087191
16. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk Factors Contributing to Type 2 Diabetes and Recent Advances in the Treatment and Prevention. *Int J Med Sci* (2014) 11(11):1185–200. doi: 10.7150/ijms.10001
17. Buijsse B, Simmons RK, Griffin SJ, Schulze MB. Risk Assessment Tools for Identifying Individuals at Risk of Developing Type 2 Diabetes. *Epidemiol Rev* (2011) 33(1):46–62. doi: 10.1093/epirev/mxq019
18. Kundu S, Kers JG, Janssens ACJW. Constructing Hypothetical Risk Data From the Area Under the ROC Curve: Modelling Distributions of Polygenic Risk. *PloS One* (2016) 11(3):e0152359. doi: 10.1371/journal.pone.0152359
19. WHO. *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia* . Available at: https://www.who.int/diabetes/publications/Definition%20and%20diagnosis%20of%20diabetes_new.pdf(2013).
20. ADA. Standards of Medical Care in Diabetes—2013. *Diabetes Care* (2013) 36(Supplement 1):S11–66. doi: 10.2337/dc13-S011
21. WHO. Appropriate Body-Mass Index for Asian Populations and its Implications for Policy and Intervention Strategies. *Lancet* (2004) 363(9403):157–63. doi: 10.1016/S0140-6736(03)15268-3
22. Saklayen MG. The Global Epidemic of the Metabolic Syndrome. *Curr Hypertens Rep* (2018) 20(2):12–. doi: 10.1007/s11906-018-0812-z
23. Janghorbani M, Adineh H, Amini M. Evaluation of the Finnish Diabetes Risk Score (FINDRISC) as a Screening Tool for the Metabolic Syndrome. *Rev Diabetes Stud* (2013) 10(4):283–92. doi: 10.1900/RDS.2013.10.283
24. Meijnikman AS, De Block CEM, Verrijken A, Mertens I, Van Gaal LF. Predicting Type 2 Diabetes Mellitus: A Comparison Between the FINDRISC Score and the Metabolic Syndrome. *Diabetol Metab Syndr* (2018) 10:12–. doi: 10.1186/s13098-018-0310-0
25. Chien K, Cai T, Hsu H, Su T, Chang W, Chen M, et al. A Prediction Model for Type 2 Diabetes Risk Among Chinese People. *Diabetologia* (2008) 52(3):443. doi: 10.1007/s00125-008-1232-4
26. He S, Chen X, Cui K, Peng Y, Liu K, Lv Z, et al. Validity Evaluation of Recently Published Diabetes Risk Scoring Models in a General Chinese Population. *Diabetes Res Clin Pract* (2012) 95(2):291–8. doi: 10.1016/j.diabres.2011.10.039
27. Mao T, Chen J, Guo H, Qu C, He C, Xu X, et al. The Efficacy of New Chinese Diabetes Risk Score in Screening Undiagnosed Type 2 Diabetes and Prediabetes: A Community-Based Cross-Sectional Study in Eastern China. *J Diabetes Res* (2020) 2020:7463082. doi: 10.1155/2020/7463082
28. Zhang M, Zhang H, Wang C, Ren Y, Wang B, Zhang L, et al. Development and Validation of a Risk-Score Model for Type 2 Diabetes: A Cohort Study of a Rural Adult Chinese Population. *PloS One* (2016) 11(4):e0152054-e. doi: 10.1371/journal.pone.0152054

29. Lindström J, Tuomilehto J. The Diabetes Risk Score: A Practical Tool to Predict Type 2 Diabetes Risk. *Diabetes Care* (2003) 26(3):725–31. doi: 10.2337/diacare.26.3.725
30. Salinero-Fort M, de Burgos-Lunar C, Mostaza Prieto J, Lahoz Rallo C, Abánades-Herranz JC, Gómez-Campelo P, et al. Validating Prediction Scales of Type 2 Diabetes Mellitus in Spain: The SPREDIA-2 Population-Based Prospective Cohort Study Protocol. *BMJ Open* (2015) 5(7):e007195. doi: 10.1136/bmjopen-2014-007195
31. Franciosi M, De Berardis G, Rossi MCE, Sacco M, Belfiglio M, Pellegrini F, et al. Use of the Diabetes Risk Score for Opportunistic Screening of Undiagnosed Diabetes and Impaired Glucose Tolerance: The IGLOO (Impaired Glucose Tolerance and Long-Term Outcomes Observational) Study. *Diabetes Care* (2005) 28(5):1187–94. doi: 10.2337/diacare.28.5.1187
32. Hellgren MI, Petzold M, Björkelund C, Wedel H, Jansson P-A, Lindblad U. Feasibility of the FINDRISC Questionnaire to Identify Individuals With Impaired Glucose Tolerance in Swedish Primary Care. A Cross-Sectional Population-Based Study. *Diabetic Med* (2012) 29(12):1501–5. doi: 10.1111/j.1464-5491.2012.03664.x
33. Makrilakis K, Liatis S, Grammatikou S, Perrea D, Stathi C, Tsiligros P, et al. Validation of the Finnish Diabetes Risk Score (FINDRISC) Questionnaire for Screening for Undiagnosed Type 2 Diabetes, Dysglycaemia and the Metabolic Syndrome in Greece. *Diabetes Metab* (2011) 37(2):144–51. doi: 10.1016/j.diabet.2010.09.006
34. Soriguer F, Valdés S, Tapia MJ, Esteva I, Ruiz de Adana MS, Almaraz MC, et al. Validación Del FINDRISC (FINnish Diabetes Risk SCore) Para La Predicción Del Riesgo De Diabetes Tipo 2 En Una Población Del Sur De España. Estudio Pizarra. *Med Clín* (2012) 138(9):371–6. doi: 10.1016/j.medcli.2011.05.025
35. Dugee O, Janchiv O, Jousilahti P, Sakhiya A, Palam E, Nuorti JP, et al. Adapting Existing Diabetes Risk Scores for an Asian Population: A Risk Score for Detecting Undiagnosed Diabetes in the Mongolian Population. *BMC Public Health* (2015) 15(1):938. doi: 10.1186/s12889-015-2298-9
36. Ku GMV, Kegels G. The Performance of the Finnish Diabetes Risk Score, a Modified Finnish Diabetes Risk Score and a Simplified Finnish Diabetes Risk Score in Community-Based Cross-Sectional Screening of Undiagnosed Type 2 Diabetes in the Philippines. *Prim Care Diabetes* (2013) 7(4):249–59. doi: 10.1016/j.pcd.2013.07.004
37. Lim HM, Chia YC, Koay ZL. Performance of the Finnish Diabetes Risk Score (FINDRISC) and Modified Asian FINDRISC (ModAsian FINDRISC) for Screening of Undiagnosed Type 2 Diabetes Mellitus and Dysglycaemia in Primary Care. *Prim Care Diabetes* (2020) 14(5):494–500. doi: 10.1016/j.pcd.2020.02.008
38. Pawar SD, Naik JD, Prabhu P, Jatti GM, Jadhav SB, Radhe BK. Comparative Evaluation of Indian Diabetes Risk Score and Finnish Diabetes Risk Score for Predicting Risk of Diabetes Mellitus Type II: A Teaching Hospital-Based Survey in Maharashtra. *J Family Med Prim Care* (2017) 6(1):120–5. doi: 10.4103/2249-4863.214957
39. Saaristo T, Peltonen M, Lindström J, Saarikoski L, Sundvall J, Eriksson JG, et al. Cross-Sectional Evaluation of the Finnish Diabetes Risk Score: A Tool to Identify Undetected Type 2 Diabetes, Abnormal Glucose Tolerance and Metabolic Syndrome. *Diabetes Vasc Dis Res* (2005) 2(2):67–72. doi: 10.3132/dvdr.2005.011
40. Getaneh A, Andres R, Brillon DJ, Findley SE. Hemoglobin A1C Criterion for Diabetes Diagnosis Among Hispanic and Non-Hispanic Populations. *Endocr Pract* (2011) 17(2):210–7. doi: 10.4158/EP10119.OR
41. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. *Biomark Insights* (2016) 11:95–104. doi: 10.4137/BMI.S38440
42. Bennett CM, Guo M, Dharmage SC. HbA1c as a Screening Tool for Detection of Type 2 Diabetes: A Systematic Review. *Diabetic Med* (2007) 24(4):333–43. doi: 10.1111/j.1464-5491.2007.02106.x
43. Gomez-Arbelaez D, Alvarado-Jurado L, Ayala-Castillo M, Forero-Naranjo L, Camacho PA, Lopez-Jaramillo P. Evaluation of the Finnish Diabetes Risk Score to Predict Type 2 Diabetes Mellitus in a Colombian Population: A Longitudinal Observational Study. *World J Diabetes* (2015) 6(17):1337–44. doi: 10.4239/wjd.v6.i17.1337
44. Salinero-Fort MA, Burgos-Lunar C, Lahoz C, Mostaza JM, Abánades-Herranz JC, Laguna-Cuesta F, et al. Performance of the Finnish Diabetes Risk Score and a Simplified Finnish Diabetes Risk Score in a Community-Based, Cross-Sectional Programme for Screening of Undiagnosed Type 2 Diabetes Mellitus and Dysglycaemia in Madrid, Spain: The SPREDIA-2 Study. *PloS One* (2016) 11(7):e0158489. doi: 10.1371/journal.pone.0158489
45. Zhang L, Zhang Z, Zhang Y, Hu G, Chen L. Evaluation of Finnish Diabetes Risk Score in Screening Undiagnosed Diabetes and Prediabetes Among U.S. Adults by Gender and Race: NHANES 1999-2010. *PloS One* (2014) 9(5):e97865. doi: 10.1371/journal.pone.0097865
46. Costa B, Barrio F, Piñol JL, Cabré JJ, Mundet X, Sagarra R, et al. Shifting From Glucose Diagnosis to the New HbA1c Diagnosis Reduces the Capability of the Finnish Diabetes Risk Score (FINDRISC) to Screen for Glucose Abnormalities Within a Real-Life Primary Healthcare Preventive Strategy. *BMC Med* (2013) 11:45–. doi: 10.1186/1741-7015-11-45
47. Witte DR, Shipley MJ, Marmot MG, Brunner EJ. Performance of Existing Risk Scores in Screening for Undiagnosed Diabetes: An External Validation Study. *Diabetic Med* (2010) 27(1):46–53. doi: 10.1111/j.1464-5491.2009.02891.x

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