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The role of diabetes in the pathophysiology and prognosis of ischemic stroke

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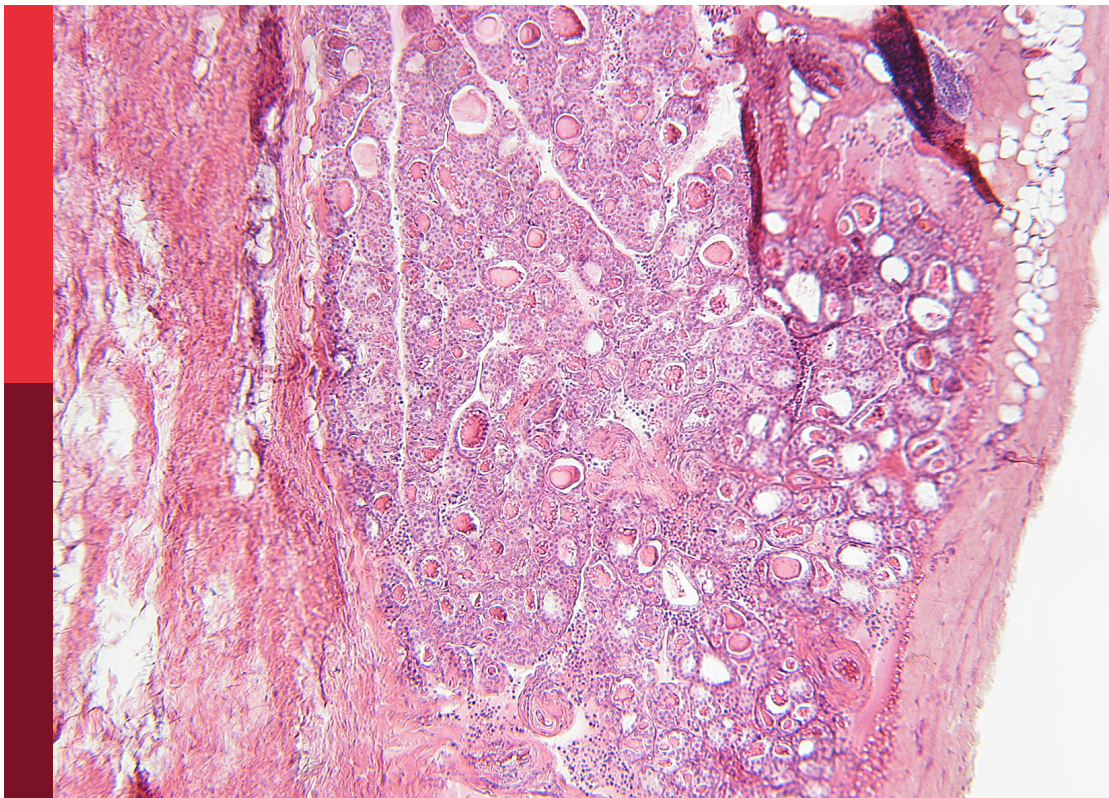

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The role of diabetes in the pathophysiology and prognosis of ischemic stroke

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Editorial: the role of diabetes in the pathophysiology and prognosis of ischemic stroke

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KEYWORDS

ischemic stroke, diabetes mellitus, insulin resistance, TyG index, reperfusion therapy

Editorial on the Research Topic
The role of diabetes in the pathophysiology and prognosis of ischemic stroke

Diabetes mellitus is a disease of abnormal carbohydrate metabolism characterized by hyperglycemia. It is associated with a relative or absolute impairment in insulin secretion, along with varying degrees of peripheral resistance to the action of insulin. Diabetes is estimated to affect 537 million adults worldwide, with a global prevalence of 10.5% among adults aged 20 to 79 years (1). Diabetes is one of the most serious and common chronic diseases of our times, causing life threatening disabling complications. Among these complications, stroke is most well-recognized and most common. Stroke is a disease with high prevalence, high disability, high mortality, and high recurrence rate. The lifetime risk of stroke for adult men and women is approximately 25% (2). Worldwide, stroke is the second most common cause of mortality and the second most common cause of disability (3). Diabetes affects 33% of patients with ischemic stroke, and 26% of those with hemorrhagic stroke (4). The Emerging Risk Factors Collaboration meta-analysis of 102 prospective studies with 8.5 million person-years of follow-up demonstrated that diabetes increased ischemic stroke 2.27-fold (5). Diabetes not only affects the onset of stroke, but also is related to the prognosis of stroke outcome. Diabetes double the risk of stroke recurrence, and increases the risk of death or disability post ischemic stroke (6). Diabetic individuals after stroke have a 25% induction in favorable outcome, such as being able to function independently in activities of daily life (7). Furthermore, diabetes has been reported to be associated with increased risk of developing cognitive impairment and dementia after stroke by 2.56-fold (8). Diabetes itself increases the production of reactive oxygen species, promotes the proinflammatory processes. These are the considered mechanisms of accelerated arthrosclerosis and increased risk of thrombus formation which finally lead the onset of ischemic stroke (7, 9). Therefore, in this special issue 'The role of diabetes in pathophysiology and prognosis of ischemic stroke' in Frontiers in Endocrinology, we focused on the roles of etiology, pathology, treatment, prognosis of stroke.

Ding et al. explored the association between insulin resistance (IR) and the mechanisms of brain injury after ischemic stroke, which revealed that IR was not a feature of diabetes, but also was essential in the development and progression of ischemic stroke. On one hand,

IR added to the risk of embolism by causing endothelial dysfunction and platelet hyperactivation. On the other hand, IR promoted atherosclerosis through inflammatory process, vascular smooth muscle cells proliferation, dyslipidemia, and hypertension. In the clinical practice, there were several well-recognized criteria for evaluating IR, such as homeostasis model assessment of IR (HOMA-IR), oral glucose tolerance tests (OGTT), C-peptide release test, triglyceride glucose (TyG) index, and metabolic score for insulin resistance (METS-IR). And each of them has their own merits and demerits. In recent years, TyG index has been studied as a novel and convenient tool for evaluating atherosclerosis of cardiovascular diseases. In a retrospective observational cohort study of 5593134 persons older than 40 years during a follow-up of 8.2 years, higher TyG was proved to increase the risk of stroke and myocardial infarction by 1.1259- and 1.282-fold (10). In the current issue, Tang et al. demonstrated in a cross-sectional study in southeast China which included 4499 participants aged ≥40 years, that higher TyG index was an independent predictor of the presence of plaque in the carotid artery among the high-stroke-risk population. But there was no correlation between TyG and increased intima-media thickness or carotid artery stenosis. METS-IR is a novel score to screen of evaluate cardiometabolic risk and could be used to screen for early IR as relative to other markers (11). Cai et al. reported that in a retrospective cohort study which included 14032 hospitalized patients, METS-IR was associated with risk of stroke (HR, 1.80; 95CI%, 1.50-2.17) and ischemic stroke (HR, 1.96; 95% CI, 1.60-2.42). Furthermore, circulating microRNAs (miRNAs) have emerged as potential biomarkers in stroke. The downregulation of miRNAs might promote expression of its target genes and their corresponding protein-associated pathways. And miRNA upregulation inhibits expression and the function of target genes and their encoded proteins. Toor et al. specifically emphasized the circulating miRNA profiling of ischemic stroke patients with or without type 2 diabetes. They discovered that Has-miR-361 -3p and -664a-5p were downregulated, whereas miR-423-3p, -140-5p, and -17-3p were upregulated.

Apart from the association of diabetes and incidence of stroke, diabetes is closely correlated to the outcome of stroke. Reperfusion therapy including thrombolysis with intravenous alteplase Tenecteplase, or mechanical thrombectomy for major artery occlusion significantly improves the prognosis of stroke patients (12, 13). Stress hyperglycemia ratio (SHR) defined as defined as the stress fasting glycemia/HbA1c ratio, is a quantitative tool for stress hyperglycemia. Previous studies suggested that high SHR was associated with early neurological deterioration (END) and poor outcome in patients treated with intravenous thrombolysis (14) and increased the risk of symptomatic intracranial hemorrhage and mortality after endovascular thrombectomy (15). In the current issue, Dai et al. demonstrated that SHR increased the incidence of END (OR, 5.77; 95%CI, 1.878-17.742) and decreased the likelihood of favorable outcome (OR, 1.96, 95%CI, 0.077-0.502) in stroke patients with endovascular thrombectomy. Gu et al. from the PostErior circulation iSchemIc Stroke (PERSIST) registry revealed that, for patients with vertebral artery occlusion receiving endovascular treatment, not only SHR, but also admission hyperglycemia, fasting blood glucose (FBG) are predictive risk factor for symptomatic intracranial hemorrhage, poor functional outcomes at 90 days and at 1 year. The effect of these perioperative glycemic indicators was significant in both the general population and the non-diabetic subgroup, but not in the diabetic subgroup. In 955 patients from Nanjing Stroke Registry Program, Wang et al. suggested that elevated TyG index was associated with stroke recurrence within 1 year follow-up (HR, 2.077; 95% CI, 1.158-3.711).

Together, in the Research Topic, we reveal that the status of hyperglycemia, or insulin resistance, increases the risk of atherosclerosis and ischemic stroke, and leads to worse outcome for stroke patients even though reperfusion therapy is applied.

Author contributions

JZ for manuscript preparation, YJ for revising and final approval.

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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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Triglyceride-glucose index and stroke recurrence in elderly patients with ischemic stroke

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Background and purpose: This study aimed to explore the association between triglyceride–glucose (TyG) index and stroke recurrence in elderly patients with ischemic stroke in China.

Methods: We enrolled ischemic stroke patients aged ≥ 65 years from the Nanjing Stroke Registry Program. The primary endpoint was defined as recurrent stroke within one year after the index stroke. We used multivariable Cox proportional hazards regression models to investigate the association between TyG index and stroke recurrence. We assessed the discriminative ability of TyG index with the receiver operative characteristic and the area under the curve.

Results: A total of 955 patients (median age, 70.0 [67.0, 75.0]; male sex, 67.2%) from the Nanjing Stroke Registry Program were enrolled. During one year follow-up, 97 (10.2%) elderly patients experienced stroke recurrence. In multivariable analyses, the association between TyG index and stroke recurrence remained significant after adjusting for confounders (quartile 4 versus quartile 1; hazard ratio, 2.073, 95% confidence interval, 1.158-3.711; P = 0.014). The restricted cubic spline showed an increasing trend for TyG index and stroke recurrence (P for non-linearity = 0.072). The area under the curve to predict stroke recurrence with TyG index was 0.719 (95% confidence interval, 0.666-0.772). Besides, TyG index slightly improved the prediction for stroke recurrence.

Conclusion: Elevated TyG index was associated with stroke recurrence in elderly patients with ischemic stroke. Further studies are warranted to assess the role of TyG index in the development of stroke recurrence in the elderly.

KEYWORDS

TyG index, ischemic, stroke, elderly, recurrence

Introduction

Stroke is a leading course of mortality and disability worldwide (1). Due to multiple risk factors, ischemic stroke increases with advancing age and is associated with poor prognosis in elderly patients (2). Prior studies reported that over 75% of strokes occurred in the elderly and added a heavy economic burden (3). Furthermore, elderly patients have an increased risk of vascular events compared with younger adults (4). With a life expectancy of a least five years ahead, it is warranted to identify elderly patients with higher risk of stroke recurrence (5).

Insulin resistance (IR) is a pathological state caused by increased insulin sensitivity and the precursor of diabetes mellitus (6). Previous studies revealed that IR promotes stroke progression and is associated with poor prognoses (7–9). The golden standard measurement for IR, hyperinsulinemic-euglycemic clamp, is not commonly used in clinical practice due to the cost and complexity (10). Notably, triglyceride–glucose (TyG) index, which is derived from fasting blood glucose and triglyceride, is a convenient surrogate marker of IR (11). Prior studies suggested that TyG index is associated with nonalcoholic fatty liver disease (12), acute coronary syndrome (13), and hyperuricemia (14) in elderly patients. However, few studies have investigated the association between TyG index and the risk of stroke recurrence in elderly patients with ischemic stroke.

Hence, we conducted this study to explore the potential role of TyG index in elderly patients with ischemic stroke.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Participants

From January 1 2013 to October 31, 2016, patients with ischemic stroke were continuously enrolled from the Nanjing Stroke Registry Program (15). This study was approved by the ethics review board of Jinling Hospital. All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Due to the retrospective nature of this study, patient consent was waived.

Patients were included according to the following criterion: (1) diagnosed as ischemic stroke within 14 days of onset, (2) aged ≥ 65 years old, (3) examined with a brain computed tomography or magnetic resonance imaging right before or during admission, (4) finished at least one year follow up or deceased before then. Patients were excluded if they (1) had recurrent events within the first 21days, (2) had missing fasting blood glucose and triglyceride values.

Baseline characteristics

Demographic data, medical history, laboratory data, imaging data, and medications at discharge were all recorded. Stroke severity was assessed with the National Institute of Health Stroke Scale score (16). Stroke subtypes were classified according to the trial of ORG 10172 in Acute Stroke Treatment classification as large-artery atherosclerosis, cardio-embolism, small vessel occlusion, and others (stroke of other determined etiology and stroke of undetermined etiology) (17). Annual family incomes (1 USD = 7.18 RMB; RMB, Chinese currency) and educational years were acquired with the face-to-face questionnaire. Smoking status was classified as non-smokers, former smokers, and current smokers according to the consumption of cigarettes (18). Fasting blood samples were collected within 24 hours after admission. TyG index was calculated as ln [triglyceride (mg/dL) × fasting blood glucose (mg/dL)/2] (19).

Follow-up and endpoints

The follow-up schedule for each patient was three, six, and twelve months and annually after the discharge. Stroke recurrence was defined as a new neurological deficit or a sudden deterioration of a previous deficit that fits the criterion of ischemic or hemorrhagic stroke, which was confirmed by clinical manifestation, neuroimaging results, death certificates, or other available data at each follow-up. The endpoint was defined as fatal or nonfatal recurrent stroke within one year of the index stroke (15).

Statistical analysis

Continuous parameters presented as mean ± SD or median (interquartile range) were compared using Student t test or Mann-Whitney U test as appropriate. Categorized parameters presented as n (%) were compared with $\chi 2$ test or Fisher exact test. Comparison of multiple values between subgroups was compared with trend tests, one-way analysis of variance, or Kruskal-Wallis H test as appropriate. Multiple imputation method with chain equations was performed to deal with missing values.

We performed univariable Cox proportional hazards regression models to explore the association between baseline characteristics and the risk of stroke recurrence. To assess the

association between TyG index and stroke recurrence, we adjusted model 1 with age and sex. Model 2 was further adjusted for hypertension, diabetes mellitus, smoking status, drinking, coronary heart disease, atrial fibrillation, antiplatelet drug, anticoagulant, statin, antihypertensive drug and hypoglycemic agent. Model 3 was adjusted for variables with the significance level of $P < 0.1$ in the univariable analysis with the back-ward selection method except for fasting blood glucose and triglyceride which were included in TyG index. We found no violations of the proportional-hazards assumption with the Schoenfeld residuals test. We also performed the competing risk analysis by accounting for the competing risk of death with the Fine and Gray method.

We explored the pattern of the association between TyG index and stroke recurrence risk with the restricted cubic spline with four knots (at 5th, 35th, 65th, and 95th percentiles) adjusted for the variables finally included in the model 3 (20). The discriminative ability of TyG index was assessed with the receiver operative characteristic and the corresponding area under the curve. Besides, we used the net improvement index (NRI) and integrated discrimination improvement (IDI) to assess the improvement of the model performance after adding TyG index into models (21).

All statistical tests were conducted with R statistical software version 4.1.0. (R Foundation, Vienna, Austria) and a two-sided P value < 0.05 was considered to be statistically significant.

Results

Baseline characteristics

A total of 955 elderly patients (median age, 70.0 [67.0, 75.0]; male sex, 67.2%) with ischemic stroke were included in the present study after excluding 63 patients without fasting blood glucose and triglyceride values and 37 patients without follow-up information or experienced recurrence within first 21 days. Patients with stroke recurrence had lower levels of high density lipoprotein ($P = 0.005$), higher levels of homocysteine ($P = 0.002$), fasting blood glucose ($P < 0.001$), systolic blood pressure ($P = 0.015$), and TyG index ($P = 0.006$), higher proportions of large-artery atherosclerosis and cardio-embolism ($P = 0.029$), lower annal family income ($P = 0.039$, Table 1. Across different quartiles of TyG index, patients with higher TyG index had higher levels of body mass index ($P < 0.001$), fasting blood glucose ($P < 0.001$) and blood urea nitrogen ($P = 0.044$), higher proportions of hypertension ($P = 0.001$), antihypertensive drug ($P < 0.001$), diabetes mellitus ($P < 0.001$), hypoglycemic agent ($P = 0.016$), dyslipidemia ($P < 0.001$), smoking ($P = 0.001$) and stroke recurrence ($P = 0.017$, Supplementary Table 1; $P = 0.036$, Figure 1), and lower proportion of male sex ($P < 0.001$, Supplementary Table 1).

Association between TyG index and stroke recurrence

During one year follow-up, 97 (10.2%) patients experienced stroke recurrence, which included 79 (81.4%) ischemic and 18 (18.6%) hemorrhagic recurrent stroke, and 164 (17.1%) patients died. Univariable analysis revealed that age (hazard ratio [HR], 1.041; 95% confidence interval [CI], 1.004-1.078; $P = 0.029$), triglyceride (HR, 1.263; 95% CI, 1.133-1.408, $P <0.001$), high density lipoprotein (HR, 0.306; 95% CI, 0.129-0.724, $P = 0.007$), homocysteine (HR, 1.024; 95% CI, 1.014-1.035, $P < 0.001$), fasting blood glucose (HR, 1.133; 95% CI, 1.067-1.203, $P < 0.001$), creatine (HR, 1.003; 95% CI, 1.000-1.007, $P = 0.041$), TyG index (HR, 1.861; 95% CI, 1.400-2.475, $P < 0.001$), TOAST (small-vessel occlusion versus large-artery atherosclerosis; HR, 0.302; 95% CI, 0.121-0.758, $P = 0.011$) and annual family income (>15021 versus 1-1502; HR, 0.259; 95% CI, 0.078-0.866, $P = 0.028$; Supplementary Table 2).

The ability of the TyG index to predict stroke recurrence

In multivariable analyses, the association between TyG index and stroke recurrence remained significant after adjusting for confounders in model 1 (quartile 4 versus quartile 1; HR, 2.220, 95% CI, 1.251-3.940; $P = 0.006$), model 2 (quartile 4 versus quartile 1; HR, 2.229, 95% CI, 1.153-4.309; $P = 0.017$), and model 3 (quartile 4 versus quartile 1; HR, 2.073, 95% CI, 1.158-3.711; $P = 0.014$; Figure 2). The restricted cubic spline showed an increasing trend for TyG index and stroke recurrence (P for non-linearity = 0.072, Supplementary Figure 1) after adjusting for covariables in the model 3. The area under the curve to predict stroke recurrence with TyG index was 0.719 (95% CI, 0.666-0.772, Figure 3). The association remained significant in the competing risk analysis accounting for the risk of death (Supplementary Table 3). Furthermore, adding TyG index into model 3 slightly improved the prediction of stroke recurrence (NRI (continuous), 0.142; 95% CI, 0.026-0.264, $P = 0.020$; NRI (categorical) 0.142; 95% CI, -0.032-0.260; $P = 0.056$; IDI, 0.028; 95% CI, 0.010-0.045; $P = 0.002$; Table 2).

Discussion

In this study, we found that TyG index was associated with stroke recurrence in elderly patients with ischemic stroke. Besides, TyG index slightly improved the prediction for stroke recurrence. These results indicated that TyG index was a surrogate marker and might help identify patients with an increased risk of stroke recurrence in the elderly.

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TABLE 1 Baseline Characteristics between Patients with or without Stroke Recurrence in the Elderly.

Characteristics	Without recurrence (N = 858)	Recurrence (N = 97)	P value
Age, years	70 [67, 75]	71 [67, 77]	0.17
Male, n (%)	576 (67.1)	66 (68.0)	0.947
BMI, kg/m (2)	24.2 [22.3, 26.3]	24.8 [22.2, 26.4]	0.517
Systolic blood pressure, mmHg	143 [130, 160]	149 [134, 170]	0.015
Diastolic blood pressure, mmHg	83 [76, 90]	87 [79, 90]	0.103
Hypertension, n (%)	683 (79.6)	81 (83.5)	0.437
Diabetes mellitus, n (%)	287 (33.4)	37 (38.1)	0.417
Dyslipidemia, n (%)	201 (23.4)	28 (28.9)	0.287
Atrial fibrillation, n (%)	120 (14.0)	15 (15.5)	0.809
Coronary heart disease, n (%)	107 (12.5)	11 (11.3)	0.874
Drinking, n (%)	158 (18.4)	15 (15.5)	0.564
Smoking, n (%)			0.804
Nonsmokers	413 (48.1)	44 (45.4)	
Former smokers	112 (13.1)	12 (12.4)	
Current smokers	333 (38.8)	41 (42.3)	
NIHSS, score	3 [1, 7]	4 [2, 8]	0.086
Laboratory results			
Total cholesterol (mmol/L)	4.1 [3.5, 4.8]	4.3 [3.4, 5.0]	0.399
Triglyceride (mmol/L)	1.3 [1.0, 1.7]	1.4 [1.0, 1.9]	0.054
Low density lipoprotein (mmol/L)	2.5 [1.9, 3.0]	2.6 [1.8, 3.1]	0.677
High density lipoprotein (mmol/L)	1.0 [0.9, 1.2]	1.0 [0.8, 1.1]	0.005
Homocysteine (mmol/L)	14.7 [10.7, 19.2]	16.8 [12.6, 20.4]	0.002
Fasting blood glucose (mmol/L)	5.2 [4.6, 6.3]	5.6 [5.0, 7.7]	<0.001
Creatine (μmmol/L)	67.0 [56.0, 80.0]	70.0 [57.0, 86.0]	0.102
Blood urea nitrogen (mmol/L)	5.3 [4.5, 6.6]	5.6 [4.8, 6.8]	0.115
Uric acid, μmol/L	326 [259, 393]	331 [277, 402]	0.577
TyG	8.6 [8.3, 9.0]	8.8 [8.3, 9.3]	0.006
TOAST, n (%)			0.029
LAA	401 (46.7)	50 (51.5)	
CE	100 (11.7)	16 (16.5)	
SVD	137 (16.0)	5 (5.2)	
Others	220 (25.6)	26 (26.8)	
Education, years, n (%)			0.869
0-6	361 (42.1)	41 (42.3)	
06-9	298 (34.7)	31 (32.0)	
09-12	114 (13.3)	13 (13.4)	
>12	85 (9.9)	12 (12.4)	
Annual family Income, $, n (%)			0.039
1-1502	153 (17.8)	22 (22.7)	
1502-4506	160 (18.6)	22 (22.7)	
4506-7510	218 (25.4)	31 (32.0)	
7510-15021	241 (28.1)	19 (19.6)	
>15021	86 (10.0)	3 (3.1)	
Medication at discharge, n (%)			
Antiplatelet drug	790 (92.1)	94 (96.9)	0.13
Anticoagulant	53 (6.2)	1 (1.0)	0.065
Statin	814 (94.9)	90 (92.8)	0.529
Antihypertensive drug	462 (53.8)	53 (54.6)	0.967
Hypoglycemic agent	256 (29.8)	36 (37.1)	0.174

BMI, body mass index; CE, cardio-embolism; LAA, large-artery atherosclerosis; NIHSS, National Institute of Health Stroke Scale; SAA, small-vessel occlusion; TyG, triglyceride-glucose index.

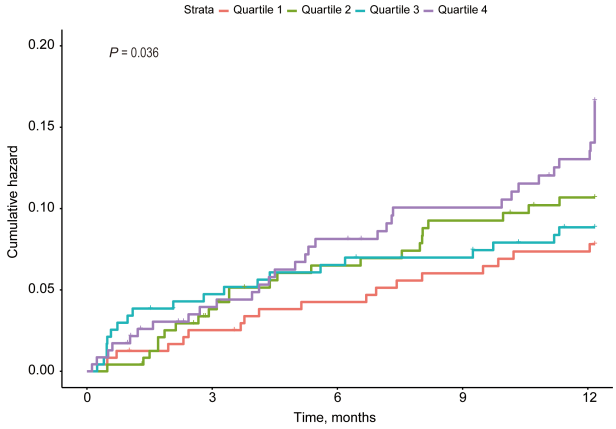

FIGURE 1
Cumulative Hazard Curves of Stroke Recurrence by TyG index.

Stroke recurrence is common and could cause cumulative disability and cognitive impairment (22). Despite the promotion of secondary preventive drugs and the decreasing trend of the recurrence incidence, approximately 11% of patients still experience recurrence in the early stage after the index stroke (4, 23). Age is the risk factor for stroke recurrence. The incidence of stroke recurrence in the elderly varies from 7.7% to 13.1% due to different exclusion criterion (24, 25). The cumulative incidence in our study (10.2%) was similar to previous reports. Prior studies indicated that elderly patients had markedly higher risks of stroke recurrence than other age groups (23, 26). Besides, elderly patients might have less-effective treatment and poorer outcomes than younger adults (3). Accurate identification of patients at high risk of stroke recurrence in elderly patients is important to deliver effective secondary prevention and reduce the recurrence risk.

IR is a syndrome linked to metabolic disorders, such as diabetes mellitus, hypertension, obesity, and lipid disorder (27). Subsequently, these disorders are important risk factors for stroke recurrence (28). Previous studies reported that IR played an important role in the development of coronary heart disease, stroke, and cognitive dysfunction, few studies had explored the potential role of IR in the prognosis of ischemic stroke in the elderly (29). TyG index is the combination of fasting glucose and triglyceride and a reliable surrogate marker of IR. In recent years, TyG index has been suggested to assess IR in clinical

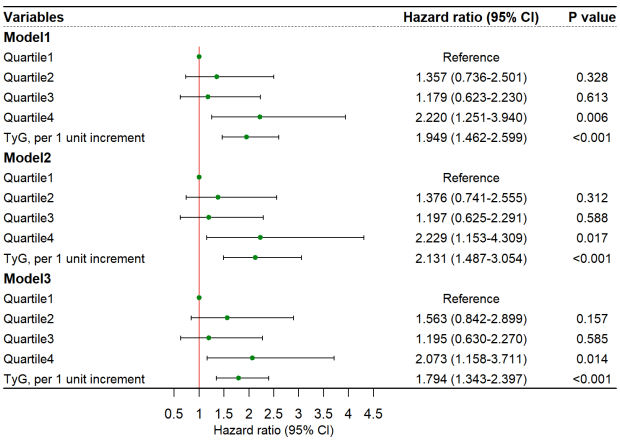

FIGURE 2
Risk of stroke recurrence in elderly patients for TyG index. CI, confidence interval, TyG, triglyceride-glucose index. Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, hypertension, diabetes mellitus, smoking status, drinking, coronary heart disease, atrial fibrillation, antiplatelet drug, anticoagulant, statin, antihypertensive drug and hypoglycemic agent. Model 3: adjusted for variables included in the back-ward selection method: age, high density lipoprotein, homocysteine, annual family income, anticoagulants and stroke subtypes.

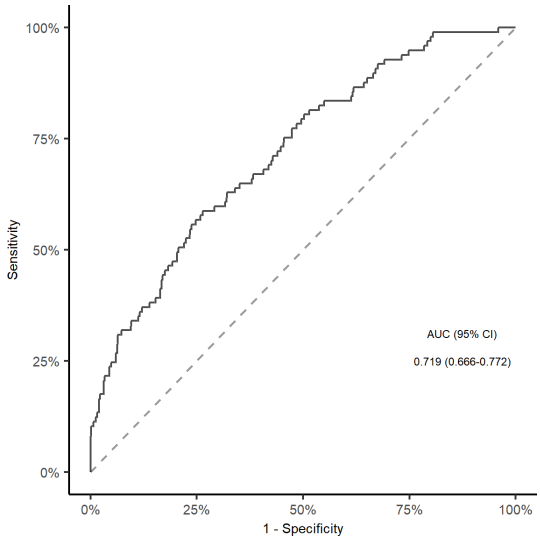

FIGURE 3
ROC curve and corresponding AUC for TyG index to predict Stroke recurrence in elderly patients. ROC, receiver operative characteristic; AUC, area under the curve; TyG, triglyceride-glucose index.

practice rather than the golden standard measurement, the hyperinsulinaemic-euglycaemic clamp test, due to the convenience and reliability (19).

Prior studies showed that TyG index was associated with ischemic stroke. A community-based cohort showed that elevated levels of TyG index could independently predict ischemic stroke during an 11-year follow-up in the general population regardless of the sampling time (9). Guo et al. explored the relationship between TyG index and platelet reactivity in patients with acute ischemic stroke and found that elevated TyG index could enhance platelet reactivity. Nam et al. found that TyG index was associated with early recurrent ischemic lesions in a small sample of patients with acute ischemic stroke (30). Unfortunately, studies focusing on the elderly population were limited. A longitudinal study performed among the elderly showed that TyG index had a superior discriminative ability for the occurrence of hypertension over lipid parameters (31). The Northern Shanghai Study revealed that TyG index was associated with macro- and microvascular damage in elderly individuals (32). In our study, we found that TyG index was associated with stroke recurrence and traditional risk factors such as hypertension and diabetes mellitus in the elderly. TyG index might help identify high risk patients who might benefit from interventions for IR including weight control, physical activity and healthy diets.

The mechanism underlying the association between TyG index and stroke recurrence might be explained as follows. First, IR could affect platelet adhesion, aggregation, and activation and was associated with artery stenosis and occlusion (33, 34). Second, IR might result in chronic inflammation (35), endothelium dysfunction (36), and the formation of foam cells (37). Previous studies suggested that TyG index was related to arterial stiffness in the elderly and thus might contribute to stroke recurrence (38). Third, beyond the specific setting of diabetes, TyG index was also associated with subclinical atherosclerosis (39), coronary atherosclerosis in the general population (40), and carotid plaque stability in nondiabetic adults (41), which were important predictors of ischemic events (42). Fourth, IR might coexist with a cluster of traditional risk factors, such as hypertension, obesity, and diabetes mellitus (9), and contribute to stroke recurrence development.

TABLE 2 Reclassification Indexes for Regression Models.

Indexes	Estimate (95% CI)	*P* value
NRI (continuous)	0.142 (0.026-0.264)	0.02
NRI (categorical)	0.142 (-0.032-0.260)	0.056
IDI	0.028 (0.010-0.045)	0.002

CI, confidence interval; IDI, integrated discrimination improvement; NRI, net reclassification improvement.

Besides, we found that homocysteine, stroke subtypes, and family income were associated with stroke recurrence in the elderly. Zhang et al. performed a prospective cohort and found that elevated homocysteine can predict stroke recurrence and mortality in patients with stroke (43). Shi et al. found that homocysteine was associated with stroke recurrence in patients with large-vessel atherosclerosis (44). Large-vessel atherosclerosis is the most common subtype in the Chinese population (45). Lange et al. found that patients with atherosclerosis in the internal carotid artery, intracranial and posterior circulation had an increased risk of stroke recurrence (46). Besides, Flach et al. reported that cardio-embolic stroke also had a higher risk of recurrence (47). Socioeconomic status was also associated with stroke recurrence. Chen et al. suggested that patients with lower socioeconomic status might have less access to acute interventions and were more disobedient to the secondary prevention treatments (48). Our results were in agreement with these previous findings.

However, our study had several limitations. First, this was a retrospective analysis of a prospective database that included patients aged ≥ 65 years, which might generate sampling bias. Second, socioeconomic status information was collected by questionnaires, which might generate information bias. Third, we selected stroke recurrence within one year as the endpoint because of the higher recurrence rate within one year and the lower rate of patients without follow-up (49), however, long-term follow-up information was still warranted in the future. Forth, limited by the study design, the time-varying change of TyG index after discharge was not provided, which might provide more information. Fifth, we lacked information about transient ischemic attacks and the patterns of adherence or persistence of medication after discharge, however, we provided medication at discharge instead. Finally, although TyG index was validated to be correlated with the hyperinsulinaemic-euglycaemic clamp test, we were unable to compare the performance of the hyperinsulinaemic-euglycaemic clamp test in our study because of the retrospective nature.

In conclusion, the results of our study showed that elevated TyG index was associated with stroke recurrence in elderly patients with ischemic stroke. Further studies are warranted to assess the role of TyG index in the development of stroke recurrence in the elderly.

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 ethics review board of Jinling Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

FW, JW, and YH contributed to conception and design of the study. FW, XS, and CH organized the database. FW and XX performed the statistical analysis. FW wrote the first draft of the manuscript. SZ and JG wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1005614/full#supplementary-material

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Effects of perioperative glycemic indicators on outcomes of endovascular treatment for vertebrobasilar artery occlusion

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Objective: Endovascular treatment (EVT) is, to date, the most promising treatment of vertebrobasilar artery occlusion (VBAO). The study aimed to determine the influence of perioperative glucose levels on clinical outcomes in patients with acute VBAO treated with EVT.

Methods: We retrospectively collected consecutive VBAO patients received EVT in 21 stroke centers in China. The associations between perioperative glycemic indicators (including fasting blood glucose[FBG], admission hyperglycemia, stress hyperglycemia ratio [SHR] and short-term glycemic variability [GV]) and various clinical outcomes were analyzed in all patients and subgroups stratified by diabetes mellitus (DM).

Results: A total of 569 patients were enrolled. Admission hyperglycemia significantly correlated with increased risk of symptomatic intracranial hemorrhage (sICH) (odds ratio [OR] 3.24, 95% confidence interval [CI]: 1.40-7.46), poor functional outcomes at 90 days (OR 1.91, 95%CI: 1.15-3.18) and 1 year (OR 1.96, 95%CI: 1.20-3.22). Similar significant correlations exist between FBG, SHR, GV and all the adverse outcomes except higher levels GV was not associated with increased risk of sICH (OR 1.04, 95% CI: 0.97-1.12). Subgroup analyses showed that admission hyperglycemia, FBG and SHR were significantly associated with adverse outcomes in non-diabetic patients, but

not in DM patients. While, GV was associated with poor functional outcomes regardless of diabetes history.

Conclusions: Admission hyperglycemia, FBG, SHR and short-term GV in VBAO patients treated with EVT were associated with adverse outcomes. The results suggested that comprehensive evaluation and appropriate management of perioperative glucose might be important for patients with VBAO and treatment with EVT.

KEYWORDS

admission blood glucose, stress hyperglycemia, glycemic variability, endovascular treatment, vertebrobasilar artery occlusions, prognosis

Introduction

Vertebrobasilar artery occlusion (VBAO) accounts for 10% to 20% of all large vessel occlusions (1). It could be devastating, resulting in severe disability and death in almost 80% of patients (2). Endovascular treatment (EVT) is currently the most effective method for recanalization of occluded large vessels. However, even if EVT treatment increased the rate of successful recanalization, the prognosis of VBAO patients has not been significantly improved (2, 3).

Many glycemic indicators, such as admission blood glucose (4), fasting blood glucose (FBG) (5) and stress hyperglycemia (6), have inconsistently been reported to be associated with adverse outcomes in patients with anterior circulation stroke (ACS) treated with EVT (7). Furthermore, the postoperative period can be extremely dangerous for patients, especially as the glycemic variability (GV) is associated with adverse clinical outcomes (8). Previous studies have suggested that admission blood glucose of patients with posterior circulation stroke (PCS) is higher than that of patients with ACS (9, 10). Considering that hyperglycemia may aggravate the oxidative stress injury induced by cerebral ischemic reperfusion injury and lead to greater neurological impairment (11, 12), we hypothesized that the prognosis of patients with PCS treated with EVT is more likely to be affected by blood glucose levels.

Using the acute PostErior ciRculation iSchemIc Stroke regisTry (PERSIST, ChiCTR2000033211) data, we aimed to investigate the comprehensive perspective of the relationships between perioperative glycemic indicators and outcomes of patients with PCS treated with EVT, and whether the impact of blood glucose on the incidence of outcomes differed between diabetic subgroups.

Methods

Patients

The PERSIST is a retrospective registered trial conducted in 21 stroke centers in China from December 2015 to December 2018, which consecutively collected patients with acute VBAO received EVT treatment. Inclusion and exclusion criteria have been described in detail in previous studies (13, 14). The inclusion criteria were as follows: 1) aged 18 years or older; 2) with acute symptomatic VBAO confirmed by imaging examination (including computed tomography angiography, magnetic resonance angiography or digital subtraction angiography); 3) treated with EVT within 24 hours (estimated occlusion to groin puncture time). The exclusion criteria were: 1) combined with anterior circulation stroke; 2) accompanied by aneurysm or arteriovenous malformation; 3) with mRS score >2 before stroke; 4) participated in any clinical trials; 4) pregnant or breastfeeding; and 5) with incomplete critical baseline data. In this analysis, we further excluded patients who had hemoglobin disorders (i.e. thalassemia), recent blood transfusion, severe hepatic disease and other factors that affected hemoglobin A1c (HbA1c) measurements.

The study was approved by the ethics committee of the First Affiliated Hospital of University of Science and Technology of China (approval number: 2020KY-40). Due to its retrospective nature, patient consent was waived.

Baseline characteristics

Baseline demographics, medical histories, stroke severity evaluated by the National Institutes of Health Stroke Scale (NIHSS) score, Glasgow Coma Scale (GCS) score, stroke etiology classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, onset patterns, treatment with intravenous thrombolysis (IVT), estimated occlusion to groin puncture time, puncture to reperfusion time and treatment profiles of EVT were retrospectively obtained by reviewing medical records. Two neuroradiologists, who were unaware of the clinical data and outcomes, retrospectively evaluated all neuroimaging data, including the posterior circulation-Alberta Stroke Program Early CT Score (pc-ASPECTS) (15), the Basilar Artery on Computed Tomography Angiography (BATMAN) score (16), and the American Society of Interventional and Therapeutic Neuroradiology/Society of Interventional Radiology (ASITN/SIR) collateral score (17). Successful recanalization was defined as modified Thrombolysis in Cerebral Infarction (mTICI) score 2b or 3.

Perioperative glucose levels and short-term GV

We collected available admission blood glucose, fasting blood glucose (FBG) within 24 hours of EVT and first-measured HbA1c. Previous diabetes mellitus (DM) was defined as a known history of DM on admission or the presence of background hyperglycemia (HbA1c ≥ 6.5% [48 mmol/mol]) (18). According to the previous studies (4), admission hyperglycemia was defined as admission blood glucose ≥ 7.8 mmol/L, regardless of the diabetic status. We calculated stress hyperglycemia ratio (SHR) with the following formula: FBG (mmol/L)/HbA1c-derived estimated average glucose, where HbA1c-derived estimated average glucose (mmol/L) = (1.59×HbA1c)-2.59 (19).

Besides, we also collected available capillary blood glucose levels during the first 36 hours after EVT. Considering the different frequency of measurement in each center, we mainly collected three specific times of each day: fasting (≥8 hours), postprandial (2 hours after the meal) and nighttime. During the monitoring period, various parameters of capillary glucose level were used to analyze the short-term GV: mean glucose level, standard deviation (SD) of the mean and coefficient of variation (CV) (8). The CV for glucose was calculated as [SD/mean glucose] × 100% (20).

Outcome assessment

Safety outcomes included symptomatic intracranial hemorrhage (sICH) and in-hospital mortality. Intracranial hemorrhage was classified according to the Heidelberg Bleeding Classification within 48 hours after EVT. SICH was defined as a new intracranial hemorrhage detected by brain imaging, which was associated with an increase by ≥ 4 points of total NIHSS, or an increase by ≥ 2 points of a NIHSS subcategory, or neurological deterioration leading to major medical/surgical intervention (21).

Functional outcomes were assessed by the mRS score at 90 days and 1 year after stroke through routine telephone interview or face-to-face visit with patients or their relatives. Poor functional outcome was defined as mRS scores of 4-6.

Statistical analysis

All statistical analyses were conducted with Stata 14.1, and a two-sided P value <0.05 was considered to be statistically significant.

Baseline characteristics, treatment profiles and outcomes were compared between patients with admission hyperglycemia and without admission hyperglycemia. Categorical variables were reported as number (percentages), and continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range [IQR]) according to normality. The percentages of categorical variables were compared using the Pearson's χ^2 tests, and continuous variables were compared using the Student's *t*-tests or the Wilcoxon rank sum test, as appropriate.

When studying the correlation between admission hyperglycemia, FBG and SHR and outcomes, we imputed 25 complete datasets with multivariate imputation by chained equations incorporating all analysis related variable. The imputation model contained 5 missing variables (GCS score, puncture to reperfusion time, admission hyperglycemia, FBG and HbA1c), of which the missing values of 3 variables are less than 10%, one variable is 10.9%, and one variable is 23.7%. We imputed continuous variables with linear regression and categorical variables with logistic regression. All estimates were obtained by averaging results across the 25 imputed datasets with inferences under multiple imputation obtained with Rubin's rules. We also performed a sensitivity analysis on patients with complete data. Considering that only some patients had capillary blood glucose monitoring and the ratio of missing value was high, we used complete data to analyze the correlation between GV and outcomes.

To ascertain the independent contribution of admission hyperglycemia, FBG and SHR to outcomes (sICH and mRS), multivariable logistic regression analyses were performed and the prespecified covariates were adjusted: age, sex, hypertension, hyperlipidemia, baseline NIHSS, GCS, TOAST classification, baseline pc-ASPECT score, collateral status, BATMAN score, treatment with IVT, time from estimated occlusion to groin

puncture, time from puncture to reperfusion, mTICI and previous DM. In exploring the association between GV and outcomes, we further adjusted for the use of tube feeding in patients.

Subgroup analyses were carried out to explore whether there are differences in the influence of blood glucose on the incidence of outcomes between diabetic subgroups. Interactions were examined by adding product terms to the logistic regression model to assess whether previous DM modified the association between glucose (admission hyperglycemia, FBG, SHR and GV) and outcomes.

In addition to the quantitative relationships, we used restricted cubic splines with 4 knots (at the 5th, 35th, 65th and 95th percentiles) to explore the nonlinear relationships between FBG, SHR and functional outcomes.

Results

Out of a total 609 consecutive VBAO patients treated with EVT, we identified 569 eligible patients (Figure I in the Data Supplement). After one year follow up, 22 patients were lost. Details on the missing data can be found in Figure II in the Data Supplement. Baseline characteristics based on the presence of admission hyperglycemia are summarized in Table 1. Compared with patients without admission hyperglycemia, patients with admission hyperglycemia were more likely to have a higher prevalence of DM ($p < 0.001$) and lower BATMAN score ($p = 0.040$). No difference in the rates of successful recanalization (mTICI score 2b or 3) was detected between the two groups (85.5% vs. 84.7%, $p = 0.806$). Patients with admission hyperglycemia also had higher rates of sICH (11.4% vs. 3.8%, $p = 0.001$), in-hospital mortality (29.1% vs. 19.2%, $p = 0.009$) and poor functional outcomes at 90 days (68.2% vs. 56.8%, $p = 0.009$) and 1 year (67.6% vs. 53.2%, $p = 0.001$, Table 1). The distribution of mRS scores at 90 days and 1 year in patients with and without admission hyperglycemia was displayed in Figure 1.

Perioperative glucose levels and outcomes

After adjustment for potential confounders, patients with admission hyperglycemia were more likely to have higher risk of sICH (odds ratio [OR] 3.24, 95% confidence interval [CI]: 1.40-7.46, $p = 0.006$, Table 2). Admission hyperglycemia was also independently associated with poor functional outcomes at 90 days and 1 year (OR 1.91, 95% CI: 1.15-3.18 for 90 days; OR 1.96, 95% CI: 1.20-3.22 for 1 year; Table 2). Multivariable analyses showed that increased FBG and SHR levels were significantly associated with worse outcomes, including sICH (OR 1.16, 95% CI: 1.05-1.30 for FBG; OR 3.53, 95% CI: 1.35-9.18 for SHR), poor functional outcomes at 90 days (OR 1.10, 95% CI: 1.02-1.20 for FBG; OR 3.06, 95% CI: 1.44-6.51 for SHR) and 1 year (OR 1.10, 95% CI: 1.02-1.19 for FBG; OR 3.04, 95% CI: 1.45-6.38 for SHR). Besides, the relationship between FBG levels and in-hospital mortality was also statistically significant (OR 1.08, 95% CI: 1.00-1.17, $p = 0.037$, Table 2).

In subgroup analysis, the correlation between perioperative glucose levels and outcomes in non-diabetic patients was similar to that in the entire patients. Nevertheless, in patients with previous DM, no significant association was observed between admission hyperglycemia, FBG, SHR levels and outcomes (Figure 2 and Figure III in the Data Supplement).

For outcome variables, such as sICH (p for interaction = 0.039), poor functional outcomes at 90 days (p for interaction = 0.008) and 1 year (p for interaction = 0.004), the interaction effect between previous DM and FBG was significant, indicating that the influence of FBG levels on these adverse outcomes in non-diabetic patients is greater than that in DM patients (Figure 2 and Figure III in the Data Supplement). No evidence of interaction effects between admission hyperglycemia or SHR and previous DM was found regardless of the outcome variables. The same results were obtained by sensitivity analyses of patients with complete data (Table I in the Data Supplement).

We observed a J-shaped association between SHR and the risk of poor functional outcomes at 90 days in multiple-adjusted restricted cubic spline regression, with the lowest point of SHR of 0.90, although not statistically significant (p for nonlinearity = 0.103, Figure 3A). There was also a nonlinear correlation between SHR and poor functional outcome at 1 year (p for nonlinearity = 0.010), showing a similar J-shaped curve (Figure 3B). That is, with the increase of SHR, the risk of poor functional outcome at 1 year first decreased and then increased. Although it can be observed that the risk of poor functional outcome increases with the increase of FBG, there is no nonlinear relationship between FBG and 90-day (p for nonlinearity = 0.056) or 1-year (p for nonlinearity = 0.132) poor functional outcomes (Figure IV in the Data Supplement).

Short-term GV and outcomes

We obtained available capillary blood glucose levels of 266 patients within 36 hours after EVT (median number of measurements 9, IQR 8-11). The median CV of all patients was 18.25% (IQR 13.23-23.16). Patients with sICH had higher CV levels than those without sICH (19.58 [18.41-21.65] vs. 17.62 [13.05-23.23], $p = 0.048$). However, after adjusting for confounding factors, there was no significant correlation between CV and sICH (OR 1.04, 95% CI: 0.97-1.12, $p = 0.275$). While, the results of multivariable analysis showed that CV was independently associated with poor functional outcomes at 90 days (OR 1.23, 95% CI: 1.14-1.33, $p < 0.001$) and 1 year (OR 1.25, 95% CI: 1.15-1.37, $p < 0.001$).

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TABLE 1 Baseline characteristics (n=507).

	Admission Hyperglycemia (n = 220)	No Admission Hyperglycemia (n = 287)	P
Age, mean ± SD, y	63.4 ± 12.1	64.0 ± 13.6	0.576
Male, n (%)	164 (74.5)	203 (70.7)	0.341
Hypertension, n (%)	158 (71.8)	183 (63.8)	0.055
Diabetes mellitus, n (%)	131 (59.5)	37 (12.9)	<0.001
Hyperlipidemia, n (%)	92 (41.8)	97 (33.8)	0.064
Previous stroke/TIA, n (%)	39 (17.7)	59 (20.6)	0.424
Coronary artery disease, n (%)	20 (9.1)	28 (9.8)	0.800
Smoking, n (%)	65 (29.5)	96 (33.4)	0.349
Baseline NIHSS, median (IQR)	22.0 (14.0-28.0)	23.0 (14.0-31.0)	0.459
Glasgow Coma Score, median (IQR), (n=506)	8 (6-11)	7 (6-12)	0.831
TOAST classification, n (%)			0.005
Large artery atherosclerosis	161 (73.2)	171 (59.2)	
Cardioembolism	35 (15.9)	67 (23.3)	
Others	24 (10.9)	50 (17.4)	
Baseline pc-ASPECT score, median (IQR)	9 (7-10)	9 (8-10)	0.163
Collateral status, n (%)			0.062
ASITN/SIR grade 0-1	173 (78.6)	214 (74.6)	
ASITN/SIR grade 2	36 (16.4)	42 (14.6)	
ASITN/SIR grade 3-4	11 (5.0)	31 (10.8)	
BATMAN score, median (IQR)	4 (3-6)	5 (3-7)	0.040
Onset patterns, n (%), (n=506)			0.139
Maximum neurological deficit from onset	97 (44.1)	143 (50.0)	
Progressive stroke	105 (47.7)	112 (39.2)	
Others	18 (8.2)	31 (10.8)	
Treatment with intravenous thrombolysis, n (%)	37 (16.8)	55 (19.2)	0.497
Time from estimated occlusion to groin puncture, median (IQR), min	332.5 (236.0-523.8)	330.0 (216.0-494.0)	0.280
Time from puncture to reperfusion, median (IQR), min, (n=476)	105.0 (76.0-165.0)	110.0 (70.0-150.0)	0.618
Type of initial application, n (%)			0.703
Stent retriever	176 (80.0)	232 (80.8)	
Aspiration	17 (7.7)	17 (5.9)	
Others	27 (12.3)	38 (13.2)	
mTICI score 2b or 3	188 (85.5)	243 (84.7)	0.806
sICH	25 (11.4)	11 (3.8)	0.001
In-hospital mortality	64 (29.1)	55 (19.2)	0.009
Poor functional outcome (mRS 4-6) at 90 days	150 (68.2)	163 (56.8)	0.009
Poor functional outcome (mRS 4-6) at 1 year (n=488)	142 (67.6)	148 (53.2)	0.001

SD, standard deviation; TIA, transient ischemic attack; NIHSS, National Institutes of Health Stroke Scale; IQR, interquartile range; TOAST, Trial of Org 10172 in Acute Stroke Treatment; pc-ASPECT score, posterior circulation-Alberta Stroke Program Early CT Score; ASITN/SIR, American Society of Interventional and Therapeutic Neuroradiology/Society of Interventional Radiology; BATMAN, Basilar Artery on Computed Tomography Angiography; mTICI, modified Thrombolysis in Cerebral Infarction; sICH, symptomatic intracranial hemorrhage; mRS, modified Rankin Scale.

The results of the subgroup analyses showed that whether in patients with or without previous DM, CV was always significantly associated with poor functional outcomes at 90 days and 1 year (Table 3). For 1-year poor functional outcome, the interaction effect between previous DM and CV was significant (p for interaction = 0.042), suggesting that CV levels had a greater impact on non-diabetic patients than DM patients.

Discussion

In this study of patients with VBAO treated with EVT, we observed the predictive significance of admission hyperglycemia, FBG and stress hyperglycemia on sICH, poor functional outcomes at 90 days and at 1 year. Furthermore, FBG was also associated with in-hospital mortality. The predictive significance

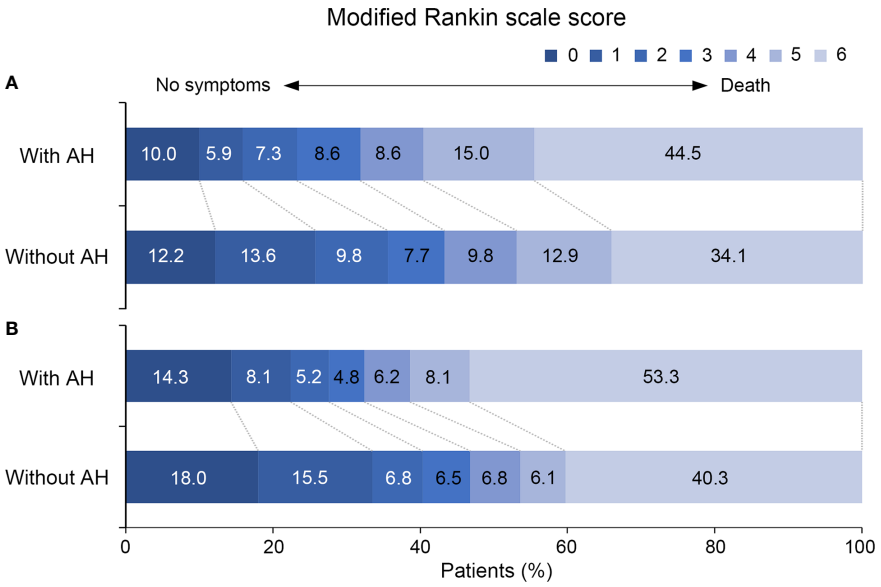

FIGURE 1
Distribution of the modified Rankin Scale Scores at **(A)** 90 days and **(B)** 1 year for patients with and without admission hyperglycemia. AH, admission hyperglycemia.

still exists in non-diabetic patients, but not in DM patients. In addition, there was a significant interaction between FBG and previous DM, indicating that FBG had a greater impact on sICH and poor functional outcomes (90 days and 1 year) of non-diabetic patients than those with previous DM. Further analysis showed that SHR was J-shaped associated with the risk of poor functional outcome at 1 year in VBAO patients treated with EVT. Our results also suggested that short-term GV after EVT was independently associated with poor functional outcomes at 90 days and 1 year, whether in patients with or without previous DM.

In our study, the median admission glucose was 7.6 mmol/L (interquartile range, 6.7-9.3), which was higher than that of patients with ACS received EVT (6.6 [5.7-7.7] mmol/L) reported in previous studies (22). In addition, the probability of previous DM (34.1% vs. 16.1%) and admission hyperglycemia (43.3% vs.

TABLE 2 The associations between perioperative glucose levels and outcomes.

Outcomes		Unadjusted model		Adjusted model*	
		OR (95%CI)	P	OR (95%CI)	P
sICH	Admission hyperglycemia	2.86 (1.39-5.88)	0.004	3.24 (1.40-7.46)	0.006
	FBG	1.12 (1.03-1.21)	0.006	1.16 (1.05-1.30)	0.006
	SHR	3.47 (1.42-8.51)	0.007	3.53 (1.35-9.18)	0.010
In-hospital mortality	Admission hyperglycemia	1.67 (1.11-2.51)	0.014	1.55 (0.93-2.60)	0.092
	FBG	1.10 (1.04-1.16)	0.001	1.08 (1.00-1.17)	0.037
	SHR	1.84 (0.97-3.49)	0.063	1.70 (0.87-3.32)	0.118
Poor functional outcome (mRS 4-6) at 90 days	Admission hyperglycemia	1.71 (1.19-2.45)	0.004	1.91 (1.15-3.18)	0.012
	FBG	1.11 (1.05-1.18)	0.001	1.10 (1.02-1.20)	0.018
	SHR	3.37 (1.85-6.13)	<0.001	3.06 (1.44-6.51)	0.004
Poor functional outcome (mRS 4-6) at 1 year (n=547)	Admission hyperglycemia	1.78 (1.24-2.55)	0.002	1.96 (1.20-3.22)	0.008
	FBG	1.11 (1.05-1.18)	0.001	1.10 (1.02-1.19)	0.016
	SHR	3.21 (1.75-5.87)	<0.001	3.04 (1.45-6.38)	0.003

*Adjusted for age, sex, hypertension, hyperlipidemia, baseline NIHSS, Glasgow Coma Score, TOAST classification, Baseline pc-ASPECT score, Collateral status, BATMAN score, treatment with intravenous thrombolysis, time from estimated occlusion to groin puncture, time from puncture to reperfusion, mTICI and DM.
OR, odds ratio; CI, confidence interval; sICH, symptomatic intracranial hemorrhage; FBG, fasting blood glucose; SHR, stress hyperglycemia ratio; mRS, modified Rankin Scale.

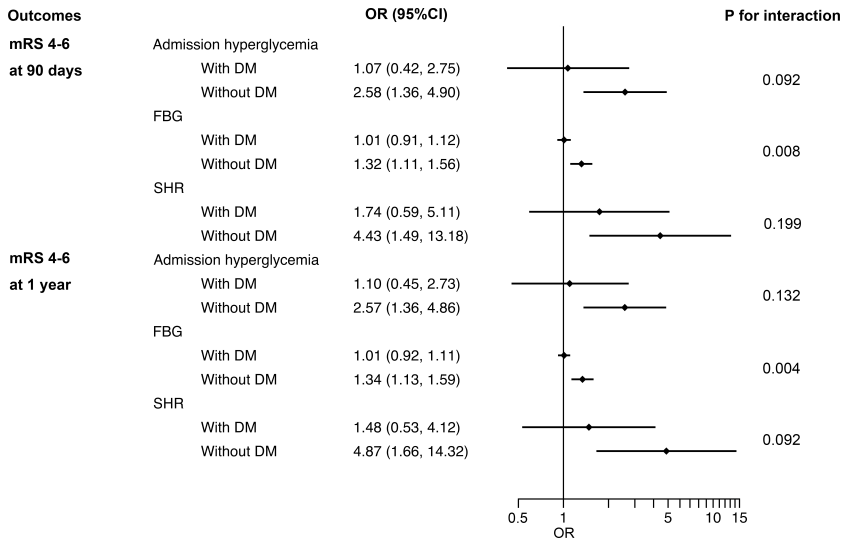

FIGURE 2
Interaction between DM and admission hyperglycemia, FBG and SHR on poor functional outcomes at 90 days and 1 year. DM, diabetes mellitus; FBG, fasting blood glucose; SHR, stress hyperglycemia ratio; OR, odds ratio; CI, confidence interval; mRS, modified Rankin Scale.

30.3%) were also higher (4). These findings are consistent with previous studies in which patients with PCS are more likely to have previous DM and higher admission blood glucose levels than patients with ACS (9, 10).

Hyperglycemia was considered to impair the efficacy of IVT by lowering recanalization rates (23). However, previous studies showed no significant correlation between admission hyperglycemia and successful reperfusion after EVT in ACS (4, 22). Consistent with this, in our study, there was also no significant difference in the rate of successful reperfusion after EVT between patients with and without hyperglycemia (85.3% vs. 84.9%), suggesting that the worse outcomes in patients with hyperglycemia after EVT may not be the consequence of reduced recanalization rate, no matter in anterior or in posterior circulation stroke.

Our results are in agreement with the findings of previous studies conducted in patients with ACS treated with EVT, showing that increased admission glucose was associated with higher risk of sICH (24), increased in-hospital mortality and worse functional outcomes at 90 days (4, 7). EVT is currently the most effective way of recanalization of the occluded vessels, which can promote oxygen re-entry into the ischemic brain (22).

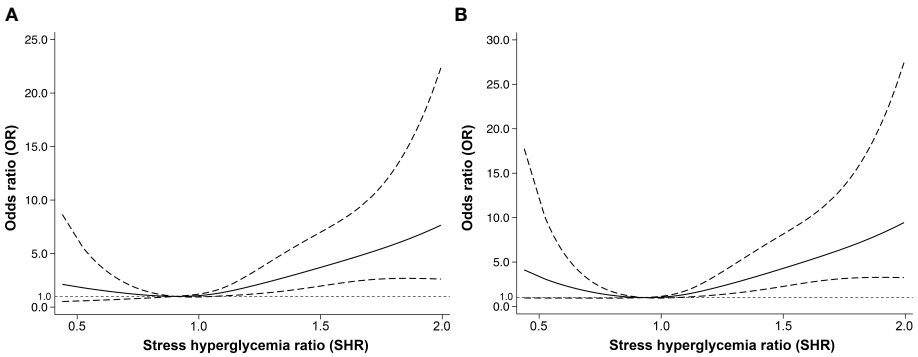

FIGURE 3
The nonlinear relationship between stress hyperglycemia ratio and adjusted odds ratios of poor functional outcomes at **(A)** 90 days and **(B)** 1 year. The nonlinear relationship was modeled by restricted cubic splines. The dashed lines indicate the 95% confidence intervals of the nonlinear solid line.

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TABLE 3 The associations between short-term GV and outcomes.

Outcomes	Subgroups	Unadjusted model		Adjusted model*		Interaction
		OR (95%CI)	P	OR (95%CI)	P	
sICH		1.04 (0.98-1.10)	0.175	1.04 (0.97-1.12)	0.275	0.567
	DM	1.05 (0.97-1.15)	0.210	1.10 (0.97-1.25)	0.140	
	Non-DM	1.03 (0.94-1.13)	0.553	1.03 (0.90-1.18)	0.678	
Poor functional outcome (mRS 4-6) at 90 days		1.16 (1.11-1.22)	<0.001	1.23 (1.14-1.33)	<0.001	0.323
	DM	1.17 (1.09-1.26)	<0.001	1.18 (1.08-1.30)	<0.001	
	Non-DM	1.23 (1.12-1.36)	<0.001	1.57 (1.20-2.05)	0.001	
Poor functional outcome (mRS 4-6) at 1 year		1.16 (1.11-1.22)	<0.001	1.25 (1.15-1.37)	<0.001	0.042
	DM	1.14 (1.07-1.22)	<0.001	1.16 (1.06-1.28)	0.002	
	Non-DM	1.30 (1.16-1.46)	<0.001	1.89 (1.30-2.74)	0.001	

*Adjusted for age, sex, hypertension, hyperlipidemia, baseline NIHSS, Glasgow Coma Score, TOAST classification, Baseline pc-ASPECT score, Collateral status, BATMAN score, treatment with intravenous thrombolysis, time from estimated occlusion to groin puncture, time from puncture to reperfusion, mTICI, DM and tube feeding.
GV, glycemic variability; OR, odds ratio; CI, confidence interval; sICH, symptomatic intracranial hemorrhage; DM, diabetes mellitus; mRS, modified Rankin Scale.

Because oxygen promotes the formation of free radicals together with glucose, patients receiving EVT may have a greater exposure to redox-mediated effects related to admission blood glucose levels (12). Hyperglycemia could increase oxidative stress, exacerbate blood-brain barrier permeability after cerebral ischemia/reperfusion injury, and increase the risk of brain edema and hemorrhage transformation (25), resulting in increased infarct volume and greater neurological deficit (26).

Stress hyperglycemia has not been specifically defined and is usually restricted to patients without previous DM (27). We used SHR as a quantitative indicator of stress hyperglycemia, because relative hyperglycemia has been proved to be a better biomarker for critical illness than absolute hyperglycemia (19). A J-shaped association between SHR and the risk of poor functional outcome at 1 year was found in our study, which means that moderate stress is beneficial. Previous studies have demonstrated a J-shaped association between serum glucose and functional outcome in patients with ischemic stroke (28). Also, in patients with ACS treated with EVT, the probability of poor functional outcome was first decreased and then increased along with the increasing of the admission blood glucose (4). Although the nonlinear relationship between SHR and poor functional prognosis has not been reported in patients treated with EVT, some studies have shown that both relative hyperglycemia and hypoglycemia are associated with higher mortality at 90 days (29), which may indicate the existence of a J-shaped correlation.

Our results suggested that elevated short-term GV was independently associated with poor functional outcomes in patients with PCS undergoing EVT, but not with increased risk of sICH. In a recent study, consistent with the results of our study, no significant correlation between CV after successful recanalization with EVT and increased risk of sICH was found (8). Besides, there was also no significant association between CV and poor functional outcomes. Considering that most of the patients enrolled in that study are patients with anterior circulation occlusion (85.7%), it can be speculated that the effect of GV on the prognosis of patients with anterior and posterior circulation may be different.

Subgroup analyses showed that hyperglycemia was significantly associated with adverse outcomes in non-diabetic patients, but not in DM patients. A possible explanation is that DM patients may be more tolerant to blood glucose variability. When blood glucose levels fluctuate in a high range, non-diabetic patients are more likely to suffer from impaired immune defense and microvascular environment disorder (6, 30). Another possible explanation is that DM patients are more likely to receive glucose-lowering therapy, which may reduce the amount of glucose diffused into the brain, thereby reducing harmful metabolic changes in the brain (31). Therefore, high-quality studies on larger samples are needed to verify the results of this study.

Besides the typical limitations inherent in retrospective analysis, our study has additional limitations. First, some patients with diseases (such as hemoglobin disorders and severe hepatic disease) that may affect HbA1c measurements were excluded from all the analyses, so our results cannot be extrapolated to these patient populations. Second, information on antidiabetic medications, course of treatment and long-term blood glucose control for DM patients before the endovascular treatment was mostly incomplete, and the impact of these factors on outcomes cannot be fully evaluated despite we used SHR to quantify stress hyperglycemia to adjust blood glucose control over the past 8-12 weeks (32). Finally, because this was a retrospective registration trial, standard meals were not used, which may have affected the accuracy of CV calculations. Only a part of patients completed GV measurement, and the measurement duration is limited to a short time, so the interpretation of the results needs to be cautious.

Conclusions

In summary, our findings indicate that admission hyperglycemia, FBG and stress hyperglycemia in VBAO patients treated with EVT were associated with adverse post-stroke outcomes both in the general population and in the non-diabetic subgroup, but not in the DM subgroup. The result also suggested that GV could be an appropriate clinical target to reduce the adverse effect of glucose fluctuation on prognosis. Since this is a retrospective observational study, our results should be interpreted with caution.

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 study was reviewed and approved by the ethics committee of the First Affiliated Hospital of University of Science and Technology of China. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

MG, JF, JZ, and WS conceived and designed the study. JF, PX, LX, JW, ML, CL, GL, QC, DL, and LY acquired the data. MG, LX and JW analyzed the data. MG drafted the manuscript. JZ and WS revised the manuscript and approved the final version of the manuscript. All authors reviewed and approved the final manuscript.

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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.

The reviewer XK declared a shared affiliation with the authors MG, JZ to the handling editor at the time of review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1000030/full#supplementary-material

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Identification of distinct circulating microRNAs in acute ischemic stroke patients with type 2 diabetes mellitus

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Stroke is the second leading cause of global mortality and continued efforts aim to identify predictive, diagnostic, or prognostic biomarkers to reduce the disease burden. Circulating microRNAs (miRNAs) have emerged as potential biomarkers in stroke. We performed comprehensive circulating miRNA profiling of ischemic stroke patients with or without type 2 diabetes mellitus (T2DM), an important risk factor associated with worse clinical outcomes in stroke. Serum samples were collected within 24 h of acute stroke diagnosis and circulating miRNAs profiled using RNA-Seq were compared between stroke patients with T2DM (SWDM; n = 92) and those without T2DM (SWoDM; n = 98). Our analysis workflow involved random allocation of study cohorts into discovery (n = 96) and validation (n = 94) datasets. Five miRNAs were found to be differentially regulated in SWDM compared to SWoDM patients. Hsa-miR-361-3p and -664a-5p were downregulated, whereas miR-423-3p, -140-5p, and -17-3p were upregulated. We also explored the gene targets of these miRNAs and investigated the downstream pathways associated with

them to decipher the potential pathways impacted in stroke with diabetes as comorbidity. Overall, our novel findings provide important insights into the differentially regulated miRNAs, their associated pathways and potential utilization for clinical benefits in ischemic stroke patients with diabetes.

KEYWORDS

microRNA, miRNA, ischemic, stroke, diabetes mellitus, T2DM

Introduction

Based on data gathered from 204 countries and territories between 1990 and 2019 (1), stroke remains the second leading cause of global mortality and has shown staggering increases in overall incidence, prevalence and death. These surges have been predominantly observed in younger populations belonging to low-income countries. At the same time, elevated blood pressure (BP), high body mass index (BMI), elevated fasting glucose levels, ambient particulate matter and smoking are considered the leading risk factors for stroke (1). In contrast, the incidence of ischemic strokes has been shown to steadily decline between 1993 and 2015 in studies conducted in the USA, with evidence of increased risk among Black and Hispanic females (≥70 years) in follow-up studies till 2019, while adults with treated BP also showed a significant reduction (42%) in stroke risk (2).

Stroke refers to a neurological deficit caused by acute focal injury of the central nervous system (CNS) due to a vascular cause (3). It is broadly classified into ischemic and hemorrhagic strokes, with the former caused by arterial occlusions, constituting most of stroke cases (4). Ischemic strokes are further divided into small-vessel occlusions, large-artery atherosclerosis, cardioembolism, or strokes of other determined or undermined etiologies (5). Treatment options for ischemic strokes are dependent on the duration from stroke onset, the extent of neurologic deficit and observations recorded by neuroimaging (3), while the clinical outcomes are widely assessed by the modified Rankin scale (mRS), which is also utilized as a robust tool for assessing the efficacy of treatment (6). Importantly, while diabetes is a known important risk factor for stroke (7), evidence has shown that it is also associated with worse clinical outcomes and fatal ischemic strokes (8, 9).

Diabetes is strongly associated with vascular diseases (10) and uncontrolled hyperglycemia can lead to ischemic or hemorrhagic strokes (11). In a 20-year follow-up data of more than 13,000 subjects, the risk of stroke was two- to threefold higher in type 2 diabetes mellitus (T2DM) patients, independently of other known risk factors (12). A meta-analysis covering ∼360,000 individuals showed that around one third of all stroke patients had diabetes and the presence of hyperglycemia/diabetes were associated with poor stroke outcomes (13). Notably, hyperglycemia included both pre-existing diabetes as comorbidity and post-stroke surges in fasting-blood glucose levels in stroke patients (13). Similarly, newly diagnosed diabetes cases were associated with more severe strokes, poorer outcomes, and increased mortality than pre-existing diabetes (14). In addition, the impact of pre-existing diabetes, acute hyperglycemic events during a stroke and post-reperfusion outcomes in stroke patients with diabetes can also have significant implications on stroke management (15). Fasting hyperglycemia recorded a day after mechanical thrombectomy in acute ischemic stroke patients was associated with worse clinical outcomes (16). Consequently, the associations between administration of anti-diabetic drugs and stroke risk have been thoroughly explored and evidence shows that selective anti-diabetic therapies such as metformin can have favorable effects on reducing stroke risk, while others may increase or pose no effect on the risk of ischemic strokes (17, 18). Additionally, investigations on the effects of cholesterol-lowering statins for reducing stroke risk showed elevated risk for diabetes due to statin use. However, cardiovascular events and clinical outcomes were favorable and exceeded the risk-to-benefit ratio for diabetes in favor of cardiovascular and mortality benefits of statin therapy (19, 20).

A stroke results in molecular imbalances due to the pathological and physiological events that take place. MicroRNAs (miRNAs) are small non-coding RNAs that form a regulatory network by affecting gene expression and are altered in various pathological conditions (21). Performing next-generation sequencing (NGS) on peripheral blood samples is a robust approach to decipher the global miRNA expression profiles (miRnome) and is increasingly utilized to improve understanding of various clinical pathologies including aneurysmal subarachnoid hemorrhages (22) and chronic kidney disease (23). Notably, miRNAs are consistently being explored as predictive, diagnostic, prognostic, and therapeutic markers in stroke (24). Accumulating evidence has shown the differential gradients in the expression levels of various miRNAs incurred transiently during ischemic strokes or with long-term alterations in peripheral blood samples from stroke patients (25–34). Likewise, the miRNA regulatory network affected in diabetes and its complications has been extensively explored and has led to the identification of various candidate miRNAs with potential

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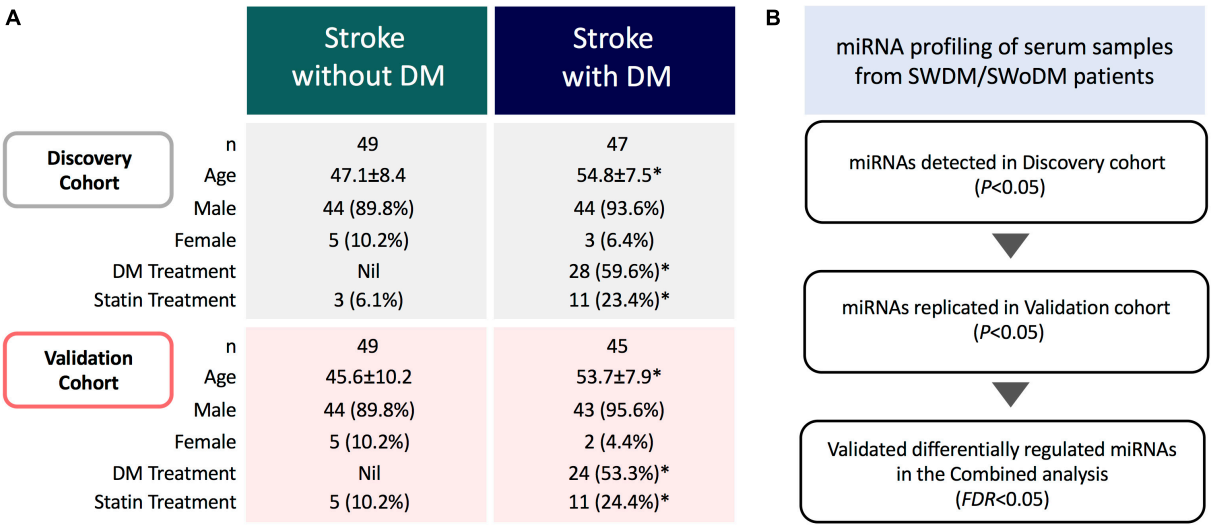

FIGURE 1
Study cohort and analysis workflow. **(A)** The study comprised acute ischemic stroke patients with and without type 2 diabetes mellitus (SWDM; n = 92, SWoDM; n = 98). Patients were randomly divided into discovery and validation cohorts with comparable distribution of covariates (age, gender, diabetes and statin treatment). Statistically significant parameters ($P < 0.05$) between SWDM and SWoDM patients are marked with an asterisk (*). **(B)** Serum samples were collected within 24 h of clinical diagnosis of acute ischemic stroke from SWDM and SWoDM patients. Differentially regulated and statistically significant miRNAs detected in the discovery dataset generated from miRNA transcripts by RNA-Seq were investigated in the validation dataset. Combined analysis was then performed to identify FDR-significant miRNAs presented as validated differentially regulated miRNAs in SWDM compared to SWoDM patients.

diagnostic and prognostic ability (35, 36). However, very few studies have explored and linked the aberrant expression of miRNAs in diabetes and stroke (37–40). Importantly, these studies have exclusively utilized targeted approaches using real-time quantitative PCR. Combined, the impact of diabetes on stroke and the associated fluctuations in the miRNA regulatory network warrant comprehensive investigations for their potential to further improve our understanding of the molecular pathways involved in diabetes-associated stroke.

In the present study, the circulating miRNAs in serum samples from acute ischemic stroke patients with or without clinically diagnosed T2DM were profiled using RNA-Seq and thoroughly investigated. We used a rigorous analysis approach for the robust identification of differentially expressed miRNAs. Using a similar approach, we have recently reported a panel of 10 differentially regulated miRNAs with remarkably high discriminatory performance between acute ischemic stroke patients and healthy controls (41). In the present study we focused our investigations on T2DM, a critical factor associated with stroke outcomes. We identified a panel of five differentially regulated miRNAs between stroke patients with T2DM (SWDM) and stroke patients without T2DM (SWoDM). We also probed the previously experimentally validated gene targets and potential pathways affected by these miRNAs. Our findings warrant further functional investigations and validations for their ultimate clinical translation.

Materials and methods

Patient samples

This study was approved by the Institutional Review Boards of Qatar Biomedical Research Institute (Approval no. 2019-013) and Hamad Medical Corporation (Approval no. 15304/15), Doha, Qatar. Written informed consents were taken from all participating individuals prior to sample collection. The study population (n = 190) comprised clinically diagnosed patients with acute ischemic stroke admitted to Hamad General Hospital (Doha, Qatar). Fresh serum samples were collected (within 24 h of stroke onset) and stored at −80°C for downstream analysis. Patients were divided into two groups based on clinical diagnosis of T2DM; SWDM (n = 92) and SWoDM (n = 98). Patients' clinical records covering information on prior treatments administered for T2DM or cholesterol-lowering drugs (statins) were also retrieved from hospital records. The characteristic features of study population are presented in **Figure 1A**.

Study design and analysis workflow

The overall study cohort comprised acute ischemic stroke patients with and without T2DM. The study cohort was randomly divided into discovery and validation cohorts with

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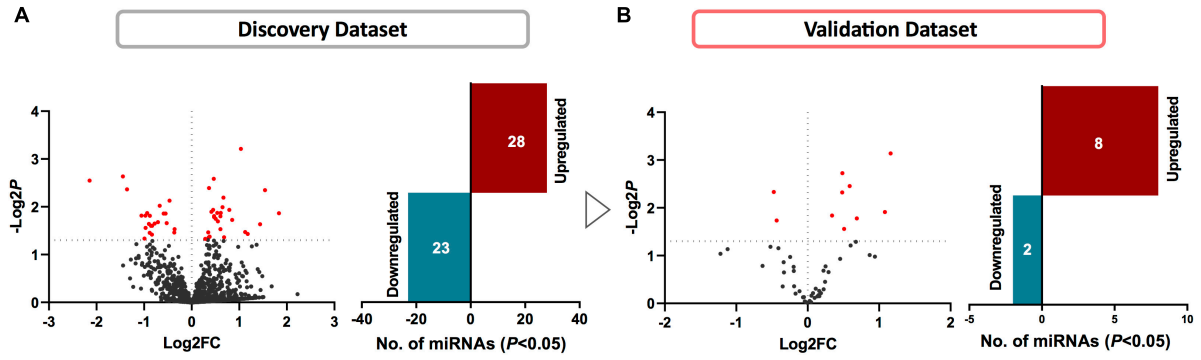


FIGURE 2
Identifying differentially regulated miRNAs in SWDM compared to SWoDM patients. Volcano plots show the number of differentially regulated miRNAs in SWDM versus SWoDM patients: red dots depict statistically significant ($P < 0.05$) and gray dots depict unsignificant miRNAs, while bar plots show the number of statistically significant miRNAs: downregulated in teal and upregulated presented in crimson color in **(A)** discovery and **(B)** validation datasets.

TABLE 1 Differentially regulated miRNAs in SWDM compared to SWoDM patients.

miRNA	Discovery		Validation		Combined	
	FC*	*P*-value	FC	*P*-value	FC	*P*-value**
hsa-miR-361-3p	−1.28	2.95×10^{-2}	−1.39	4.67×10^{-3}	−1.34	1.57×10^{-4}
hsa-miR-423-3p	1.45	1.34×10^{-2}	1.40	4.76×10^{-3}	1.37	5.53×10^{-4}
hsa-miR-664a-5p	−1.38	7.43×10^{-3}	−1.35	1.85×10^{-2}	−1.29	3.44×10^{-3}
hsa-miR-140-5p	1.39	1.57×10^{-2}	1.40	1.88×10^{-3}	1.24	5.20×10^{-3}
hsa-miR-17-3p	1.60	4.35×10^{-2}	1.61	1.67×10^{-2}	1.45	5.23×10^{-3}

*Fold change. **FDR < 0.05.

comparable distribution of covariates (number, gender, age, and treatment for T2DM and statin administration), and analyzed by RNA-Seq ($n = 190$) (Figure 1A). Briefly, the miRNA profiles of SWDM patients were first compared with SWoDM patients in the discovery cohort. The panel of differentially regulated and statistically significant ($P < 0.05$) miRNAs identified in the discovery dataset was validated in the validation cohort (Figure 1B). Combined analysis was then performed using more stringent analysis criteria (false discovery rate; FDR < 0.05) to identify robust differentially regulated miRNAs. This panel of miRNAs represented the differentially regulated miRNAs in SWDM compared to SWoDM and further downstream analyses were performed to explore the potential pathways affected by their targets.

MicroRNA purification and sequencing

RNA-Seq was performed on collected samples as previously described (41). Briefly, circulating miRNA from serum samples (200 µl) were extracted using miRNeasy Serum/Plasma Advanced Kit (Qiagen, Hilden, Germany) and RNA concentrations were measured by Qubit RNA Broad Range Assay Kit (Invitrogen, CA, USA). Library preparation was carried out using QIAseq miRNA NGS Library Kit (Qiagen) and indexing was done using QIAseq miRNA NGS 96 Index IL kit (Qiagen). The quality control measures for generated libraries were performed using Qubit dsDNA HS assay kit (Invitrogen) and Agilent 2100 Bioanalyzer DNA1000 chip (Agilent Technologies, Santa Clara, CA, USA). The pooled libraries were clustered using TruSeq PE Cluster Kit v3-cBot-HS (illumina, San Diego, CA, USA). Sequencing was performed on illumina HiSeq 4000 system (10 million reads per sample) using HiSeq 3000/4000 SBS kit (illumina).

Data processing

The NGS data generated as single reads (at 75 cycles) were aligned to the human miRbase v22 reference genome in CLC Genomics Workbench (v.21.0.5, Qiagen). The expression levels of miRNA transcripts were presented as counts per million (CPM) of the total count of mapped miRNA reads. Calibration for RNA spike-in (RNA transcript of known sequence and quantity) was also performed. The differential miRNA expression analyses were carried out on RStudio

(version 4.1.1; RStudio, MA, USA) utilizing the DSEq2 method (V. 1.32.0) (42), while adjusting for covariates (age, gender, diabetes, and statin treatment). Statistical analyses and data visualization were performed using GraphPad Prism 9.1.2 (GraphPad Software, MD, USA).

MicroRNA target and pathway analysis

The miRNA targets were identified from the miRTargetLink 2.0 (43). The gene enrichment and functional protein association network analysis of the target gene panel was performed by STRING (44), while functional pathway analyses were performed using QIAGEN Ingenuity Pathway Analysis (IPA) software (QIAGEN Inc.[1]) (45).

Results

Identification of differentially regulated microRNAs in stroke patients with type 2 diabetes mellitus

Our study cohort comprised clinically diagnosed stroke patients with or without T2DM as a comorbidity (**Figure 1A**). The study population was predominantly comprised of males, while SWDM patients were also significantly older than SWoDM patients. In addition, T2DM and statin therapies were administered to significantly higher proportions of SWDM compared to SWoDM patients, as expected. Considering these differences in characteristics of study cohorts, we corrected for these covariates in our analysis model and workflow, which involved random allocation of SWDM and SWoDM patients into discovery and validation datasets for the identification of replicated and statistically significant (FDR < 0.05) differentially regulated miRNAs (**Figure 1B**).

We first compared the circulating miRNA profiles of SWDM patients with SWoDM patients in the discovery cohort datasets (**Figure 2A**). We found that 51 miRNAs were differentially regulated between the two groups ($P < 0.05$) and showed varying degrees of fold change (FC) (**Supplementary Table 1**). We then tested these miRNAs in the validation cohort (**Figure 2B**) and out of the 51 miRNAs, 10 miRNAs showed significant dysregulation in the validation dataset ($P < 0.05$). Next, we performed combined analysis and five miRNAs remained statistically significant at FDR < 0.05 for the expression levels of the identified miRNA panel between SWDM and SWoDM patients with consistent direction of effect as shown in **Table 1**.

1 https://digitalinsights.qiagen.com/IPA

Differentially regulated microRNAs validated in stroke patients with type 2 diabetes mellitus

Five miRNAs; hsa-miR-361-3p, hsa-miR-423-3p, hsa-miR-664a-5p, hsa-miR-140-5p, and hsa-miR-17-3p were dysregulated between SWDM and SWoDM patients (**Figure 3A**). Out of these, two showed downregulation, while the remaining showed upregulation in SWDM versus SWoDM patients. Although these miRNAs showed moderate dysregulation in terms of FC, they showed high statistical significance (FDR < 0.05). Hsa-miR-361-3p was the most significant differentially regulated miRNA (**Figure 3A**). In addition, we also compared the CPM values of the five validated miRNAs, which also revealed significant differences between SWDM and SWoDM patients (**Figure 3B**).

Identifying potential microRNA-mediated pathways affected in stroke patients with type 2 diabetes mellitus

To explore the potential pathways affected by the panel of the statistically dysregulated five miRNAs in SWDM compared to SWoDM, we retrieved the experimentally validated molecular targets of these miRNAs in the miRTargetLink 2.0 database with strong experimental evidence. We compiled a list of 47 gene targets (**Table 2**). We first performed protein–protein interaction (PPI) and functional enrichment analysis of the proteins encoded by the 47 genes using STRING (**Figure 4**). The generated PPI network showed high statistical significance ($P = 1.0 \times 10^{-16}$) of the protein associations with strong involvement of VEGFA, STAT1, CDKN1A, and PTEN, among others (**Figure 4A**). Moreover, Gene Ontology (GO), biological process (BP), and molecular function (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway gene enrichment analysis predominantly showed vasculature-related pathways and the involvement of molecular pathways associated with disrupted homeostasis also observed in cancer (**Figure 4B**).

Next, investigating the clinical pathologies associated with these gene targets showed marked associations with cardiac anomaly-, hepatic-, and renal damage-related annotations (**Figure 5A**). In addition, the Ingenuity pathway and network analysis for the genes affected by dysregulated miRNAs in SWDM patients showed significant enrichment for two major pathways; histone H3 variants and *TP53* canonical pathways. Gene networks related to histone H3 highlighted enrichment in gastric development and function, neurological disease and organismal injury and abnormalities, which include edema, hemorrhage and lesions (**Figure 5B**). Additionally,

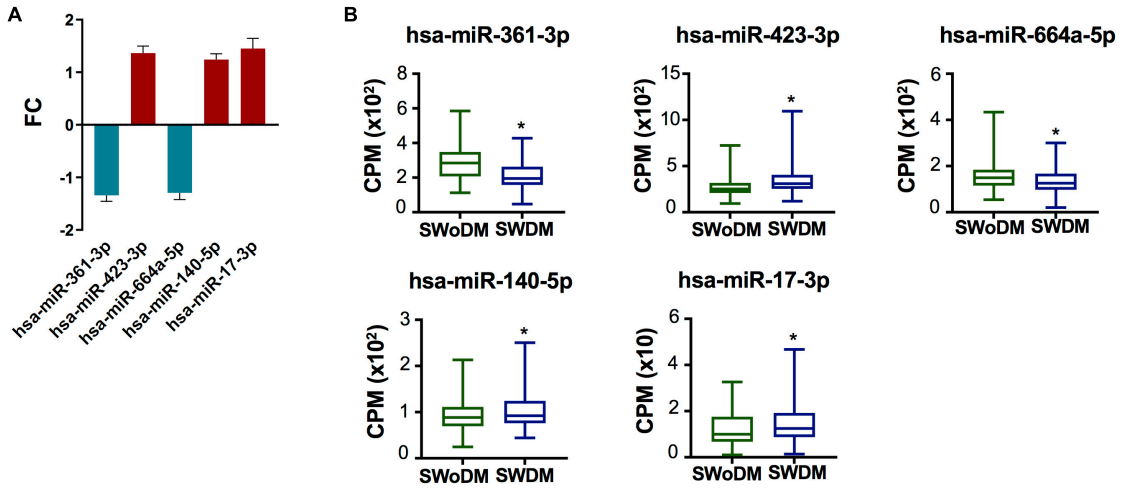


FIGURE 3
Validated miRNAs in SWDM patients compared to SWoDM patients. **(A)** Column plot shows the fold change (FC) and standard error of the mean (SEM) of FDR-significant validated, differentially regulated miRNAs (n = 5) in SWDM versus SWoDM patients: downregulated in teal and upregulated in crimson color. **(B)** Box and whiskers plots show the difference in counts per million (CPM) of the five validated miRNAs in SWDM and SWoDM patients. Mean with minimum and maximum values, upper and lower quartiles, and statistical significance ($P < 0.05$) marked by an asterisk (*) are shown for each dataset.

cancer, hematological and immunological disease-related pathways, mediated by *TP53* gene network, were also annotated with interactions between miRNA panel gene targets (Figure 5C).

Discussion

In this study, we identified five differentially regulated circulating miRNA in SWDM compared to SWoDM patients. While some of the miRNAs have been previously explored in relation to stroke or T2DM separately, their contribution to the impact of T2DM on stroke remains largely unexplored. SWDM patients have been previously reported to show worse disease outcomes, high recurrence and mortality compared to SWoDM patients (46). Deciphering the changes in the miRNA regulatory network in SWDM patients has, therefore, a potential therapeutic and prognostic significance.

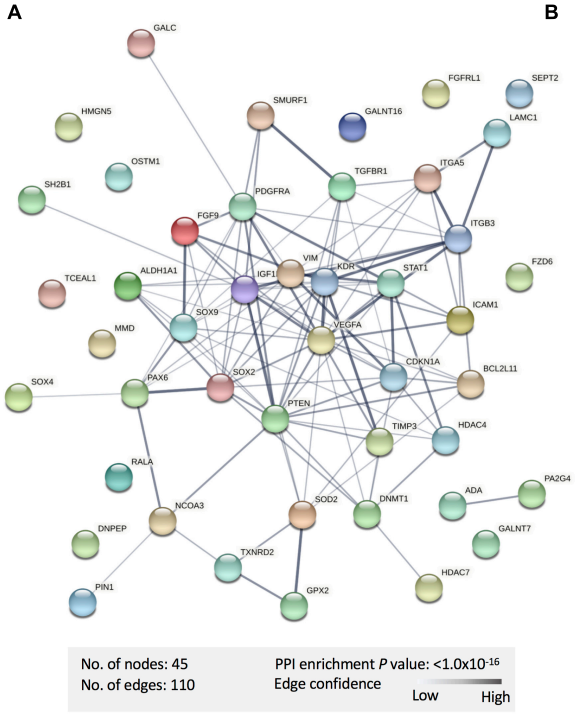
TABLE 2 The experimentally validated miRNA targets of the identified panel of differentially regulated miRNAs in SWDM compared to SWoDM patients.

miRNA	Target
hsa-miR-361-3p	*SH2B1*
hsa-miR-423-3p	*TCEAL1, CDKN1A, PA2G4, BCL2L11*
hsa-miR-664a-5p	*
hsa-miR-140-5p	*ALDH1A1, DNMT1, DNPEP, SOX2, HDAC4, VEGFA, PDGFRA, OSTM1, FGF9, TGFBR1, SOX9, FZD6, SEPT2, IGF1R, RALA, MMD, PAX6, HDAC7, LAMC1, ADA, SNORD12C, STAT1, PIN1, MEG3, GALC, GALNT16, SOX4, HMGN5, FGFRL1, SMURF1*
hsa-miR-17-3p	*ICAM1, KDR, VIM, SOD2, GPX2, TXNRD2, GALNT7, TIMP3, ITGA5, ITGB3, NCOA3, PTEN*

*No experimentally validated gene target.

Our data showed significant downregulation of miR-361-3p in SWDM compared to SWoDM. The downregulation of hsa-miR-361-3p has been previously reported in a cerebral artery occlusion-induced ischemic stroke murine model and presented as a potential therapeutic target following cerebral ischemic reperfusion injury (47). miR-361-3p has also been previously linked with vascular hemostasis. Upregulation of miR-361-3p was observed in patients with hereditary hemorrhagic telangiectasia (HHT) (48). Moreover, Huang et al., showed the involvement of miR-361-3p in inhibiting high-glucose induced vascular endothelial injury (49). Notably, the sole experimentally validated gene target of hsa-miR-361-3p, *SH2B1* is identified as a crucial protein involved in regulating energy balance, body weight, insulin sensitivity and glucose metabolism/homeostasis (50, 51). *SH2B1* is also associated with myocardial infarction in diabetic patients (52) while, Genome-Wide Association Studies (GWAS) have associated variants in *SH2B1* with BMI (53, 54). The dysregulation of miR-361-3p in SWDM patients indicates the impact on glucose metabolism but further studies are required to investigate this relation. Similarly, miR-664a-5p has been shown to promote neuronal differentiation (55) and its downregulation in SWDM patients indicates the suppression of neuroprotective machinery. Of note, dysregulation of miR-664a-5p has also been associated with the senescence of vascular smooth muscle cells and it has been proposed as a potential diagnostic marker and therapeutic

A



B

GO-Term BP	Description	FDR
GO:1905930	Regulation of vascular associated smooth muscle cell differentiation involved in phenotypic switching	$3.07x10^{-5}$
GO:0038091	Positive regulation of cell proliferation by VEGF-activated platelet derived growth factor receptor signaling pathway	$2.80x10^{-3}$
GO:1903984	Positive regulation of trail-activated apoptotic signaling pathway	$2.80x10^{-3}$
GO:0036324	VEGFR-2 signaling pathway	$4.90x10^{-3}$
GO:0038033	Positive regulation of endothelial cell chemotaxis by VEGF-activated VEGFR signaling pathway	$4.90x10^{-3}$
GO:2000739	Regulation of mesenchymal stem cell differentiation	$6.10x10^{-3}$
GO:0032808	Lacrimal gland development	$7.40x10^{-3}$
GO:0061042	Vascular wound healing	$8.90x10^{-3}$
GO:0060982	Coronary artery morphogenesis	$1.04x10^{-2}$
GO:1905459	Regulation of vascular associated smooth muscle cell apoptotic process	$1.04x10^{-2}$

GO-Term MF	Description	FDR
GO:0005161	Platelet-derived growth factor receptor binding	$2.47x10^{-6}$
GO:0005021	VEGF-activated receptor activity	$3.30x10^{-2}$
GO:0038085	Vascular endothelial growth factor binding	$3.30x10^{-2}$
GO:0032041	NAD-dependent histone deacetylase activity (H3-K14 specific)	$4.68x10^{-2}$
GO:0070411	I-SMAD binding	$4.72x10^{-2}$
GO:0004714	Transmembrane receptor protein tyrosine kinase activity	$5.20x10^{-3}$
GO:0019199	Transmembrane receptor protein kinase activity	$1.70x10^{-3}$
GO:1990841	Promoter-specific chromatin binding	$4.76x10^{-2}$
GO:0070851	Growth factor receptor binding	$1.50x10^{-3}$
GO:0005178	Integrin binding	$4.72x10^{-2}$

KEGG ID	Description	FDR
Hsa01521	EGFR tyrosine kinase inhibitor resistance	$2.06x10^{-6}$
Hsa05206	MicroRNAs in cancer	$1.06x10^{-12}$
Hsa05218	Melanoma	$2.97x10^{-5}$
Hsa00512	Mucin type O-glycan biosynthesis	$2.41x10^{-2}$
Hsa05212	Pancreatic cancer	$2.97x10^{-5}$
Hsa05214	Glioma	$6.20x10^{-4}$
Hsa05210	Colorectal cancer	$9.40x10^{-4}$
Hsa05219	Bladder cancer	$4.09x10^{-2}$
Hsa04068	FoxO signaling pathway	$2.39x10^{-5}$
Hsa00514	Other types of O-glycan biosynthesis	$4.35x10^{-2}$

FIGURE 4
Functional enrichment analysis of the proteins encoded by the gene targets of dysregulated miRNAs in SWDM versus SWoDM patients. **(A)** The protein–protein interaction (PPI) network generated for the 47 gene targets of the identified miRNA panel is shown. Network nodes represent proteins, while edges depict protein–protein associations. The key network statistics are also presented. **(B)** The top functional enrichment annotations from Gene Ontology (GO), biological process (BP)/molecular function (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways are listed.

target for cardiovascular diseases (56, 57). Moreover, Kim et al., reported upregulation of exosomal miR-664a-5p in obese T2DM patients compared to healthy controls (58). The dysregulation of miR-664a-5p could be associated with disease complications in SWDM patients but further investigations are required to confirm this association and also to experimentally validate its gene targets.

The downregulation of hsa-miR-423-3p has been previously reported as a biomarker for acute ischemic stroke patients (30) and has been associated with worse overall survival in patients with heart failure (59). However, its upregulation has been linked with onset and severity of Type 1 diabetes (60, 61) and it also showed high predictive potential in identifying T2DM remission after sleeve gastrectomy (62). We found that miR-423-3p was upregulated in SWDM patients compared to SWoDM patients, which suggests its association with diabetes. Importantly, upregulation of *CDKN1A*, an experimentally validated gene target of mir-423-3p, is presented as a specific marker of ischemic brain (63). GWAS also showed that variants in *CDKN1A* are associated with ischemic strokes (64), atrial fibrillation and cardioembolic stroke (65), while variants in other experimentally validated gene targets of mir-423-3p; *PA2G4* are associated with BMI (66) and *BCL2L11* with T2DM and cholesterol levels (67).

Ortega et al., reported upregulation of hsa-miR-140-5p in T2DM patients compared to healthy controls and showed its high discriminant capacity for T2DM (68). miR-140-5p was also upregulated in a blood stasis syndrome (BSS) model with diabetes compared to diabetes without BSS (69). Notably, it was reported that miR-140-5p could nullify the high glucose-induced inflammation and apoptosis in renal tubular cells (70) and mediate neuroprotection in ischemic strokes *via* exploitation of TLR4/NF-κB pathway (71). Of note, miR-140-5p has been also presented as an early biomarker for late-onset post-stroke depression (72). Our data showed upregulation of miR-140-5p in SWDM patients, which is in agreement with the findings of Ortega et al. (68) and suggests its involvement in diabetes-related pathways. Elevated activity of the experimentally validated gene target of mir-140-5p, *ALDH1A1* was associated with severity of T2DM (73). Among other experimentally validated miR-140-5p gene targets, *DNMT1*, *HDAC4*, and *HDAC7* are involved in epigenetic machinery. The associations between DNA methylation patterns and increased risk of various pathologies including diabetes, cancer, hypertension and atherosclerosis are

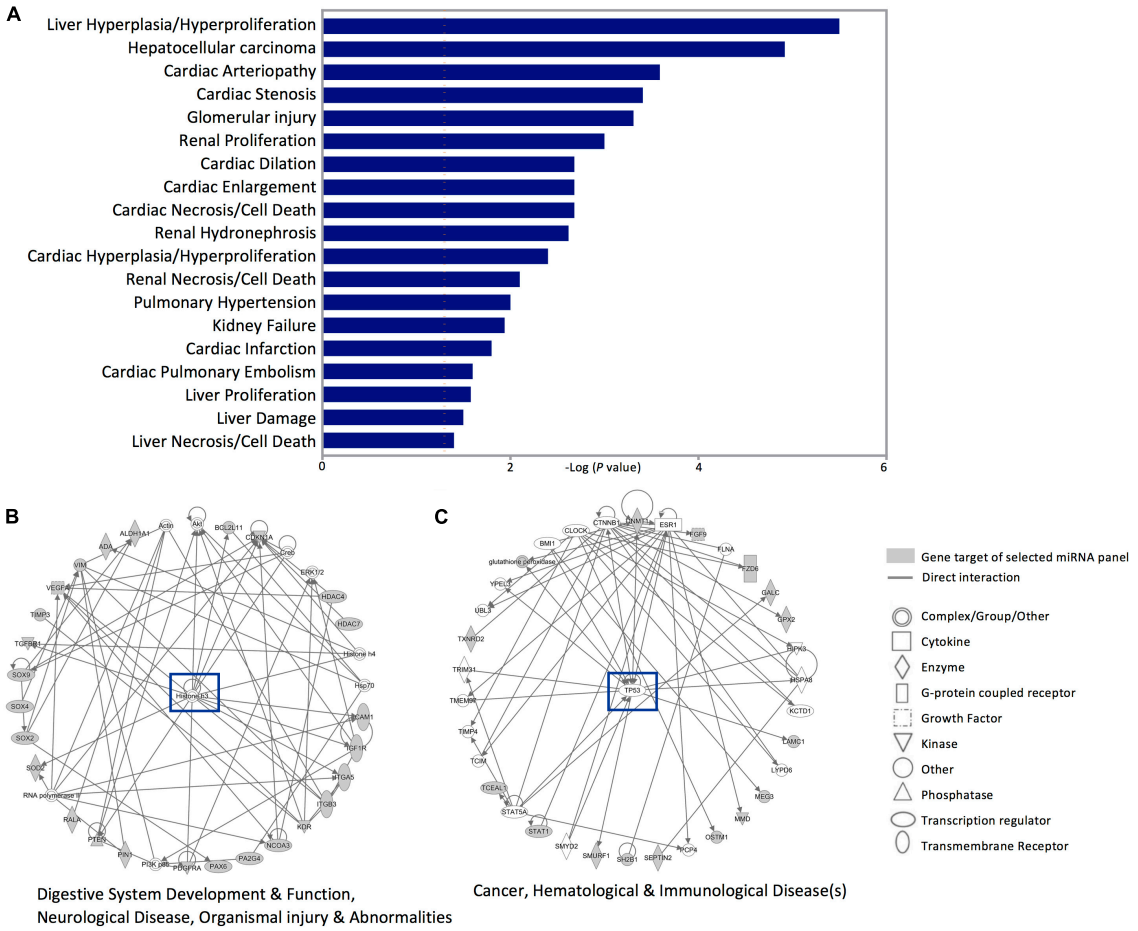


FIGURE 5
Disease annotation and Ingenuity pathway analysis for the gene targets of dysregulated miRNAs in SWDM versus SWoDM patients. The gene targets of the five dysregulated miRNAs in SWDM patients compared to SWoDM patients were analyzed for disease/function annotation and network analysis. **(A)** Bar plot shows the diseases annotations. **(B,C)** Ingenuity pathway network analysis of the gene targets of dysregulated miRNAs in SWDM patients are shown.

well documented (74). The miRNA modulation of these gene targets coupled with modulation of additional gene targets associated with vasculature such as *VEGFA* and *MMD* by miR-140-5p indicates the potential involvement of another mechanism responsible for worse disease outcomes in SWDM patients *via* atherosclerotic vascular diseases or *via* epigenetic mechanisms (75). Additionally, GWAS have associated single nucleotide polymorphisms (SNPs) in miR-140-5p gene targets such as *SEPT2* (*SEPTIN2*) with T1DM (76), *DNMT1* with CVD (77) and *HDAC4* with increased susceptibility to myocardial infarction following coronary artery bypass surgery (78).

The upregulation of hsa-miR-17-3p was previously identified as a diagnostic and potential biomarker for acute ischemic strokes in two independent studies but it was not replicated in the validation cohorts (31, 79). Herein, we validated that miR-17-3p is upregulated in SWDM patients. miR-17-3p has been also associated with diabetic retinopathy (80, 81), coronary artery disease, cardiac ischemia (82–84) and has been previously presented as a circulating biomarker for T1DM (85, 86). Importantly, the experimentally validated gene target of miR-17-3p, *ICAM1* is strongly associated with poor prognosis in acute ischemic strokes (87). Changes in ICAM-1 serum concentration were reported in ischemic stroke patients with cerebral microbleeds and were associated with increased risk of hypertension and diabetes (88). Additionally, variants in *ICAM1* and serum ICAM-1 levels are also associated with the development of diabetes and diabetic nephropathy (89). However, targeting ICAM-1 in ischemic stroke patients is not a *via*ble therapeutic strategy as using anti-ICAM-1 antibody led to worse clinical outcomes in a clinical trial of 625 ischemic stroke patients (90). Among other gene targets of miR-17-3p, *VIM* has been associated with total/LDL-cholesterol measurement (91) and *PTEN* with T2DM (92) in GWAS. Combined, these data reflect the potential significance of miR-17-3p in ischemic strokes with T2DM and highlights pathways related to diabetic complications such as diabetic retinopathy.

To understand the potential clinical implication of the dysregulation in our miRNA panel in SWDM patients, we performed protein interaction network, functional gene enrichment and disease annotation and pathway analysis. The integration of functional annotations and disease mapping is widely followed and provides crucial understanding related to genes involved. miRNAs primarily regulate gene expression *via* repression during translation or degradation of target mRNA. The downregulation of miRNAs can promote expression of its target genes and consequently their protein-associated pathways. Conversely, miRNA upregulation can impede expression and function of target genes and their encoded proteins. However, since gene expression is influenced by various factors, functional validation of the effects of miRNAs on their targets is essential for confirmation.

Investigating the predicted interactions between proteins encoded by the gene targets of differentially regulated miRNAs in SWDM patients showed strong interaction enrichment. These interactions predominantly corresponded to vascular processes, mediated by *VEGFA*. Atherosclerosis is a known factor for impaired life expectancy, while diabetic nephropathy and retinopathy lead to renal diseases and blindness in diabetic patients (93). However, the disease complications observed in diabetic patients are multifaceted and involve modulation of multiple homeostasis-associated processes, also observed in various human malignancies. For instance, FoxO signaling regulates multiple processes such as cell cycle, apoptosis and metabolism, and is dysregulated in both cancer and diabetes (94). Of note, T2DM patients are at a higher risk of developing certain cancers including pancreatic and kidney cancer (95), potentially attributed to metabolic imbalances or genetic susceptibility. Our findings indicate the influence of diabetes-related complications and imbalances/exploitation of cancer-related pathways in SWDM patients.

Linking gene enrichment with disease and function annotations revealed associations with cardiac-, hepatic-, and renal-related pathologies, which are strongly associated with the clinical complications of diabetes. Physiological changes in cardiac and hepatorenal functions are also associated with stroke and imbalances in hematological indicators are commonly observed in diabetes and stroke patients. Moreover, the Ingenuity pathway analysis of the gene targets of dysregulated miRNAs in SWDM patients revealed gastric-, neurological-, and organismal injury-related pathways and cancer-, hematological-, and immunological disease-related pathways in SWDM patients. These networks encompass gastrointestinal disturbances, neurological deficits, inflammatory and immune imbalances in wound healing, atherosclerosis and vascular anomalies and reiterate the significance of underlying pathways, which are potentially exploited in SWDM patients and affect disease outcomes. Importantly, we identified several gene/protein targets which may be explored in future studies.

Investigating the delineation of the miRNA profiles of the ischemic brain from the healthy brain can potentially disclose critical pathways affected in stroke. However, the difficult accessibility to brain tissue renders investigating circulating miRNAs as the most feasible approach for use as disease biomarkers. Our findings provide insights into the differentially expressed miRNAs and their potential effects in SWDM compared to SWoDM patients. Importantly, our panel of differentially regulated miRNAs highlights the critical pathways potentially involved in the neuronal, cardiac, and diabetes-related complications observed in stroke patients with diabetes comorbidity and worse clinical outcomes. However, functional studies are warranted to investigate the biological significance of the identified dysregulated miRNAs and their associated pathways. Additionally, the relatively modest sample size of our study requires validation in a larger sample size in an external dataset. Of note, females represented a small proportion of our overall study cohort and repeating the analysis by excluding females generated essentially the same results. Overall, the gene targets of the panel of differentially expressed miRNAs and protein interactions uncovered in our study can be explored further for their clinical utilization for therapeutic benefits.

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 below: NCBI SRA, accession no. PRJNA879740 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA879740/).

Ethics statement

The studies involving human participants were reviewed and approved by the Qatar Biomedical Research Institute and Hamad Medical Corporation, Doha, Qatar. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ST: formal analysis, visualization, and writing—original draft. EKA: data curation, formal analysis, and investigation. AP and NA: resources, investigation, and sample preparation. YA-S: investigation. EMA, AA, OE-A, and PT: resources. SP, GP, and RK: investigation and sample preparation. AS: resources, investigation, and writing—review and editing. NMA: formal analysis and writing—review and editing. OMEA: conceptualization, funding acquisition, project administration,

resources, investigation, supervision, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1024790/full#supplementary-material

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Relationship between triglyceride–glucose index and carotid plaques in a high-stroke-risk population in southeast china: A population-based cross-sectional survey

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Background: Cervical arterial atherosclerosis (CAA) is an important risk factor of stroke in China. The triglyceride–glucose (TyG) index is a simple and low-cost marker for ischemic stroke. Whether the TyG index predicts cervical arterial atherosclerosis remains uncertain. This study aimed to investigate the relationship between the TyG index and cervical arterial atherosclerosis.

Methods: This cross-sectional study was conducted in residents aged ≥40 years in the general population of southeast China. All participants completed a detailed questionnaire and provided blood samples. The high-stroke-risk groups further completed cervical artery ultrasonography. The TyG index was calculated using a well-established formula and analyzed in quartiles (Q1–Q4). Multivariate logistic regression was used to investigate the relationship between the TyG index and cervical arterial atherosclerosis.

Results: A total of 4,499 participants aged ≥40 years were finally included, with 23.47% comprising the high-stroke-risk population. The prevalence rates of increased intima–media thickness (IMT), carotid plaque, and cervical artery stenosis (CAS) in the high-stroke-risk population were 21.97%, 39.3%, and 6.1%, respectively. Subjects with higher TyG were still more likely to have carotid plaque. After adjusting for several established risk factors, compared with the TyG-Q1 group, the TyG-Q2, TyG-Q3, and TyG-Q4 groups were more likely to have carotid plaque (OR = 1.85, 95%CI = 1.28–2.67; OR = 1.51, 95%CI = 1.05–2.18; and OR = 1.29, 95%CI = 0.90–1.84). TyG was an independent predictor of the presence of plaque in the carotid artery of the high-stroke-risk population.

Conclusions: An elevated TyG index is a potential predictor of carotid plaques in the high-stroke-risk population older than 40 years.

KEYWORDS

triglyceride–glucose index, cervical arterial atherosclerosis, carotid plaque, stroke screening, insulin resistance

Introduction

Stroke ranks as the third cause of death and disability in China, which has been a threat to human health (1). China has the highest estimated lifetime risk of stroke, which increases the total cost of stroke. Reducing the incidence of stroke ensures quality of life for adults and has a positive impact on individuals, families, and the society. Stroke is not an accident. Stroke screening for the early detection of a high-risk subject and managing the risk factors are of great significance to the prevention of stroke. Primary stroke prevention can reduce the incidence of stroke and lessen its financial burden (2, 3).

There are numerous causes of stroke, including prolonged hypertension, arteriosclerosis, and emboli (4). Atherosclerosis is a chronic condition that causes an accumulation of fatty streaks in arterial walls, which develop into atheromas and plaque. One-third of all strokes are related to cervical carotid disease. The risk factors for coronary and systemic atherosclerosis, including age, sex, hypertension, hyperlipidemia, unhealthy lifestyle habit, and family history, apply to this patient population. Cervical arterial atherosclerosis includes increased intima–media thickness (IMT), carotid plaque, and cervical artery stenosis (CAS). The mechanism of cervical carotid stroke is usually embolization from the carotid bifurcation plaque and/or hemodynamic compromise from stenosis (5). Ultrasonography is a good method for detecting ceramic arterial atherosclerosis. However, due to the requirement of professional equipment, the need for experienced ultrasound physicians, and the higher cost of ultrasonography, it is impossible for the general population of grassroots hospitals in villages and towns to complete ultrasound examinations.

The triglyceride–glucose (TyG) index is a product of triglyceride and fasting blood glucose (FBG) and has several related parameters, such as the product of TyG and waist circumference (TyG-WC), TyG and waist-to height ratio (TyG-WHtR), TyG and waist-to-hip ratio (TyGWHpR), and TyG and body mass index (TyG-BMI) (6). The measurement of TyG is inexpensive and easily obtained, and it is suitable for use as a screening indicator for the general population. The TyG index is a novel surrogate indicator of insulin resistance (IR). Therefore, initial studies have suggested the TyG index as a novel marker for multiple IR-related clinical diseases, such as metabolic syndrome (MS) (6). It provides good discrimination of people with prediabetes and diabetes (7). In recent years, the TyG index has been studied as a novel tool for evaluating atherosclerosis of cardio-cerebrovascular diseases (CVDs) in different populations. This index may serve as a marker for subclinical atherosclerosis and arterial stiffness in lean and overweight postmenopausal women (8). Researchers found that the TyG index could predict the IMT of the common carotid artery (CCA) in hypertensive individuals, which is an important risk factor for stroke (9). Jin et al. found the TyG index to be positively associated with future cardiovascular events and may be a useful marker for predicting clinical outcomes in patients with coronary artery disease (CAD) (10, 11).

Previous findings also suggested the potential value of TyG and the TyG-related parameters in optimizing the risk stratification of ischemic stroke. In a cross-sectional study that included 10,900 subjects from rural areas of northeast China, the prevalent ischemic stroke correlated proportionally with the increment of TyG, implicating the linearity of TyG as an indicator of ischemic stroke (12). In another rural Chinese cohort study, an elevated TyG also predicted the risk of incident ischemic stroke (13). Sun et al. found a robust correlation between TyG-BMI and ischemic stroke, independently of a host of conventional risk factors in populations of northeast China (14). In addition to this, the TyG index can predict functional outcomes and mortality after acute ischemic stroke (15–17). It is also a potential predictor of hospital and intensive care unit (ICU) mortality in critically ill stroke patients, especially in ischemic stroke patients (15). On the other hand, Hou et al. suggested that the TyG index may not be beneficial in understating the metabolic mechanisms responsible for the stroke obesity paradox (18). In summary, the correlations between the TyG index and stroke are inconsistent. To date, only a few studies have focused on the TyG index and cervical arterial atherosclerosis. The causal link between the TyG index and atherosclerosis remains unclear. Moreover, the most relevant studies in China have focused on the population of the northeast area.

Therefore, we aimed to investigate the associations between the TyG index and the occurrence of cervical arterial atherosclerosis in the general population of eastern China. Our hypothesis was that the TyG index is associated with and may be a predictor of cervical arterial atherosclerosis. Assessment of this relationship will help verify that TyG, a more convenient and low-cost index, has potential value in improving the risk

stratification of stroke. For this reason, the present study examined the TyG index in different risk populations of eastern China based on the Stroke Screening and Prevention Program. On the other hand, the study further explored the association between the TyG index and carotid ultrasound indices in the high-stroke-risk population.

Methods

Study design and participants

The study population was from the Stroke Screening and Prevention Program of the National Health and Family Planning Commission of China, which was supervised by the Chinese National Center for Stroke Care Quality Control and Management (Stroke Prevention Project, National Health Commission). The project implemented stroke screening for urban and rural residents in Suzhou, Jiangsu Province, China. As a representative city, Suzhou is located in southeast China, southeast Jiangsu, and in the middle of the Yangtze River Delta. One urban and one rural location were selected randomly in Suzhou. The study was conducted from December 2018 to June 2019. The number of the final screening subjects accounts for more than 85% of the residents in this location. Finally, a total of 4,705 permanent residents aged ≥40 years who had lived in Suzhou City for more than 6 months were randomly selected, of which 4,499 individuals successfully completed the face-to-face survey. The sample selection framework is presented in Figure 1. This study was conducted according to the guidelines of the Helsinki Declaration. Ethical approval was obtained from the Ethics Committee of the First Affiliated Hospital of Soochow University before the start of the study. Written informed consent was obtained from all the participants.

Data collection and laboratory Analysis

Data were collected during face-to-face interviews at a central survey site in the area. The participants completed a structured, pre-

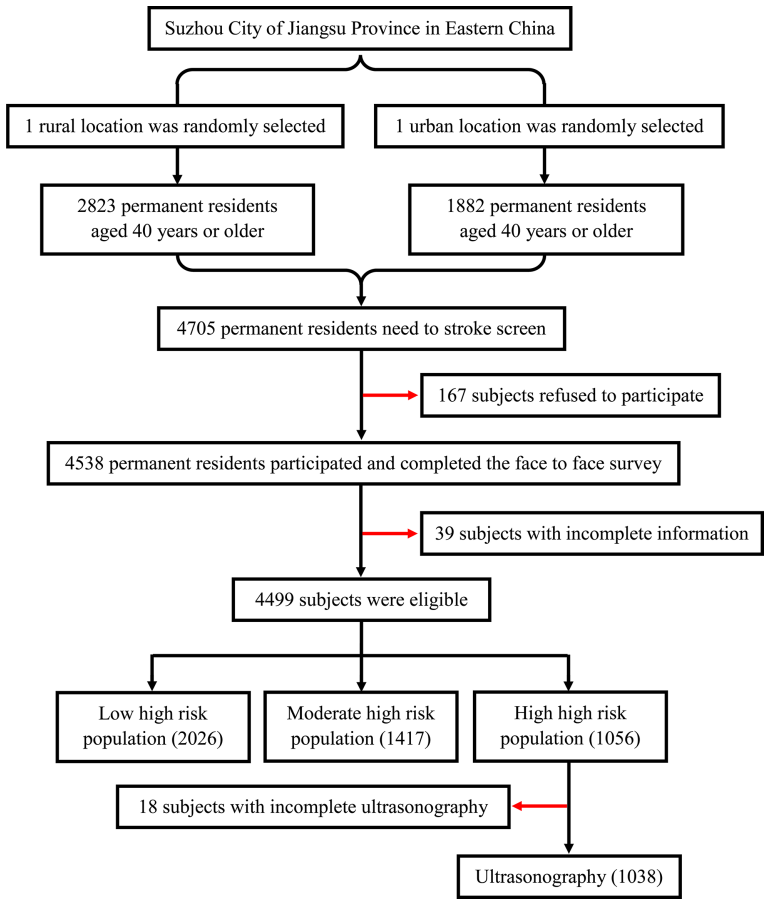

FIGURE 1
Flowchart of the enrollment of subjects.

coded questionnaire including demographic details, personal lifestyle, and personal and family medical history of stroke and chronic diseases. Anthropometric measurements were taken following standardized protocols. Blood pressure was measured twice and the average taken by trained professionals. Venous blood samples were collected from an antecubital vein in the morning after an overnight fast for laboratory examinations. These indices included FBG, hemoglobin A_{1C}, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and homocysteine (Hcy). All data were double entered and validated.

Definition of terms and groups

The TyG index and related parameters were calculated as follows (6):

1) TyG index=Ln[TG (mg/dl)×fasting blood glucose (mg/dl)/2]
2) TyG and body mass index: TyG-BMI=TyG index×BMI
3) TyG and waist circumference: TyG-WC=TyG index×WC
4) TyG and waist-to height ratio: TyG-WHtR=TyG index×WHtR

The visceral adiposity index (VAI) was calculated using an established formula (19).

In men: VAI=(WC/(39.68+(1.88×BMI))×(TG/1.03)×(1.31/HDL)

In women: VAI=(WC/(36.58+(1.89×BMI))×(TG/0.81)×(1.51/HDL)

Low-, medium-, and high-risk populations of stroke were defined according to the "8+2" Stroke Risk Screening Program: 2 main risk factors and 8 general risk factors for stroke (20).

Eight general risk factors were described, as follows:

1) Hypertension: defined as a history of high blood pressure (≥140/90 mmHg) or current use of antihypertensive medication;
2) Atrial fibrillation (AF) or heart valve diseases: either reported by the respondent or defined as an irregular pulse during physical examination;
3) Smoking: defined as current or former practice of smoking ≥6 months;
4) Dyslipidemia: defined as current use of anti-lipidemic medication, TC≥6.22 mmol/L, or serum TG≥2.26mmol/L, or HDLC<1.04mmol/L;
5) Diabetes mellitus: defined as a previous diagnosis, treatment with insulin or oral hypoglycemic medications, fasting plasma glucose ≥126 mg/dl, or glycosylated hemoglobin≥6.5%;
6) Physical inactivity: defined as physical exercise less than three times a week for<30 min each (moderate-intensity exercise such as brisk walking; industrial and agricultural labor was considered as a form of exercise);
7) Overweight or obesity: defined as BMI ≥24 kg/m^2; and
8) Family history of stroke

The additional two main stroke risks were:

1) Personal history of stroke and
2) Personal history of transient ischemic attack (TIA)

Subjects with at least three of these risk factors or a previous history of stroke or TIA were classified into the high-risk population. Subjects with up to three of these risk factors or with hypertension or diabetes mellitus (DM) or AF or heart valve diseases were classified into the medium-risk population. Subjects with three or less of these risk factors without hypertension or DM or AF or heart valve diseases were classified into the low-risk population.

Ultrasonography

The high-risk groups further underwent cervical artery ultrasonography (MyLabSix, Esaote, Italy) examinations by experienced ultrasound physicians with at least 5 years of ultrasound experience. The bilateral CCA, internal carotid artery (ICA), the subclavian artery, and the vertebral artery were examined and recorded. IMT was measured manually three times in a plaque-free area of each CCA and the averaged thickness recorded. Both common carotid arteries were examined, and an increased IMT was defined as IMT ≥1.0 mm in either the left or the right carotid artery. Carotid plaque was defined as IMT ≥1.5 mm or a focal narrowing of the vessel wall of >50% relative to adjacent segments. The incidences of plaque morphology, ulcer, and echo were recorded. CAS included intracranial vascular stenosis and occlusion (19).

Statistical analysis

Statistical analysis was completed using SPSS 22.0 (International Business Machine, West 31 Grove, PA, USA). Continuous variables were presented as the mean ± SD. Categorical variables were presented as frequency (percentage). Differences between groups were tested using one-way ANOVA with *post-hoc* analysis (Bonferroni) for continuous variables and chi-square test, corrected chi-square test, or Fisher's exact probability method for categorical variables. Baseline characteristics of the high-risk groups were sorted by TyG

quartiles. Multivariate logistic regressions were conducted to explore the relationship between carotid plaque and all risk factors. A chi-square goodness-of-fit test was applied and represented the goodness of fit in the logistic regression. We mainly included age, sex, and the "8+2" stroke risk factors in the final logistic regression models. Furthermore, the correlations between TyG and carotid plaque were calculated using logistic regression analysis in different models: model 2 was adjusted for age and sex; model 3 was adjusted for age, sex, smoking, drinking, and physical inactivity; model 4 was adjusted for age, sex, smoking, drinking, physical inactivity, hypertension, DM, heart disease and dyslipidemia, personal history of stroke/TIA, and family history of stroke; and model 5 was adjusted for the predictors that showed significant differences in the univariate analysis. We also performed several subgroup analyses for men, women, DM, non-DM, overweight or obese, non-overweight or obese, sweet tooth, non-sweet tooth, physical inactivity, and non-physical inactivity. All of the subgroup analyses were adjusted using the same parameters as described in model 4 in the main analysis. Receiver operating characteristic (ROC) analysis was performed to construct the prediction of cervical artery atherosclerosis. All reported *p*-values were two-sided, and a $p< 0.05$ was considered as statistically significant.

Results

Demographics and baseline characteristics of all participants

In total, 4,499 participants in the stroke screening (1,556 men and 2,943 women; mean age, 57.88 ± 9.79 years) were included in the analysis. Nearly 61.52% of the participants were from townships and 38.48% from communities. The smoking rate of the participants in this study was 17.54%. Among the included participants, 38.50% reported physical inactivity, 12.05% reported being overweight or obese, 49.3% had hypertension, 11.3% had DM, 1.07% had AF and/or valvular heart disease (VHD), and 35.45% had dyslipidemia. In addition, 6.53% of the participants had a family history of stroke. Regarding personal medical history, 1.53% had a personal history of stroke and 0.07% had a history of TIA (Figure 2).

According to the aforementioned diagnostic criteria of the "8+2" Stroke Risk Screening Program, the target population of stroke screening was divided into three groups: low-, medium-, and high-risk groups. All the demographic and baseline characteristics were sorted based on the risk of stroke and are shown in Table 1. The results showed the percentages of the three groups as 45.03%, 31.50%, and 23.47%. Of the participants, 61.75% have a sweet tooth. There were significant differences between the groups regarding family history of hypertension, diabetes, and coronary heart disease. The high-risk group was more likely to have a higher systolic blood pressure (SBP) ($p< 0.001$), weight ($p = 0.002$), waist circumference ($p< 0.001$), and BMI ($p< 0.001$). Concerning laboratory features, the high-risk group was more likely to have a higher VAI ($p< 0.001$), FBG ($p< 0.001$), hemoglobin A1c ($p< 0.001$), TG ($p< 0.001$), TC ($p< 0.001$), LDL-C ($p = 0.008$), and homocysteine ($p< 0.001$). In addition, the high-risk group had significantly higher TyG and values of the TyG parameters TyG-BMI, TyG-WC, and TyG-WHtR (all $p< 0.001$).

Demographics and baseline characteristics of the high-stroke-risk population

All the demographic and baseline characteristics were sorted by TyG quartiles and are shown in Table 2. Ultimately, a total of 1,038 high-stroke-risk participants were included. The mean age was 58.66 ± 9.77 years. Among the included participants, 55.88% have a sweet tooth, 89.98% had hypertension, 27.55% had DM, 2.89% had AF and/or VHD, 64.07% had dyslipidemia, 6.64% had a personal history of stroke, and 0.29% had a personal history of TIA. The smoking rate was 38.34%. In addition, 70.13% reported physical inactivity and 32.47% reported being overweight or obese. Moreover, 14.93% had a family history of stroke. Regarding the prevalence of cervical arterial atherosclerosis, 21.97% of the participants had increased IMT, 37.09% had carotid plaque, and 6.17% had CAS. The TyG-Q4 group showed a significantly increased proportion of men ($p = 0.006$) and was more likely to have higher SBP

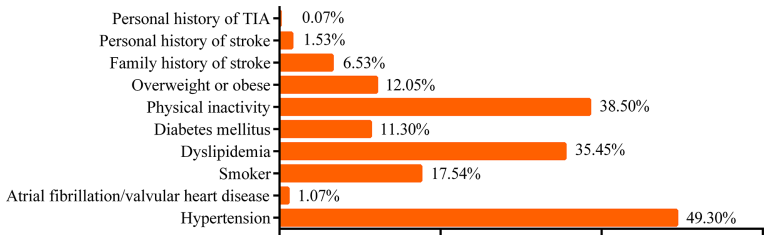

FIGURE 2
Risk factors according to the "8+2" Stroke Risk Screening Program. *TIA*, transient ischemic attack.

TABLE 1 Baseline characteristics of all participants.

	Total	Low-risk population	Medium-risk population	High-risk population	*p*-value
N	4,499	2,026 (45.03%)	1,417 (31.50%)	1,056 (23.47%)	
Region					0.46
Urban, *n* (%)	2,768 (61.52)	1,237 (61.06)	864 (60.97)	667 (63.16)	
Rural, *n* (%)	1,731 (38.48)	789 (38.94)	553 (39.03)	389 (36.84)	
Age (years)	57.88 ± 9.79	57.52 ± 9.87	57.84 ± 9.63	58.62 ± 9.84	0.01*
Gender, male, *n* (%)	1,556 (34.59)	798 (39.39)	404 (28.51)	354 (33.52)	<0.001***
Eating habits					<0.001***
High-salt diet	595 (13.23)	265 (13.08)	181 (12.77)	149 (14.11)	
Oil-heavy diet	1,126 (25.03)	428 (21.13)	383 (27.03)	315 (29.83)	
Sweet tooth	2,778 (61.75)	1333 (65.79)	853 (60.20)	592 (56.06)	
Meats and vegetables					<0.001***
Balanced	321 (7.13)	126 (6.22)	105 (7.41)	90 (8.52)	
Carnivorous diet	895 (19.90)	350 (17.28)	302 (21.31)	243 (23.01)	
Plant-based diet	3,283 (72.97)	1,550 (76.50)	1,010 (71.28)	723 (68.47)	
Daily vegetable intake, *n* (%)	2,551 (56.70)	1,223 (60.37)	776 (54.76)	552 (52.27)	<0.001***
Daily fruit intake, *n* (%)	1,717 (38.16)	828 (40.87)	535 (37.76)	354 (33.52)	0.001**
Alcohol consumption, *n* (%)					<0.001***
Never	3,619 (80.44)	1,572 (77.59)	1,194 (84.26)	853 (80.78)	
Heavy drinking	720 (16.0)	368 (18.16)	187 (13.20)	165 (15.62)	
Light/moderate drinking	160 (3.56)	86 (4.25)	36 (2.54)	38 (3.60)	
Family history of CHD, *n* (%)	163 (3.62)	83 (4.10)	48 (3.39)	32 (3.03)	0.006**
Family history of hypertension, *n* (%)	1,527 (33.94)	720 (35.53)	488 (34.44)	319 (30.21)	0.002**
Family history of diabetes, *n* (%)	466 (10.36)	186 (9.18)	128 (9.03)	152 (14.39)	<0.001***
SBP (mmHg)	131.23 ± 14.47	128.88 ± 14.76	132.01 ± 13.76	134.68 ± 14.05	<0.001***
DBP (mmHg)	81.20 ± 8.88	81.05 ± 9.18	81.00 ± 8.57	81.75 ± 8.70	0.07
Height (cm	161.10 ± 7.95	161.90 ± 8.07	160.39 ± 7.62	160.52 ± 7.99	<0.001***
Weight (kg)	63.50 ± 9.95	63.6 ± 9.95	62.83 ± 9.84	64.22 ± 10.06	0.002**
Waist circumference (WC) (cm)	80.32 ± 9.08	80.32 ± 8.84	79. 66 ± 9.37	81.20 ± 9.08	<0.001***
Body mass index (BMI) (kg/m^2)	24.46 ± 3.35	24.25 ± 3.21	24.40 ± 3.29	24.93 ± 3.66	<0.001***
FBG (mmol/L)	5.31 ± 1.22	4.51 ± 0.35	5.29 ± 0.20	6.89 ± 1.57	<0.001***
Hemoglobin A1c (%)	5.52 ± 0.94	5.24 ± 0.56	5.38 ± 0.62	6.28 ± 1.38	<0.001***
Triglyceride (TG) (mmol/L)	1.55 ± 0.96	1.46 ± 0.88	1.55 ± 0.97	1.73 ± 1.06	<0.001***
Total cholesterol (TC) (mmol/L	4.71 ± 1.09	4.63 ± 1.04	4.76 ± 1.09	4.82 ± 1.18	<0.001***
HDL-C (mmol/L)	1.36 ± 0.38	1.37 ± 0.38	1.37 ± 0.38	1.32 ± 0.37	0.001**
LDL-C (mmol/L)	2.14 ± 1.00	2.10 ± 0.94	2.18 ± 1.01	2.19 ± 1.07	0.008**
Homocysteine (μmol/L)	9.68 ± 6.63	8.49 ± 5.71	9.93 ± 5.81	11.60 ± 8.53	<0.001***
VAI	2.07 ± 1.76	1.89 ± 1.62	2.08 ± 1.73	2.40 ± 2.01	<0.001***
TG/HDL-C	1.32 ± 1.12	1.22 ± 1.02	1.31 ± 1.10	1.52 ± 1.27	<0.001***
TyG	8.63 ± 0.57	8.42 ± 0.50	8.63 ± 0.52	8.99 ± 0.58	<0.001***
TyG-BMI	211.40 ± 35.05	204.68 ± 31.89	211.12 ± 33.70	224.89 ± 38.74	<0.001***
TyG-WC	693.96 ± 99.42	677.98 ± 92.88	689.05 ± 97.93	731.85 ± 103.87	<0.001***
TyG-WHtR	4.31 ± 0.63	4.19 ± 0.58	4.30 ± 0.62	4.57 ± 0.66	<0.001***

Daily vegetable intake: eats 300 g of vegetables a day. Daily fruit intake: eats 200 g of fruits a day.
LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CHD, coronary heart disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TyG, triglyceride–glucose; WHtR, waist-to height ratio; VAI, visceral adiposity index.
*p< 0.05; **p< 0.01; ***p< 0.001.

(p< 0.001), diastolic blood pressure (DBP) (p< 0.001), weight (p< 0.001), WC (p< 0.001), BMI (p< 0.001), VAI (p< 0.001), FBG (p< 0.001), hemoglobin A1c (p< 0.001), TG (p< 0.001), TC (p< 0.001), LDL-C (p< 0.001), and homocysteine (p = 0.047). In addition, the TyG-Q4 group had significantly higher values of the TyG parameters TyG-BMI, TyG-WC, and TyG-WHtR (all p< 0.001). The TyG-Q4 group also had a significantly higher proportion of carotid plaque (p = 0.021).

TABLE 2 Baseline characteristics by triglyceride–glucose (TyG) quartiles in the high-stroke-risk population.

	Total	Q1	Q2	Q3	Q4	*p*-value
TyG	–	≤8.570243	8.570243–8.946032	8.946032–9.369544	≥9.369544	–
N	1,038	260	260	259	259	–
Region						0.64
Urban, *n* (%)	656 (63.20)	164 (63.08)	160 (61.54)	172 (66.41)	160 (61.78)	
Rural, *n* (%)	382 (36.80)	96 (36.92)	100 (38.46)	87 (33.59)	99 (38.22)	
Age (years)	58.66 ± 9.77	59.11 ± 9.27	58.32 ± 10.30	58.61 ± 10.03	58.63 ± 9.50	0.83
Gender, male, *n* (%)	350 (33.72)	76 (29.23)	77 (29.62)	88 (33.98)	109 (42.08)	0.006**
Eating habits						0.72
High-salt diet	147 (14.16)	35 (13.46)	43 (16.54)	36 (13.90)	33 (12.74)	
Oil-heavy diet	311 (29.96)	76 (29.23)	84 (32.31)	73 (28.19)	78(30.12)	
Sweet tooth	580 (55.88)	149 (57.31)	133 (51.15)	150 (57.92)	148 (57.14)	
Meats and vegetables						0.36
Balanced	89 (8.57)	20 (7.69)	27 (10.38)	18 (6.95)	24 (9.27)	
Carnivorous diet	240 (23.12)	54 (20.77)	65 (25.00)	54 (20.85)	67 (25.88)	
Plant-based diet	709 (68.30)	186 (71.54)	168 (64.62)	187 (72.20)	168 (64.86)	
Daily vegetable intake, *n* (%)	543 (52.31)	136 (52.31)	135 (51.92)	135 (52.12)	137 (52.90)	0.99
Daily fruit intake, *n* (%)	347 (33.43)	87 (33.46)	93 (35.77)	81 (31.27)	86 (33.20)	0.78
Alcohol consumption, *n* (%)						0.88
Never	837 (80.64)	217 (83.46)	210 (80.77)	207 (7.92)	203 (78.38)	
Heavy drinking	164 (15.80)	35 (13.46)	40 (15.38)	43 (16.60)	46 (17.76)	
Light/moderate drinking	37 (3.56)	8 (3.08)	10 (3.85)	9 (3.47)	10 (3.85)	
Family history of CHD, *n* (%)	32 (3.08)	11 (4.23)	11 (4.23)	4 (1.54)	6 (2.32)	0.11
Family history of hypertension, *n* (%)	314 (30.25)	69 (26.54)	69 (26.54)	79 (30.50)	97 (37.45)	0.01*
Family history of diabetes, *n* (%)	151 (14.54)	25 (9.62)	32 (12.31)	44 (17.00)	50 (19.31)	0.004**
SBP (mmHg)	134.80 ± 14.06	132.48 ± 14.23	133.30 ± 12.96	136.35 ± 13.95	137.09 ± 14.59	<0.001***
DBP (mmHg)	81.79 ± 8.74	80.50 ± 8.29	80.69 ± 8.08	82.37 ± 9.32	83.63 ± 8.88	<0.001***
Height (cm)	160.54 ± 8.00	160.36 ± 0.15	160.30 ± 7.32	160.05 ± 7.80	161.46 ± 8.65	0.229
Weight (kg)	64.29 ± 10.08	60.44 ± 9.00	63.59 ± 9.62	65.44 ± 8.87	67.69 ± 11.27	<0.001***
Waist circumference (WC) (cm)	81.24 ± 9.12	77.58 ± 8.37	80.71 ± 8.25	83.02 ± 9.12	83.65 ± 9.49	<0.001***
BMI (kg/m^2)	24.96 ± 3.67	23.55 ± 3.43	24.74 ± 3.26	25,61 ± 3.85	25.95 ± 3.66	<0.001***
FBG (mmol/L)	6.89 ± 1.58	6.26 ± 0.65	6.55 ± 0.89	6.92 ± 1.58	7.84 ± 2.21	<0.001***
Hemoglobin A1c (%)	6.33 ± 1.42	5.81 ± 0.98	6.06 ± 1.08	6.40 ± 1.25	7.05 ± 1.90	<0.001***
Triglyceride (TG) (mmol/L)	1.75 ± 1.09	0.97 ± 0.12	1.12 ± 0.32	1.80 ± 0.41	3.14 ± 1.22	<0.001***
Total cholesterol (TC) (mmol/L)	4.82 ± 1,21	4.54 ± 1.27	4.76 ± 1.13	4.88 ± 1.19	5.12 ± 1.20	<0.001***
HDL-C (mmol/L)	1.28 ± 0.47	1.56 ± 0.55	1.33 ± 0.48	1.18 ± 0.39	1.07 ± 0.27	<0.001***
LDL-C (mmol/L)	2.20 ± 1.08	2.05 ± 1.05	2.35 ± 1.03	2.30 ± 1.09	2.10 ± 1.11	0.002**
Homocysteine (μmol/L)	11.64 ± 8.59	12.69 ± 10.01	11.37 ± 8.33	10.63 ± 7.01	11.89 ± 8.64	0.047*
VAI	2.41 ± 2.02	0.91 ± 0.36	1.59 ± 0.57	2.42 ± 0.91	4.71 ± 2.60	<0.001***
TG/HDL-C	1.53 ± 1.27	0.56 ± 0.19	0.98 ± 0.30	1.51 ± 0.49	3.01 ± 1.60	<0.001***
TyG-BMI	224.89 ± 38.74	195.36 ± 28.55	216.75 ± 28.70	234.25 ± 35.19	253.33 ± 36.09	<0.001***
TyG-WC	731.85 ± 103.87	644.29 ± 72.61	707.42 ± 72.93	758.77 ± 83.77	817.37 ± 96.53	<0.001***
TyG-WHtR	4.57 ± 0.66	4.03 ± 0.48	4.42 ± 0.46	4.75 ± 0.57	5.07 ± 0.61	<0.001***
Increased IMT (%)	228 (21.97)	58 (22.31)	57 (21.92)	50 (19.31)	63 (24.32)	0.59
Carotid plaque (%)	385 (37.09)	80 (30.77)	92 (35.38)	100 (38.61)	113 (43.63)	0.02*
CAS (%)	64 (6.17)	12 (4.62)	12 (4.62)	18 (6.95)	22 (8.49)	0.18

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CHD, coronary heart disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TyG, triglyceride–glucose; WHtR, waist-to height ratio; VAI, visceral adiposity index.
*p< 0.05; **p< 0.01; ***p< 0.001.

Relationship between TyG and cervical arterial atherosclerosis

Table 3 shows the multivariate logistic analyses between TyG quartiles and carotid plaque in different models. The omnibus tests of model coefficients are shown in Supplementary Table S2. Compared with those in the TyG-Q1 group, participants in the TyG-Q2, TyG-Q3, and TyG-Q4 groups were more likely to have carotid plaque (model 4: OR = 1.85, 95%CI = 1.28–2.67; OR = 1.51, 95%CI = 1.05–2.18; OR = 1.29, 95%CI = 0.90–1.84; p = 0.01). The TyG-Q2 group had a significant increased risk of cervical atherosclerosis in all models, while no significant statistical difference was found for the TyG-Q4 group. After adjustment for age, sex, smoking, drinking, physical inactivity, hypertension, DM, AF and/or VHD, dyslipidemia, personal history of stroke/TIA, and family history of stroke, TyG was also found to be independent of all covariates. Logistic analysis between the TyG quartiles and increased IMT/CAS showed no statistical difference. In addition, ROC analysis was performed to examine the accuracy of the prediction of cervical artery atherosclerosis. As displayed in Supplementary Figure S1, TyG showed a significantly high area under the ROC curve (AUC) of 0.62 with a permutation $p< 0.001$.

Relationship between TyG and carotid plaque in subgroup analysis

We further performed subgroup analysis using several daily habit and body fat indices. For the participants with DM, those in the TyG-Q2, TyG-Q3, and TyG-Q4 groups were more likely to have carotid plaque than those in TyG-Q1 (OR = 3.21, 95% CI = 1.52–6.80; OR = 2.20, 95%CI = 1.10–4.40; OR = 1.69, 95% CI = 0.84–3.39; p = 0.018) (Table 4). For participants in the non-overweight or obese group, those in TyG-Q2, TyG-Q3, and TyG-Q4 were more likely to have carotid plaque than those in TyG-Q1 (OR = 2.03, 95%CI = 1.28–3.20; OR = 1.56, 95% CI = 1.01–2.42; OR = 1.65, 95%CI = 1.05–2.58; p = 0.02). For subjects in the non-sweet tooth group, those in TyG-Q2, TyG-Q3, and TyG-Q4 were more likely to have carotid plaque than those in TyG-Q1 (OR = 2.60, 95%CI = 1.43–4.71; OR = 1.93, 95%CI = 1.11–3.36; OR = 1.38, 95%CI = 0.79–2.42; p = 0.01).

Discussion

In the current study, we found that the prevalence of a high stroke risk in the general population older than 40 years in eastern China was 23.47%. The TyG index in this population was higher than that in the low-stroke-risk population. Furthermore, it was found that the TyG index is an independent risk factor for carotid plaques in the high-stroke-risk population. Cervical arterial atherosclerosis is a notoriously pathophysiological process of ischemic stroke. These results suggest that the TyG index, a simple measure reflecting carotid plaques, is potentially useful in the early screening of individuals at high risk of stroke.

China faces the greatest challenge from stroke in the world (1). Screening of the risk factors of stroke was implemented in eastern China to improve management of the high-stroke-risk population. In the current study, a large percentage of the high-risk population emphasized the importance of stroke screening. The main contributors to stroke include behavioral risk factors (e.g., eating habits, exercise habits, smoking, and alcohol use) and preexisting conditions (hypertension, DM, dyslipidemia, and AF). All these factors may contribute to the regional high prevalence of cardiovascular disease (CVD) and cervical artery atherosclerosis.

Recently, several general population-based studies have investigated the correlation between TyG and cervical arterial atherosclerosis. The relationship between these two has been proven in postmenopausal women. The study compared the association between the structural and functional indices of subclinical atherosclerosis [i.e., carotid artery IMT, flow-mediated dilation of the brachial artery, and pulse wave velocity (PWV)] and the TyG index, separately for lean and overweight/obese women. The results showed that the TyG

TABLE 3 Odds ratios (95% confidence intervals) for triglyceride–glucose (TyG) and carotid plaque in the high-stroke-risk population.

	TyG quartiles				
Model	**Q1**	**Q2**	**Q3**	**Q4**	***p*-value**
1	Reference	1.74 (1.22–2.50)*	1.41 (0.99–2.01)	1.23(0.87–1.75)	0.021*
2	Reference	1.77 (1.23–2.54)*	1.42 (1.00–2.04)*	1.24 (0.87–1.76)	0.017*
3	Reference	1.79 (1.24–2.58)*	1.41 (0.99–2.02)	1.23 (0.86–1.76)	0.016*
4	Reference	1.85 (1.28–2.67)*	1.51 (1.05–2.18)*	1.29 (0.90–1.84)	0.010*
5	Reference	1.99 (1.28–3.09)*	1.51 (1.02–2.25)*	1.25 (0.87–1.81)	0.021*

Model 1: crude model; model 2: adjusted for age and sex; model 3: adjusted for age, sex, smoking, drinking, and physical inactivity; model 4: adjusted for age, sex, smoking, drinking, physical inactivity, hypertension, diabetes mellitus, atrial fibrillation/valvular heart disease, dyslipidemia, personal history of stroke/transient ischemic attack (TIA), and family history of stroke; model 5: adjusted for gender, family history of hypertension, family history of diabetes, systolic blood pressure (SBP), diastolic blood pressure (DBP), weight, waist circumference, body mass index (BMI), fasting blood glucose (FBG), hemoglobin A1c, triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and homocysteine. *p<0.05.

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TABLE 4 Odds ratios (95% confidence intervals) of the subgroup analysis for triglyceride–glucose (TyG) and carotid plaque in the high-stroke-risk population.

Groups	TyG quartiles				*p*-value
	Q1	Q2	Q3	Q4	
Female	Reference	1.78 (1.12–2.81)	1.40 (0.89–2.19)	1.22 (077–1.92)	0.09
Male	Reference	2.03 (1.07–3.88)	1.80 (0.96–3.41)	1.38 (0.76–2.49)	0.12
DM	Reference	3.21 (1.52–6.80)*	2.20 (1.10–4.40)*	1.69 (0.84–3.39)	0.02
Non-DM	Reference	1.51 (0.98–2.32)	1.31 (0.84–2.02)	1.20 (0.78–1.84)	0.30
Overweight or obese	Reference	1.56 (0.82–2.96)	1.53 (0.77–3.03)	0.89 (0.47–1.69)	0.22
Non-overweight or obese	Reference	2.03 (1.28–3.20)**	01.56 (1.01–2.42)*	1.65 (1.05–2.58)*	0.02
Sweet tooth	Reference	1.48 (0.92–2.39)	1.23 (0.75–2.02)	1.22 (0.76–1.96)	0.46
Non-sweet tooth	Reference	2.60 (1.43–4.71)**	1.93 (1.11–3.36)*	1.38 (0.79–2.42)	0.01
Physical inactivity	Reference	1.67 (1.07–2.60)	1.54 (0.99–2.39)	1.33 (0.85–2.07)	0.12
Non-physical inactivity	Reference	2.28 (1.15–4.52)	1.33 (0.68–2.61)	1.16 (0.61–2.20)	0.11

Adjusted for age, sex, smoking, drinking, physical inactivity, hypertension, diabetes mellitus, atrial fibrillation/valvular heart disease, dyslipidemia, personal history of stroke/transient ischemic attack (TIA), and family history of stroke.
*p< 0.05; **p< 0.01.

index is associated with carotid atherosclerosis and arterial stiffness mainly in lean postmenopausal women. The TyG index may serve as a useful marker for the identification of high-risk women in the normal-weight postmenopausal population (8). High blood pressure is a major cause of atherosclerosis that leads to stroke and myocardial infarction. Data from 77 hypertensive and 199 normotensive individuals were analyzed in a case–control study in Taiwan. The TyG index, IMT, and plaque presence were higher in hypertensive individuals compared to the control group. The TyG index was also significantly correlated with the carotid plaque score and the IMT of the CCA, ICA, and external carotid artery (ECA). Researchers have suggested that the TyG index is significantly associated with the carotid IMT, but could only predict early-stage subclinical atherosclerosis independent of hypertension history, age, sex, and BMI (9).

Other studies have focused on the TyG index and atherosclerotic CVD (ASCVD) (10, 11, 13, 15, 21–26). A retrospective observational cohort study addressed TyG and ASCVD in a large-scale population dataset (5,593,134 participants) older than 40 years. During 8.2 years of mean follow-up, a high TyG index was found to be associated with a significantly increased risk of future ASCVD events, including stroke, myocardial infarction, and both stroke and myocardial infarction. These studies showed that a high TyG index could be a significant predictor of future cardiovascular events. One study applied intravascular optical coherence tomography (OCT) to investigate the prognostic value of the TyG index combined with the morphological characteristics of vulnerable culprit coronary plaques in predicting cardiovascular outcomes. OCT, a cross-sectional and high-resolution intravascular imaging technique, allows the acquisition of detailed *in vivo* images of coronary plaque morphology characteristics, including plaque rupture (PR) and plaque erosion (PE). Researchers have found that the TyG index, combined with plaque characteristics, is a novel biomarker for cardiovascular outcomes (27).

In the present study, we investigated the association between the TyG index and carotid plaque in a high-stroke-risk population in China. The results showed that TyG is independently associated with carotid plaques in individuals at high risk of stroke. After adjusting for several established risk factors, subjects with higher TyG were still more likely to have carotid plaque. The TyG-Q2 group showed a significantly increased risk of cervical atherosclerosis, but the statistical difference was not significant for the TyG-Q4 group. Further studies may require bigger sample sizes and more rigorous statistical methods. In the subgroup analysis, the statistical differences were more significant for individuals with DM, those who are non-overweight or obese, and for those without a sweet tooth. We also found a significant effect of gender on TyG and of DM on carotid plaque. However, logistic analysis did not show much statistical difference between TyG and IMT or CAS. Compared with theTyG-Q1 group, subjects in the TyG-Q2, TyG-Q3, and TyG-Q4 groups were all more likely to have CAS, but showed no statistics difference. Our study also specifically confirmed the correlation between TyG and cervical arterial atherosclerosis. As previously mentioned, the measurement of TyG is inexpensive and readily available, and it is suitable for use as a screening indicator for the general population. It is easier to promote the use of this method in the general population as not all community hospitals have ultrasound equipment. Therefore, TyG is more advantageous compared to traditional parameters.

Previous general population-based studies also investigated the correlation between TyG or its related parameters and stroke. Several studies have focused on the value of TyG to estimate the risk of ischemic stroke (12, 13, 28). An 11-year follow-up study showed that elevated levels at both baseline and long-term updated cumulative average TyG index can independently predict ischemic stroke, but not intracerebral hemorrhage, in the general population (28). Du et al. estimated the prevalence of ischemic stroke from TyG-BMI in two general populations in Liaoning, northeast China. They discovered the potential usefulness of TyG-BMI to improve the risk stratification of ischemic stroke (14). Other studies found that the TyG index could predict the clinical outcomes of stroke, including poor outcomes after reperfusion therapy (17), an increased risk of stroke recurrence (16, 29), all-cause mortality (15, 16), and neurological worsening in patients with ischemic stroke (16, 18). As previously mentioned, most of the relevant studies in China have focused on the population of northeast area. Moreover, few studies have focused on the TyG index and the carotid ultrasound indices in stroke.

Our study, in accordance with other studies, showed that the TyG index is significantly higher in the high-stroke-risk population. In this study, we found that the prevalence rates of increased IMT, carotid plaque, and CAS in the high-stroke-risk general population older than 40 years were 21.97%, 39.3%, and 6.1%, respectively. We also discovered that the TyG index can be an independent predictor of carotid plaque in the high-stroke-risk population, which is a marker of cervical arterial atherosclerosis. However, we did not find a correlation between TyG and an increased IMT or CAS. A possible reason may be the relatively lower prevalence of CAS (6.1%) in this general population. Furthermore, a number of non-CAS participants still had accompanying carotid plaque when they were compared with the CAS group; as a result, some differences may have been obscured. Long-term follow-up studies are needed to further confirm the relationship between TyG and an increased IMT or CAS. In conclusion, our study provided more reference for the application of TyG as a clinically useful marker in the identification of individuals at high risk of carotid plaques or even cervical arterial atherosclerosis.

Why would TyG be associated with cervical arterial atherosclerosis? It might be linked to IR, while the TyG index is a marker of IR. IR is a common pathological condition in which cells are impaired in their ability to respond to the hormone insulin. IR contributes to the development of atherosclerosis *via* multiple mechanisms. Firstly, IR facilitates the formation of atherosclerosis through increased systemic inflammation (30) and endothelial dysfunction (31, 32) and promotes vulnerable plaques. Secondly, IR promotes platelet adhesion, activation, and aggregation, leading to the occlusion of cerebral arteries (33). Thirdly, IR could modify and influence the role of the modifiable stroke risk factors and contribute to the occurrence of atherosclerosis (34, 35). The association between TyG and cervical arterial atherosclerosis based on population levels is still controversial. Analyzing the composition of plaques and careful consideration of the mechanisms of IR would help clarify the exact mechanisms by which TyG contributes to cervical arterial atherosclerosis.

The present study has several limitations. Firstly, its cross-sectional design only allowed assessment of the associations between TyG and carotid plaques rather than their causal links. We could not establish cause-and-effect relationships between the observed associations. In addition, this cross-sectional survey could not provide data on the dynamic changes of TyG. Long-term follow-up studies are needed to further explore the predictive value of TyG for the estimation of ischemic stroke. Secondly, the general population used in the study is from Suzhou City. Varied populations from other cities in eastern China should be enrolled in future research to further confirm these initial findings. Thirdly, several other factors are potentially related to carotid atherosclerosis, but the current study mainly focused on age, sex and the "8+2" stroke risk factors in the final logistic regression models. Future research should further confirm the contribution of the other factors.

In conclusion, the TyG index was significantly associated with carotid plaques and is a convenient, reliable, and practical for clinical use. This index could be a practical indicator for ultrasound-detected cervical artery atherosclerosis, especially for carotid plaques. Furthermore, the statistical differences were more significant for non-overweight or obese and non-sweet tooth individuals, implying that, even for individuals with normal anthropometric features, TyG could still present a potential risk of cervical arterial atherosclerosis. Primarily, TyG may serve as a simple and low-cost marker for the screening of high-stroke-risk groups who need further ultrasonography. It is beneficial to early primary stroke prevention.

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

This study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. Patients/participants provided written informed consent to participate in this study. Written informed consent was obtained from individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XT and LZ conceived the research and wrote the main manuscript text. YL and YZ participated in the recruitment of the sample population. XT, LZ, and YL acquired the data and analyzed the results. XC helped in the interpretation of the results and revised the manuscript. YY and QF guided the process, interpreted the results, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1023867/full#supplementary-material

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Change of serum uric acid and progression of cardiometabolic multimorbidity among middle aged and older adults: A prospective cohort study

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Background: Hyperuricemia is prevalent and associated with individual cardiometabolic diseases, highlighting the potential role of serum uric acid (SUA) in the development and progression of cardiometabolic multimorbidity (CMM, the coexistence of diabetes, heart disease, or stroke). This study aimed to examine the role of SUA change in the progression of CMM.

Methods: This prospective cohort study used data from the China Health and Retirement Longitudinal Study, included 4,820 participants aged 45 years or above with three complete surveys at 2011 (baseline), 2015, and 2018. SUA level at survey 2011 and 2015 was used to measure SUA change as keeping or rising to hyperuricemia, and keeping or declining to non-hyperuricemia. CMM progression was defined as the first report of CMM or additional report of cardiometabolic diseases during survey 2015 and 2018. We used logistic regression models to estimate the odds ratios (ORs) and 95% confidence intervals (95% CIs) of SUA change on CMM progression.

Results: During the follow-up of around 7 years, 519 (10.8%) of the participants kept or rose to hyperuricemia from survey 2011 to 2015, and 311 (6.5%) experienced CMM progression from survey 2015 to 2018. Participants who kept or rose to hyperuricemia had 1.86 (95% CI, 1.29, 2.68) increased odds of CMM progression compared with those who kept or declined to non-hyperuricemia. Specifically, keeping or rising to hyperuricemia (vs. keeping or declining to non-hyperuricemia) was associated with 2.01 times higher odds (95% CI, 1.18, 3.43) of incident diabetes and 1.67 times higher odds (OR:1.67; 95% CI, 1.15, 2.43) of incident cardiovascular diseases following diabetes.

Conclusion: Keeping or rising to hyperuricemia was associated with CMM progression, particularly with incident cardiovascular diseases

following diabetes. These findings suggest that monitoring SUA change may provide innovative insights into the prevention of CMM, especially in the secondary prevention of CMM (i.e., preventing further progression to cardiovascular diseases among patients with diabetes).

KEYWORDS

cardiometabolic multimorbidity, diabetes mellitus, cardiovascular disease, serum uric acid, progression, prospective cohort study, hyperuricemia

Introduction

Cardiometabolic multimorbidity (CMM), the coexistence of diabetes, heart disease, or stroke (1), is the most common multimorbidity pattern (2, 3) and constitutes the major disease burden worldwide including China, particularly in the era of COVID-19 pandemic (4, 5). A pooled analysis of 91 cohort studies showed that the mortality risk of any combinations of cardiometabolic diseases was substantially greater than that of each individual diseases (1). Due to disease-disease interactions, CMM complicates the treatment regimen and greatly challenges healthcare systems configured for single diseases (6). Therefore, evidence informing strategies for prevention and management the development and progression of CMM is urgently needed.

Uric acid is an end product of purine metabolism (7) and traditionally implicated in gout and kidney stones formation (8). Several studies showed that elevated serum uric acid (SUA) was a significant risk factor for incident diabetes (9, 10) and cardiovascular diseases (CVD) mortality (11). Furthermore, UA was suggested as a potential therapeutic target for CVD (12). However, the mechanism by which SUA affecting cardiometabolic diseases is still inconclusive and few studies have ever examined the effect of SUA change. Many studies suggest an independent association between SUA and cardiovascular diseases or mortality, but the association may be explained by its complex relationship with other cardiovascular risk factors (13), for example, a study used data from Framingham cohort found that SUA level was associated with risk for incident coronary heart disease, CVD death, or all-cause death among women, but in fully adjusted multivariate Cox models, the association was no longer significant (14). Recently, rapid development in detection methods of uric acid has made it easy to acquire information on change of uric acid (15). Assessing the effect of uric acid change on CMM progression may help informing risk prediction and intervention targets for the efficient management and prevention of CMM. Using longitudinal data of representative Chinese middle-aged and elderly adults, the present study aimed to explore the association between SUA change and CMM progression.

Methods

Study participants

The China Health and Retirement Longitudinal Study (CHARLS) is a nationally representative prospective cohort which recruited adults aged 45 or older at baseline and followed up regularly to collect information on the demographics, socioeconomics, physical and psychological health. The baseline survey (wave 1) was conducted in 2011–12, with wave 2 in 2013, wave 3 in 2015, and wave 4 in 2018. In order to ensure sample representativeness, the CHARLS baseline survey covered 150 countries/districts, 450 villages/urban communities, across the country, involving 17,708 individuals in 10,257 households, reflecting the mid-aged and older Chinese population collectively. Details of the study have been described elsewhere (16). As of writing, four surveys of 2011, 2013, 2015, 2018 were publicly available. Because blood samples were only collected at survey 2011 and 2015, we chose three surveys of 2011, 2015, 2018 for the present study. Participants aged 45 or above at 2011 and with complete information on exposures and outcomes of interest across the three surveys were included. Participants were excluded if they (a) did not complete the three selected surveys; (b) had missing data on serum uric acid at survey 2011 or 2015; (c) had missing data on diabetes, heart disease, or stroke at survey 2015 or 2018 (Figure 1).

Assessment of SUA change

Blood samples were collected from each participant after fasting overnight and the methods of laboratory assay for the SUA have been described elsewhere (17). Hyperuricemia was defined as SUA level >7 mg/dl in men and >6 mg/dl in women (18). SUA levels at survey 2011 and 2015 was used to measure SUA change. In the present study, SUA change had two forms: (1) the continuous change of SUA concentration between survey 2011 and 2015; (2) the categorical variable of keeping or rising to hyperuricemia, and keeping or declining to non-hyperuricemia from 2011 to 2015.

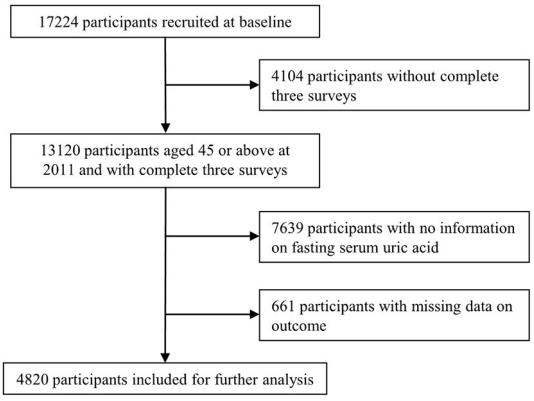

FIGURE 1
Flowchart showing the selection of participants.

TABLE 1 Definition of progression of CMM.

No. of cardiometabolic diseases			CMM progression
Survey 2015	Survey 2018	Change of No.	
0	0	0 to 0, stable	×
0	1	0–1	×
0	2	0–2	√
0	3	0–3	√
1	1	1 to 1, stable	×
1	2	1–2	√
1	3	1–3	√
2	2	2 stable	×
2	3	2–3	√
3	3	3–3, stable	×

√indicates situations which can be considered as CMM progression. X indicates situations which can not be considered as CMM progression.

Assessment of CMM and CMM progression

At each survey, participants were asked whether or not a doctor has told them they had diabetes, heart disease (including heart attack, coronary heart disease, angina, congestive heart failure, or other heart problems) or stroke, to which they could respond "yes" or "no." Where "Yes" was reported, the timing of diagnoses, current medications and treatments for each specific condition were further collected. Thus, the self-report diagnosis can be validated by diagnosis time or medications. CMM referred to the coexistence of two or more diseases from diabetes, heart disease, and stroke. From survey 2015 to 2018, the first report of individual diseases or CMM after survey 2015 was considered as incidence. The progression of CMM, as defined in previous studies (2), indicated the first report of CMM or additional report of cardiometabolic disease among participants who were already with CMM after survey 2015 (Table 1).

Assessment of covariates

Covariates were assessed based on self-reported data at survey 2011 (baseline). There were four groups of covariates: demographic information including age and sex (male, female); health behaviors including physical activity (inactive, active), alcohol consumption (less than weekly drinking, weekly drinking or more), smoking status (never, ever), body mass index (BMI, weight divided by the square of height), and history of other chronic conditions including systolic blood pressure (SBP), liver disease (yes, no), kidney disease (yes, no), and dyslipidaemia (yes, no); and other serum biomarkers including high-density lipoprotein (HDL, <40 mg/dl, ≥40 mg/dl), low-density lipoprotein (LDL, <160 mg/dl, ≥160 mg/dl), total cholesterol (TC, <240 mg/dl, ≥240 mg/dl), fasting blood glucose (FBG, <126 mg/dl, ≥126 mg/dl). Notably, BMI was based on physically measured height and weight and categorized as underweight (<18.5 kg/m^2), healthy (18.5–24.9 kg/m^2), overweight or obese (≥25 kg/m^2). Details can be seen in Table 2.

Statistical analysis

Baseline characteristics were described by SUA change (i.e., keeping or rising to hyperuricemia, keeping or declining to non-hyperuricemia). Mean and standard deviation, or frequency and percentages were used to describe continuous or categorical variables, respectively. Differences between groups were examined using *t*-tests or chi-squared tests.

Main analysis

The primary aim of this study was to explore the role of SUA change on CMM progression. We firstly used multivariable logistic regression models (adjusted for age, sex, and health behaviors) to examine the associations of keeping hyperuricemia (vs. declining to non-hyperuricemia), and rising to hyperuricemia (vs. keeping non-hyperuricemia) with CMM progression among participants with or without hyperuricemia at baseline, respectively. Secondly, we further examined the effect of four categorized group of SUA change with "keeping non-hyperuricemia" as reference in the whole population. Thirdly, we aggregated the exposure (keeping or rising to hyperuricemia vs. keeping or declining to non-hyperuricemia) and investigated their association with CMM progression. Furthermore, we analyzed the change of SUA as a continuous variable and used restricted cubic splines with five knots to visualize the shape of the association. Wald chi-square tests were used to test whether the null hypothesis of linear association can be rejected. Lastly, we restricted population

TABLE 2 Baseline characteristics (2011) according to serum uric acid change.

Characteristics	Serum uric acid		*P*
	Keeping or rising to hyperuricemia ($n = 4,301$)	Keeping or declining to non-hyperuricemia ($n = 519$)	
Age, years (median [IQR])	59.0 [53.0, 67.0]	58.0 [52.0, 64.0]	<0.001
Sex (%)			0.001
Male	1,882 (43.8)	266 (51.3)	
Female	2,419 (56.2)	253 (48.7)	
SBP, mmHg (median [IQR])	135.2 [122.5, 148.7]	128.3 [116.7, 142.0]	<0.001
Body mass index, kg/m² (%)			<0.001
Underweight (<18.5)	213 (5.7)	9 (2.0)	
Healthy (18.5–23.9)	2,017 (53.6)	183 (40.5)	
Overweight (≥24)	1,534 (40.8)	260 (57.5)	
Marital status (%)			0.854
Married	3,861 (89.8)	464 (89.4)	
Separated/divorced/widowed/never married	440 (10.2)	55 (10.6)	
Education (%)			0.058
less than upper secondary education	2,092 (48.6)	229 (44.1)	
Upper secondary & vocational training & tertiary education	2,209 (51.4)	290 (55.9)	
Smoking (%)			0.762
Never	3,035 (71.4)	364 (70.7)	
Ever/now	1,214 (28.6)	151 (29.3)	
Alcohol drinking (%)			0.032
Less than weekly drinking	3,410 (83.7)	389 (79.7)	
Weekly drinking or more	666 (16.3)	99 (20.3)	
Physical activity* (%)			0.242
Inactive	572 (31.4)	82 (36.9)	
Active	1,249 (68.6)	140 (63.1)	
Dyslipidaemia (%)			<0.001
No	3,779 (89.2)	420 (82.5)	
Yes	458 (10.8)	89 (17.5)	
Liver disease (%)			1.000
No	4,126 (96.3)	498 (96.3)	
Yes	160 (3.7)	19 (3.7)	
Kidney disease (%)			0.847
No	4,019 (93.8)	484 (93.6)	
Yes	265 (6.2)	33 (6.4)	
HDL (%)			<0.001
<40 mg/dl	3,324 (77.3)	327 (63.0)	
≥3. mg/dl	977 (22.7)	192 (37.0)	
LDL (%)			0.007
≤.00 mg/dl	3,873 (90.0)	447 (86.1)	
>160 mg/dl	428 (10.0)	72 (13.9)	
FBG (%)			0.001
<126 mg/dl	3,803 (88.4)	434 (83.6)	
≥3.6 mg/dl	498 (11.6)	85 (16.4)	
TG (%)			<0.001
<240 mg/dl	3,839 (89.3)	436 (84.0)	
≥4.0 mg/dl	462 (10.7)	83 (16.0)	

SBP, Systolic Blood Pressure; HDL, High Density Liptein Cholesterol; LDL, Low Density Liptein Cholesterol; FBG, Fasting Blood Glucose; TG, Triglyceride. *In CHARLS wave 1–3 (2011, 2013, 2015), the questions on physical activity were only asked to a random subsample of half the sample, and those who were not selected were assigned a special missing value.

TABLE 3 Associations between change of serum uric acid and progression of cardiometabolic multimorbidity.

Populations	Exposure	No. and % of case	ORs (95% CIs)	*P*-value
Participants with hyperuricemia at baseline	Declining to non-hyperuricemia ($n = 137$)	7 (7.95%)	1.00 (ref)	0.248
($n = 225$)	Keeping hyperuricemia ($n = 88$)	14 (10.22%)	2.46 (0.53, 11.42)	
Participants without hyperuricemia at baseline	Keeping non-hyperuricemia ($n = 4,213$)	249 (5.91%)	1.00 (ref)	<0.001
($n = 4,595$)	Rising to hyperuricemia ($n = 382$)	41 (10.73%)	1.98 (1.32, 2.95)	
The whole population ($n = 4,820$)	Declining to non-hyperuricemia ($n = 137$)	7 (7.95%)	0.63 (0.19, 2.13)	0.21
	Keeping hyperuricemia ($n = 88$)	14 (10.22%)	1.37 (0.65, 2.90)	0.58
	Keeping non-hyperuricemia ($n = 4,213$)	249 (5.91%)	1.00 (ref)	
	Rising to hyperuricemia ($n = 382$)	41 (10.73%)	2.00 (1.34, 2.98)	0.01
The whole population ($n = 4,820$)	Keeping or declining to non-hyperuricemia ($n = 4,301$)	256 (5.95%)	1.00 (ref)	<0.001
	Keeping or rising to hyperuricemia ($n = 519$)	55 (10.60%)	1.86 (1.29, 2.68)	
The whole population ($n = 4,820$)	Continuous change of SUA	311 (6.45%)	1.23 (1.08, 1.39)	0.001

Case here indicates participants experiencing progression of cardiometabolic multimorbidity. Details on the definition of progression can be seen in Table 1. Model: Adjust for age, sex, and health behaviors (smoking, alcohol consumption, physical activity).

to those without CMM at survey 2015 and used multinomial logistic regression models to examine the association of the SUA change with different transitions (Supplementary Figure 1) of cardiometabolic diseases (19): (1) transition from no conditions to incident diabetes only, (2) transition from no conditions to incident CVD only, (3) transition from no conditions to CMM, (4) cardiovascular diseases (heart disease or stroke) followed-by diabetes, (5) diabetes followed-by cardiovascular diseases. In this multinomial analysis, participants with none of the three cardiometabolic diseases from survey 2015 to 2018 were the reference group. All the analyses above reported associations as odds ratios (ORs) with 95% confidence intervals (CIs). For each analysis, models adjusting for age at baseline, sex, BMI, SBP and behaviors.

Subgroup analysis and sensitivity analysis

We performed subgroup analysis stratified by age, sex, BMI, smoking, alcohol, and physical activates, respectively, to examine the variations in the associations of the aggregated SUA change with CMM progression. To check the robustness of our main findings, we run models with additional adjustment for history of chronic conditions and other serum biomarkers to examine the association between the aggregated SUA change and progression of CMM.

All analysis was performed using SAS (version 9.4, SAS Institute Inc.) and R (version 4.0.5). All statistical tests were two-side, and $P < 0.05$ was considered to be statistically significant.

Results

Characteristics of participants

Of the 4,820 participants included, participants who kept or rose to hyperuricemia were more likely to be older, male, overweight, consume alcohol more frequently, have dyslipidaemia, have higher SBP, LDL and lower HDL, TG, FBG (Table 2).

Associations of SUA change with CMM progression

From survey 2011 to 2015, 88 (1.8%) of the participants kept hyperuricemia and 382 (7.9%) rose to hyperuricemia, while 4,213 (87.4%) kept non-hyperuricemia and 137 (2.8%) declined to non-hyperuricemia (Table 3). Over approximate 3-year follow-up, 311 (6.5%) participants experienced CMM progression. Among participants without hyperuricemia at baseline, rising to hyperuricemia (vs. keeping non-hyperuricemia) was associated with 1.98 elevated odds (95% CI, 1.32, 2.95, $P < 0.001$) of CMM progression. Among participants with hyperuricemia at baseline, keeping hyperuricemia (vs. declining to non-hyperuricemia) was associated with 2.46 elevated odds (OR, 2.46; 95% CI, 0.53, 11.42, $P = 0.25$) of CMM progression. In terms of the four categorized group with keeping non-hyperuricemia as reference, rising to hyperuricemia was significantly associated with higher odds (OR, 2.00; 95% CI, 1.34, 2.98, $P = 0.01$) of CMM progression in the whole population. With For participants who kept or rose to hyperuricemia, the odds of CMM progression was 1.86 (95% CI, 1.29, 2.68, $P < 0.001$) times higher than participants who kept or declined to non-hyperuricemia. In model with continuous change of SUA as exposure, we found each unit increase in SUA was associated with 1.23 times higher odds (95%CI, 1.08, 1.39, $P = 0.001$) of CMM progression. According to the restricted cubic splines, there was no evidence demonstrate that nonlinear association between SUA concentration change and CMM progression (P for non-linear trend = 0.20) (Figure 2).

Associations of SUA change with transitions of cardiometabolic diseases

Of the 4,576 participants without CMM at survey 2015, 123 (2.7%) participants developed diabetes only from survey 2015 to 2018, 341 (7.5%) developed CVD only, 57 (1.2%) developed CMM, 306 (6.7%) developed cardiovascular diseases following diabetes and 76 (1.7%) developed diabetes following cardiovascular diseases. Compared to participants who kept or declined to non-hyperuricemia, participants who kept or rose to hyperuricemia had 2.04 higher odds (95% CI, 1.20, 3.47, $P = 0.009$) of developing diabetes only, and 1.67 higher odds (95% CI, 1.15, 2.43, $P = 0.007$) of developing cardiovascular diseases following diabetes. We found no significant associations between SUA change and developing CVD only, CMM, or diabetes following cardiovascular diseases (Table 4).

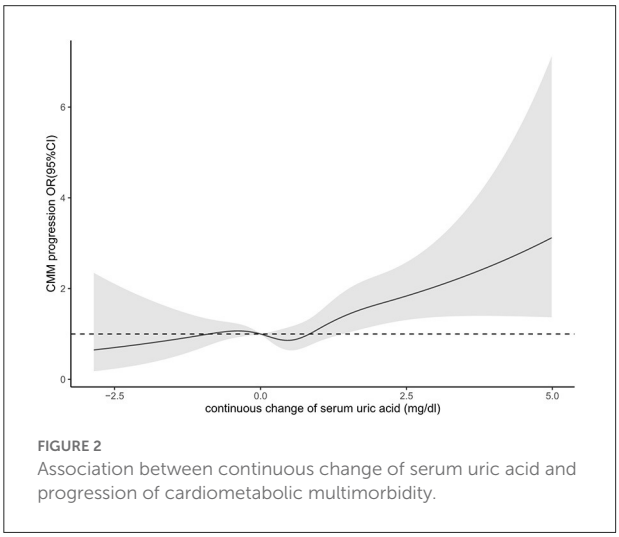

FIGURE 2
Association between continuous change of serum uric acid and progression of cardiometabolic multimorbidity.

Subgroup analysis and sensitivity analysis

The subgroup analysis and sensitivity analysis were consistent with our main results. However, we also observed variations in the associations of the aggregated SUA change with CMM progression in different subgroups (Supplementary Figure 1). For example, the ORs of the aggregated SUA change on CMM progression was larger in males than females. In models with additional adjustment for history of chronic conditions and serum biomarkers (Supplementary Tables 1, 2), we found that keeping or rising to hyperuricemia (vs. keeping or declining to non-hyperuricemia) was significantly associated with the progression of CMM (OR, 1.77; 95% CI, 1.18, 2.64, $P = 0.006$).

TABLE 4 Multinomial logistic regression analysis of the associations between change of serum uric acid and different transitions of cardiometabolic diseases.

Transitions of cardiometabolic diseases		The population without CMM at survey 2015 ($n = 4,576$)	
		Keeping or declining to non-hyperuricemia ($n = 4,096$)	Keeping or Rising to hyperuricemia ($n = 480$)
No conditions to diabetes ($n = 123$)	Observed prevalence	2.39% (98/4,096)	5.21% (25/480)
	OR (95% CI)	1.00 (ref)	2.04 (1.20, 3.47)
	P-value	0.009	
No conditions to CVD ($n = 341$)	Observed prevalence	7.54% (309/4,096)	6.66% (32/480)
	OR (95% CI)	1.00 (ref)	0.97 (0.64, 1.49)
	P-value	0.905	
No conditions to CMM ($n = 57$)	Observed prevalence	1.12% (46/4,096)	2.29% (11/480)
	OR (95% CI)	1.00 (ref)	2.02 (0.98, 4.18)
	P-value	0.057	
Diabetes to CVD ($n = 306$)	Observed prevalence	6.15% (252/4,096)	11.25% (54/480)
	OR (95% CI)	1.00 (ref)	1.67 (1.15, 2.43)
	P-value	0.007	
CVD to diabetes ($n = 76$)	Observed prevalence	1.59% (65/4,096)	2.29% (11/480)
	OR (95% CI)	1.00 (ref)	1.51 (0.74, 3.09)
	P-value	0.257	

Models were adjusted for age, sex, and health behaviors (smoking, alcohol consumption, physical activity). CMM, cardiometabolic multimorbidity; CVD, cardiovascular disease, the diagnosis of either heart disease or stroke.

Discussion

We analyzed 4,820 middle-aged or elderly Chinese adults and found keeping or rising to hyperuricemia was associated with higher odds of CMM progression. Specifically, keeping or rising to hyperuricemia was associated with higher odds of incident cardiovascular diseases among patients with diabetes.

Interpretation and comparison with other studies

To our knowledge, this is the first study to delineate the role of SUA change in the progression of diabetes, heart disease, stroke, and CMM. In our study, keeping or rising to hyperuricemia was associated with diabetes (OR, 1.86; 95% CI, 1.29, 2.68, $P < 0.001$), compared with keeping or declining to non-hyperuricemia; but showed no effects on heart disease (OR, 1.05; 95% CI, 0.62, 1.78, $P = 0.86$) and stroke (OR, 0.88; 95% CI, 0.45, 1.73, $P = 0.68$), which is consistent with prior studies. For example, a meta-analysis (42,834 participants) suggests that SUA level is positively associated with the development of type 2 diabetes regardless of various study characteristics (18), and evidence from the British Regional Heart Study, which included middle-aged 7,735 men (average 16.8 years follow-up), found that SUA was strongly correlated with many CVD risk factors and was positively associated with risk for fatal and non-fatal CHD events. However, after full adjustment for potentially confounding clinical factors, this relationship was no longer significant (20). However, as for CVD, other studies demonstrated the associations between SUA and CVD, for example, a prospective study, found that compared with individuals in the bottom third of baseline measurements of serum uric acid in the Reykjavik study, those in the top third had an age- and sex-adjusted odds ratio for CHD of 1.39 (95% CI, 1.23–1.58) (20). Evidence from the Third National Health and Nutrition Examination Survey revealed an increased risk of CV mortality with increasing SUA levels, hazard ratio (95% CI) per 59.5 mmol/l of SUA was 1.32 (1.25–1.38), and remained 1.15 (1.08–1.21) even after adjusted for demographic factors, comorbidities and other risk factors (21). The role of SUA as a risk factor for developing cardiovascular disease is controversial (22), and the association between SUA and CVD may be explained by its complex relationship with other cardiovascular risk factors (23).

Besides, evidence from previous studies indicated that high uric acid is independently associated with an increased risk of new-onset CVD in patients with diabetes (21, 22). Which is consistent with our finding that keeping or rising to hyperuricemia was significantly associated with the incidence of cardiovascular diseases among patients with diabetes (OR, 1.67; 95% CI, 1.15, 2.44, $P = 0.007$). Our data for incidence and transition of diabetes to heart disease added to new evidence by suggesting that keeping or rising to hyperuricemia is strongly linked to cardiometabolic multimorbidity, particularly among those who had already diagnosed with diabetes.

The findings reflected that cardiometabolic conditions are not simply co-occurring through chance. Zemedikun et al. used a combination of cluster analysis and data mining techniques identified that diabetes might be the epicenter of disease clusters for multimorbidity (23). There is evidence that certain conditions are more likely to cluster due to shared pathological pathways or networks, whereby the onset of one condition increases the risk of another (24), previous studies showed that associations of diabetes with chronic disease outcomes are largely independent of major cardiovascular risk factors (25, 26) and diabetes is a well-established risk factor for heart disease and stroke. Although the underlying mechanisms for higher risks of CVD among participants with diabetes remain unclear, previous studies have suggested hyperuricemia in patients with diabetes causes incremental variations in uric acid levels over time, thus increasing oxidative stress and generating free radicals, which contribute to endothelial dysfunction and RAAS activation, ultimately leading to CVD (13, 21). But studies also suggested that the apparent association might be explained that SUA caused CVD is primarily a function of SUA being strongly collinear with established CVD risk factors (14, 27).

Cardiometabolic multimorbidity, one of the most common multimorbidity pattern, could accelerate the development and progression of other conditions like mental or musculoskeletal disorders (28). Exploring the association between SUA change and the progression of CMM may help us to develop methods to interrupt the progression; furthermore, it can also provide evidence for secondary prevention of developing other diseases. Hence, clinicians should pay attention to the SUA levels and changes over time, particularly among patients with diabetes. Besides, studies also suggested that some lifestyle factors (e.g., smoke, alcohol consumption, less healthy dietary habits) played important roles in the progression of CMM (29, 30); therefore, lifestyle modifications those targeted for keeping or declining serum uric acid level and the development of cardiometabolic diseases should be emphasized especially among patients with diabetes.

Strengths and limitations

Using representative sample of Chinese middle-aged and elderly adults and prospective study design, that study may provide reliable associations between uric acid and cardiometabolic diseases. In addition, we measured the longitudinal change of SUA, instead of cross-sectional status, and comprehensively explored the associations between SUA change with different transition patterns of cardiometabolic

diseases, adding novel insights into the role of SUA in the networks of cardiometabolic diseases. This study also has several limitations. First, most variables of interest were self-reported and thus susceptible to reporting errors. Second, CMM progression was a chronic process, and 3-year follow-up may not long enough to capture the long-term progression. Third, information about medications affecting SUA change, such as diuretics and beta blockers were potential confounders and not adjusted in models because of limited data availability. Forth, we used frequency, instead of amount (e.g., volume or weight) to assess drinking behavior, which may be not accurate enough. Finally, due to the small sample size and short-time period, the progression from no conditions to diabetes and then to CVD, and the progression from no conditions to CVD and then to diabetes cannot be captured; which guaranteed further studies.

Conclusions

Keeping or rising to hyperuricemia was associated with CMM progression, particularly associated with higher odds of developing CVD after diabetes. Our study highlighted the role of SUA change in interpreting the mechanisms for the progression of CMM, and the need for healthcare professionals to monitor SUA change for efficient management of CMM, especially in the secondary prevention of diabetes (i.e., preventing further progression to CMM).

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 Biomedical Ethics Committee of Peking University (No. IRB00001052–11015). The patients/participants provided their written informed consent to participate in this study.

Author contributions

XX conceptualized the study, supervised the whole project, had full access to all the data in the study, and had final responsibility for the decision to submit for publication. DL, DW, and XX made the analysis plan. DL conducted the statistical analyses. DL and DW wrote the initial draft of the manuscript. YN and DW verified the underlying data. XD provided valuable support on statistical methods, drafting, and revisions of the manuscript. All authors contributed to the article and approved the final manuscript.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1012223/full#supplementary-material

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The impact of socioeconomic deprivation on the risk of atrial fibrillation in patients with diabetes mellitus: A nationwide population-based study

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Objective: To evaluate the relationship between socioeconomic status and the risk of atrial fibrillation (AF) in patients with diabetes mellitus (DM).

Research design and methods: From the National Health Insurance Service (NHIS) database, we identified 2,429,610 diabetic patients who underwent national health check-ups between 2009 and 2012. Tracing back the subjects for 5 years from the date of health check-up, we determined the subjects' income and whether they received medical aid (MA) during the past 5 years. Subjects were divided into six groups according to the number of years of receiving (MA groups 0 through 5) and into four groups according to socioeconomic status change during the past 5 years. We estimated the risk of AF for each group using the Cox proportional-hazards model.

Results: During a median follow-up of 7.2 ± 1.7 years, 80,257 were newly identified as AF. The MA groups showed a higher risk of AF than the non-MA group with the hazard ratios (HRs) and 95% confidence interval (CI) 1.32 (1.2–1.44), 1.33 (1.22–1.45), 1.23 (1.13–1.34), 1.28 (1.16–1.4), and 1.50 (1.39–1.63) for MA groups 1 through 5, respectively. Dividing subjects according to socioeconomic condition change, those who experienced worsening socioeconomic status (non-MA to MA) showed higher risk compared to the persistent non-MA group (HR 1.54; 95% CI 1.38–1.73).

Conclusion: Low socioeconomic status was associated with the risk of AF in patients with diabetes. More attention should be directed at alleviating health inequalities, targeting individuals with socioeconomic deprivation to provide timely management for AF.

KEYWORDS

socioeconomic status, atrial fibrillation, social medicine, diabetes mellitus, medical aid beneficiaries

Introduction

The prevalence of atrial fibrillation (AF) and AF-related morbidity and mortality is increasing globally. It is associated with an increasingly older adult population, more prevalent comorbidities, and unhealthy lifestyles (1, 2). For example, the Framingham study identified aging and cardiovascular comorbidities such as hypertension, diabetes mellitus (DM), coronary artery disease, and valvular heart disease as the most potential risk factors for the development of AF (3).

Diabetes mellitus is known to increase the risk of AF by 28% (4, 5) and is an important factor in the prognosis of AF. In addition, the risk of AF is increased in patients with poorly controlled DM and macrovascular and microvascular complications (6). Prevention of incident AF and appropriate management of the prevalent AF in patients with diabetes would lower the risk of morbidity and mortality. Indeed, this holistic or integrated care approach to AF care is being increasingly promoted (7, 8), given that adherence to holistic management is associated with improved clinical outcomes (9, 10).

Among the non-disease risk factors for AF, many studies have been conducted on whether the risk of AF changes according to health inequalities and socioeconomic status (11–16). Although socioeconomic status shows an inverse relationship with overall morbidity, mortality, and cardiovascular diseases (17, 18), the results on the relationship between socioeconomic status and AF remain controversial. Previous studies have shown that a lower income is associated with a higher risk of AF (11, 12). However, a weak relationship between a low socioeconomic status and the risk of AF has also been reported (13). Nevertheless, low socioeconomic status notably elevates the prevalence of various diseases, including DM, obesity, and depression (19, 20), and these diseases are risk factors for AF (3, 21).

We investigated the association between socioeconomic status and AF risk in patients with diabetes using a population-based nationwide cohort study.

Materials and methods

Data source and study population

We used data from the National Health Insurance Service (NHIS) database. The Korean NHIS is a compulsory public medical assistance system with over 51 million Koreans currently participating. In addition, the Korean National Health Insurance Corporation provides annual or biennial national health examinations for people over the age of 20, and these data are linked to the NHIS database.

We identified 2,429,610 subjects with DM and without prevalent AF, who underwent a national health examination at least once between 2009 and 2012. Patients younger than 20 years of age, those with missing values among covariates, and those diagnosed with AF within 1 year after the health examination were excluded. **Figure 1** shows the flowchart of study enrolment. This study was exempt from review by the Seoul National University Hospital Institutional Review Board (E-2105-141-1220).

Definition of medical aid beneficiary

Medical aid (MA) is a public assistance system that the state guarantees for the medical problems of low-income people who cannot sustain life or have difficulties in living (22). People who are unable to work and homeless are usually recipients of the MA; the recipient household's income must be less than 40% of the median national household income to benefit from the MA (23).

By tracing back 5 years from the index date of the subject's health examination, we obtained the participants' household income status and categorized the study population into six groups according to the number of years the subject was a beneficiary of the MA program (0 through 5; named non-MA, MA 1, MA 2, MA 3, MA 4, and MA 5 groups, respectively). The detailed study design is illustrated in **Figure 1**.

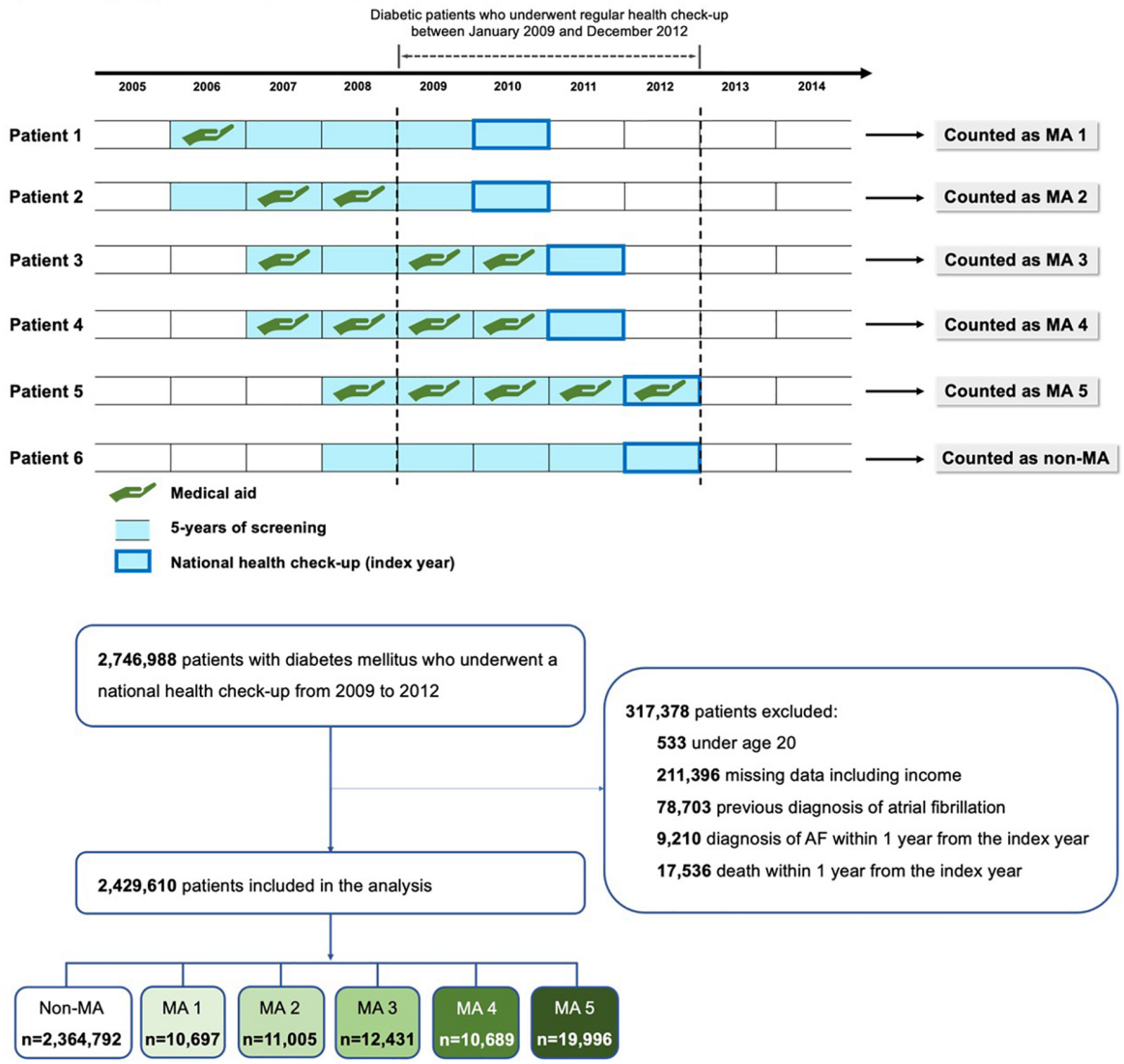

FIGURE 1
Study design and flowchart of enrolment.

Assessment of covariates

Covariates included age, sex, smoking status, drinking status, regular exercise, comorbidities of hypertension and dyslipidaemia, body mass index (BMI), waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose level, total cholesterol, and high and low-density lipoprotein (HDL and LDL) cholesterol (24). The details of the subjects' DM were analyzed to determine whether they had been diagnosed with DM 5 years or more, whether the subject was taking DM medication, and whether the number of diabetic medications was three or more (25). Smoking status (never, ex, or current smoker), drinking status, and physical activity were assessed using a self-report questionnaire completed as part of the national health examination (26). Alcohol consumption of less than 30 g per day was defined as mild drinking, and 30 g or more was defined as heavy drinking. Regular physical activity was defined as performing moderate-intensity exercise more than five times a week or vigorous-intensity exercise more than three times a week (26). Detailed definitions of the diagnoses, including AF and comorbidities, such as hypertension and dyslipidaemia, are presented in **Supplementary Table 1** (24).

Study outcome and follow-up

The primary outcome was the occurrence of incident AF during the follow-up period. AF was defined as at least one

hospitalization or at least two outpatient clinic visits with diagnostic codes of AF (I480-I484 and I489), according to the International Classification of Disease, 10th Revision (ICD-10) (6, 27, 28). Subjects were followed up from the index date of the national health examination until the occurrence of AF, death, or the end of the study period (31 December 2018), whichever came first.

Statistical analysis

Baseline characteristics were described across groups with different numbers of years of receiving MA. Continuous variables were expressed as mean ± standard deviation, and categorical variables were presented as numbers and percentages. Differences among the groups were examined using analysis of variance (ANOVA). The crude incidence rate (IR) of incident AF was calculated as the number of events per 1,000 person-years (PY). To analyze the association between the number of years of receiving MA and the risk of incident AF, we used univariate and multivariate Cox proportional hazard regression models. The outcomes for the groups were presented as hazard ratios (HRs) and 95% confidence intervals (CIs). Adjustments were made for the covariates of age, sex, hypertension, dyslipidaemia, BMI, fasting blood glucose level, smoking, drinking status, and regular physical activity.

The level of significance was set at 0.05 and all analyses were two-sided. Statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Complementary analyses

We further conducted multiple complementary analyses to investigate the associations between various indicators of SES and the risk of incident AF. First, to check whether there is a relationship between various income levels and AF risk, participants were divided into 21 groups according to health insurance premiums paid in the index year (the year of national health examination): the MA group and income level 1 through 20 groups, with higher numbers indicating higher insurance premiums. We cross-sectionally analyzed the effect of income level on the risk of AF in the index year. Second, to check whether low income–applying a more lenient definition than MA beneficiary–defined as paying the bottom 20% of health insurance premiums also affects the risk of AF, we divided the participants into six groups according to the number of years of low income. Lastly, to check whether changes in socioeconomic status affect AF risk, whether the subjects received MA in the index year and 4 years ago from the index year were investigated. Subjects were classified into four groups: initial MA recipients and MA recipients later (persistent MA group), initial non-MA recipients but later becoming MA recipients (non-MA to MA group), initial MA recipients but later becoming non-MA recipients (MA to non-MA group), and initial non-MA recipients and non-MA recipients later (persistent non-MA group). In these three complementary analyses, the multivariable-adjusted HRs of incident AF among the groups were estimated.

Subgroup analyses

We performed subgroup analyses and interaction tests to evaluate the potential impact of age, sex, duration of DM, insulin use, use of three or more antidiabetic medications, and comorbidities, including hypertension and dyslipidaemia, on the relationship between socioeconomic status and the risk of AF. P for interaction less than 0.1 was considered significant.

Results

Among a total of 2,429,610 subjects (mean age 56.9 ± 12.4, 60% men), 2,364,792 did not have a history of MA (97.3%, non-MA group), and 10,697 subjects had a history of MA at least for 1 year (0.4%, MA 1 group), 11,005 for 2 years (0.5%, MA 2 group), 12,431 for 3 years (0.5%, MA 3 group), 10,689 for 4 years (0.4%, MA 4 group), and 19,996 subjects benefited from MA for 5 years (0.8%, MA 5 group) (**Figure 1**). **Table 1** shows the baseline characteristics of each group.

Compared with the non-MA group, the MA group had a higher prevalence of hypertension and dyslipidaemia and a lower prevalence of regular physical activity. The proportion of obese people with BMI ≥ 25 kg/m^2 was significantly higher in the MA 5 group (48.6% in the non-MA group, 46.6% in the MA 1, 47.2% in the MA 2 group, 47.3% in the MA 3 group, 47.3% in the MA 4 group, and 50.2% in the MA 5 group; $p < 0.0001$). The proportion of patients with a DM duration of 5 years or longer was significantly higher in the MA group than in the non-MA group (29.9 vs. 32.9 to 45.4% in the MA ≥ 1 group, $p < 0.0001$). The proportion of subjects taking three or more antidiabetic drugs was higher in the MA group than in the non-MA group (18.6 to 24.7% in the MA group vs. 13.7% in the non-MA group, $p < 0.0001$).

The baseline characteristics based on the occurrence of AF during the follow-up period are summarized in **Supplementary Table 2**. Patients who developed new AF during follow-up (AF group) were older than those who did not develop the arrhythmia (non-AF group) (65.1 ± 10.32 years in AF group vs. 56.59 ± 12.39 years in non-AF group, $p < 0.0001$), while the sex ratio between groups was similar (men 59.93% in AF

TABLE 1 Baseline characteristics of the subjects grouped by the number of times receiving medical aid.

	Cumulative medical aid burden						
	0	1	2	3	4	5	*P*-value
	n = 2,364,792	*n* = 10,697	*n* = 11,005	*n* = 12,431	*n* = 10,689	*n* = 19,997	
Age	56.82 ± 12.44	58.5 ± 13.18	59.82 ± 13.11	60.52 ± 12.39	60.1 ± 12.41	56.49 ± 8.73	<0.0001
<40	199,126 (8.42%)	705 (6.59%)	545 (4.95%)	417 (3.35%)	376 (3.52%)	326 (1.63%)	<0.0001
40–64	1,486,221 (62.85%)	6,042 (56.48%)	6,091 (55.35%)	7,022 (56.49%)	6,049 (56.59%)	14,616 (73.09%)	
≥65	679,445 (28.73%)	3,950 (36.93%)	4,369 (39.7%)	4,992 (40.16%)	4,264 (39.89%)	5,054 (25.28%)	
Male sex	1,429,887 (60.47%)	4,938 (46.16%)	4,642 (42.18%)	5,068 (40.77%)	4,341 (40.61%)	9,195 (45.98%)	<0.0001
Smoking							<0.0001
Never	1,305,493 (55.21%)	6,633 (62.01%)	7,133 (64.82%)	8,220 (66.13%)	7,104 (66.46%)	11,920 (59.61%)	
Former	428,469 (18.12%)	1,260 (11.78%)	1,250 (11.36%)	1,355 (10.9%)	1,106 (10.35%)	2,332 (11.66%)	
Current	630,830 (26.68%)	2,804 (26.21%)	2,622 (23.83%)	2,856 (22.97%)	2,479 (23.19%)	5,744 (28.73%)	
Drinking							
Non-MA	1,333,833 (56.4%)	7,390 (69.08%)	7,965 (72.38%)	9,132 (73.46%)	7,866 (73.59%)	14,878 (74.4%)	
Mild	787,339 (33.29%)	2,464 (23.03%)	2,341 (21.27%)	2,476 (19.92%)	2,158 (20.19%)	3,813 (19.07%)	
Heavy	243,620 (10.3%)	843 (7.88%)	699 (6.35%)	823 (6.62%)	665 (6.22%)	1,305 (6.53%)	
Regular exercise	488,724 (20.67%)	1,645 (15.38%)	1,706 (15.5%)	1,922 (15.46%)	1,627 (15.22%)	3,159 (15.8%)	<0.0001
Hypertension	1,295,148 (54.77%)	6,502 (60.78%)	7,033 (63.91%)	8,184 (65.84%)	6,970 (65.21%)	12,848 (64.25%)	<0.0001
Dyslipidemia	935,703 (39.57%)	4,720 (44.12%)	5,081 (46.17%)	5,980 (48.11%)	5,306 (49.64%)	10,978 (54.9%)	<0.0001
BMI, kg/m^2	25.06 ± 3.88	24.92 ± 3.79	24.96 ± 3.88	25.01 ± 3.87	24.98 ± 3.91	25.29 ± 4.13	<0.0001
BMI < 18.5	36,462 (1.54%)	323 (3.02%)	309 (2.81%)	354 (2.85%)	303 (2.83%)	616 (3.08%)	
18.5 ≤ BMI < 23	588,690 (24.89%)	2,941 (27.49%)	3,124 (28.39%)	3,441 (27.68%)	3,039 (28.43%)	5,231 (26.16%)	
23 ≤ BMI < 25	589,915 (24.95%)	2,442 (22.83%)	2,383 (21.65%)	2,758 (22.19%)	2,295 (21.47%)	4,119 (20.6%)	
25 ≤ BMI < 30	917,367 (41.08%)	4,001 (37.4%)	4,147 (37.68%)	4,667 (37.54%)	4,023 (37.64%)	7,582 (37.92%)	
30 ≤ BMI	178,358 (7.54%)	990 (9.25%)	1,042 (9.47%)	1,211 (9.74%)	1,029 (9.63%)	2,448 (12.24%)	
Waist circumference, cm	85.34 ± 8.88	84.93 ± 9.43	85.16 ± 12	85.1 ± 9.53	85.02 ± 12.14	85.85 ± 10.03	<0.0001
SBP, mmHg	129.01 ± 15.87	128.42 ± 16.65	128.51 ± 16.57	128.59 ± 16.76	128.26 ± 16.57	126.42 ± 16.37	<0.0001
DBP, mmHg	79.11 ± 10.29	78.49 ± 10.49	78.32 ± 10.33	78.38 ± 10.4	78.2 ± 10.4	77.73 ± 10.42	<0.0001
Glucose, mg/dL	137.98 ± 47.89	141.09 ± 54.27	141.33 ± 54.79	140.62 ± 54.06	141.22 ± 55.33	141.23 ± 54.78	<0.0001
Total cholesterol, mg/dL	197.53 ± 46.02	195.32 ± 44.51	193.52 ± 49.97	192.12 ± 45.62	192.77 ± 49.37	187.7 ± 44.84	<0.0001
HDL, mg/dL	52.49 ± 31.91	52.46 ± 28.36	52.01 ± 25.33	52.03 ± 47.87	51.99 ± 24.56	51.21 ± 39.87	<0.0001
LDL, mg/dL	113.81 ± 92.14	111.24 ± 63.33	110.58 ± 62.65	110.49 ± 132.27	108.6 ± 51.75	104.61 ± 43.59	<0.0001
Diabetes mellitus							<0.0001
Disease duration ≥5 year	706,448 (29.87%)	3,522 (32.93%)	4,140 (37.62%)	5,021 (40.39%)	3,787 (35.43%)	9,069 (45.35%)	<0.0001
OnDM medication	1,280,126 (54.13%)	7,054 (65.94%)	7,661 (69.61%)	9,240 (74.33%)	7,835 (73.3%)	16,345 (81.74%)	<0.0001
≥ 3DM medications	323,077 (13.66%)	1,988 (18.58%)	2,211 (20.09%)	2,762 (22.22%)	2,393 (22.39%)	4,931 (24.66%)	<0.0001

group vs. 60.02% in non-AF group, $p = 0.6272$). The incidence of underlying hypertension and dyslipidaemia was higher in the AF group (hypertension 72.34% in AF group vs. 54.42%, $p < 0.0001$; dyslipidaemia 42.21% in AF group vs. 39.75%, $p < 0.0001$). Finally, the proportion of patients who were diagnosed with diabetes more than 5 years ago and who used three or more antidiabetics was also higher in the AF group (duration of diabetes ≥5 years 39.31% in the AF group vs. 29.81% in the non-AF group, $p < 0.0001$; ≥three antidiabetics 16.52% in the AF group vs. 13.8% in the non-AF group, $p < 0.0001$).

The number of years of receiving medical aid and the risk of incident atrial fibrillation

During the mean of 7.2 ± 1.7 years of follow-up (17,436,758 PY), AF was newly diagnosed in 80,257 patients (3.30%). The crude IR and the unadjusted and adjusted HRs for each group are summarized in Figure 2. The risk of AF was higher by 23% to 50% in the MA groups than in the non-MA group: the adjusted HRs (95% CI) in the MA 1 group 1.32 (1.20–1.44); 1.33 (1.22–1.45) in the MA 2 group; 1.23 (1.13–1.34) in the MA 3 group;

1.28 (1.16–1.4) in the MA 4 group, and 1.50 (1.39–1.63) in the MA 5 group. Notably, the MA 5 group showed the highest risk of AF compared to the non-MA group.

Income levels at the index year and the risk of incident atrial fibrillation

To understand the relationship between income level and the risk of AF, we divided the participants who did not receive MA as of the index year (the year of the national health examination) into 20 groups according to their income level. The income level was estimated using the amount of health insurance premiums paid. We analyzed the risk of AF among 21 groups, comprising the MA beneficiary group (MA group) and income level 1–20 groups.

Compared with group 20, the group of subjects estimated to have the highest income, the adjusted HRs of groups 1 to 19 showed an increasing trend of AF risk, whereas the MA group showed a 57% higher risk of AF (adjusted HR 1.57, 95% CI, 1.47–1.68, **Supplementary Figure 1**).

The number of years with low income and the risk of incident atrial fibrillation

To check whether low income, which is defined as patients with income levels of less than 20% of the entire Korean population, also affects the risk of AF, we divided the total population into six groups in the same way according to the number of years of low income (**Supplementary Figure 2**). This applies a more lenient definition than that of the MA beneficiary criterion.

Patients with low income for 2, 3, 4, and 5 years were associated with a higher risk of AF compared to subjects without low income for 5 years: the adjusted HRs (95% CI) in patients with low income for 2 years 1.04 (1.01–1.07), for 3 years 1.07 (1.03–1.10), for 4 years 1.04 (1.01–1.08), and for 5 years 1.09 (1.06–1.12), whereas those with low income for 1 year did not show a significant difference. The association between the number of years with low income and incident AF was significantly attenuated compared with the association between the number of years with MA, and the risk of AF.

Changes of socioeconomic status assessed with or without receiving medical aid and the risk of atrial fibrillation

To determine whether changes in socioeconomic status affected the risk of AF, we investigated whether the subject received MA in the index year and 4 years before the index year (**Figure 3**). Subjects who were not MA beneficiaries both in the index year and 4 years before the index year (persistent non-MA group) were regarded as the reference group.

The AF risk was higher in subjects who became the new MA group in the index year or who continuously belonged to MA groups (non-MA to MA group, adjusted HR 1.54, 95% CI 1.38–1.73; and persistent MA group 1.51, 95% CI 1.40–1.63). Subjects in the non-MA group at index year (MA to non-MA group) still had a 20% higher AF risk than those who were persistently in the non-MA group (adjusted HR 1.22, 95% CI 1.16–1.30).

Subgroup analyses

Subgroup analyses were performed for age, sex, diabetes duration, insulin use, three or more antidiabetic drugs, hypertension, and dyslipidaemia (**Supplementary Table 3**). There was no significant interaction between the subgroups for each item, except for age (p for interaction <0.001). Among the age groups classified as <40, 40–64, ≥ 65 years, the 40–64-year age group and ≥ 65-year group showed the same trend as the main result, whereas the group under 40-year-old of age did not show an association between the risk of AF and the number of MA history. According to MA, the increase in AF risk was slightly attenuated in those aged ≥ 65 years and was most pronounced in the 40–64-year-old group.

Discussion

In this study, we investigated the effect of SES on the risk of incident AF. The main findings of the study were as follows: (1) the MA group showed a 23–50% higher risk of AF compared to the non-MA group; (2) the cumulative burden of MA exposure up to five times showed the highest risk of AF compared to the non-MA group; (3) the risk of incident AF increased by 1.57 times in the MA group compared to the group with the highest income; and (4) those who became non-MA group during follow-up still had an increased risk of AF compared to the non-MA group, suggesting a legacy effect of MA on the risk of AF.

To the best of our knowledge, this is the first study to report the impact of the cumulative burden of MA exposure on AF risk, especially in patients with diabetes. Our study supports the alleviation of health inequalities by targeting individuals with socioeconomic deprivation in order to provide timely management of AF.

Diabetes is associated with worsening of AF prognosis and is a potent risk factor for AF (29, 30). Patients with AF and underlying DM also showed a higher risk of stroke

Cumulative burden of medical aid	No. of patients	AF	IR (1000PY)	HR (95% CI) Crude model	Adjusted model
0	2364792	77695	4.56	1 (Ref.)	**1 (Ref.)**
1	10697	456	6.48	1.44 (1.32,1.58)	**1.32 (1.2, 1.44)**
2	11005	495	7.07	1.58 (1.45,1.73)	**1.33 (1.22, 1.45)**
3	12431	526	6.74	1.52 (1.39,1.65)	**1.23 (1.13, 1.34)**
4	10689	452	6.80	1.53 (1.40,1.68)	**1.28 (1.16, 1.4)**
5	19996	633	5.78	1.35 (1.24,1.45)	**1.50 (1.39, 1.63)**

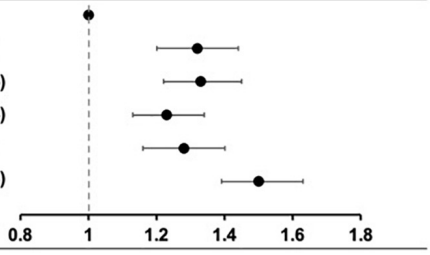

FIGURE 2
The risk of atrial fibrillation according to the cumulative burden of medical aid. Adjusted model corrected for age, sex, body mass index, blood glucose level, smoking, drinking, regular physical activity, hypertension, dyslipidemia, insulin use, ≥3 antidiabetic medication, and ≥5 years of diabetes mellitus duration. AF, atrial fibrillation; IR, incidence rate; PY, person-year; HR, hazard ratio; CI, confidence interval.

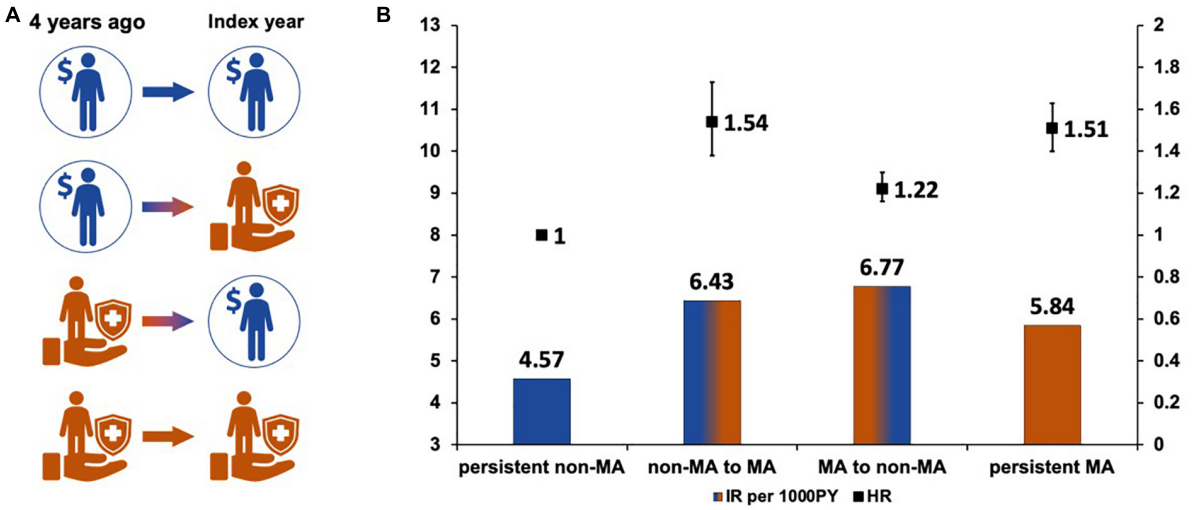

FIGURE 3
Changes in socioeconomic status and the risk of atrial fibrillation. **(A)** Changes of socioeconomic status. **(B)** Hazard ratios according to the changes of socioeconomic status.

than those without diabetes (29, 30). Thus, the diagnosis and appropriate management of AF in patients with diabetes are important for improving clinical outcomes. Health inequalities and socioeconomic status are also important factors influencing the outcome of patients with diabetes. Socioeconomic status has been associated with knowledge of diabetes, self-care, and clinical outcomes in patients with type 2 DM (31). In particular, diabetes outcomes comprising multiple components, such as HbA1c level, LDL level, blood pressure, and the physical and mental components of the QOL score, are correlated with socioeconomic factors (32–35). In our study, we further investigated the association between SES and the risk of AF in patients with diabetes and found a strong correlation between low SES and the risk of AF.

The literature shows that many studies on SES and various cardiovascular outcomes have shown an inverse correlation. A previous study reported an inverse relationship between socioeconomic status and almost all risk factors for CVD, including diabetes, obesity, smoking, and physical activity (36). More recently, in a population-based cohort study using data from the US National Health and Nutrition Examination Survey (US NHANES) and UK Biobank, adults with low socioeconomic status and no healthy lifestyle factors showed a higher risk of all-cause mortality and incident CVD higher (3.53 times and 2.09 times, respectively) than those with high socioeconomic status and healthy lifestyle factors (37). This study also reported that the influence of unhealthy lifestyle was smaller than that of socioeconomic inequity.

However, regarding the association between socioeconomic status and AF risk, previous studies have reported controversial results (11, 12, 38–40). Lower family income was associated with a higher risk of AF (11). Residents of lower socioeconomic status also had a higher risk of incident AF (12) and higher mortality when hospitalized for AF (38). Regarding studies that reported conflicting results, AF-related mortality was higher in European countries with higher GDP (39). The inverse relationship between socioeconomic status and AF risk was not evident in older adult individuals with the highest prevalence of AF (40).

Our study defined the cumulative burden of MA, enabling us to longitudinally identify the subjects' socioeconomic status in the previous 5 years. Our study, which more comprehensively determines the subjects' socioeconomic status using the concept of cumulative MA burden, confirms once again that there is an inverse correlation between low socioeconomic status and the risk of AF.

Becoming a beneficiary of MA is accepting the status of the socially underprivileged. The stigmatization of welfare beneficiaries has been studied sociologically for decades. Previous studies have reported an association between living on welfare, increased mental stress, and negative emotions (33, 34). The problem of poor self-care with health in people of low socioeconomic status is also a frequently studied topic (31, 41). The individual's psychological stress and neglect of healthcare accompanying the process of accepting a new status as underprivileged might have increased the risk of AF. In addition, we found that an unhealthy lifestyle was more prevalent in the MA group than in the non-MA group. Patients in the MA group had a higher prevalence of current smoking and non-regular exercise than those in the non-MA group. This is consistent with the study results that socioeconomic inequity in various health outcomes is highly associated with lifestyle factors, such as smoking, alcohol consumption, physical activity, and diet (42–44). A previous study reported that smoking, alcohol use, and physical activity were significantly associated with new-onset AF (42). These findings imply that lifestyle factors may have a therapeutic value in patients with diabetes. For example, abstinence from alcohol is associated with a lower risk of developing AF in patients with newly diagnosed T2DM (43). In patients with newly diagnosed AF, current alcohol intake is associated with an increased risk of ischemic stroke, whereas alcohol abstinence after AF diagnosis is associated with a lower risk of ischemic stroke (44).

Interestingly, we found that AF risk differed according to socioeconomic status change. First, the risk of AF was similarly higher in those who received MA at the index year, regardless of a previous history of MA. Current SES had a more significant impact on the risk of AF than past SES. A worsening socioeconomic status would have resulted in greater psychological pressure on the subjects, increasing the risk of AF. Second, patients in the non-MA group at the index year showed different risks of AF according to their previous socioeconomic status. The group of patients who experienced an improvement in socioeconomic status (MA to non-MA group) still showed a 22% higher AF risk than the persistent non-MA group. This suggests a prophylactic effect of MA on the risk of AF, emphasizing that SES still has a considerable impact on clinical outcomes in patients with diabetes.

Strengths and limitations

In our study, the socioeconomic status of the subjects was identified longitudinally by screening for 5 years, not at any single time point. This method has the advantage of being able to grasp the burden of the low socioeconomic status experienced by the subjects during the period, and further being able to determine whether there was a change in the socioeconomic status during the period, so that the situation of the subjects could be evaluated in a more diversified way. In addition, we limited the subjects of this study to patients with diabetes. Since DM is one of the notable risk factors for AF, setting this risk factor as the subject's prerequisite allowed us to focus more on the influence of socioeconomic status. In addition, one of the strengths of our study is that we identified the socioeconomic status of the participants using the NHIS database (24). Instead of collecting information on income through self-questionnaires reported by participants, we improved the reliability of the results by using a database that records the exact amount of health insurance premium payments. The NHIS database holds information on all citizens residing in Korea in all age groups, reducing the possibility of selection bias, and the resident registration number jointly recorded further ensures the accuracy of information.

This study had some limitations. As mentioned, the subjects were limited to patients with diabetes at enrolment to focus more on the impact of socioeconomic status on incident AF. Therefore, this study alone cannot explain whether the results can be equally applied to the non-diabetic population. However, since all subjects had diabetes, the correction for diabetes was more reliable than when the entire population was enrolled. We believe that even when conducting further research on whether socioeconomic status affects the increase in AF risk among non-diabetic individuals, only non-diabetics should be included as subjects, since DM is a clear risk factor for AF (3, 5, 34) and the correction of its impact might not be complete in the coexistence of subjects with diabetes and those without diabetes in the analysis.

Low socioeconomic status is associated with the risk of AF in patients with diabetes. More attention should be directed at alleviating health inequalities and targeting individuals with socioeconomic deprivation to provide timely management of AF.

Data availability statement

This study used publicly available datasets from Korean National Health Insurance Service Database https://nhiss.nhis.or.kr/bd/ab/bdaba021eng.do.

Ethics statement

This study was exempt from review by the Seoul National University Hospital Institutional Review Board (E- 2105-141-1220). Written informed consent was not required for this study in accordance with the local legislation and institutional requirements.

Author contributions

MH, S-RL, E-KC, SO, and GL initially conceptualized the subject of this study. S-RL and E-KC designed the study. MH, S-HP, HL, JC, and JMC performed the literature research and data collection. MH, S-RL, and K-DH analyzed the data. MH and S-RL were major contributors on writing the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

Author E-KC research grants or speaking fees from Bayer, BMS/Pfizer, Biosense Webster, Chong Kun Dang, Daiichi-Sankyo, Dreamtech Co., Ltd., Medtronic, Samjinpharm, Sanofi-Aventis, Seers Technology, and Skylabs. Author GL consultant and speaker for BMS/Pfizer, Boehringer Ingelheim, and Daiichi-Sankyo. No fees are received personally.

The remaining 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1008340/full#supplementary-material

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Relationship of the metabolic score for insulin resistance and the risk of stroke in patients with hypertension: A cohort study

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Background: The current status of the dose-response relationship between the metabolic score for insulin resistance (METS-IR) and new-onset stroke in hypertensive patients and its subtypes is unclear. This study aimed to determine the association between METS-IR and incident stroke and its subtypes within a cohort of Chinese hypertensive patients.

Methods: A total of 14032 hospitalized patients with hypertension from January 1, 2010, to December 31, 2021, were included in this retrospective cohort study. Cox models and restricted cubic splines were applied to determine the association between METS-IR and the risk of stroke.

Results: During a median follow-up of 4.80 years, 1067 incident stroke cases occurred. Patients in the highest quartile group of METS-IR levels exhibited a higher risk of stroke (HR, 1.80; 95% CI, 1.50-2.17) and ischemic stroke (HR, 1.96; 95% CI, 1.60–2.42) than those in the lowest quartile group. However, no significant associations were observed between METS-IR and the risk of hemorrhagic stroke. Restricted cubic spline analysis suggested a nearly J-shaped association between METS-IR and risk of stroke and ischemic stroke (P for nonlinearity < 0.001). METS-IR did produce a significant improvement in the C statistic when added to the basic model (from 0.637 to 0.664, P < 0.001). Notably, the addition of METS-IR to the basic model resulted in a significant improvement in predicting incident total stroke and ischemic stroke.

Conclusions: This cohort study suggests a relationship between METS-IR and the risk of stroke and ischemic stroke. Further studies are required to elucidate the underlying mechanisms.

KEYWORDS

metabolic score for insulin resistance, hypertension, stroke, ischemic stroke, cohort study

Introduction

Stroke has developed into a significant global health problem (1–3). According to the latest annual report in 2019, there are currently more than 20 million stroke patients in China (4). Available evidence suggests that hypertension is the most significant risk factor for stroke (5, 6). Therefore, identifying hypertensive patients with a high risk of stroke is clinically essential to improve risk stratification.

Abnormalities in glucose and lipid metabolism are common in hypertensive patients, and insulin resistance (IR) serves an essential function in this biological procedure (7–9). IR is not only an important contributor to the progression of arterial stiffness, endothelial dysfunction, and metabolic syndrome but also a risk factor for stroke development. Therefore, early discovery and control of IR may help in the early prevention of stroke (9–12). Currently, there are several methods available to assess IR. First, in the 1970s, the euglycaemic-hyperinsulinaemic clamp (EHC) was proposed as the gold standard for the assessment of IR (13). However, this method is challenging to apply in large-scale clinical and epidemiological studies due to its drawbacks such as complexity, cost, and invasiveness (14). Secondly, given its accessibility and low cost, the triglyceride glucose index (TyG), which is generated from fasting blood glucose (FPG) and fasting triglycerides (TG), is presently the most widely used marker of IR (15). Nevertheless, the index only includes two metabolic variables and does not take into account how diet and cholesterol affect cardiovascular disease. As a result, TyG might not accurately depict how IR affects the cardiovascular system (16). Fortunately, a novel IR marker called the metabolic score for IR (METS-IR) has just been created by Bello-Chavolla et al. (17). The most potent IR measure outside of EHC, METS-IR, combines FPG, TG, high-density lipoprotein cholesterol (HDL-C), and body mass index (BMI), which represents nutritional status (18, 19). So far, numerous studies have found METS-IR to be associated with various cardiometabolic diseases, including hypertension, diabetes, non-alcoholic fatty liver disease, and ischemic heart disease (18, 20–22). Therefore, METS-IR may be clinically important for risk stratification of new-onset stroke in hypertensive patients. In addition, the current status of the dose-response relationship between METS-IR and new-onset stroke in hypertensive patients and its subtypes is unclear.

In this study, we sought to determine the association between baseline METS-IR and stroke and its subtypes among Chinese hypertensive patients.

Material and methods

Study population

We conducted a cohort study of hypertensive patients followed at a hypertension center (the People's Hospital of Xinjiang Uygur Autonomous Region). Deidentified patient data retrieved from electronic medical records was used, including the date of birth, sex, physical measurements, diagnostic codes according to the International Classification of Diseases, 10th Revision (ICD-10), medication prescriptions, and laboratory results. A total of 18609 patients with hypertension were recruited from January 1, 2010, to December 31, 2021. After strict exclusion criteria, a total of 14032 patients were included (Figure 1). A comparison of baseline characteristics of participants included and excluded from this study may be found in Table S1. The ethics application was approved by the Ethics Committee of the People's Hospital of Xinjiang Uygur Autonomous Region. Informed consent was

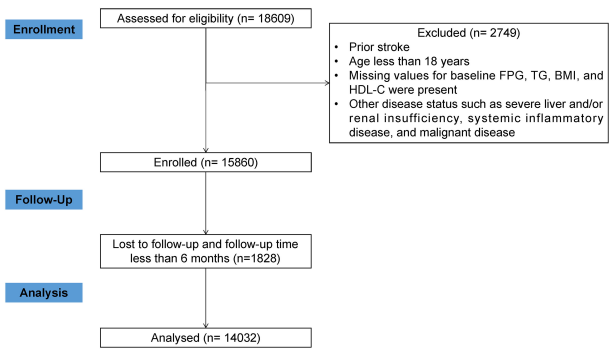

FIGURE 1
A study flowchart.

waived owing to the retrospective nature of the study. Moreover, this study followed STROBE reporting guidelines.

Data collection and definitions

The information provided by the electronic medical record includes demographic data, lifestyle factors, laboratory measurements, medical history, and medication history. BMI was calculated as body weight (in kilograms) divided by height (in meters squared). Blood pressure (BP) and heart rate were measured by standard procedures. Smoking status was categorized as non-smokers and current smokers. Alcohol consumption status was divided into non-drinkers and current drinkers. Blood samples were collected after an overnight fast. Fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), high-sensitivity C-reactive protein (hsCRP), hemoglobin A1c (HbA1c), uric acid (UA), and cystatin C (Cys C) were measured. The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation. The participants' prior medical histories were evaluated using ICD-10 codes. To ensure the accuracy of diagnoses, diabetes (E10-E14) and dyslipidemia (E78) were regarded as present if a participant was treated ≥ 2 times. Coronary heart disease (CHD) (I24 and I25) was considered present if a participant was treated ≥ 1 time. The Charlson Comorbidity Index (CCI) was determined from claims data during a lookback period of 2 years before the baseline. The CCI (using ICD-10 codes) was calculated, as reported previously (23). The list of medications included in the study is available in Table S2. METS-IR was calculated as previously reported, and is presented as follows: METS-IR = Ln[(2 × FPG (mg/dL))+TG (mg/dL)] × BMI (kg/m^2))/(Ln[HDL-C (mg/dL)]) (20).

Follow-up and assessment of outcomes

The primary outcome was the first occurrence of stroke (ischemic or hemorrhagic), either nonfatal or fatal. Secondary outcomes included the first ischemic stroke and the first hemorrhagic stroke. The outcomes of events since participants enrolled in the study at baseline were determined through medical records, contact with local disease and death registries, or access to the database of basic medical insurance. These data sources are linked using an individual national identification number assigned to each Chinese person for life. This number is replaced by a series number when provided for personal data analysis to anonymize the individual participant's data. Patients were followed from the date of enrollment to the end of the observation period, defined as the date of the last follow-up visit, the date of the first appearance of any study outcome, the date of death, or the end of the study period (December 31, 2021).

Statistical analysis

We compared the METS-IR quartile characteristics of the participants. The cumulative incidence of total stroke and its subtypes was estimated using the Kaplan-Meier method. Covariates with variance inflation factors (VIF) ≥ 5 were omitted to avoid multicollinearity (Table S3). Hazard ratios (HR) and confidence intervals (CI) were derived from the Cox regression models. Restricted cubic spline (RCS) curves were created at the 10th, 50th, and 90th percentiles using three default knots. In addition, we performed subgroup analyses stratified by age, sex, eGFR, Hcy, hyperlipidemia, diabetes, and CCI. Interactions between METS-IR and each of these variables were tested. We conducted several sensitivity analyses to test the robustness of our findings. First, we excluded events occurring in the first two years of follow-up to minimize potential reverse causality. In the second sensitivity analysis, we additionally excluded any participants older than 80 years. Third, the same analyses were repeated after excluding participants under treatment with glucose-lowering or lipid-lowering medications. Fourth, competing risk analyses were performed using the Fine and Gray method, and non-stroke deaths were treated as competing risk events. Fifth, a sensitivity analysis without adjustment for diabetes and hyperlipidemia was used to exclude potential bias. Finally, we also performed a sensitivity analysis using an E-value approach. Details of the statistical analysis are provided in the Supplementary Material. All analyses were done with R software version 4.1.1 at a two-tailed alpha level of 0.05.

Results

Characteristics of the study population

The METS-IR was normally distributed in the population (Figure S1). Participants were divided into four groups based on METS-IR quartiles at baseline (Table 1). Among the 14032 participants eligible for analysis, individuals with higher METS-IR levels were younger, more likely to be current smokers and drinkers, had a higher BMI, and had higher rates of hyperlipidemia, CHD, and diabetes. Furthermore, participants with higher METS-IR levels used glucose-lowering medications and statins more frequently during treatment (Table S4).

Association of METS-IR with total stroke and its subtypes

During a median follow-up of 4.80 years (interquartile range, 1.80-7.60), among the eligible participants, 1067

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TABLE 1 Baseline characteristics of the study population according to quartiles of METS-IR.

Characteristics	METS-IR quartiles				*P*-value
	Q1 (<37.32)	Q2 (37.32-42.47)	Q3 (42.48-48.22)	Q4 (>48.22)	
Participants, n	3507	3507	3508	3510	
Age, year	52.36 ± 12.20	51.57 ± 12.07	52.31 ± 11.74	51.90 ± 11.93	0.018
Men, n (%)	1904 (54.29%)	1879 (53.58%)	1931 (55.05%)	1915 (54.56%)	<0.001
Body mass index, kg/m^2	22.61 ± 1.61	24.74 ± 1.45	26.61 ± 1.86	29.59 ± 2.89	<0.001
Heart rate, bpm	80.41 ± 10.85	80.67 ± 10.20	81.22 ± 10.18	82.82 ± 10.78	<0.001
SBP, mmHg	143.97 ± 20.51	145.43 ± 20.57	145.92 ± 20.46	148.07 ± 20.96	<0.001
DBP, mmHg	87.53 ± 14.35	89.22 ± 14.28	90.34 ± 14.17	92.61 ± 14.67	<0.001
Current smoking, n (%)	615 (17.54%)	1082 (30.85%)	1300 (37.06%)	1526 (43.48%)	<0.001
Current drinking, n (%)	572 (16.31%)	1013 (28.89%)	1234 (35.18%)	1390 (39.60%)	<0.001
Laboratory parameters					
UA, mmol/L	293.85 ± 81.26	330.34 ± 84.67	355.05 ± 91.51	377.28 ± 99.33	<0.001
eGFR, mL/min/1.73 m^2	97.14 ± 18.64	96.47 ± 17.55	96.68 ± 17.51	97.35 ± 18.55	0.153
Cys C, mg/L	0.90 ± 0.34	0.92 ± 0.33	0.93 ± 0.34	0.95 ± 0.31	<0.001
TC, mmol/L	4.45 ± 0.95	4.42 ± 0.94	4.48 ± 1.00	4.53 ± 1.04	<0.001
TG, mmol/L	1.19 ± 0.52	1.60 ± 0.83	1.96 ± 1.04	2.74 ± 2.02	<0.001
HDL-C, mmol/L	1.31 ± 0.30	1.09 ± 0.22	0.99 ± 0.20	0.88 ± 0.18	<0.001
LDL-C, mmol/L	2.65 ± 0.82	2.78 ± 0.81	2.75 ± 0.85	2.71 ± 0.83	<0.001
HbA1c, %	6.01 ± 1.22	6.17 ± 1.25	6.18 ± 1.24	6.22 ± 1.26	<0.001
FPG, mmol/L	4.68 ± 0.94	5.03 ± 1.51	5.36 ± 2.02	5.84 ± 2.36	<0.001
Hcy, mmol/L	14.40 ± 6.92	14.58 ± 6.79	14.96 ± 7.04	15.08 ± 7.33	<0.001
hsCRP, mg/L	3.14 (1.23-7.45)	3.02 (1.15-7.17)	3.15 (1.15-7.65)	3.27 (1.17-7.80)	0.319
Medical histories, n (%)					
Hyperlipidemia	1982 (56.52%)	2010 (57.31%)	2072 (59.06%)	2140 (60.97%)	<0.001
Coronary heart disease	489 (13.94%)	435 (12.40%)	541 (15.42%)	708 (20.17%)	<0.001
Diabetes	965 (27.52%)	923 (26.32%)	1001 (28.53%)	1127 (32.11%)	<0.001
Charlson comorbidity index					0.005
0	1622 (46.25%)	1637 (46.68%)	1602 (45.67%)	1532 (43.65%)	
1	944 (26.92%)	1029 (29.34%)	1037 (29.56%)	1051 (29.94%)	
2 or more	941 (26.83%)	841 (23.98%)	869 (24.77%)	927 (26.41%)	
Follow-up duration, years	4.80 (1.80-7.50)	4.70 (1.70-7.60)	4.80 (1.80-7.50)	5.00 (1.80-7.60)	0.332

Data are mean (standard deviation), n (%), or median (interquartile range).
METS-IR, metabolic score for insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular fltration rate; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose; Hcy, homocysteine; UA, uric acid; hsCRP, high-sensitivity C-reactive protein; Cys C, cystatin C.

patients had a total stroke, including 842 incident ischemic strokes and 225 incident hemorrhagic strokes. The incidence rates of total stroke, ischemic stroke, and hemorrhagic stroke were 15.45 (95% CI: 14.54–16.41), 12.47 (95% CI: 11.89–13.07), and 3.98 (95% CI: 3.66-4.33) per 1000 person-years, respectively. The Kaplan-Meier curve showed that participants in the Q4 group had a higher risk of total stroke and ischemic stroke instead of hemorrhagic stroke than those in other groups (log-rank test, P < 0.001, Figures 2A, B; P = 0.880, Figure 2C) (Peto-Peto test, P < 0.001, Figures 2A, B; P = 0.361, Figure 2C). The cumulative incidence of total stroke increased with increasing METS-IR (Figure 2A). This trend remained significant even after adjusting for potential confounders in model 3 (P trend < 0.001). Compared with the Q1 group, the HRs were 0.97 (95% CI, 0.80-1.18), 1.34 (95% CI, 1.12-1.61), and 1.80 (95% CI, 1.50-2.17) for the Q2, Q3, and Q4 groups, respectively (Table 2). It appeared that the risk of total stroke was higher per 1 SD increase of METS-IR (HR, 1.33; 95% CI, 1.25-1.42; Table 2). Similar results were seen in ischemic stroke, but the risk of hemorrhagic stroke was not significantly increased (Table 2). To visualize the relationship between the METS-IR and total stroke and its subtypes, we fitted 3 RCS curves (Figure 3). The multivariable-adjusted spline regression model showed a nearly J-shaped dose-response relationship between the METS-IR and the risk of total stroke (P for nonlinearity < 0.001). A similar association has been found in ischemic stroke. In contrast, the association

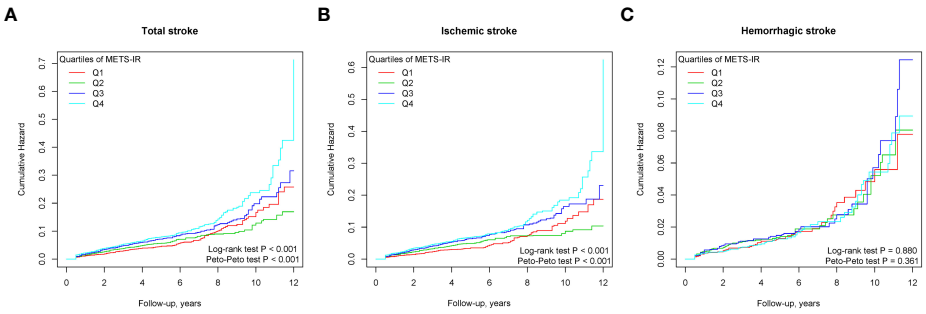

FIGURE 2
Cumulative incidence of outcomes stratified by the quartile of the METS-IR. **(A)** total stroke; **(B)** ischemic stroke; **(C)** hemorrhagic stroke.

between METS-IR and incident hemorrhagic stroke risk was nonlinear (P for nonlinearity = 0.861). As METS-IR increased beyond 42.48, the HRs for both total stroke (HR per SD 1.60, 95% CI 1.46-1.75) and ischemic stroke (HR per SD 1.62, 95% CI 1.46-1.79) increased significantly.

Stratified analyses

Stratified analysis was conducted to evaluate the association of METS-IR (per SD increase) with the risk of total stroke in each subgroup (Figure 4). None of the factors significantly altered the association between METS-IR and the risk of total stroke (all P for interactions > 0.05). A stratified analysis of the association between METS-IR (per SD increase) and the risk of ischemic stroke found similar trends (Figure 4B).

Sensitivity analysis

We performed sensitivity analyses to confirm the effect of METS-IR on total stroke and its subtypes in patients with hypertension. Tables S5–S10 present results from our sensitivity analyses. In the sensitivity analyses, the associations of METS-IR with the risk of total stroke and its subtypes did not change significantly after excluding participants who had an outcome event during the first two years of follow-up (Table S5), excluding participants aged 80 and older (Table S6), excluding participants under treatment with glucose-lowering medications (Table S7), or excluding participants receiving lipid-lowering therapy (Table S8). In analyses with non-stroke death as a competing risk, there was no significant change in the primary outcome (Table S9). The primary outcome was not changed using multiple regression analysis without adjustment for diabetes and hyperlipidemia (Table S10). The E-values demonstrated that the observed correlations were at least moderately robust to potential unmeasured confounding (Table S11).

Incremental predictive value of METS-IR

As demonstrated in Table 3, METS-IR did produce a significant improvement in the C statistic when added to the basic model (from 0.637 to 0.664, P < 0.001). Notably, the addition of METS-IR to the basic model resulted in a significant improvement in predicting incident total stroke, with increments in continuous NRI (0.114, P < 0.001) and IDI (0.007, P = 0.007). In ischemic stroke, similar findings were observed.

Discussion

In this large retrospective cohort study, the risk of stroke and its subtypes based on the METS-IR, a novel surrogate marker of IR, was assessed. We consistently found that higher levels of METS-IR at baseline were associated with an increased risk of future stroke and ischemic stroke, even after adjusting for confounders. However, there was no significant correlation between baseline METS-IR and hemorrhagic stroke. Additionally, we observed a nearly J-shaped association between levels of METS-IR and the risk of stroke and ischemic stroke.

IR is an essential indicator of metabolic abnormalities (24). In the long term, IR can lead not only naturally to pathophysiological disorders such as abnormal glucolipid metabolism, elevated blood pressure, hyperuricemia, raised signatures of inflammation, and thrombotic states, but also indirectly to diseases associated with metabolic disorders (25–27). Recently, a new non-insulin metabolic score based on conventional clinical indicators such as FPG, TG, HDL-C, and BMI, namely METS-IR, was developed and has been shown to have a high accuracy similar to that of EHC (17, 20). Bello-Chavolla et al. analyzed the advantages of METS-IR versus markers such as EHC and TyG in the diagnosis of impaired insulin sensitivity and demonstrated that METS-IR was significantly better than the other markers (17, 20). Research to indicate that METS-IR may be used to screen for early insulin

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TABLE 2 Associations between METS-IR and clinical outcomes.

Exposure	Unadjusted HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Model 3 HR (95% CI)
Total stroke				
Per SD increment	1.26 (1.19, 1.33)	1.26 (1.19, 1.34)	1.29 (1.21, 1.37)	1.33 (1.25, 1.42)
Categories				
Q1-Q2	Reference	Reference	Reference	Reference
Q3-Q4	1.49 (1.32, 1.68)	1.48 (1.31, 1.67)	1.52 (1.34, 1.72)	1.57 (1.38, 1.79)
Quartiles				
Q1	Reference	Reference	Reference	Reference
Q2	0.95 (0.79, 1.15)	0.97 (0.80, 1.18)	0.96 (0.80, 1.17)	0.97 (0.80, 1.18)
Q3	1.30 (1.09, 1.55)	1.29 (1.08, 1.54)	1.30 (1.09, 1.55)	1.34 (1.12, 1.61)
Q4	1.60 (1.35, 1.90)	1.63 (1.38, 1.93)	1.71 (1.43, 2.03)	1.80 (1.50, 2.17)
P for trend	<0.001	<0.001	<0.001	<0.001
Ischemic stroke				
Per SD increment	1.32 (1.23, 1.41)	1.32 (1.24, 1.41)	1.35 (1.26, 1.44)	1.39 (1.29, 1.49)
Categories				
Q1-Q2	Reference	Reference	Reference	Reference
Q3-Q4	1.61 (1.40, 1.85)	1.60 (1.39, 1.84)	1.63 (1.41, 1.87)	1.68 (1.45, 1.95)
Quartiles				
Q1	Reference	Reference	Reference	Reference
Q2	1.01 (0.81, 1.25)	1.03 (0.83, 1.28)	1.03 (0.83, 1.28)	1.04 (0.84, 1.30)
Q3	1.47 (1.21, 1.79)	1.46 (1.20, 1.78)	1.47 (1.20, 1.80)	1.52 (1.24, 1.87)
Q4	1.75 (1.45, 2.12)	1.79 (1.48, 2.17)	1.85 (1.52, 2.26)	1.96 (1.60, 2.42)
P for trend	<0.001	<0.001	<0.001	<0.001
Hemorrhagic stroke				
Per SD increment	0.98 (0.87, 1.11)	0.98 (0.87, 1.11)	1.02 (0.90, 1.15)	1.01 (0.89, 1.15)
Categories				
Q1-Q2	Reference	Reference	Reference	Reference
Q3-Q4	1.06 (0.83, 1.34)	1.05 (0.83, 1.33)	1.12 (0.87, 1.42)	1.12 (0.87, 1.44)
Quartiles				
Q1	Reference	Reference	Reference	Reference
Q2	1.02 (0.73, 1.44)	1.02 (0.73, 1.44)	1.01 (0.71, 1.42)	0.96 (0.68, 1.35)
Q3	1.13 (0.81, 1.57)	1.12 (0.80, 1.56)	1.13 (0.81, 1.59)	1.12 (0.79, 1.58)
Q4	1.01 (0.72, 1.42)	1.01 (0.72, 1.42)	1.10 (0.78, 1.57)	1.07 (0.74, 1.55)
P for trend	0.817	0.835	0.462	0.530

Model 1, adjusted for age; sex, Model 2, adjusted for heart rate, SBP, DBP, current smoker, current drinker, hyperlipidemia, Charlson comorbidity index, diabetes, and coronary heart disease based on model 1, Model 3; included variables in model 2 and further adjusted for uric acid, eGFR, cystatin C, TC, TG, LDL-C, HbA1c, FPG, Hcy, hsCRP, use of statins, use of aspirins, use of insulins, use of oral antidiabetic drugs, and antihypertensive drugs.
SD, standard deviation; HR, hazard ratio; CI, confidence interval. Other abbreviations as presented in Table 1.

sensitivity and metabolism-related illnesses (17). In a large cohort study, Lee et al. demonstrated that METS-IR was superior to HOMA-IR in predicting the incidence of NAFLD and that METS-IR may be a more accurate index of IR than HOMA-IR (28). In another large epidemiological study, Liu et al. identified elevated METS-IR with a concomitant increased risk of hypertension (29). In a community-based population without cardiovascular disease, a J-shaped association was found between METS-IR and subclinical myocardial injury (16). The results of Wu et al. suggest that METS-IR is a significant predictor of the presence and severity of CHD and may serve as a quality indicator for the prevention and management of CHD (22). A cohort study in Korea also demonstrated that elevated METS-IR predicted the future risk of ischemic heart disease in a community-based population without diabetes and served as a useful predictive marker for ischemic heart disease (30). In addition, studies revealed that METS-IR is also strongly associated with many risk factors for stroke, such as hyperuricemia, atherosclerosis, and early renal insufficiency (18, 31–34). In summary, METS-IR may be an economical

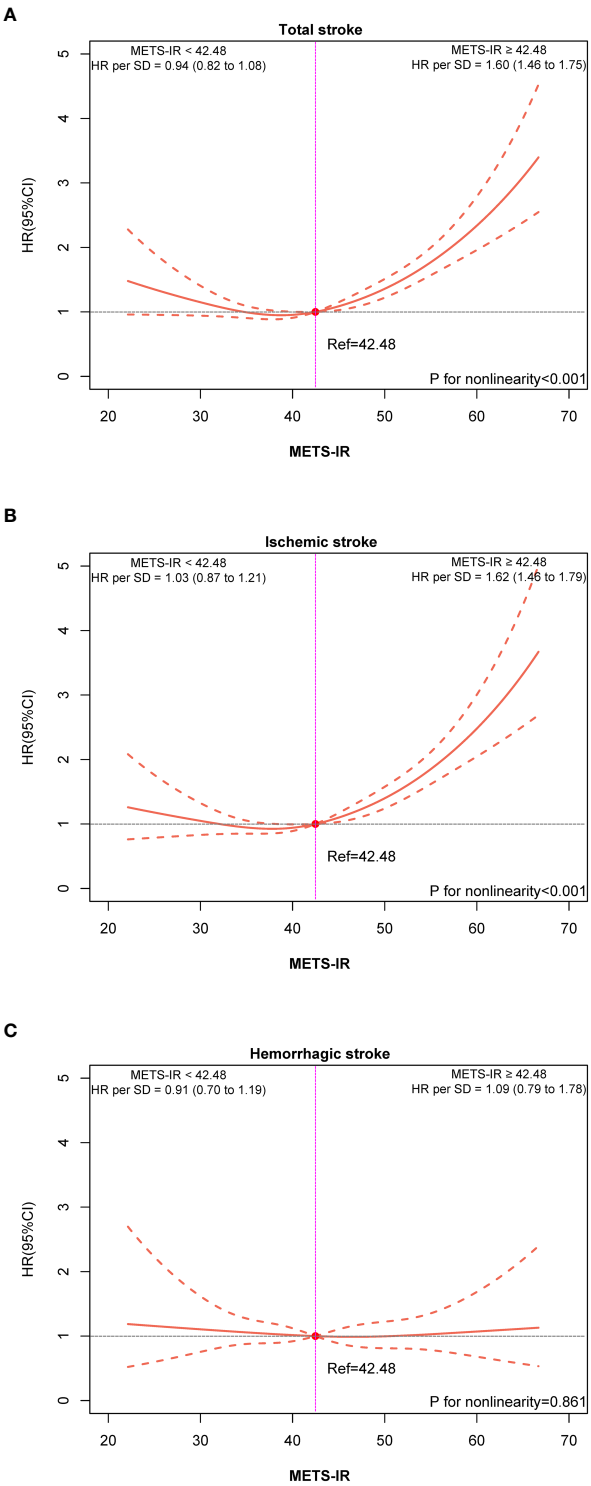

FIGURE 3
Dose-response associations of METS-IR with total stroke **(A)**, ischemic stroke **(B)**, and hemorrhagic stroke **(C)**.

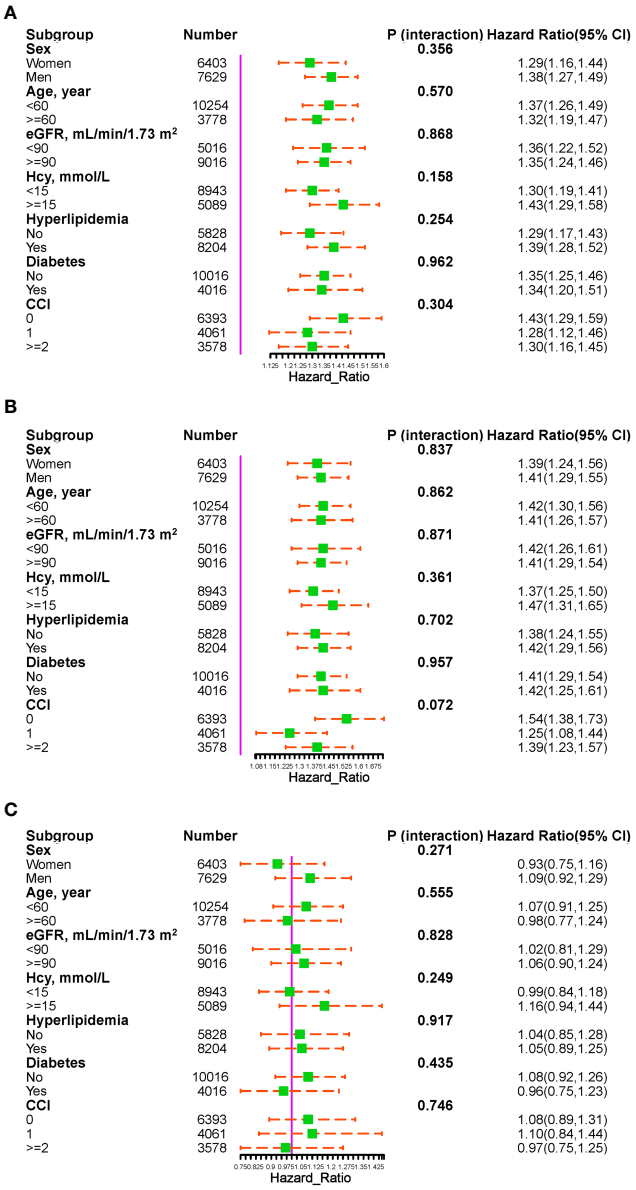

FIGURE 4
The association of METS-IR (per SD increment) with the risk of total stroke **(A)**, ischemic stroke **(B)**, and hemorrhagic stroke **(C)** in various subgroups.

and convenient index for IR screening. Our findings suggest that elevated METS-IR may be useful in identifying people at high risk for developing stroke. In terms of clinical applications, contemporary electronic medical records have the potential to automatically calculate METS-IR in order to better stratify individuals by risk based on METS-IR. A high METS-IR can also alert people to establish early lifestyle changes that can reduce disease progression or morbidity.

Mechanisms linking METS-IR and stroke and ischemic stroke remain incompletely understood. There are several potential interpretations for this observation. First, IR enhances the atherosclerotic process. IR enhances the pathophysiological processes of vascular endothelial cells, smooth muscle cells, and macrophages *via* inflammation, promoting the formation of atherosclerosis-associated foam cells and vulnerable plaques. In addition, IR may have atherogenic effects through impaired fibrinolysis and dyslipidemia (35–38). Second, it has been shown that IR plays an instrumental function in platelet adhesion, activation, and aggregation (39–41). IR may increase platelet count and volume

TABLE 3 Incremental predictive value of METS-IR.

	C statistic Estimate (95% CI)	*P*-value	NRI (continuous) Estimate (95% CI)	*P*-value	IDI Estimate (95% CI)	*P*-value
Total stroke						
Basic model	0.637 (0.621–0.654)		Reference		Reference	
Basic model + METS-IR	0.664 (0.648–0.681)	<0.001	0.114 (0.028–0.157)	<0.001	0.007 (0.001–0.010)	0.007
Ischemic stroke						
Basic model	0.664 (0.646–0.683)		Reference		Reference	
Basic model + METS-IR	0.686 (0.669–0.704)	<0.001	0.132 (0.030–0.167)	<0.001	0.005 (0.001–0.008)	0.033
Hemorrhagic stroke						
Basic model	0.596 (0.564–0.628)		Reference		Reference	
Basic model + METS-IR	0.625 (0.593–0.657)	0.066	0.055 (-0.110–0.128)	0.698	0.001 (-0.004–0.003)	0.944

The basic model included age, sex, heart rate, SBP, DBP, current smoker, current drinker, hyperlipidemia, Charlson comorbidity index, diabetes, coronary heart disease, uric acid, eGFR, cystatin C, TC, TG, LDL-C, HbA1c, FPG, Hcy, and hsCRP.
IDI, integrated discrimination improvement; NRI, net reclassification index. Other abbreviations as presented in Table 1.

and promote platelet activation. Moreover, IR is tightly linked to vascular endothelial dysfunction, which further promotes platelet adhesion and aggregation (41–43). All of the above are intimately correlated with cerebral vascular stenosis or occlusion and are involved in ischemic stroke events. Third, IR predisposes to hemodynamic disturbances. Previous studies have found significantly reduced cerebrovascular reserve in insulin-resistant patients (44–46). Finally, IR may accelerate the progression of atherosclerosis by altering risk factors and disrupting brain metabolism through oxidative stress and inflammatory mechanisms (47–49). Further examinations are warranted to clarify the precise role of METS-IR in stroke and ischemic stroke in the future. Nevertheless, no association between METS-IR and hemorrhagic stroke was observed in this study. Although hypertension is an independent risk factor for hemorrhagic stroke, it has been proposed that lipid metabolism disorders can produce a protective effect against cerebrovascular hemorrhage (50). Thus, the combination of two opposing effects may have contributed to the statistically nonsignificant association between METS-IR and hemorrhagic stroke in hypertensive patients.

This study has several strengths that distinguish it from previous studies. First, to our knowledge, this is the first large cohort study to assess the association between METS-IR and the risk of stroke and its subtypes in patients with hypertension. Second, this study reports the results derived from real-world clinical practice. Our findings are more likely to reflect real-world conditions. Several potential limitations are also noteworthy. First, the observational, retrospective study design limits inferences of causality. Second, the participants in this study were mainly Chinese hypertensive patients, so it is uncertain whether the obtained results would be generalizable to other populations. Although we controlled for confounders, we cannot rule out the possibility that unmeasured (e.g., genetic susceptibility and environmental exposure) or poorly measured confounders could explain our observations. Thus, further prospective studies are needed to confirm these findings. Finally, this study does not use repeated measurements of METS-IR. Longitudinal studies using repeated measurements of METS-IR are necessary to investigate more accurate associations between METS-IR and outcomes than a single measurement.

Conclusion

In summary, a relationship between METS-IR and the risk of stroke and ischemic stroke was observed in patients with hypertension. It will require further studies to clarify this potential mechanism.

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 People's Hospital of Xinjiang Uygur Autonomous Region. Written informed consent for participation was not

required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

XC and JHu analyzed the data and wrote the manuscript. XC, QZ, MW, and YD helped with copyediting. XC and NL audited the data. SL, JHo, and XC conducted research. NL had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1049211/full#supplementary-material

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Insulin resistance in ischemic stroke: Mechanisms and therapeutic approaches

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The pathological condition of insulin resistance prevents the neuroprotective effects of insulin. Numerous studies have demonstrated that insulin resistance, as an independent risk factor for ischemic stroke, accelerates the formation of thrombosis and promotes the development of atherosclerosis, both of which are major mechanisms of ischemic stroke. Additionally, insulin resistance negatively affects the prognosis of patients with ischemic stroke regardless of whether the patient has diabetes, but the mechanisms are not well studied. We explored the association between insulin resistance and the primary mechanisms of brain injury in ischemic stroke (inflammation, oxidative stress, and neuronal damage), looking for potential causes of poor prognosis in patients with ischemic stroke due to insulin resistance. Furthermore, we summarize insulin resistance therapeutic approaches to propose new therapeutic directions for clinically improving prognosis in patients with ischemic stroke.

KEYWORDS

ischemic stroke, insulin resistance, atherosclerosis, embolism, therapeutic approaches

Introduction

Stroke incidence and patient prognosis have not changed significantly over the past few decades, despite significant advances in clinical interventions aimed at reducing stroke risk factors like hypertension, smoking, and diabetes. Stroke remains the second-leading cause of disability and death globally (1). Since energy metabolism is a prerequisite for life activity, many studies have examined disorders of energy metabolism in brain tissue, particularly insulin. Insulin protects brain tissue development by preventing ischemia, oxidative stress, and apoptosis-induced brain tissue damage, regulating cholesterol metabolism in neurons and astrocytes. Insulin

also can effectively alleviate cognitive dysfunction caused by Alzheimer's disease (2). Insulin resistance (IR) has long been linked to ischemic stroke, which makes up 87% of strokes and is increasing (3). IR, which is present in the majority of type II diabetes (T2D) patients, promotes the development of ischemic stroke and is associated with a poor prognosis (4, 5). One of the best-known effects of IR is the presence of hyperglycemia, which may negatively affect brain function through various mechanisms (6). This review evaluates studies on IR as an independent risk factor for ischemic stroke, with a particular focus on the mechanisms and therapy associated with IR and ischemic stroke. Firstly, we discuss the relationship between IR and the risk factors for ischemic stroke (hypertension, hyperlipidemia, etc.). Secondly, we discuss the potential causes of IR leading to poor outcomes in ischemic stroke patients. Lastly, we synthesize the research on IR inhibitors and ischemic stroke currently available and look ahead to a day when reducing IR may be a useful strategy for both preventing and treating ischemic stroke.

Normal brain insulin signaling and IR

Insulin is a well-known hormone secreted by pancreatic β-cells, which regulates peripheral glucose metabolism. Insulin signaling from the central nervous system (CNS) regulates energy balance *via* complex mechanisms (7). The concentration of insulin in cerebrospinal fluid is correlated with the concentration in circulating plasma. Although it is still debated whether insulin can be produced in the CNS, circulating insulin can enter brain tissue *via* the (BBB) (8).

Neurons are the ultimate beneficiaries of brain tissue glucose uptake, consuming 85% of the energy needed by brain tissue (9). Brain tissue accounts for only about 2% of an adult's body weight but consumes about 20% of total body energy, and glucose metabolism produces even more than 95% of the ATP required by brain tissue, making glucose metabolism in brain tissue especially important (10). Glucose transporter 3 (GLUT3) and glucose transporter 4 (GLUT4) are found on the neuronal cell membrane, with GLUT4 serving as a critical determinant of glucose homeostasis and being highly dependent on insulin (11, 12). Under insulin stimulation, GLUT4 translocation from the cytoplasm to the cell membrane in neurons in the hippocampus and cortex has a glucose-promoting transport effect (13). Insulin not only maintains the balance of energy metabolism in brain tissue but also stimulates neurite outgrowth, modulates catecholamine release and uptake, regulates the expression and localization of N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and γ-aminobutyric acid (GABA) receptors, and modulates synaptic plasticity to enhance neuronal survival by inhibiting apoptosis (14).

Insulin needs to bind to insulin receptors (INSR) on plasma membranes to exert its known biological effects. Insulin receptor, which consists of two extracellular α subunits and β subunits, are found in the brain and on most cells. In brain tissue, insulin receptors are mainly distributed in the hypothalamus, olfactory bulb, hippocampus, striatum, cerebral cortex, and cerebellum (14). Insulin first binds to the extracellular α subunits of insulin receptors, which induces the autophosphorylation of intracellular β subunits (15). INSR has two isoforms, A and B. Evidence shows that the brain only expresses the shorter form INSR-A rather than the full-length INSR-B, which differs from many peripheral tissues (16). The most classic INSR scaffold is the insulin receptor substrate (IRS) family, which has six isoforms (IRS1-6). IRS1 and IRS2 are assumed to mediate most of the metabolic effects of INSR activation (17). In brain tissue, insulin acts through IRS1, and IRS2 is strongly related to the activity of insulin-like growth factor 1(IGF-1) (16). By phosphorylating several IRS tyrosine residues, INSR attracts downstream signaling effectors to relay and enhance insulin responses. Tyrosine-phosphorylated IRS proteins active phosphoinositide 3-kinase (PI3K) and then phosphorylate AKT to exert the known physiological effects of insulin (Figure 1) (17).

IR, as the name implies, is the failure of tissues to the normal response to insulin stimulation (18). IR appears earlier in brain tissue than in the periphery, suggesting that brain tissue is more vulnerable to IR, particularly in brain diseases such as ischemic stroke (14). There are four clinically accepted criteria for detecting the presence of IR in patients: (1) the gold standard assessment, homeostasis model assessment of IR (HOMA-IR); (2) oral glucose tolerance tests (OGTT); (3) C-peptide release test; and(4) triglyceride glucose (TyG) index, each with advantages and disadvantages (Table 1) (19–22). In addition, the TyG index combined with obesity indices, including body mass index (BMI), waist circumference (WC), and waist height ratio (WHtR) were found to be more accurate than the TyG index alone. TyG-BMI had the best ability to detect IR and the best consistency with HOMA-IR among them (23). However, TyG-BMI, TyG-WC, and TyG-WHtR share the same advantages and disadvantages as the TyG index. At the molecular level, while abnormalities in INSR number, receptor kinase activity, and various receptors for insulin action can all lead to IR production in tissues but abnormal serine phosphorylation of IRS blocking the PI3K/AKT pathway is thought to be the main cause of IR (17, 24). As a result, the ratio of serine phosphorylation to total phosphorylated IRS can be used as a marker of IR in either the brain or peripheral tissues, with a higher percentage indicating increased IR (25).

How IR leads to ischemic stroke

Ischemic stroke mechanisms are classified as embolism, large vessel disease, and small vessel disease (26). In up to two-thirds of acute stroke patients, abnormalities in glucose

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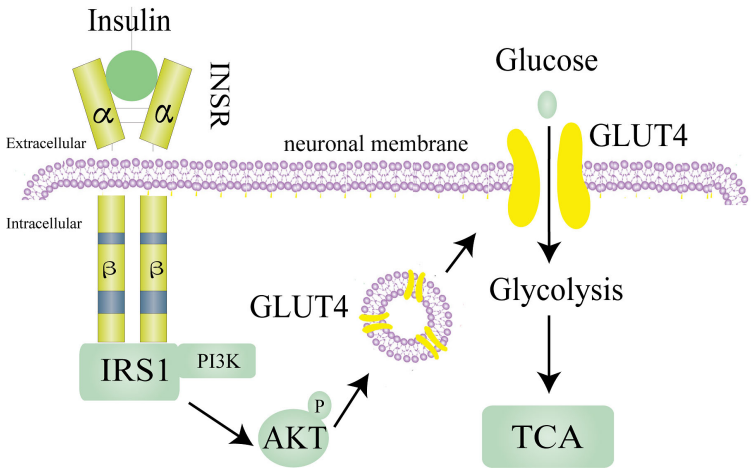

FIGURE 1
Normal insulin pathway in the neuron.

TABLE 1 Four normal methods for assessment of IR in clinical.

Name	Method	advantage	Disadvantage
HOMA-IR	(Plasma insulin during fasting × plasma glucose during fasting)/22.5	Simple, minimally invasive, predicts fasting steady-state glucose and insulin levels	limitations because of significant heterogeneity of cut-off value and IR definitions; HOMA-IR may not be appropriate in patients with severely impaired or absent β-cell function
OGTT	After the overnight fast, fasting blood glucose levels were measured and then remeasured at the time point after drinking 75 g of glucose solution.	Simple, minimally invasive	Relatively crude measurement of glucose tolerance without measuring insulin sensitivity and insulin secretion components
C-peptide release test	After the overnight fast, fasting blood C-peptide levels were measured and then remeasured at the time point after drinking 75 g of glucose solution.	Simple, minimally invasive; Long half-life; It is not interfered with by insulin antibodies and can more accurately reflect the patient's β-cell function.	Relatively crude; Needs to be analyzed in conjunction with blood glucose and insulin
TyG index	Fasting triglyceride [mg/dL] × fasting glucose [mg/dL]/2	Simple, minimally invasive; Suitable for clinical and epidemiological studies	limitations because of significant heterogeneity of cut-off value and IR definitions

HOMA-IR, Homeostasis model assessment of IR; OGTT, oral glucose tolerance tests; TyG index, triglyceride glucose index. IR, insulin resistance.

regulation (of which diabetes is a manifestation) are observed, and because ischemic stroke accounts for 87% of strokes, there have been numerous studies on T2D and ischemic stroke (3, 27). According to research, ischemic stroke and T2D share many causative factors, including IR and IR-associated syndrome. An earlier study found that 50% of 72 nondiabetic patients with transient ischemic attack (TIA) or ischemic stroke had significant IR (28). And in non-diabetic ischemic stroke patients, the value of HOMA-IR ≥2. 5, suggesting the presence of IR, in more than 20% of cases (29). Numerous studies show that IR is a risk factor for ischemic stroke and can lead to the incidence of ischemic stroke (30). Based on past reviews and contemporary research, this section will explain the relationship between IR and the two main causes of ischemic stroke—embolism and atherosclerosis—and how it influences the occurrence and progression of ischemic stroke (Figure 2).

IR promotes ischemic stroke through embolism

Ischemic stroke is most frequently caused by embolism. Most embolisms are blood clots that arise from the heart due to heart disease (cardiogenic embolism). Atrial fibrillation, heart

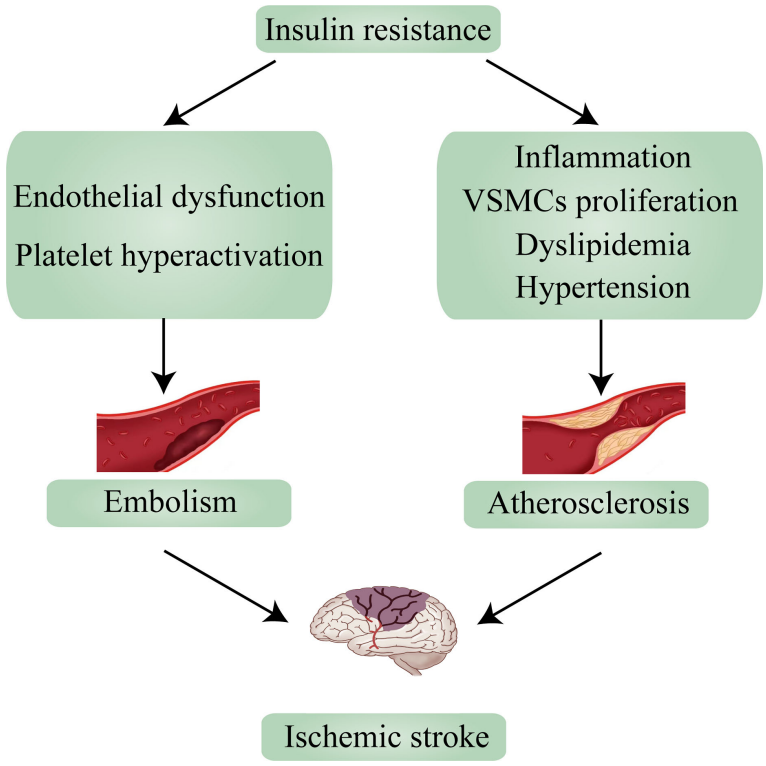

FIGURE 2
Insulin resistance leads to ischemic stroke *via* embolism and atherosclerosis. VSMCs, vascular smooth muscle cells.

valve disease, and myocardial infarction or cardiomyopathy brought on by excessive blood pressure are common cardiac disorders that cause stroke (26). Thrombosis is commonly considered to be a pathological hemostatic deviation caused by coagulation and platelet activation. The formation of an intravascular thrombus (clot) and vascular occlusion constitute thrombosis. Ischemic stroke occurs when dislodged clots move and block cerebral blood vessels (31). Interventions using antiplatelet agents to prevent platelet activation and thrombosis are known to reduce the incidence and severity of ischemic strokes. The metabolic environment of T2D includes IR, hyperglycemia, excessive release of free fatty acids, and other metabolic abnormalities affecting blood vessel walls as a result of a variety of events such as endothelial dysfunction and platelet hyperactivation. Compared to non-diabetes, T2D had a two-to four-fold higher risk of recurrent atherosclerotic thrombotic events and vascular complications. The activation of these events causes even more vasoconstriction and promotes thrombosis (32). IR, the key role of T2D, has also attracted the interest of researchers. A rising number of studies are focusing on the connection between IR and thrombosis. Researchers found that IR can impair endothelial cell function and enhance platelet adhesion, activation, and aggregation, resulting in the formation of thrombosis (33). Endothelial cells serve several functions, including pro- and anti-coagulation, which are balanced under normal conditions. When endothelial cell function is impaired, the risk of thrombosis increases (34). In IR, the PI3K pathway is impaired, resulting in decreased NO (vasodilator) production, whereas the MAPK pathway is activated, leading to increased ET-1 (vasoconstrictor) production and ultimately endothelial dysfunction (35). Excessive free fatty acids (FFA) release caused by IR and excess FFA may cause lipotoxicity, which leads to increased expression of coagulation tissue factors (e.g., PAI-1) *via* the same mechanism as glucotoxicity, resulting in a prothrombotic state (36). Furthermore, FFA can set off a vicious cycle by activating several pathways which increases the percentage of IRS1 serine phosphorylation (37). Chronic hyperglycemia and decreased serum paraoxonase/arylesterase 1 (PON-1) activity may impair high-density lipoprotein's (HDL) anti-inflammatory capacity. Eventually, serum tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP), which respond to inflammatory levels, were eventually elevated, indicating a link between the inflammatory response caused by IR and endothelial dysfunction (38). In addition, IR, assessed by HOMA-IR, was associated with endothelial dysfunction in patients with chest pain and non-diabetic patients with normal myocardial perfusion (39).

Several studies have shown that IR promotes vascular occlusion and cardiovascular disease (CVD) development by influencing platelet adhesion and aggregation (32). Platelets' major function in the body is to support primary hemostasis and intravascular blood flow. Platelet adhesion, aggregation, and release are the three steps in the platelet activation process. Adhesion to the subendothelial extracellular matrix occurs through the initial interaction of the matrix with specific receptors on the platelet, including the GP1b/V/IX complex bound to the Von Willebrand factor and the GPVI and αIIβ1 receptors bound to the collagen component of the extracellular matrix on the platelet surface. Firm adhesion leads to initial clot or thrombus formation, and activated platelets bound within the thrombus will begin to take up new platelets from the circulation *via* platelet-platelet interactions mediated by integrin receptor αIIbβ3. Platelets express two purinergic receptors, P2Y1 and P2Y12, which have been shown to play an important role in platelet activation, one of which (P2Y12) is a target for antiplatelet therapy (40). Treatment with clopidogrel and aspirin improves prognosis in patients with cerebral infarction without increasing the risk of moderate to severe bleeding (41). The effect of hyperinsulinemia on platelets is complex and varies between insulin-resistant patients and healthy individuals. In healthy individuals, insulin reduces platelet aggregation and the release of pro-aggregation agents by facilitating the transfer of magnesium to platelets (such as thromboxane B2) (42). Insulin naturally inhibits platelet hyperactivity. It makes platelets more sensitive to prostacyclin (PGI2) and increases endothelial cell production of PGI2 and nitric oxide (NO), allowing platelets to maintain normal function. Platelet hyperactivation favors macrovascular and microvascular events when IR occurs, which may explain why platelets adhere to the vascular endothelium more frequently in T2D patients than in healthy individuals (43). In addition, hyperglycemia may activate platelets through miR-144 and miR-223 to downregulate IRS1 and upregulate P2Y12 expression in the platelets *via* IRS1/PI3K/AKT pathways (44).

IR facilitates ischemic stroke *via* atherosclerosis

Atherosclerosis is another common underlying cause of ischemic stroke (26). Atherosclerosis is a chronic inflammatory disease of large and medium-sized arteries that can lead to ischemic heart disease, stroke, and peripheral vascular disease collectively referred to as CVD (45). Increasing evidence has demonstrated that atherosclerosis is a major cause of ischemic stroke (46). Furthermore, there is strong evidence that IR or metabolic syndrome caused by IR contributes to the pathogenesis of ischemic stroke by promoting the formation of atherosclerotic and advanced plaques in the progression of atherosclerosis (47). According to epidemiological studies, hyperinsulinemia is an independent risk factor for atherosclerosis. Hyperinsulinemia caused by IR can exacerbate atherosclerosis by promoting vascular inflammation, vascular smooth muscle cells (VSMCs) development, a pathological cholesterol profile, hypertension, and immune cell recruitment to the endothelium (48). The relationship between inflammation and T2D can be traced back to the 1950s. At the molecular level, IR is promoted when macrophage polarization shifts from an alternative M2 (anti-inflammatory) activation state maintained by STAT6 and PPARs to a classical M1 (pro-inflammatory) activation state driven by NF-B, AP1. In short, inflammation promotes the development of IR (49). In contrast, researchers gave a 4-hour insulin intervention to healthy volunteers with normal glucose tolerance and no history of diabetes, took biopsies of their lateral femoral muscles, and discovered noticeably elevated levels of related inflammatory genes (50). We are aware of a mutual relationship between IR and inflammation. With both protective and pathogenic roles, monocyte-macrophage lineage cells and VSMCs are two key participants in the atherosclerotic process. Proliferation of VSMCs promotes plaque growth while also forming the atheroma's fibrous cap (51). Hyperinsulinemia, which is caused by IR, is a potential growth factor that promotes its growth through the MAPK pathway, which catalyzes the phosphorylation of transcription factors that stimulate VSMCs growth, proliferation, and differentiation (52). It has been demonstrated that IR-related inflammation can also promote the development of VSMCs (53). C-peptide, produced by the cleavage of proinsulin in the β-cell. It is secreted equimolarly with the other cleavage product, insulin (54). C-peptide study extends to our knowledge of the mechanisms that promote VSMCs proliferation in hyperinsulinemia conditions (55). PI3K/AKT and ERK1/2-MAPK are thought to be critical signaling pathways controlling VSMCs. Through the activation of the protein tyrosine kinase Src, which can function as an intermediary in signaling networks that link G-protein-coupled receptors with downstream signaling cascades like the PI3K/Akt and the Ras/MAPK pathway, C-peptide can stimulate the growth of VSMCs (56, 57).

Atherosclerosis develops as a result of a disturbed cholesterol homeostasis balance (58). Numerous studies have shown that IR leads to disorders of lipid metabolism (59). In comparison to the normal metabolic state, insulin resistance promotes excess *de novo* lipogenesis as well as the production and secretion of very-low-density lipoprotein (VLDL) (60). Additionally, IR can accelerate the production of connective tissue in blood vessel walls and the aggregation of LDL cholesterol into arterial smooth muscle, both of which directly accelerate the development of atherosclerosis and, eventually, the occurrence of an ischemic stroke (47).

Hypertension is frequently regarded as a major cause of hemorrhagic stroke, as well as a risk factor for ischemic stroke (3). IR and hypertension are both independent risk factors for CVD, and a growing number of studies are beginning to recognize

the link between these two diseases that promote the formation and progression of atherosclerosis, which together lead to CVD (61). Despite the fact that a short period of insulin stimulation did not significantly increase blood pressure in non-diabetic subjects, the researchers acknowledge that IR is directly correlated with the severity of hypertension (62). In a subsequent study, the researchers prolonged the insulin intervention and discovered that chronic insulin administration can significantly raise the blood pressure of lean rats (63). Leptin is crucial for controlling body weight, plays a role in the development of the IR syndrome, and is associated with cardiovascular disease. Increased sympathetic activity mediated by leptin may cause short- and long-term alterations in blood pressure through central and peripheral effects, which may help to explain how IR affects blood pressure (64). Although studies in humans appear controversial, the role of insulin in promoting antidiuretic effects and stimulating sympathetic nervous system activation has been confirmed as a potential mechanism by which insulin may elevate blood pressure (65). Furthermore, animal studies have shown that long-term insulin administration accelerates the development of atherosclerosis (47).

How IR affects the progression of ischemic stroke

Stroke prevention and treatment are divided into two parts. Primary prevention includes modifying one's lifestyle and treating risk factors such as hypertension, diabetes mellitus, etc. Secondary prevention includes surgical intervention and treatment of IR, etc. (66). In clinical studies, researchers have found that IR is independently associated with poor functional outcomes after acute ischemic stroke, regardless of whether the patient has T2D (5, 67). However, at the mechanistic level, it has not been well studied how IR negatively affects the prognosis of patients with ischemic stroke. Starting with the potential damage caused by IR, we summarize the potential mechanisms by which IR contributes to the poor prognosis of ischemic stroke patients in this section.

IR and inflammation

The inflammatory response is believed to play a crucial part in the cerebral damage caused by an ischemic stroke (68). Microglia are important immune cells in brain tissue. Brain microglia are activated in response to ischemia. On the one hand, activated microglia secrete pro-inflammatory factors such as TNF-α and interleukin-1β (IL-1β), which cause cellular damage; on the other hand, activated microglia have phagocytic and major histocompatibility complex (MHC) class II-restricted antigen-presenting properties, which help remove dead tissue and debris after ischemia. Furthermore, activated microglia can promote the production of neurotrophic growth factors such as brain-derived neurotrophic factor (BDNF) (69, 70). This is related to the state of microglia polarization. Microglia polarization refers to the development of a classically activated (M1, pro-inflammatory) or alternatively activated (M2, anti-inflammatory) phenotype of activated microglia (71). M1 and M2 are in a dynamic equilibrium under normal physiological conditions, but in ischemic stroke, M2 is converted to M1 (72). Severe ischemic injury accompanied by a pro-inflammatory environment can produce and release large amounts of inflammatory factors causing ischemic damage to brain tissue, among which IL-6 and TNF-α can also lead to an increase in neutrophils in circulating dead brain tissue. Increased neutrophil counts not only correlate with infarct size but also can disrupt the BBB (73). Obesity can induce activation of IKKβ, leading to nuclear translocation of NF-κB and, as a result, the production of various inflammatory markers and potential mediators, and obesity can also co-promote phosphorylation of IRS1 at serine sites (ser302 and ser307) through JNK activation, which together leads to the development of IR (74). A majority of studies have found that inflammation plays a significant role in the development of IR, some other research has also shown that IR actually promotes the development of an inflammatory response. Both sides agree that inflammation and IR feed off each other in a vicious cycle (49). In both diet-induced obesity and genetically (mTORC2-knockout) induced adipose-specific IR, researchers discovered that IR causes local accumulation of pro-inflammation macrophages. IR produces the chemokine monocyte chemoattractant protein 1 (MCP1), which recruits monocytes and activates pro-inflammatory macrophages (75). Researchers discovered that blocking glucose oxidative metabolism not only prevented macrophage polarization to the M2 phenotype but also drove macrophages into the M1 type. Simultaneously, forcing an M1 macrophage to undergo oxidative metabolism boosts the M2 phenotype (76, 77). IR prevents glucose from entering neurons for oxidative phosphorylation, which explains the potential cause of IR-induced inflammation in terms of macrophage metabolic reprogramming.

IR and oxidative stress

Oxidative stress and inflammation are two key points in ischemic stroke, and there has been considerable evidence in recent years that oxidative stress, associated with the overproduction of reactive oxygen species (ROS), is the underlying mechanism of brain injury in stroke (78). In ischemic stroke, excessive ROS production disrupts the balance of oxidant and antioxidant composition, resulting in oxidative stress (79). ROS are naturally occurring small molecule by products of oxygen metabolism that include superoxide anion radicals ($O2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (-OH). The most reactive substance, -OH, can damage

polyunsaturated fatty acids, causing loss of biofilm integrity, proteins, and enzymes, as well as affecting nucleic acids' functional qualities and producing mutations that eventually result in cellular senescence or death (80). ROS are beneficial in certain physiological processes such as cell signaling, induction of pro-mitotic responses, immune defense, cellular senescence, apoptosis, and breakdown of toxic compounds. However, most studies indicate that ROS causes cellular damage and impaired function during biological stress (81). ROS can react with lipids to form peroxides, which are then degraded to aldehydes (e.g., hydroxynonenal) that are toxic to neurons and white matter in the brain, induce apoptosis, and are significantly associated with focal ischemia in rats (82). Increased ROS production after transient global ischemia upregulates p53 upregulated apoptosis regulator (PUMA) and Bcl-2 and Bax in neurons, and PUMA, along with anti-apoptotic Bcl-2 or pro-apoptotic Bax, plays an important role in ischemic neuronal death (83, 84). The effects of IR and oxidative stress also appear to be reciprocal. Oxidative stress-induced oxidative damage markers such as malondialdehyde (MDA), advanced glycation end products (AGEs), and 8-hydroxy-2′-deoxyguanosine (8-OH-dG) were found to reduce insulin sensitivity in skeletal muscle cells and adipocytes (85). Some investigators believe that oxidative stress can phosphorylate IRS proteins by activating the IKKβ/NF-κB and JNK pathways, resulting in IRS degradation (86). Furthermore, some researchers believe that metabolic disturbances caused by peripheral IR may be the source of oxidative stress in brain tissue. IR increases FFA and promotes glucotoxicity and lipotoxicity and promotes NF-κB nuclear translocation to increase ROS production (87). Mitochondria are the main source of ROS, and in recent years, researchers have recently begun to study the relationship between mitochondrial function and IR. On the one side, researchers think in some cases, the reduced mitochondrial function may be a major cause of IR. They found that increased intracellular fatty acyl CoA and diglycerides in myocytes as a result of reduced β-oxidation due to mitochondrial dysfunction or increased plasma transport, activating serine/threonine kinases such as protein kinase C (PKC) in skeletal muscle. This, in turn, activates IRS1 serine residues, resulting in IR (88). Obesity is a common cause of IR. The 75-kDA glucose regulatory protein (GRP75) is downregulated in mice fed a high-fat diet (HFD); however, increasing GRP75 prevents HFD-induced obesity and IR. GRP75 is required for mitochondrial homeostasis because it is a component of the mitochondrial mass control system and mitochondrial-associated membranes. It can improve insulin sensitivity by regulating mitochondrial function by controlling the turnover of the mitochondrial-supercomplex (89). Furthermore, insulin is required for the maintenance of mitochondrial function. Researchers recently discovered that insulin deprivation not only resulted in decreased efficiency of ATP production by isolated muscle mitochondria but also increased degradation of mitochondrial proteins in streptozotocin (STZ)-induced diabetic mice treated continuously with insulin implants and mice that had their insulin implants removed after re-establishing healthy glycemic control. This increase in protein degradation could be attributed to increased expression of the mitochondrial autophagy marker protein (Beclin) (90). To better understand the connection between IR and mitochondrial function, researchers used IRS1 and IRS2 double-knockout mice to mimic IR. This study revealed increased expression of several forkhead box O1 (Foxo1) target genes in the liver of double knockout mice, including heme oxygenase-1 (Hmox1), which disrupts complexes III and IV in the respiratory chain and reduces the NAD+/NADH ratio and ATP production (91).

IR and neuronal injury

Neurons are the most fundamental structural and functional elements of the nervous system. There is widespread agreement that prompt and effective neuroprotection following an ischemic stroke can significantly improve patients' prognoses. In clinical practice, drugs (e.g., gangliosides) are widely used as neuroprotective agents in patients with ischemic stroke. However, the actual mechanism of neuronal death after ischemic stroke remains unknown. We discuss the association of IR with these mechanisms based on a recent review of the mechanisms of neuronal death after ischemic stroke (92). Ischemic stroke causes a large release of the neuroexcitatory transmitter glutamate, which leads to excessive activation of NMDA receptors and allows a large inward flow of calcium ions. Excessive intracellular calcium ion accumulation activates many calcium-dependent proteases, lipases, and deoxyribonucleases, leading to cell death. Moreover, calcium overload also causes the release of apoptotic factors from the mitochondria, which induces apoptosis (93). Excitotoxicity and inflammatory responses can cause not only receptor-interacting protein kinase 1 (RIPK1) activation to promote neuronal cell necrosis, but also DNA damage. Once DNA is damaged, p53 can promote ferroptosis by inhibiting SLC7A11 expression or promoting spermidine/spermine N1-acetyltransferase 1(SAT1) and glutaminase 2 (GLS2) expression (94, 95). After an ischemic stroke, oxygen and glucose transport are restricted, anaerobic oxidative metabolism takes over as the primary source of neuronal ATP, and energy supply exceeds demand, resulting in neuronal apoptosis (96). oxygen-glucose deprivation also inhibits the AKT pathway leading to decreased mTORC1 activity, a classical pathway that blocks autophagy activation (97). Excessive ROS accumulation due to mitochondrial dysfunction activates FOXO3, increasing the abundance of LC3 to promote autophagosome generation, and excessive autophagy can increase neuronal apoptosis (98). Autophagy response dysfunction causes adipocyte dysfunction, and the development of IR. IR induces glucotoxicity, which exacerbates

oxidative stress, inflammation, and endoplasmic reticulum stress caused by lipotoxicity, further impairing the autophagy response (99). Furthermore, our team found that IR inhibits GLUT4 membrane translocation, leading to the apoptosis of neurons due to insufficient glucose uptake. Inhibiting neuronal IR attenuates neuronal apoptosis (100). All of this suggests that IR could cause neuronal death. According to research, ischemic stroke patients' declining memory and cognitive function may be caused by neural synaptic plasticity dysfunction (101, 102). Synaptic plasticity is an activity-dependent change in the strength of neuronal connections and has long been recognized as an important component of learning and memory (103). Considering that insulin is required for neurosynaptic function, IR also has an impact on neuronal function. It has the ability to alter synaptic plasticity in neurons (104). Neuronal injury may be responsible for poor clinical outcomes such as worsening neurological function and a poorer functional outcome at 3 months in patients with ischemic stroke due to IR. A new study discovered that maternal HFD-dependent IR impairs multigenerational synaptic plasticity, learning, and memory (105). By inducing IR in female mice with HFD and evaluating hippocampus-dependent synaptic plasticity and memory in female offspring (no difference between females and males), researchers found that offspring novelty recognition experimental preference indices were lower than controls and that only BDNF was reduced in all three generations of offspring mice. Epigenetic inhibition of exon-specific BDNF expression in offspring may explain how HFD-induced IR multi-generationally impairs synaptic plasticity, learning, and memory. Furthermore, maternal administration of BDNF or lack of pro-IR gene p66Shc abrogated the transmission of HFD-dependent cognitive dysfunction to offspring.

How to treat IR in ischemic stroke patients

T2D is an established risk factor for ischemic stroke, however, numerous research has revealed that many ischemic stroke patients who do not have T2D but do have IR also have a significantly worse prognosis than those who do not have IR. Insulin is important for maintaining the functional integrity of the brain, and peripheral and central insulin dysfunction due to IR may be an independent risk factor for stroke. IR treatment is an important component of the secondary prevention of ischemic stroke (66). Increasing CNS insulin concentrations or CNS insulin sensitivity may be effective not only in preventing ischemic stroke but also in improving the prognosis of ischemic stroke patients. We discuss the treatment of IR in terms of non-pharmacological modalities, insulin therapy, and increasing insulin sensitivity (Table 2).

Non-pharmacological modalities

Lifestyle changes, including a healthy diet, weight loss, smoking cessation, and appropriate physical activity are well-known ways to improve peripheral insulin sensitivity and are considered primary prevention of ischemic stroke (66). Obesity is associated with an increased prevalence of vascular risk factors, and obesity is typically the primary cause of IR. In the

TABLE 2 The treatment of IR in ischemic stroke.

Therapeutic approach	Method	Mechanism
	Healthy diet	Improve insulin sensitivity
Non-pharmacological modalities	Smoking cessation	Improve insulin sensitivity
	Exercise	Improve insulin sensitivity
Intranasal insulin	Intranasal insulin	Activate AKT
	Thiazolidinediones	Activate PPAR-γ
	Metformin	Activate AMPK
Insulin sensitizers	Statins	Regulate lipid metabolism
	Astaxanthin, α-ketoglutarate	Inhibit mTOR/S6K1
GLP-1 receptor agonists	Semaglutide, Exendin-4, etc.	Active GLP-1 receptor

PPAR-γ, peroxisome proliferator-activated receptor γ; AMPK, AMP-activated protein kinase; GLP-1, glucagon-like peptide-1. IR, insulin resistance.

INTERSTROKE research, obesity was responsible for 82% and 90% of the population-attributable risk for ischemic and hemorrhagic stroke, respectively (106). The researchers discovered that a healthy vegetarian diet was substantially associated with a lower risk of overall stroke and that vegetarian diet was unrelated to stroke in a follow-up of 3,015 ischemic strokes and 853 hemorrhagic strokes (107). Compared with the control condition, folic acid was associated with a lower risk of stroke [relative risk (RR)= 0.80, confidence interval (CI) =0.67-0.96], whereas combined calcium plus vitamin D intake was associated with an increased risk (RR=1.17, CI=1.05-1.30) (108). Dietary control in diabetic patients is a simple, effective, safe, and efficient way to improve IR (109). Researchers identified smoking and exposure to secondhand smoke as definite risk factors for stroke in a comprehensive review of the Global Burden of Disease Study 2019 (110). Researchers found that both male and female smokers (cigarettes + e-cigarettes and cigarettes or e-cigarettes) were more likely to develop IR than non-smokers (male, dual: odds ratio (OR)=2.19, CI=1.39–3.44; single: OR=1.78, CI=1.43–2.22; female, dual: OR=2.32, CI=1.01–5.34; single: OR=1.76, CI=1.28–2.42) (111). There is sufficient evidence to incorporate cardiopulmonary and mixed training involving walking into post-stroke rehabilitation programs. Cardiopulmonary training, as well as, to a lesser extent, mixed training, improves movement and balance and thus reduces disability during or after conventional stroke treatment (112). Exercise and IR have a well-established link. IRS1 and GLUT4 mRNA levels were lower in the proliferating endometrium of patients with polycystic ovaries compared with BMI-matched controls. Lifestyle changes combined with diet and exercise resulted in improved menstrual patterns in 65% of overweight/obese women with polycystic ovary syndrome, and significantly higher IRS1 and GLUT1 mRNA levels were found in the endometrium of these women with improved menstrual function, suggesting that lifestyle interventions can improve insulin sensitivity in patients (113). Recent studies have consistently shown that moderate repetitive exercise for 30 minutes or longer at least three times per week for at least eight weeks improves insulin sensitivity in patients with diabetes, obesity, and metabolic syndrome. Improved insulin sensitivity may be associated with weight loss (114). In summary, lifestyle changes are not only an important part of the primary prevention of ischemic stroke, but they can also play an important role in the treatment of IR in the secondary prevention of ischemic stroke.

Intranasal insulin

IR is essentially a decrease in insulin cellular utilization, and exogenous insulin supplementation is also an effective way to increase intracellular insulin concentrations. Insulin administration in stroke patients has shifted from intravenous to intranasal. In a meta-analysis including 9 studies (1,491 patients), there was no statistically significant difference in the incidence of mortality (OR=1.16, CI= 0.89-1.49) and improvement in neurological function (OR=1.01, CI=0.81-1.26) in patients treated with intravenous insulin compared to controls. However, the odds of any hypoglycemia (OR=8.19, CI=5.60-11.98) and symptomatic hypoglycemia (OR=6.15, CI=1.88-20.15) were significantly higher in patients receiving intravenous insulin therapy (115). The reason for this phenomenon may be that although peripheral insulin injections are the classical way to treat T2D, they may induce hypoglycemia in stroke patients with only an insulin-resistant state that has not yet developed T2D, and this hypoglycemia can cause secondary brain damage, and peripheral insulin injections may be ineffective due to impaired insulin transport by BBB. As a result, researchers have concentrated on intranasal insulin administration as a neuroprotective treatment for ischemic stroke. Intranasal administration is a safe and effective method of bypassing the BBB and increasing distribution to the CNS without the downsides of systemic side effects or first-pass metabolism while reducing IR (116). Investigators discovered that the incidence of vascular disease was not significantly higher in the insulin group than in the placebo group in a clinical study examining the safety of intranasal insulin administration for the treatment of Alzheimer disease dementia, also demonstrating that intranasal insulin administration is safe (117). Intranasal insulin administration reduced cerebral infarction and neurological deficits and increased phosphorylation of Akt and endothelial nitric oxide synthase (eNOS) proteins in STZ-induced diabetic rats with focal cerebral ischemia-reperfusion injury. When insulin is combined with N-iminoethyl-L-ornithine, an eNOS inhibitor, the beneficial effects of insulin on infarct volume and neurological deficits in ischemia-reperfusion diabetic rats are inhibited, but the hypoglycemic effect of insulin is not affected (118). Another hemorrhagic stroke study found that administering 1 IU of intranasal insulin significantly reduced hematoma volume, brain edema, and BBB permeability in intracerebral hemorrhage-induced mice. The researchers discovered that after intranasal insulin administration, the expression of AKT (Ser473) and GSK3 (Ser9) in perihematomal tissue was significantly increased. Insulin binding to its receptor activated AKT through serine phosphorylation at position 437, and the activated AKT promoted GSK3β phosphorylation at Ser9, which inactivated GSK3β. GSK3β was associated with neuronal death, and GSK3β over-activation induces neuronal degeneration and reduces the expression of claudin-1 and claudin-3, the major components of BBB tight junctions. The activation of the AKT/GSK3 signaling pathway by intranasal insulin may be the mechanism by which intranasal insulin administration improves neurological function in mice with cerebral hemorrhage (119). The evidence for intranasal insulin administration in protecting neurological function is sufficient, and intranasal insulin administration is a potential therapeutic modality for improving IR and restoring

neurological function in ischemic stroke. In short, AKT is an essential pathway for insulin to exert its physiological effects, and although clinical trials of intranasal insulin administration for ischemic stroke need to be supplemented, the evidence for intranasal insulin administration in protecting neurological function is sufficient.

Insulin sensitizers

Another viable strategy is to use insulin sensitizers to directly combat IR. Despite the fact that there have been few studies on insulin sensitizers in ischemic stroke, we have identified a number of useful insulin sensitizers based on previous research in T2D patients. Thiazolidinediones (TZDs) reduce insulin resistance directly by activating peroxisome proliferator-activated receptor γ (PPAR-γ), which promote mesenchymal stem cell differentiation into adipocytes, promote lipogenesis in peripheral adipocytes, lower hepatic and peripheral triglycerides, reduce visceral adipocyte activity, and enhance adiponectin (120). Two common TZDs are pioglitazone and rosiglitazone, both of which are PPAR-γ agonists. 3,876 patients who had recently experienced an ischemic stroke or TIA and had HOMA-IR > 3.0 but no T2D were split into two groups in a multicenter, double-blind experiment (pioglitazone or placebo). They discovered pioglitazone not only lowers the risk of T2D (hazard ratio (HR)=0.48, CI=0.33-0.69) but also the incidence of stroke and myocardial infarction (HR=0.76, CI=0.62-0.93) (121). 5,039 people with stroke or TIA participated in a meta-analysis of 5 RCTs, of which 4 evaluated the medication pioglitazone and 1 rosiglitazone (122). Additionally, it was discovered that PPAR-γ agonists, as opposed to a placebo, lower the incidence of recurrent stroke (RR=0.66, CI=0.44-0.99). Researchers discovered that PPAR-γ agonists decreased the composite outcome of major vascular events, such as cardiovascular death, non-fatal myocardial infarction, or non-fatal stroke total occurrences (RR=0.73, CI=0.54-0.99) in a single experiment with 984 patients. Since it doesn't result in hypoglycemia, metformin, the most often used medication to treat T2D, is arguably the subject of the most research. Metformin exerts its activity through two main mechanisms: AMP-activated protein kinase (AMPK)-dependent and AMPK-independent modalities. When metformin activates AMPK, it can increase fatty acid oxidation by inhibiting the phosphorylation of acetyl coenzyme A carboxylase, ultimately improving lipid metabolism and insulin sensitivity (123). Metformin also has neuroprotective benefits in ischemic stroke by activating the AMPK pathway. Activated AMPK reduces neuroinflammation by inhibiting the release of inflammatory markers, decreasing ROS generation to reduce oxidative stress, and promoting autophagy. Furthermore, by reducing glutamate release, AMPK activation prevents glutamate excitotoxicity-induced apoptosis (124). Statins are lipid-lowering medications that are primarily used to treat cardiovascular disorders caused by high blood lipid levels, such as hyperlipidemia, hypertension, atherosclerotic heart disease, and others. Previous research has shown that long-term pravastatin plus captopril treatment improves the progression of IR and associated risk factors (hyperinsulinemia, hypercholesterolemia) (125). Another study came to the same conclusion, showing that in ob/ob mice—which developed obesity and IR due to a loss of functioning leptin, the infarct volume was considerably higher after middle cerebral artery occlusion (MCAO) than in wild-type or lean mice. Short-term treatment with rosuvastatin (10 mg/kg/day for 3 days) did not reduce infarct volume in wild-type and lean mice, but significantly reduced infarct volume in ob/ob mice with IR (126). However, ten weeks of intensive atorvastatin (40 mg/d) treatment increased the emergence of insulin resistance and insulin secretion in participants without diabetes (127). This conflicting outcome indicates that statins for insulin resistance still require additional experimental confirmation. Apart from this, several medicines (e.g., α-ketoglutarate, astaxanthin) can reduce IR and increase neuronal survival by decreasing aberrant serine phosphorylation of IRS1 directly *via* inhibiting mTOR/S6K1 (100, 128). In summary, there is substantial evidence that insulin sensitizers can boost brain insulin sensitivity, successfully treat ischemic stroke, and improve neurological function.

GLP-1 receptor agonists

Glucagon-like peptide-1 (GLP-1) maintains glucose metabolism stability by increasing insulin production and suppressing glucagon secretion, enhancing cell proliferation and growth, and avoiding cell excitotoxicity and death (129). GLP-1 receptors (GLP-1Rs) are found throughout the CNS (130). GLP-1 receptor agonists (GLP-1RAs) not only be used to treat T2D by increasing peripheral insulin sensitivity but have also been found to increase neuronal sensitivity to insulin (131, 132). In a meta-analysis of 33,457 subjects, GLP-1RAs treatment was found to reduce the incidence of major adverse cardiovascular events (cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke) compared to placebo (HR= 0.90, CI=0.82-0.99) and did not cause adverse effects such as severe hypoglycemia (133). Another meta-analysis, which included 35 preclinical studies, 11 retrospective database studies, 7 cardiovascular outcomes trials, and 4 prospective clinical studies, found that administration of GLP-1RAs after stroke reduced infarct volume, apoptosis, inflammatory responses, and oxidative stress, while also promoting neurogenesis, angiogenesis, and increased cerebral blood flow to exert neuroprotective effects. Furthermore, in cardiovascular outcome trials, dulaglutide and semaglutide were observed to minimize the risk of stroke (134). Exendin-4, another GLP-1RAs, reduces the activation of astrocyte-derived matrix metalloproteinase-9 (MMP-9), vascular endothelial

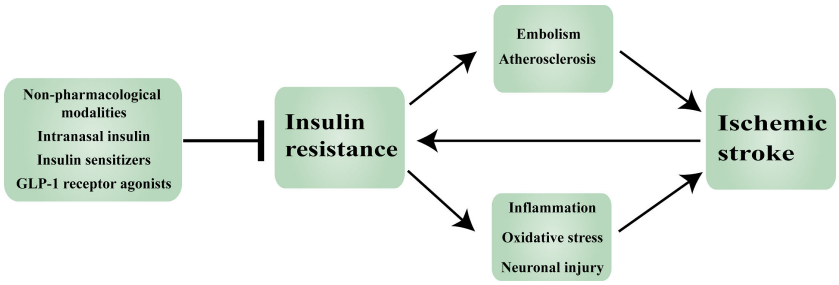

FIGURE 3
Insulin resistance in ischemic stroke: mechanisms and therapeutic approaches. GLP-1: Glucagon-like peptide-1.

growth factor (VEGF-A), MCP1, and chemokine C-X-C motif ligand 1 (CXCL-1) by oxygen-glucose deprivation (OGD), as well as the activation of the JAK2/STAT3 signaling pathway by OGD. Exendin-4 dramatically improved neurological function scores and reduced infarct volume in MCAO rats, yielding similar outcomes (135). Taken together, GLP-1RAs are a very promising medicine for the treatment of IR in ischemic stroke, but additional clinical trial evidence is required.

Conclusions and perspectives

Insulin is essential for brain function. IR, which leads to insulin dysregulation, can cause neurological damage *via* a variety of mechanisms. Numerous studies have shown that IR is not just a feature of T2D, but it also plays a key role in the development and progression of ischemic stroke. IR is an independent risk factor for ischemic stroke, IR contributes to the development of ischemic stroke by promoting thrombosis and atherosclerosis formation. IR, which is associated with a poor prognosis in ischemic stroke patients, exacerbates the inflammatory response, oxidative stress, and neuronal damage. Current IR treatment in patients with ischemic stroke is based on medicine selection based on T2D treatment experiences, such as insulin, insulin sensitizers, and GLP-1RAs. In many studies, insulin sensitizers and GLP-1RAs had no major side effects in patients with ischemic stroke, however, the use of insulin has attracted controversy. Although peripheral insulin injection therapy is the preferred treatment for T2D, it has a number of negative effects in ischemic stroke patients, and safer and more effective intranasal insulin administration is becoming more common (Figure 3). However, these studies still have some limitations, the first of which is the dispute over how to identify IR. For instance, while some authors consider HOMA-IR >3 to validate the diagnosis of IR, others define IR as HOMA-IR ≥2.5. Although ethnicity may be a factor in the cut-off value heterogeneity, a consistent scoring standard can boost the credibility of experimental findings. Secondly, further research is needed to fully understand the variety of roles that IR plays in ischemic stroke. Aside from the inhibitory effects of insulin on the regulation of glucose metabolism, inflammatory response, oxidative stress, neuronal function, and vascular function, there could be additional mechanisms that modulate ischemic stroke that is independent of the insulin signaling pathway. Overall, there is mounting proof that IR is an independent risk factor for ischemic stroke and a major component in poor patient prognosis. The prevention and treatment of ischemic stroke may benefit from a systematic understanding of the mechanisms behind IR in ischemic stroke.

Author contributions

P-FD wrote the manuscript. H-SZ, JW, C-HH and WL contributions to design of the work. Y-YG and J-NM made suggestions for some of the work. C-HH and WL revised the work. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

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Double-edged sword of diabetes mellitus for abdominal aortic aneurysm

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Introduction: Diabetes mellitus (DM) has been proved to contribute to multiple comorbidities that are risk factors for abdominal aortic aneurysm (AAA). Remarkably, evidences from epidemiologic studies have demonstrated a negative association between the two disease states. On the other hand, hyperglycemic state was linked to post-operative morbidities following AAA repair. This review aims to provide a thorough picture on the double-edged nature of DM and major hypoglycemic medications on prevalence, growth rate and rupture of AAA, as well as DM-associated prognosis post AAA repair.

Methods: We performed a comprehensive search in electronic databases to look for literatures demonstrating the association between DM and AAA. The primary focus of the literature search was on the impact of DM on the morbidity, enlargement and rupture rate, as well as post-operative complications of AAA. The role of antidiabetic medications was also explored.

Results: Retrospective epidemiological studies and large database researches associated the presence of DM with decreased prevalence, slower expansion and limited rupture rate of AAA. Major hypoglycemic drugs exert similar protective effect as DM against AAA by targeting pathological hallmarks involved in AAA formation and progression, which were demonstrated predominantly by animal studies. Nevertheless, presence of DM or postoperative hyperglycemia was linked to poorer short-term and long-term prognosis, primarily due to greater risk of infection, longer duration of hospital stays and death.

Conclusion: While DM is a positive factor in the formation and progression of AAA, it is also associated with higher risk of negative outcomes following AAA repair. Concomitant use of antidiabetic medications may contribute to the protective mechanism of DM in AAA, but further studies are still warranted to explore their role following AAA repair.

KEYWORDS

abdominal aortic aneurysm, diabetes mellitus, hyperglycemia, mortality, rupture

1 Introduction

Abdominal aortic aneurysm (AAA) is defined as an abnormal focal expansion of the abdominal aorta with a diameter greater than 3 cm or 50% larger than its normal size (1). AAA is one of the main vascular complications that pose a rising health burden worldwide (2) especially in low-income and middle-income countries due to aging population, increase in cigarette smoking and rising challenges of chronic comorbidities (3). Patients with AAA carries a high risk of mortality due to rupture, and current intervention for AAA relies exclusively on surveillance or surgical repair (4), which underlines the need to identify effective pharmacological target.

Important risk factors contributing to AAA comprise advanced age, male gender, smoking, as well as comorbidities such as history of other vascular complications, coronary artery disease, atherosclerosis, hypercholesterolemia, and hypertension (1). Diabetes mellitus (DM) is a major risk factor to atherosclerotic lesions and macrovascular diseases that are linked to AAA presence, including cardiovascular disease (CAD) and peripheral vascular disease (PAD) (5). Surprisingly, instead of accelerating the formation and progression of AAA, DM was found to have an inverse association with AAA development in previous epidemiological reports (6, 7). DM exerts a protective effect potentially by suppressing major pathological hallmarks of AAA (4, 8).

On the other hand, DM is a known risk factor for higher perioperative or postoperative morbidity and mortality. Post-operative hyperglycemia is associated with delayed would healing and higher infectious complication. Therefore, the protective effect of DM to AAA might be attenuated following AAA repair. This study aims to provide insight to standard of care for patients with both AAA and DM by reviewing the paradoxical effects of DM and hypoglycemic agents on prevalence, growth rate and rupture of AAA, as well as DM-associated morbidity and mortality post AAA operations.

2 Features of AAA pathogenesis targeted by DM

The mechanism underlying the pathogenesis of AAA was found related to the biology of abdominal aortic wall. Diabetic patients were shown to have decreased levels of matrix metalloproteinases (MMPs) and matrix degradation, enhanced matrix and collagen synthesis, resulting in a thicker abdominal aortic wall and a larger volume of extracellular matrix (ECM) (7, 9, 10).

ECMs in AAA patients are characterized by extensive proteolysis, leading to the destruction of collagen and elastin. Diabetes mellitus induces glycation of the ECM, which subsequently stimulates the formation of advanced glycation end products (AGE) (7). AGEs are covalently cross-linked with elastin and collagen in the blood vessel wall, promoting protection against mechanical structure loss and contributing to arterial stiffness (7, 9, 11, 12). Although enhanced arterial stiffness is a risk factor for atherosclerotic diseases, it provides resistance towards aneurysm growth delays AAA progression (12, 13). Nevertheless, the influence of AGEs on AAA was found conflicting. Stimulation of the AGEs receptor (RAGE) leads to upregulation of inflammatory cytokines and MMPs, thus promoting the formation of AAA (7, 14, 15). Through a murine elastase-induced AAA model, Raaz et al. discovered that segmental aortic stiffening enhanced aortic wall stress and promoted aneurysmal growth. In contrast, homogenous stiffening reduced aneurysm growth (12, 16).

MMPs are calcium-dependent zinc endopeptidases secreted by vascular endothelial cells and macrophages; they play a key role in the pathogenesis of AAA. Both MMP-2 (released by smooth muscle) and MMP-9 (released by macrophages) were found involved in the process of matrix destruction and vessel wall degradation in aortic aneurysms (9). Expression of MMP-9 was observed elevated at the site of AAA rupture, and was additionally associated with ruptured aneurysm related 30-day mortality (17, 18). The expressions of pro-MMPs and MMPs in diabetic patients are significantly attenuated. Hyperglycemic state inhibits the expression of MMP-9 messenger RNA and protein expression in macrophage cell lines by stimulating glucose sensitive nuclear receptor Nr1h2 (19), which may explain the aortic wall thickening and matrix loss deceleration in diabetic aneurysms (11).

In addition, inflammatory processes exert a significant influence on aortic wall remodeling in AAA pathogenesis. The underlying mechanisms by which diabetes affects inflammatory process may include activation of T cell insulin receptors, the monocyte-macrophage system, and through C-peptides production (9, 14). DM patients often have elevated circulating C-peptide and macrophage levels in aortic tissues. A previous study demonstrated that C-peptide impairs high glucose-induced proliferation and nuclear factor kappa B (NF-κB) nuclear translocation in vascular smooth muscle cells (VSMCs) (20). In the presence of C-peptide, the expression of various pro-inflammatory cytokines is reduced *via* the NF-κB pathway (9, 14).

Homeostasis of VSMC is another protective factor towards AAA. In most cases, VSMC exists in contractile phenotype, which contribute to vascular remodeling (1, 9). When exposed to oxidative stress, inflammation or injury, VSMC of contractile phenotype will differentiate into a synthetic phenotype. Synthetic VSMC phenotype is implicated with decreased expression of contractile protein, increased MMPs and vascular calcification, thereby potentiating AAA progression and rupture (9, 21, 22). Transforming growth factor (TGF)-β was found essential for the induction and maintenance of environmental balance and differentiation in VSMC.

Hyperglycemic state triggers TGF-β signaling pathway, thereby downregulating the expression of MMP-2 and exerting protective effect on aortic VSMC (14, 23). Moreover, an *in vitro* study discovered significant morphological differences between Type II DM (T2DM) and non-T2DM VSMC. The study found vinculin, a local adhesive protein that couples the ECM to the cytoskeleton, was upregulated in T2DM VSMC (24). Increased vinculin-positive focal adhesions in VSMC may account for the vascular stiffness of the abdominal aortic wall in DM patients, leading to retard of AAA formation (7, 10).

Intraluminal thrombus (ILT) is another increasingly recognized feature of AAA growth, remodeling, and rupture over the past decades (25). Tissues of AAA subjects were found with greater concentrations of tissue plasminogen activator (TPA) and hypoactive plasminogen activator inhibitor (PAI-1), implying a hypercoagulable state that stimulates ILT deposition and reduces ILT renewal (26). ILT is implicated with cellular inflammation, arterial wall hypoxia and ECM apoptosis, prompting aneurysm growth and eventual rupture (25). In addition, plasmin converts pro-MMPs to their active form; by inhibiting conversion of plasminogen to plasmin, PAI-1 attenuates fibrinolysis and decreases MMP production. Downregulation of PAI-1 in AAA tissues thereby results in augmented MMPs production in ILT and in the AAA wall (27–29). Dua et al. investigated the effect of hyperglycemia on endogenous PAI-1 in AAA-induced mice. Immunohistochemistry confirmed increased intensity of PAI-1 and suppressed MMP-9 expression in the diabetic mice, corresponding to a reduced AAA formation (30).

Moreover, thicker regions of ILT are linked to localized hypoxia in AAA, subsequently induces a variety of angiogenesis control factors such as vascular endothelial growth factor (VEGF); these changes induce localized mural neovascularization and inflammation, in addition to regional wall weakening (31). Hyperglycemia inhibits neovascularization by downregulating the expression of VEGF and angiogenesis response (9, 32). The protective mechanisms of DM against AAA are summarized in Figure 1.

3 Protective effect of DM to AAA

3.1 Effect on prevalence rate

AAA is most commonly diagnosed in male among the age of 60 to 80 (33). Currently known risk factors for AAA include male, smoking, family history, advancing age, hypertension, as well as obesity (34). While most of the risk factors are also linked to DM, previous meta-analysis and epidemiological studies identified DM diagnosis as a negative predictor for AAA (35, 36). The presence, growth, and probably rupture of AAA were found to be higher among non-diabetics comparing to diabetic patients (6, 7).

The negative association between DM and AAA was also demonstrated by pooled data analysis from large population prevalence studies and smaller studies in selected populations. Prospective studies showed significantly lower number of new AAA diagnosis in DM patients (37). When diabetic patients were stratified into advanced and uncomplicated groups based on existing DM comorbidities, it was demonstrated that advanced diabetes exerts a stronger protection against AAA

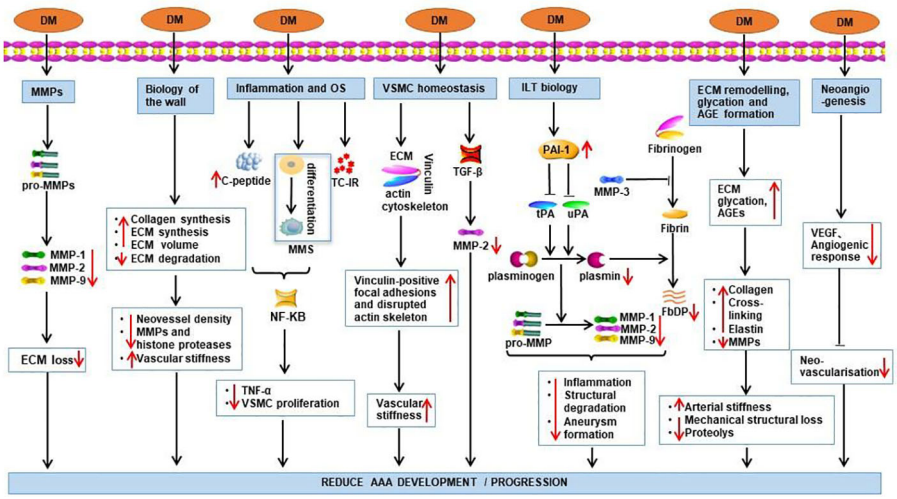

FIGURE 1
Potential mechanisms by which DM exerts its protective effect on AAA. Effects of DM are demonstrated by red arrows. TC-IR, T-cell insulin receptors; DM, diabetes mellitus; MMS, monocyte-macrophage system; OS, oxidative stress; FbDP, Fibrin degradation products; VSMC, Vascular smooth muscle cells; ILT, intraluminal thrombus; PAI-1, Plasminogen activator inhibitor-1.

without rupture, with risk of AAA decreased by near a half in the advanced DM group (6). Le et al. also revealed a decreasing risk of AAA with longer diabetes duration in addition to the independent inverse association between DM and AAA. The odds ratio of AAA was 0.50 at 3–5 years of follow-up (95% CI 0.27, 0.89), which declines further to 0.37 after 12 years of follow-up (95% CI 0.19, 0.70) (38). This outcome was compatible to the conclusion of another study, which discovered no difference in aortic diameter and AAA prevalence between those newly diagnosed type 2 diabetes and those without diabetes among 65-year-old men (39). In addition, an inverse association was established between fasting serum glucose level and aortic diameter even among non-diabetic men (38).

3.2 Effect on growth rate

Rabben et al. identified 4 independent AAA risk factors: smoking, hypertension, BMI >30, and DM, with DM being an inverse factor for AAA. The growth rate of AAA was slower in DM patients comparing to normoglycemic patients (34). Astrand et al. found the aortic intima-media thickness (IMT) to be markedly greater in diabetic patients comparing to healthy controls, adjusting for age and sex. The thick aortic wall in diabetes resulted in a 20% reduction in aortic wall stress, which proposed a potential protective mechanism against AAA (40). Another multicenter randomized study enrolling patients with small AAAs (4.1-5.4 cm) revealed a remarkably lower probability of aneurysm growth > 5 mm at 36 months in diabetic participants comparing to nondiabetics (40.8% versus 85.1%). For patients who did not undergo open repair at baseline, the need for repair at 30 months was also lower for diabetics. However, this study also demonstrated a higher hazard ratio (HR) for all-cause mortality at 36 months among diabetic patients, which could be attributed to higher obesity and cardiovascular disease rate (41).

A sub-analysis study of VIVA (Viborg Vascular) Randomized Screening Trial found that the median growth rate significantly slower in subjects with DM comparing to those without (1.7 versus 2.7 mm/year). When participants were stratified by glycosylated hemoglobin (HbA1C), the aorta growth was smaller in the highest HbA1c-tertile group compared with the lowest HbA1c-tertile group, adjusting for covariates (42).

3.3 Effect to RAAA

Despite advancements in surgical technologies, RAAA is still the major cause of AAA-related death, resulting in approximately ninety percent of mortality rate (33). The impact of DM on development and outcome of RAAA is controversial. Overall, there are three conflicting theories regarding the relationship between DM and RAAA: no association, negative association and positive association.

A large Danish register based matched case control study by Kristensen et al. (35) included 5395 cases with ruptured AAA (RAAA) matched 1:1 with patients undergoing elective AAA repairs by sex, age, and year of diagnosis. Outcomes of the study indicated that presence of DM did not protect against the risk of RAAA or influence overall 30-day mortality. A retrospective multivariate analysis study by Gokani et al. also found no statistically significant association between DM status and risk of aneurysm rupture, adjusting for cofactors (43).

In some other studies, DM is reported to be reversely associated with RAAA. A nationwide multicenter study in France reported a significantly lower prevalence of DM in ruptured AAA comparing to non-ruptured AAA (44). However, this study also demonstrated no significant difference between DM and non-DM patients in terms of in-hospital mortality in the ruptured AAA cohort. A meta-analysis by Takagi et al. also described significantly lower prevalence/incidence of RAAA in diabetic patients (odds ratio/hazard ratio, 0.71; 95% Confidence Interval, 0.56 to 0.89; p=0.003) (45). Theivacumar et al. investigated the relationship between DM and any aortic aneurysm rupture at a single center, and concluded that aortic aneurysm rupture and aneurysm rupture-related death are less likely to occur among diabetic patients (46).

On the other hand, epidemiological research of the National Health Fund and the Central Statistical Office in Poland in 2012 found significantly higher incidence of RAAA in diabetic population, regardless of gender stratification (47). Regrettably, data limitations inhibited the evaluation of the type or duration of diabetes, as well as the relationship of RAAA with the presence of cofounding factors such as hypertension, cigarette smoking and lipid values. Based on the paradoxical research outcomes regarding this issue, further prospective epidemiological research with standardized methodology is warranted to determine the link between DM and RAAA.

3.4 Gender-specific effect of DM

Previous studies have demonstrated significantly lower AAA incidence and rupture rate in women comparing to men (47). Intriguingly, the protective effect of diabetes was found attenuated among female patients. In a population-based data analysis, the sex-specific analysis showed no difference in AAA incidence rates between DM and non-DM cohorts for females, whereas the incidence rate was significant lower in males with type 2 DM (6). Another subgroup analysis of a register-based matched case control study also found increased risk of AAA rupture in female patients (35). Tsai et al. hypothesized that the diminished protective effect of DM in women was prompted by higher oestrogen level (6), which needs further investment based

on paradoxical evidences in previous animal (48, 49) and clinical studies (50, 51).

4 Effect of hypoglycemic medications to AAA

Diabetes counteracts formation and progression of AAA; therefore, it has been hypothesized that antidiabetic medicines may reverse the protective role of diabetes against AAA. Intriguingly, both animal and clinical studies have demonstrated protective effect of antidiabetic medications such as metformin, thiazolidinedione, and dipeptidyl peptidase 4 inhibitors (DPP-4i) against aortic aneurysms (14, 52). These major antidiabetics may help to reduce the prevalence, incidence and enlargement rate of AAA (53, 54) in a dose-response pattern (11, 55).

4.1 Metformin

Metformin is the first-line oral antidiabetic medicine for mild to moderate type 2 DM patients. It is also the most well-studied hypoglycemic drug in AAA. Previous studies illustrated the negative association between metformin and development of AAA. Metformin was shown to retard the formation and growth rate of AAA (56), even in normoglycemic mice (52, 57). Golledge et al. revealed that patients with diabetes prescribed metformin were associated with significantly lower AAA repair and rupture-related mortality comparing to patients with diabetes not prescribed metformin or patients with no diabetes. In contrary, diabetic patients not prescribed metformin did not show reduced incidence of AAA events comparing to patients with no diabetes (58), which raised the question that whether the protective effect of DM comes from anti-diabetic medicines. Itoga et al. found that patients with a metformin prescription had a 0.20 mm reduction in yearly AAA enlargement comparing to those without metformin prescription, adjusting for other AAA-related variables (59). While it was hypothesized that by attenuating arterial accumulation of matrix molecules, metformin could reverse the protective mechanism of diabetes and lead to increased AAA incidences, Kristensen et al. demonstrated a statistically nonsignificant protective effect of long-term metformin against ruptured AAA (RAAA) (55).

Studies mentioned above showed the promising clinical effect of metformin in limiting AAA progression. Utilization of metformin markedly reduced the maximum aortic diameter and formation of aortic aneurysm (14). Metformin activates the AMPK signal pathway, thereby downregulating the expression of proinflammatory cytokines (IL (interleukin)-1β, IL-6, MCP (monocyte chemotactic protein)-1 and TNF (tumor necrosis factor)-α), vascular growth cytokines (VEGFA, Flt-1 and CD31) and MMPs (60). The underlying mechanisms of metformin in AAA pathology eventually lead to preservation of smooth muscle cell (SMC), suppression of matrix remodeling and inhibition of inflammation pathways (61, 62). Treatment with metformin in normoglycemic mice also showed suppression of AAA formation and progression *via* restoration of the perivascular adipose tissue (PVAT) and vascular endothelial function (63). Some other potential biological pathways involved in the protective mechanism of metformin against AAA include: reduction of ECM volume, augmented arterial wall matrix formation through advanced glycation end products (8) and suppression of inflammation and oxidative stress (14). The potential mechanisms of metformin are illustrated in Figure 2.

Metformin is excreted through kidney, thereby clearance of metformin decreases during acute or chronic renal function impairment. Given the risk of contrast-induced nephropathy (CIN) after endovascular aneurysm repair (EVAR) (64) and the risk of metformin-induced lactic acidosis in patients with renal insufficiency (65), perioperative administration of metformin should be cautiously monitored. The stop and reinitiating criteria of metformin as recommended by Society for Vascular Surgery (SVS) practice guideline is presented in Table 1.

4.2 Thiazolidinedione

TZD hypoglycemic agents (such as pioglitazone and rosiglitazone) were found to active peroxisome proliferator-activated receptor-γ (PPARγ). In animal studies, PPARγ was proved to balance the aortic inflammatory conditions and to delay the progression and rupture of AAA (66). TZDs are PPARγ agonists that inhibit inflammatory response and ECM remodeling, thereby ameliorating AAA development and rupture in angiotensin II (Ang II)-induced mouse model (67–70).

Que et al. discovered that pioglitazone activates PPARγ and antagonizes the nuclear factor of activated T-lymphocytes (NFAT)/NF-κB, thus decreasing the protein expression of SMC phenotypic modulation markers. Downregulation of these modulation markers prevents cell proliferation, migration, and macrophage adhesion to SMCs, which ameliorates angiotensin II-induced aortic aneurysms (70). The expression of PPARγ in bone marrow mesenchymal stem cells of AAA patients was found notably upregulated after the administration of pioglitazone (71). The role of pioglitazone is also related to the reduction of macrophages infiltration in aortic wall and retroperitoneal periaortic fat. In a study involving sixteen patients with AA (> 5 cm in diameter) awaiting open surgical repair (OSR), after 2 months of pioglitazone pretreatment, the macrophages infiltration was diminished (72).

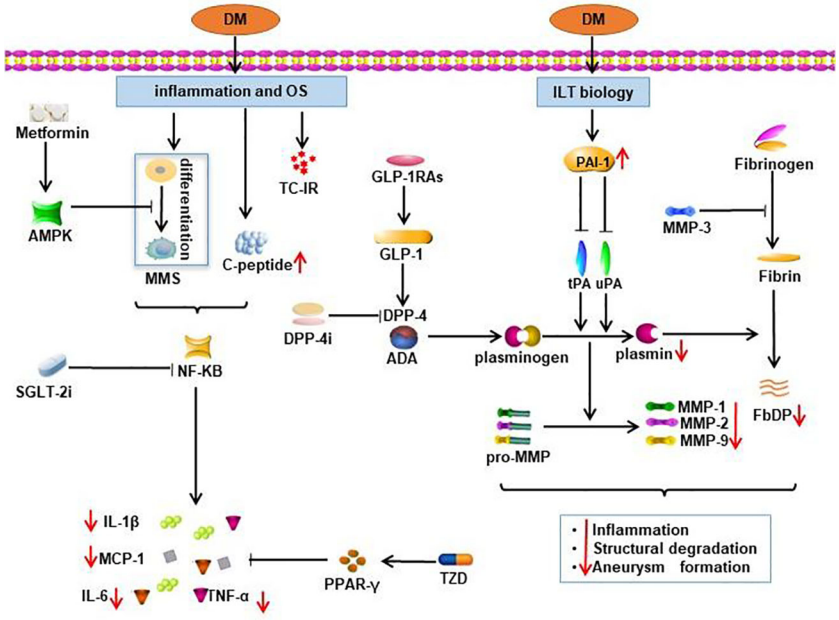

FIGURE 2
Potential mechanisms of the protective effect of oral antidiabetics on AAA. Effects of oral antidiabetics are demonstrated by red arrows. TC-IR, T-cell insulin receptors; DM, diabetes mellitus; MMS, monocyte-macrophage system; OS, oxidative stress; FbDP, Fibrin degradation products; DPP-4i, dipeptidyl peptidase 4 inhibitors; SGLT-2i, sodium-glucose cotransporter 2 inhibitors; GLP-1RAs, glucagon-like peptide 1 receptor agonists.

PPARγ activation by rosiglitazone was also reported to reduce the availability of inflammatory mediators, thereby influencing aneurysm formation and AT1a Ang II receptor expression (66, 67). Rosiglitazone can reduce the expression of TNF-α and MMP-9 in abdominal aortic aneurysm wall and retroperitoneal abdominal aortic fat (66). By increasing the production of collagen, rosiglitazone also leads to thickening of the aortic wall to reduce aortic dilation and late aneurysm rupture (66). Moreover, rosiglitazone also plays a potential protective role in the formation and rupture of aneurysms through the inhibitory effect on c-Jun N-terminal kinase (JNK) phosphorylation and toll-like receptor 4 (TLR4) expression at the site of lesion formation (68).

4.3 Dipeptidyl peptidase 4 inhibitors

DPP-4 is a glycoprotein involved in cleavage and inactivation of a variety of substrates, including incretins such as glucagon-like peptide1 (GLP-1) (4). The expression of DPP-4 in AAA media and adventitia is positively correlated with typical aneurysmal disease processes, including numerous immune responses, ECM degradation and peptidase activity, angiogenesis and reactive oxygen species (73). DPP-4 also increases plasmin levels by binding to adenosine deaminase, which results in degradation of ECM and activation of MMPs *via* activation of plasminogen-2 (74). Moreover, DPP-4 and the DPP-4-like enzyme attractin were found to induce inflammation cascades that are critical to AAA development (4).

DPP-4 inhibitors (such as sitagliptin, alogliptin and linagliptin) delay GLP-1 degradation *via* DPP-4 activity suppression, thereby improving glucose control (11). DPP-4Is also antagonize functions of DPP-4 in previous mentioned AAA pathological pathways, which consequently reduces the formation and development of AAA (4, 74, 75). in a rat-based AAA model by Bao et al., alogliptin administrations in both low-does (1 mg/kg/d) and high-dose (3 mg/kg/d) groups were associated with significant reduction of reactive oxygen species

TABLE 1 Association between renal function status and duration of metformin administration.

eGFR(mL/min)	Cessation of Metformin	Re-initiation of Metformin
<60	At the time of contrast administration	No sooner than 48 hours after contrast administration (if renal function remains stable)
<45	Up to 48 hours before contrast administration	

(ROS) expression comparing to the control group on day 7. However, the effect became only significant in high-dose group on day 28. All the other observed effects such as decreased MMP level and dilation in aortic aneurysm wall were also more prominent in the high-dose group. This dose-dependent pattern could be further explored in clinical study (76). Lu et al. investigated the function of another DPP-4I sitagliptin in Ang II-infused mice. Their results demonstrated that sitagliptin may attenuate AAA formation by restraining microphage filtration, MMPs production as well as elastin destruction (77). Other DPP4-Is, teneligliptin and vildagliptin also showed similar protection mechanisms in AAA formation and development (4, 78).

4.4 Glucagon-like peptide 1 receptor agonists

GLP-1 is the main insulin-stimulating hormone that induces insulin secretion after nutrient intake. It involves in islet beta cell proliferation and survival, glucagon secretion regulation, and gastrointestinal motility control, leading to the development of effective pharmacological treatment against DM and obesity (75). The positive effects of GLP-1RAs on AAA formation is similar to DPP-4Is, as they share same targets in pathological pathways of AAA (75).

In animal-based AAA models, GLP-1RAs (such as liraglutide and lixisenatide) inhibit AAA development through ECM preservation and through antioxidant and anti-inflammatory effects (75). Lixisenatide attenuates ROS expression and oxidative DNA damage, which in turn retards the inflammatory process of macrophage filtration, pro-inflammatory cytokine release, and eventual MMPs expression (79). Lu et al. also proved that liraglutide reduced Ang II-treated ROS production in U937 human mononuclear cell lines, confirming its antioxidative effect (77). Lixisenatide was also found to decrease levels of extracellular signal-regulated kinase (ERK), which plays a crucial role in the regulation of MMP secretion, thereby ameliorates aortic dilatation (79).

4.5 Sodium-glucose cotransporter 2 inhibitors

SGLT-2I is a novel class of hypoglycemic agent that has been investigated beyond its anti-glycemic indication. SGLT-2Is, such as dapagliflozin and empagliflozin, were found to improve the cardiovascular outcomes in patients with heart failure. In an Ang II-induced dissecting AAA mouse model, cotreatment with empagliflozin resulted in significant reduction in maximal suprarenal aortic diameter. Immunohistochemistry study further confirmed that empagliflozin disrupted elastin degradation, neovascularization, and macrophage infiltration in the AAA formation process (80). p38 mitogen-activated protein kinase (p38 MAPK) was previously proved to promote MMP production, while nuclear factor-κB (NF-κB) was known to upregulate cytokines/chemokines in the vascular wall, leading to AAA growth. Empagliflozin blunted p38 MAPK and NF-κB phosphorylation, thereby restricting AAA growth (80). Another study discovered that dapagliflozin markedly impairs medial SMC loss and alleviated aneurysmal aortic expansion in mice treated with intra-aortic porcine pancreatic elastase (PPE) infusion (54).

5 Effects of hyperglycemia on morbidity and mortality post AAA repair

Although DM and hypoglycemia medications are reported to have a protective effect against formation and progression of AAA, the role of hyperglycemia in post AAA repair might not be as beneficial. Inadequately controlled blood glucose is known to result in worse operative outcomes, with a higher incidence of infections, delayed wound healing, as well as increased mortality and length of hospital stays. Raffort et al. reported significantly higher total in-hospital mortality among DM patients with unruptured AAA, regardless of open or endovascular repair in a retrospective nationwide multicenter study (44). In addition, survival analysis identified a worse post-operative long-term mortality rate in insulin-dependent Type I DM (T1DM) comparing to normoglycemia, while T2DM was not concluded as a significant risk factor.

In a retrospective database analysis of post-discharge outcomes following elective EVAR, Gupta et al. revealed that DM patients had higher overall post-discharge morbidity, mostly linked to higher wound infection rates (81). Another database-based retrospective study by Tarbunou et al. evaluated the association between post-operative hyperglycemia and prognosis following non-ruptured AAA repair (82). Approximately one in six patients undergoing elective AAA repair developed postoperative hyperglycemia and were associated with greater risk of infection and death and longer duration of hospital stay. Patients with hyperglycemia following EVAR had nearly 2 times the odds of infection and 7.5 times the odds of in-hospital mortality. Hyperglycemic patients underwent OSR had 3 times the odds of in-hospital mortality (82).

A prospective study enrolling 66 patients undergoing elective AAA repair reported DM defined by HbA1c ≥ 6.5% as an independent risk factor for mortality (83). Huang et al.

conducted a meta-analysis that comprised 12 cohort studies involving a total 20,210 patients following AAA repair. A significantly higher long-term mortality was reported in diabetic patients comparing to normoglycemic group (5). Another meta-analysis by De Rango et al. found similar result of lower long-term survival rates and higher complication rates at 2-5 years following AAA repair among diabetic patients. This study also indicated an augmented 30-day/in-hospital operative mortality after AAA repair in DM patients (84). Based on these poor prognosis outcomes of DM following AAA repair, more attention should be paid to DM and related complications post AAA operations.

6 Conclusion

Although contribution of DM to AAA risk factors such as CAD and PAD have been well-established, an inverse relationship was revealed between DM and AAA. DM and hypoglycemic medications were found to target pathological hallmarks of AAA, thereby decreasing AAA incidence and prevalence rate, limiting AAA growth and preventing AAA rupture. Despite the protective effect of DM on AAA formation and progression, presence of DM or post-operative hyperglycemia was still found related to worse prognosis and higher long-term mortality rate post AAA repair. The double-edged nature of DM in AAA warranted further well-designed, prospective clinical investigations to formulate the standard of care for patients with AAA and DM, and to discover novel pharmacological target.

Author contributions

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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.

Publisher's note

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Impacts of stress hyperglycemia ratio on early neurological deterioration and functional outcome after endovascular treatment in patients with acute ischemic stroke

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Background and Purpose: Hyperglycemia has been associated with unfavorable outcome of acute ischemic stroke, but this association has not been verified in patients with endovascular thrombectomy treatment. This study aimed to assess the impact of stress hyperglycemia ratio on early neurological deterioration and favorable outcome after thrombectomy in patients with acute ischemic stroke.

Methods: Stroke patients with endovascular thrombectomy in two comprehensive centers were enrolled. Early neurological deterioration was defined as ≥4 points increase of National Institutes of Health Stroke Scale (NIHSS) at 24 hours after endovascular procedure. Favorable outcome was defined as modified Rankin Scale (mRS) score of 0-2 at 90 days of stroke onset. Multivariate regression analysis was used to identify the predictors for early neurological deterioration and favorable outcome.

Results: Among the 559 enrolled, 74 (13.2%) patients developed early neurological deterioration. The predictors for early neurological deterioration were high stress hyperglycemia ratio at baseline (OR =5.77; 95% CI, 1.878-17.742; *P* =0.002), symptomatic intracranial hemorrhage (OR =4.90; 95% CI, 2.439-9.835; *P* <0.001) and high NIHSS score after 24 hours (OR =1.11; 95% CI, 1.071-1.151; *P* <0.001). The predictors for favorable outcome were stress hyperglycemia ratio (OR =0.196, 95% CI, 0.077-0.502; *P* =0.001), age (OR =0.942, 95% CI, 0.909-0.977; *P* =0.001), NIHSS score 24 hours after onset (OR =0.757, 95% CI =0.693-0.827; P <0.001), groin puncture to recanalization time (OR =0.987, 95% CI, 0.975-0.998; *P* =0.025), poor collateral status before treatment (ASITN/SIR grade 0-3, OR =62.017, 95% CI,

25.920-148.382; P <0.001), successful recanalization (mTICI 2b or 3, OR =7.415, 95% CI, 1.942-28.313; P =0.001).

Conclusion: High stress hyperglycemia ratio may be related to early neurological deterioration and decreased likelihood of favourable outcomes after endovascular thrombectomy in patients with acute ischemic stroke.

KEYWORDS

acute ischemic stroke, early neurological deterioration, endovascular thrombectomy, large artery occlusion, stress hyperglycemia ratio

Introduction

Endovascular thrombectomy has been involving as the first-line treatment for acute ischemic stroke caused by large artery occlusion (1–3). However, mechanical recanalization not always necessarily resulted in favorable outcome even when patients were treated within 6 hours of stroke onset (4, 5). Exploring the possible factors associated with early neurological deterioration (END), a strong predictor for functional outcomes, is of vital importance for continuously improving the efficacy of endovascular thrombectomy in stroke patients (6, 7).

Hyperglycemia was associated with END in patients with acute ischemic stroke (8–10). Hyperglycemia could destruct blood-brain barrier, aggravate ischemic lesion, increase risk of hemorrhage transformation after cerebral infarction, and reduce duration of ischemic penumbra existence (11–14). Glycated hemoglobin (HbA1c) is more stable than blood glucose level in patients with acute ischemic stroke, and stress hyperglycemia ratio, defined as the stress fasting glycemia/HbA1c ratio (SHR), may be more feasible for evaluating the functional outcome. Some studies observed that stroke patients with high SHR had decreased likelihood of favorable functional outcome and increased likelihood of recurrence and intracranial hemorrhage after recanalization treatment (15–17); others missed these phenomena (18). Therefore, the relationship between SHR and END or functional outcome after endovascular recanalization treatment in patients with acute ischemic stroke is far from determined. This study aimed to investigate the effects of SHR on END and functional outcome in patients with acute ischemic stroke and treated with endovascular thrombectomy.

Methods

Study population

Stroke patients with endovascular thrombectomy in two comprehensive centers were screened for eligibility during November 1, 2018 and May 31, 2022. Local ethic review board approved the study protocol. Due to its retrospective nature, patient consent was waived.

Patients were treated with endovascular thrombectomy if they: 1) aged 18 years or old; 2) had ischemic stroke caused by large artery occlusion in anterior or posterior circulation; 3) pre-stroke mRS score ≤2; and 4) had arterial sheath being placed in 6 hours of stroke onset or met the DAWN or DEFUSE criteria (19, 20). Patients were not treated with endovascular thrombectomy if they: 1) had a life expectancy <12 months; 2) had severe cardiopulmonary failure; 3) had a platelet count of <55×1000/mm^3; or 4) had anemia (hemoglobin <100 g/l) or other conditions which may affect HbA1c measurement.

Recanalization treatment and baseline assessment

All thrombectomy procedures were performed with Solitaire (Covidien, Irvine, CA) and Catalyst6 devices (Stryker, Kalamazoo, MI) alone or in combination. Successful recanalization was defined as grade 2b-3 in modified thrombolysis in cerebral infarction (mTICI). Collateral circulation was evaluated using the American Society of Interventional and Therapeutic Neuroradiology/Society of Interventional Radiology collateral vessel grading system (ASITN/SIR), and categorized into grade 0 or 1, 2, and 3 or 4. The door to groin puncture time (DPT), groin puncture to final recanalization time (PRT), number of retriever passes, intravenous thrombolysis, and rescue treatment (including angioplasty, stenting, intra-artery thrombolysis) were recorded. Possible pre-procedure infarction were quantified using the Alberta Stroke Program Early CT Score (ASPECTS) or ASPECTS for posterior circulation (pc-ASPECTS) on non-contrast CT. CT was performed immediately and 24 hours after the endovascular procedures to detect possible intracranial hemorrhage. An extra CT scan was arranged whenever as the novel symptoms indicated.

Follow-up assessment

Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS) score. Stress hyperglycemia ratio was defined as the stress fasting glycemia/HbA1c ratio (SHR). END was defined as an increase of 24-hour NIHSS score of ≥4 points after endovascular procedure (21). Favorable outcome was defined as a mRS score of 0-2 at 90 days of stroke onset. Symptomatic intracranial hemorrhage (sICH) was defined and classified according to the European Cooperative Acute Stroke Study (ECASS-III) criteria (22).

Malignant brain edema was defined and classified according to the SITS-MOST (Safe Implementation of Thrombolysis in Stroke-Monitoring Study) protocol, and grade 3 was defined as malignant edema (23, 24).

Statistical analysis

Categorical variables were expressed as frequencies and percentages, and analyzed with $\chi 2$ or Fisher's exact test. Quantitative variables were expressed as medians and interquartile ranges (IQRs), and were analyzed with Mann-Whitney U test. Receiver Operating Characteristic Curve (ROC) was constructed to explore the cutoff value of SHR for predicting favorable outcome. Multivariable logistical regression model was used to assess the potential factors associated with favorable outcome. Parameters with P <0.05 in univariate analysis entered in multivariate analysis. The covariates included in the multivariable logistical regression were mTICI score (2b or 3), MCE, sICH, NIHSS 24 hours after procedure, Pre-procedure ASPCET score, PTR, Pre-procedure ASITN/SIR score, homocysteine, retriever passes >3 times, lymphocyte, HbA1c, fasting blood glucose, glycosylated hemoglobin, SHR. Model 1 and Model 2 were diabetic group and non-diabetic group, respectively. Model 3 included fasting blood glucose and glycosylated hemoglobin as confounders, and model 4 excluded fasting blood glucose and glycosylated hemoglobin as confounders. P value of <0.05 was considered as statistically significant. Statistical analyses were performed using SPSS 25.0 (IBM, Armonk, NY).

Results

A total of 559 stroke patients were enrolled. The median (IQR) age was 70 (63–77) years, and NIHSS score after 24 hours of thrombectomy was 12 (6-19). There were 357 (63.9%) male patients. Among the enrolled, 74 (13.2%) patients developed END. There were 69 (12.3%) patients occurred sICH in 24 hours, and 81 patients (14.5%) died in 90 days. Favorable outcome was obtained in 284 (50.8%) patients. There were 190 (34.0%) patients had high SHR. The ROC curve showed that the optimal cutoff value of SHR for predicting favorable outcome was 1.07, the sensitivity was 84.7%, the specificity was 52.5%, and the Youden index was 0.372 (Figure 1).

Compared with patients without END, those with END had higher median NIHSS scores after 24 hours (29 vs. 11, P <0.001), higher homocysteine (16.4 vs. 14.6, P =0.029), higher fasting glucose levels (8.7 vs. 6.9, P <0.001), higher glycated hemoglobin (6.4 vs. 5.9, P =0036), higher SHR (1.4 vs. 1.1, P <0.001), lower pre-procedure ASPECTS score (8 vs. 9, P =0.001), lower pre-procedure ASITN grade (1 vs. 3, P <0.001), longer PRT (80 vs. 67, P =0.014), higher proportion of multiple retriever passes (16.2% vs. 8.2%, P =0.028), lower proportion of successful recanalization (83.8% vs. 91.3%, P =0.040), and higher proportion of malignant brain edema (28.4% vs. 15.5%, P =0.006). The proportion of sICH was higher (48.6% vs. 6.8%, P <0.001) in patient with END than that in patient without. Proportion of favorable outcome was lower (10.8% vs. 56.9%, P <0.001), and mortality (33.8% vs. 11.5%, P <0.001) was higher in patients with END. Moreover, subgroup analysis showed that proportion of favorable outcome was lower in patients with diabetes mellitus. (Table 1, Figure 2).

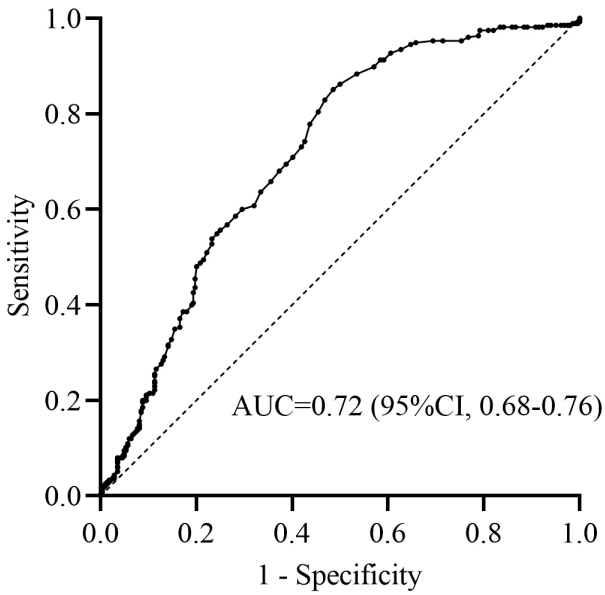

FIGURE 1
Sensitivity and specificity of SHR in predicting favorable outcome.

In Model 3 with unadjusted fasting blood glucose and glycosylated hemoglobin being adjusted, SHR (OR =4.78; 95% CI, 1.38-16.60; P =0.014), sICH (OR =5.04; 95% CI, 2.49-10.21; P <0.001), and NIHSS score at 24 hours (OR =1.10; 95% CI, 1.06-1.15; P <0.001) were related to END. In Model 4 with fasting blood glucose and glycosylated hemoglobin being adjusted, SHR (OR =5.77; 95% CI, 1.88-17.74; P =0.002), sICH (OR =4.90; 95% CI, 2.44-9.84; P <0.001) and NIHSS score at 24 hours (OR =1.11; 95% CI, 1.07-1.15; P <0.001) were related to END (Figure 3).

When patients were stratified as with and without diabetes mellitus (DM) and adjusted for major confounding factors, multivariant analysis detected that sICH (OR =6.06; 95% CI, 2.63-13.96; P <0.001), 24-hour NIHSS score (OR =1.12; 95% CI, 1.07-1.17; P <0.001), and high SHR (OR =8.84; 95% CI, 1.83-42.70; P =0.007) could influence the development of END in patients without diabetes mellitus. High NIHSS score after 24 hours (OR =1.10; 95% CI, 1.03-1.18; P =0.006) could influence the development of END in patients with diabetes mellitus (Figure 3).

Multivariant analysis detected that SHR (OR =0.20, 95% CI, 0.08-0.50; P =0.001), age (OR =0.94, 95% CI, 0.91-0.98; P =0.001), baseline NIHSS score (OR =1.15, 95% CI, 1.05-1.25; P =0.002), NIHSS score after 24 hours (OR =0.76, 95% CI, 0.69-0.83; P <0.001), PRT (OR =0.99, 95% CI, 0.98-1.00; P =0.025), pre-procedure ASITN grade (OR =62.02, 95% CI, 25.92-148.38; P <0.001) and successful recanalization (OR =7.42, 95% CI, 1.94-28.31; P =0.001) were associated with favorable outcome (Table 2).

Discussion

This study observed that stroke patients with high SHR had increased incidence of END and decreased likelihood of favorable outcome after endovascular treatment.

SHR was determined as a better quantitative indicator for stress hyperglycemia than blood glucose level when evaluating the outcomes

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TABLE 1 Baseline characteristics and clinical outcomes according to END.

Variable	END		P value
	with	without n=74	n=485
Age, y, median (IQR)	69 (61-76)	70 (63-77)	0.696
Male, n (%)	49 (66.2)	308 (63.5)	0.651
NIHSS at baseline, median (IQR)	17 (10-20)	15 (11-19)	0.943
Hypertension, n (%)	53 (71.6)	333 (68.7)	0.608
Diabetes, n (%)	24 (32.4)	120 (24.7)	0.159
CHD, n (%)	5 (6.8)	61 (12.6)	0.148
Smoking, n (%)	30 (40.5)	179 (36.9)	0.547
Alcohol Drinking, n (%)	15 (20.3)	98 (20.2)	0.990
IV rTPA, n (%)	33 (44.6)	197 (40.6)	0.517
Lymphocyte (1000/mm3)	1.3 (1.0-1.8)	1.1 (0.8-1.6)	0.011
HbA1c (%)	6.4 (5.7-7.1)	5.9 (5.5-6.8)	0.036
HCY (mmol/l)	16.4(13.3-20.2)	14.6(12.1-18.7)	0.029
Pre-procedure ASPCET score	8 (7-9)	9 (8-9)	0.001
Pre-procedure ASITN/SIR score	1 (1-2)	3 (2-3)	<0.001
retriever passes >3 times, n (%)	12 (16.2)	40 (8.2)	0.028
TOAST, n (%)			
Large artery atherosclerosis	36 (48.6)	235 (48.5)	0.148
Cardioembolism	26 (35.1)	205 (42.3)	
Other	12 (16.2)	45 (9.3)	
Puncture-to-recanalization time, min, median (IQR)	80 (55-105)	67 (49-95)	0.014
mTICI score 2b or 3, n (%)	62 (83.8)	443 (91.3)	0.040
NIHSS 24 hours after procedure, median (IQR)	29 (17-35)	11 (5-17)	<0.001
sICH, n (%)	36 (48.6)	33 (6.8)	<0.001
MCE, n (%)	21 (28.4)	75 (15.5)	0.006
Favorable outcome, n (%)	8 (10.8)	276 (56.9)	<0.001
Mortality, n (%)	25 (33.8)	56 (11.5)	<0.001

NIHSS, National Institutes of Health Stroke Scale; CHD, coronary atherosclerotic heart disease; IV, intravenous thrombolysis; HbA1c, glycated hemoglobin; HCY, homocysteine; ASPECT, Alberta Stroke Program Early CT Score; ASITN/SIR, American Society of Interventional and Therapeutic Neuroradiology/Society of Interventional Radiology; TOAST, Trial of Org 10172 in Acute stroke treatment; mTICI, modified Thrombolysis in Cerebral Infarction; sICH, symptomatic intracranial hemorrhage; MCE, malignant cerebral edema.

of critical illness (25). High SHR has been associated with increased risk of END and poor outcome in patients with intravenous thrombolysis. But no study on relationship between SHR and END has been reported in patients with endovascular thrombectomy (26). The underlying mechanism for SHR influencing END may be multifactorial. First, increased lactate productions may deteriorate ischemic condition, and disrupt neuron metabolism in penumbra areas (27). Second, stress hyperglycemia could aggravate hemorrhagic transformation after ischemic stroke by inducing mitochondrial dysfunction and endothelial cell apoptosis (28). Third, stress hyperglycemia may have adverse effects on collateral circulation (29). Fourth, the prothrombotic effect of stress hyperglycemia could result in thrombus extension and blood-brain barrier destruction (30).

Previous study confirmed that patients with high SHR had an increased risk of symptomatic intracranial hemorrhage and mortality after endovascular thrombectomy (15). This study associated SHR and unfavorable outcome in patients treated with endovascular thrombectomy. Several explanations may account for the association between SHR and unfavorable outcome after endovascular thrombectomy. First, acute stress response may lead to enhance inflammation reaction, which in turn leads to increased hepatic glycogenolysis, insulin resistance, cell endothelial injury, platelet aggregation, and mitochondrial dysfunction (11, 31). Second, stress hyperglycemia may directly damage ischemic brain tissue through lactic acid accumulation and intra-cellular acidosis, and aggravate ischemic injury (32). Third, stress hyperglycemia could generate reperfusion

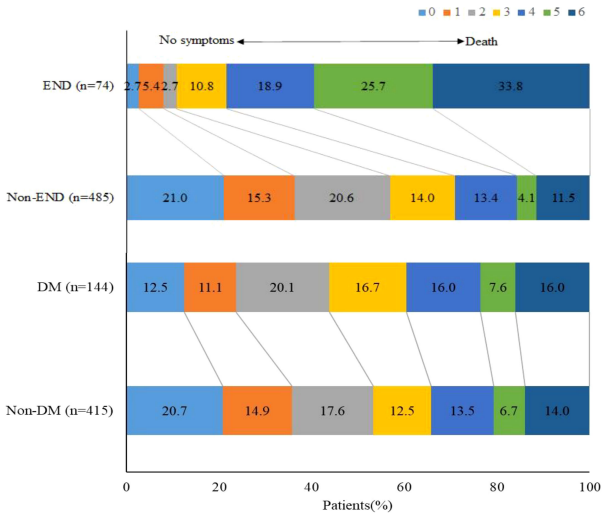

FIGURE 2
Functional outcomes according to END and DM. Distribution of modified Rankin Scale scores at 90 days.

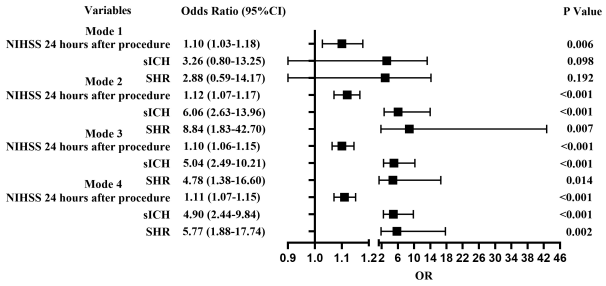

FIGURE 3
Forest plots for predictors of END. Model 1, Diabetic group; Model 2, Non-diabetic group; Model 3, Including fasting blood glucose and glycosylated hemoglobin as confounders; Model 4, Excluding fasting blood glucose and glycosylated hemoglobin as confounders.

TABLE 2 Multivariable analysis for favorable outcome.

Variable	OR	95% CI	P-value
SHR	0.20	0.08-0.50	0.001
age	0.94	0.91-0.98	0.001
NIHSS at baseline	1.15	1.05-1.25	0.002
NIHSS 24 hours after procedure	0.76	0.69-0.83	<0.001
PRT	0.99	0.98-1.00	0.025
Pre-procedure ASITN/SIR	62.02	25.92-148.38	<0.001
mTICI, 2b-3	7.42	1.94-28.31	0.001

SHR, stress hyperglycemia ratio; NIHSS, National Institutes of Health Stroke Scale; PRT, puncture-to-recanalization time; ASITN/SIR, American Society of Interventional and Therapeutic Neuroradiology/Society of Interventional Radiology; mTICI, modified Thrombolysis in Cerebral Infarction.

injury *via* oxidative stress and inflammatory process with increased expression of endothelial adhesion molecules and monomeric C-reactive protein (33). Fourth, stress hyperglycemia may disrupt blood-brain barrier and promote hemorrhagic transformation (34).

Several limitations of this study should be address when interpreting the results. END was defined as NIHSS score increase within 24 hours after endovascular procedures, but this condition could occur a few days later. We did not monitor dynamics changes of SHR. The effects of antidiabetic agents and anesthesia were not assessed.

High stress hyperglycemia ratio may be related to early neurological deterioration and decreased likelihood of favorable outcomes after endovascular thrombectomy in patients with acute ischemic stroke.

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 ethics committees of the Affiliated Wuxi People's Hospital of Nanjing Medical University and the Affiliated Nanjing Hospital of Nanjing Medical University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

ZD and HC contributed equally to the conception of the research and drafted the manuscript. LL, HJ, and HG acquired the data. XZ, JZ, and FW analyzed the data. YJ revised the manuscript and made contribution to the revision, GX and DL revised the manuscript and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

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Blood glucose level affects prognosis of patients who received intravenous thrombolysis after acute ischemic stroke? A meta-analysis

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Background and objectives: Intravenous recombinant tissue plasminogen activator (rtPA) thrombolysis is an effective treatment for acute ischemic stroke. Hyperglycemia is a major risk factor for the occurrence, development, and prognosis of ischemic stroke. This meta-analysis purposefully estimates the association between hyperglycemia and poor prognosis in acute ischemic stroke patients receiving intravenous rtPA thrombolytic therapy.

Materials and methods: According to the predefined inclusion criteria, we searched PubMed, Web of Science, and Cochrane Library databases. The association of high blood glucose(>140mg/dl) with symptomatic intracranial hemorrhage (sICH), poor clinical outcome and mortality at 90 days post-rtPA thrombolysis was studied using both a common effects model and a random effects model. Odds ratios (ORs) were plotted on forest plots.

Results: Of a total cohort of 2565 patients who received intravenous thrombolytic therapy, 721 had higher blood glucose. High glucose level significantly increased the odds of sICH (OR 1.80; 95% confidence interval(95% CI): 1.30- 2.50) and poor clinical outcome at 90 days (OR 1.82; 95%CI: 1.52-2.19), and all-cause mortality at 90 days (OR 2.51; 95%CI:1.65-3.82).

Conclusions: In our meta-analysis, high blood glucose was significantly associated with sICH, poor clinical outcome and higher mortality at 90 days.

KEYWORDS

blood glucose, acute ischemic stroke, intravenous thrombolytic therapy, functional outcome, symptomatic intracranial hemorrhage

Introduction

Acute ischemic stroke (AIS) is a common cerebrovascular disease around the world, with the characteristics of high morbidity and mortality. Hence, prompt AIS treatment is extremely important. Commonly used treatments for AIS include intravenous thrombolysis treatment (IVT), endovascular interventions, as well as antiplatelet and fiber-lowering treatments. IVT, as a widely used treatment for AIS, is required to administer recombinant tissue plasminogen activator (alteplase) to patients within 4.5h or urokinase (UK) within 6h (1). Previous studies have found that IVT decreases morbidity and mortality of patients after AIS. IVT can dissolve the fibrin in the thrombus to achieve the purpose of unclogging blood vessels. However, IVT brings up risks of complications such as symptomatic intracranial hemorrhage (sICH), post-thrombolytic cerebral edema, and allergy (2). Among them, sICH is the most serious complication. According to the National Institute of Neurological Disorders and Stroke (NINDS) Stroke Intravenous Thrombolysis Study criteria, sICH was defined as any manifestation of clinical deterioration within 36 h after revascularization therapies like IVT, along with CT showing cerebral hemorrhage. Although sICH accounts for only 6% of post-thrombolytic complications, it consists of 50%~80% of mortality caused by post-thrombolytic complications (3, 4).

More than half of patients with ischemic stroke have hyperglycemia at the time of admission (5). Previous studies have found that clinical outcomes in patients with AIS undergoing IVT are associated with hyperglycemia on admission to hospital. It was found that hyperglycemia may partially offset the beneficial effects of early recovery blood flow by IVT (6). It has been convinced that patients receiving intravenous alteplase may suffer worse clinical outcomes if admission hyperglycemia occurs (7–10). Although IVT induced partial infarct recanalization, patients with higher blood glucose (blood glucose on admission > 140mg/dl) had worse outcomes and would be more likely to have worse functional outcomes, sICH and even death than those with non-high blood glucose (blood glucose on admission < 140mg/dl) (11–13). As a result, the effect of high blood glucose on prognosis of AIS patients after IVT has received considerable critical attention.

Therefore, we conducted this meta-analysis in order to clarify the relationship between blood glucose levels and the prognosis of patients with AIS after IVT. We analyzed the 3 main indicators after IVT including rate of sICH, modified Rankin Score(mRS) and mortality. With this study, we hope to provide new perspectives for future clinical research and treatment for AIS.

Methods

The study was designed, conducted, and reported per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (14, 15).

Search strategies

We conducted article searches in PubMed, Web of Science, and Cochrane Library databases up to October 20, 2022. The search strategies were as follows: ("Blood Sugar" OR "Sugar, Blood" OR "Glucose, Blood") AND ("Stroke" OR "Transient Ischemic Stroke" OR "TIA" OR "Cerebral Infarction" OR "Cerebrovascular Infarction") AND ("Therapeutic Thrombolysis" OR "Therapeutic Thrombolyses" OR "Thrombolyses, Therapeutic" OR "Thrombolysis, Therapeutic" OR "Therapy, Fibrinolytic" OR "Fibrinolytic Therapies" OR "Therapies, Fibrinolytic" OR "Therapy, Thrombolytic" OR "Therapies, Thrombolytic" OR "Thrombolytic Therapies" OR "Fibrinolytic Therapy"). No language restriction was applied to the search of human studies. Additionally, we manually screened the references for possible related studies in the relevant original and review articles.

Inclusion and exclusion criteria

Following the recommended PICOS criteria, we developed inclusion criteria according to the meta-analysis' aim: (1) Patients had to be older than 18 years of age received IVT for AIS; (2) Patients were divided into high and non-high groups based on their admission blood glucose, and their cut-off value was set at 140 mg/dl. (3) The outcomes to be observed are the incidence of sICH, poor clinical outcome assessed by mRS at 90 days and mortality at 90 days in AIS patients treated with intravenous tissue plasminogen activator. A poor clinical outcome is defined as a mRS> 2 at 90 days (16). (4) The study design had to be a randomized controlled trial or a longitudinal observational study.

The exclusion criteria follow the following points: (1) A review, editorial, meta-analysis, studies enrolling patients with hemorrhagic stroke, and studies not analyzing blood glucose or reporting the outcomes of interest were excluded. (2) Grey literature, including conference abstracts and unpublished data, was excluded. Studies from these sources are not peer-reviewed, so including them in a meta-analysis might cause results to be inconsistent.

There were 2 authors who independently completed the database search and screening, data collection, and quality assessment of the study. If the 2 authors are in dispute, we will contact the corresponding author to discuss the results. We collected data on research information, diagnosis, definition of hyperglycemia, follow-up duration, outcomes when the associations between high blood glucose and outcomes of interest were presented. An assessment of study quality was conducted by using the Newcastle-Ottawa Scale (NOS), which included scoring relating to the selection criteria and comparability of the groups (17). The scale ranged from 1 to 9, with more stars indicating higher study quality. We considered studies that had long enough follow-up periods as those with a mean follow-up period of at least 3 months (90 days).

Statistical analyses

We standardized risk factors across studies whenever possible in order to compare data from different studies. According to the original study, we accepted all criteria for risk factor categories. We extracted data on the rate of sICH incidence, occurrence rate of poor clinical outcomes (mRS > 2)and mortality in the high glucose and non-high glucose groups of patients with AIS receiving intravenous tissue plasminogen activator, and expressed the relative risks between the 2 groups as odds ratios (ORs) and 95% confidence intervals (CIs). Our first step was to estimate the heterogeneities between studies. Heterogeneity between studies was estimated using the I^2 statistic, with I^2 above 50% reflecting significant heterogeneity. If the results of the heterogeneity analysis were significant, we used random effect models to combine the results by including random effects and we used fixed effect models to conduct meta-analysis oppositely when I^2<50 (18).

To assess the impact of individual studies on this meta-analysis, sensitivity analyses were performed by excluding 1 dataset at a time. In addition, publication bias was estimated by constructing funnel plots based on visual judgements of the symmetry of the funnel plots. We conducted the above analysis using R v4.1.2 and the 'meta' R package.

Results

Study search

A flowchart of the literature search and study inclusion procedure is presented in Figure 1. We found 327 articles in our initial database search. After eliminating 25 duplicates, we screened 302 studies left based on their titles and abstracts. Eleven studies were excluded mainly because they were reviews or meta-analysis, 7 articles were excluded because they were animal experiments, and 222 researches were excluded because they were not relevant to the objective of our meta-analysis. In the end, 62 studies were reviewed in full-text, and 57 were excluded for the reasons listed in Figure 1. Finally, 5 studies were included in this meta-analysis.

Study characteristics

Five studies were included in the meta-analysis and characteristics of these studies were presented in Table 1 (6, 8, 19–21). In all studies, stroke patients received intravenous tissue plasminogen activator, with mean ages ranging from 67 to 73 years old. Random blood glucose values at admission were measured, and the definitions of hyperglycemia were consistent across studies or varied so little that they could be put together for statistical analysis. The duration of observation and definition of the outcome indicators and scores for poor clinical outcome were consistent across the studies. What's more, the NOS for all included studies was 8 to 9 stars, indicating good study quality (Table 2).

Higher blood glucose levels increase the risk of symptomatic intracranial hemorrhage

Five cohort studies including 2565 stroke patients who received intravenous tissue plasminogen activator evaluated the association between hyperglycemia and sICH. During the follow-up period, a

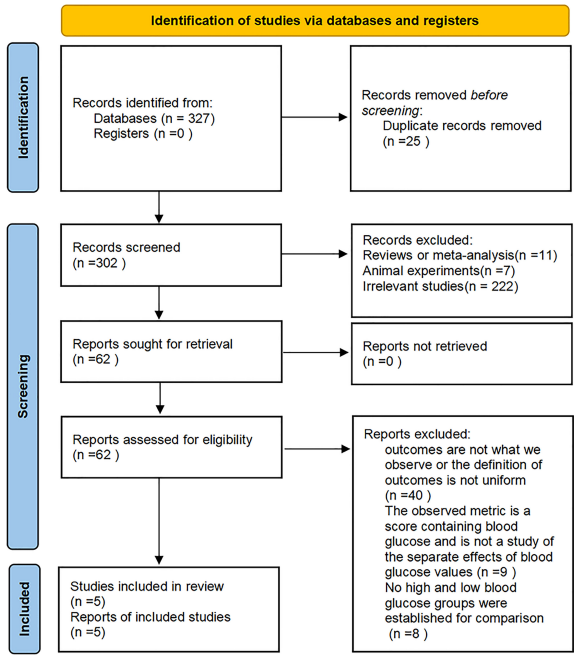

FIGURE 1
Flowchart of study search.

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TABLE 1 Characteristics of the included cohort studies.

Study	Contries	Design	Diagnosis	Definition of hyperglycemia	Mean age (years)	Male (%)	Outcome events
Alvarez-Sabín, 2003	Spain	prospective	IV-tPA–treated stroke patients	Hyperglycemia was defined as a blood glucose level >140 mg/dL(7.7mmol/l)	70.4	49.3	sICH;The modified Rankin Scale>2 at 90 days.
Poppe,2009	Canada	prospective	IV-tPA–treated stroke patients	Hyperglycemia was defined as a glucose level >144mg/dl (8.0mmol/l)	73	55	sICH, functional outcome at 90 days, and death
Putaala, 2011	Finland	retrospective	acute ischemic stroke treated with intravenous thrombolysis	Hyperglycemia was defined as a blood glucose level of >144mg/dl (8.0 mmol/l).	70	55.2	unfavorable 3-month outcome (mRS>2), death, and sICH according to NINDS criteria.
Yaghi, 2012	America	prospective	IV-tPA–treated stroke patients	Hyperglycemia was defined as a blood glucose level of > 144mg/dl (8.0mmol/l)	67.2	55	sICH, and outcome at 3 months defined by mRS.
Saqqur, 2015	Canada	retrospective	IV-tPA–treated stroke patients	Hyperglycemia was defined as a glucose level ≥140 mg/dl (7·7 mmol/l).	68.4	54.6	poor clinical outcome (3month mRS > 2), sICH

IV, intravenous injection; tPA, tissue plasminogen activator;
ICH, intracranial hemorrhage; sICH, symptomatic intracerebral hemorrhage;
NINDS, National Institute of Neurological Disorders and Stroke

total of 169 patients developed sICH. The results from the meta-analysis indicated that blood glucose level at admission was independently associated with a higher risk of sICH (High Glucose *vs* Non-high Glucose, OR: 1.80, 95% CI: 1.30-2.50, I2 = 0%; Figure 2A). The results from sensitivity analyseswere consistent (overall OR: 1.80; 95%CI: 1.30-2.50; p <0.01; Figure 3A).

Higher blood glucose levels increase the risk of poor clinical outcome at 90 days

Four studies including 2492 stroke patients who received intravenous tissue plasminogen activator evaluated the association between blood glucose level and poor clinical outcome. A total of 1272 patients had 90-day mRS scores > 2 during the follow-up period. The results from the meta-analysis indicated that blood glucose level at admission was independently associated with a higher risk of 90-day mRS scores > 2 (High Glucose *vs* Non-high Glucose, OR: 1.82, 95% CI: 1.52-2.19, I2 = 0%; Figure 2B). The results from sensitivity analyses, which excluded one data set at a time, were consistent (OR: 1.82 95%CI:1.52-2.19, p all <0.0; Figure 3B).

Higher blood glucose levels increase the morality at 90 days

Three cohort studies including 2144 stroke patients who received intravenous tissue plasminogen activator evaluated the association

TABLE 2 Quality evaluation of the included cohort studies *via* the NOS.

Study	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Outcome not present at baseline	Control for age and sex	Control for other confounding factors	Assessment of outcome	Enough long follow-up duration	Adequacy of follow-up of cohort	Total
Alvarez-Sabín, 2003	1	1	1	1	1	1	1	1	0	8
Poppe, 2009	1	1	1	1	1	1	1	1	0	8
Putaala, 2011	1	1	1	1	1	1	1	1	1	9
Yaghi, 2012	1	1	1	1	1	1	1	1	1	9
Saqqur, 2015	1	1	1	1	1	1	1	1	1	9

NOS: Newcastle-Ottawa Scale.

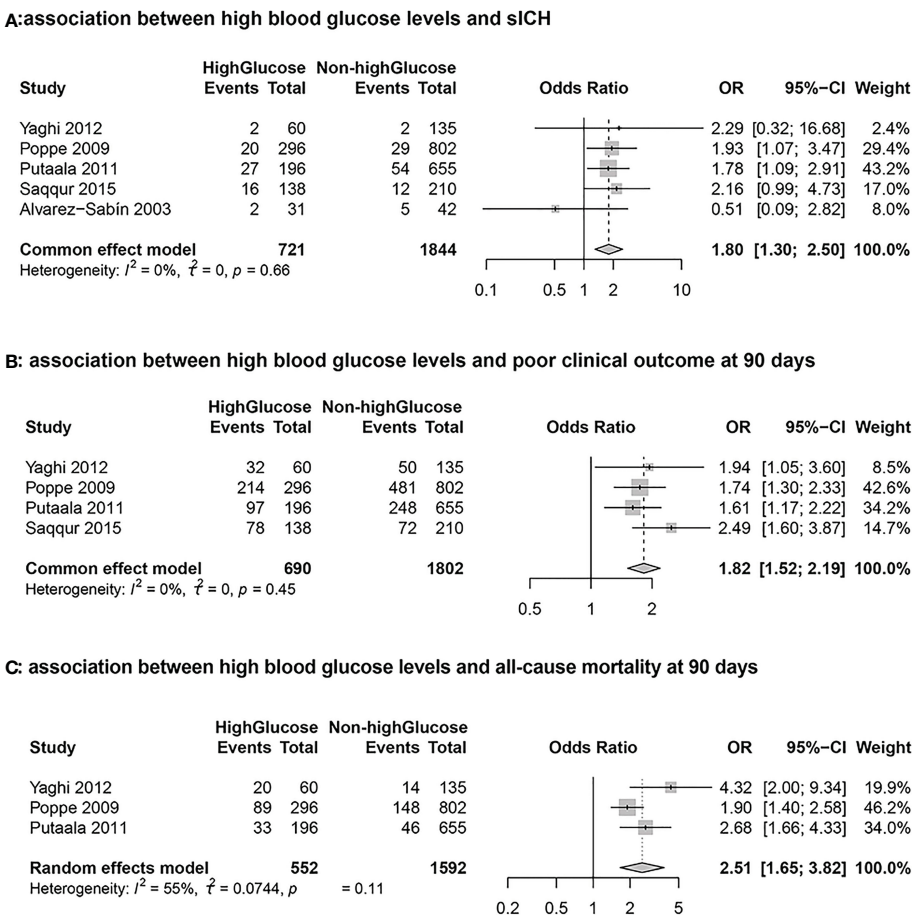

FIGURE 2
Forest plots for the meta-analyses of the outcomes between high glucose and non-high glucose groups. **(A)**: Association between hyperglycemia and symptomatic intracranial hemorrhage (sICH) **(B)**: Association between hyperglycemia and poor clinical outcome at 90 days **(C)**: Association between hyperglycemia and all-cause mortality at 90 days.

between hyperglycemia and all-cause morality. A total of 350 patients died during the follow-up period. The results from the meta-analysis indicated that blood glucose level at admission was independently associated with a higher risk of death (High Glucose *vs* Non-high Glucose, OR: 2.51, 95% CI:1.65-3.82, I2 = 55%; Figure 2C). The results from sensitivity analyses, which excluded one data set at a time, were consistent. (OR: 2.51 95%CI:1.65-3.82; Figure 3C).

Publication bias

These funnel plots show the association between blood glucose level and poor clinical outcomes in AIS patients receiving intravenous tissue plasminogen activator Figure 4. According to visual inspection, the plots were symmetrical, indicating a low risk of publication bias.

Discussion

Currently, IVT is an essential and widely used method of treatment for AIS. In order to illustrate the relationship between blood glucose level and prognosis of AIS patients who received intravenous thrombolysis, we specifically searched for 5 studies and conducted a meta-analysis on this topic.

The increased risk of sICH after intravenous thrombolysis in high glucose level group compared with non-high glucose level points to an effect of blood glucose levels on the risk of sICH. Previous studies have also shown that admission blood glucose is one of the predictors of sICH (22–24), even after controlling for HbA1c (25). Possible mechanisms include higher blood glucose impairing cellular metabolism, reducing vascular reactivity, increasing blood-brain barrier permeability and exacerbating acidosis in reperfused brain tissue. As well, the prevalence of atherosclerosis is also higher in the diabetic population compared to the non-diabetic population. This can indirectly increase the risk of sICH after thrombolysis. Thirdly, from a pathophysiological point of view, studies have established a rat model of stroke, which proved that alteplase treatment increased cerebral hemorrhage after stroke, and blood-brain barrier (BBB) leakage increased (26, 27). IVT may further aggravate BBB leakage by destroying (28).

Among patients with poor clinical outcomes, the high glucose group also showed a more significant risk than the non-high glucose

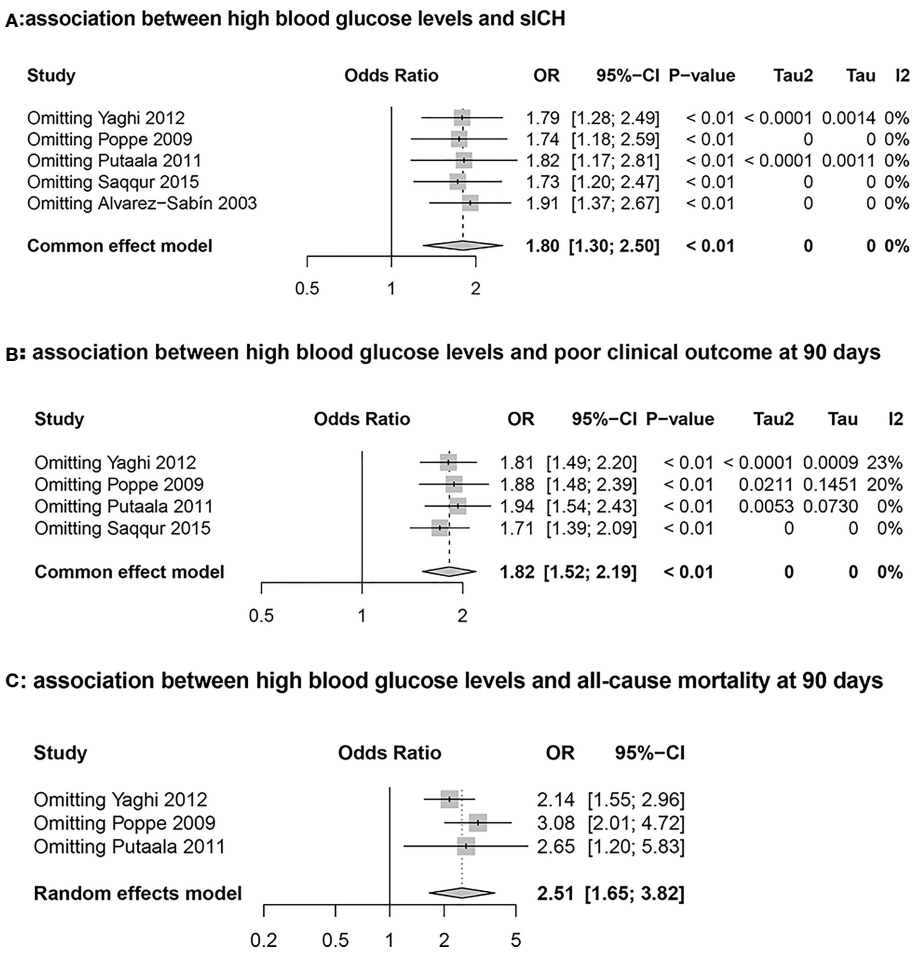

FIGURE 3
Sensitivity analysis graph for the high glucose and non-high glucose groups. **(A)**: Association between hyperglycemia and symptomatic intracranial hemorrhage (sICH) **(B)**: Association between hyperglycemia and poor clinical outcome at 90 days **(C)**: Association between hyperglycemia and all-cause mortality at 90 days.

group. The mRS is a measure of a patient's functional recovery after a stroke and is graded from 0 to 6. Therefore, in this study we defined poor clinical outcome as mRS > 2 (29). There are studies suggesting that high blood glucose level affects the thrombolytic effect of alteplase and its effect on the evolution of cerebral infarction (7). On this issue, studies have also confirmed that the deep hemispheric white matter is part of the clinically relevant penumbra and shown that hyperglycemia exacerbates the appearance of irreversible ischemic damage in this region within 24 hours (30). This may be one of the mechanisms contributing to poor clinical outcomes. In addition, it has also been shown in recent years that higher blood glucose levels stimulate the thrombophilia cascade response, which amplifies Downstream microvascular thrombo-inflammation (DMT) caused by middle cerebral artery occlusion. Then DMT exacerbates the damage to reperfusion and precipitates a range of functional and structural neurological impairments (31, 32). Therefore, irreversible damage to neurological function and structure caused by hyperglycemia can have adverse clinical outcomes. On the clinical side, there are also studies that have statistically shown a high rate of combined hyperglycemia in stroke patients with the potential to affect long-term outcomes (33). Therefore, these may be clinically relevant mechanisms that are hypothesized to explain why hyperglycemia exacerbates adverse clinical outcomes.

As for mortality, it is clear from our analysis that patients with AIS with higher blood glucose levels are also at greater risk of death after receiving intravenous tissue plasminogen activator. In addition to the neurological impairment mentioned above that may lead to death, patients with higher blood glucose levels are also prone to infection, which is a possible cause of death for patients with AIS after intravenous thrombolysis who need to stay in bed for a long time. Therefore, blood glucose levels should be assessed in AIS patients receiving intravenous tissue plasminogen activator for risk stratification and clinical decision-making to reduce mortality.

Furthermore, the higher prevalence of co-morbidities in the high glucose group may be due to older age and a higher proportion of diabetes. When patients were classified according to whether they had admission hyperglycemia, patients with high glucose were more likely to have hypertension, hyperlipidemia, coronary artery disease, and known diabetes. Concerning the covariate factors affecting the prognosis of patients after thrombolysis, our initial study showed that increasing age, history of diabetes, admission glucose ≥140 mg/

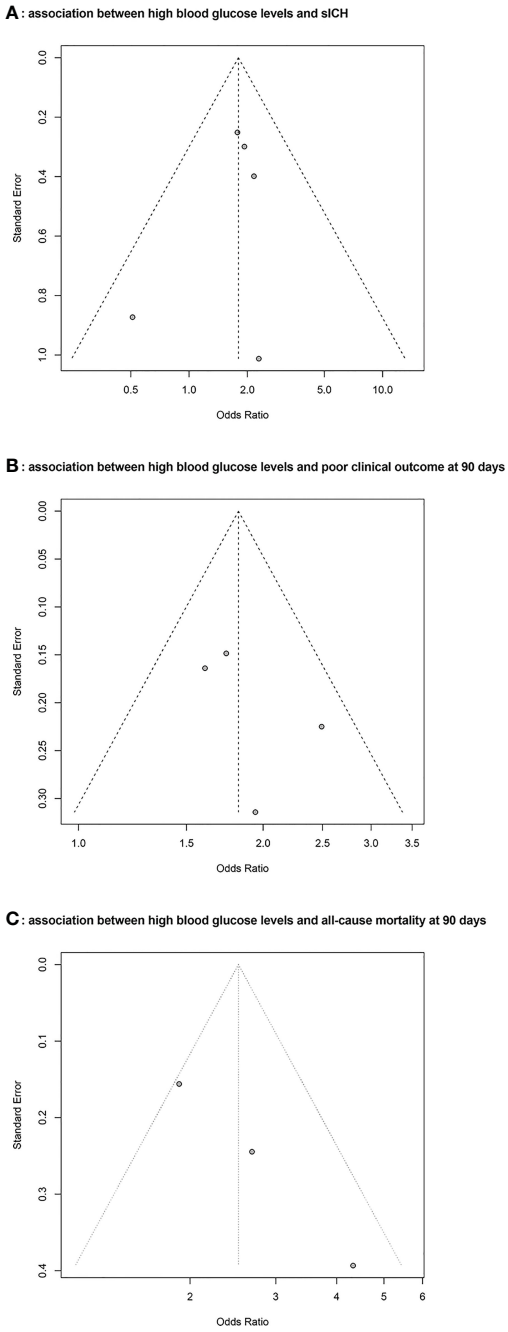

FIGURE 4
Funnel plots for the publication bias underlying the meta-analyses. **(A)**: Association between hyperglycemia and symptomatic intracranial hemorrhage (sICH) **(B)**: Association between hyperglycemia and poor clinical outcome at 90 days **(C)**: Association between hyperglycemia and all-cause mortality at 90 days.

dL, and early embolism were associated factors of poor clinical outcomes in reperfused patients. However, logistic regression models showed that only admission glucose ≥140 mg/dL emerged as an independent predictor of poor clinical outcome (6). According to previous studies, the odds of having a sICH increase with increasing blood glucose on admission (34). Therefore, it is reasonable to speculate that extremely high blood glucose would produce a different clinical outcome than moderate high levels of blood glucose.

Although we rigorously conducted data retrieval, screening, and analysis, the following limitations of our study remain. Firstly, three of the studies included in the meta-analysis were retrospective. Data collection in retrospective studies is not subject to investigator control and assessment and may result in some bias. Secondly, regarding cut-off values for the high and non-high glucose groups, we had 2 primary studies defined 140 mg/dl and the other three primary studies defined 144 mg/dl. This may contribute to heterogeneity. Each original study referred to the American

Diabetes Association criteria of hyperglycemia at admission or previous relevant studies to develop cut-off values for the high blood glucose and non-high blood glucose groups (10, 35, 36). The definitions of hyperglycemia are very close between the various studies, and there are further studies that suggest that a glucose level of approximately 140 mol/dl may indicate a watershed level and that patients with glucose above this cut-off have worse clinical outcomes (37, 38). Since there was no significant difference in the cut-off values of blood glucose among the studies, for the purpose of data analysis, we used 140 mg/dl as the cut-off value for the blood glucose group in this meta-analysis. Thirdly, In the analysis regarding mortality, we found heterogeneity between the studies through the test of heterogeneity. Regarding the heterogeneity, in addition to the possible reasons mentioned above, another possible reason is that the sample size of one study is a little small compared to the other 2 studies. Therefore, we used a random effects model to estimate the combined effect size for the data of this outcome to partially correct for meta-analysis heterogeneity in order to improve the precision of the estimated confidence intervals and to increase the test efficacy at the same time. Moreover, our study did not include grey literature such as conference abstracts, which may have led to an incomplete analysis. In addition, we did not observe whether the fluctuation of blood glucose level during hospitalization would have an impact on AIS after intravenous thrombolysis, which may lead to a lack of rigorous analysis. In addition, considering that the original study we included was based only on a single admission randomized glucose value and did not measure glycated hemoglobin in patients, this may have led to less comprehensive monitoring of our blood glucose. This is an issue that needs to be improved in further studies.

Based on the results of 5 studies, we found that higher blood glucose level could have a poor prognosis of AIS patients treated with intravenous tissue plasminogen activator. The results of the sensitivity analysis were consistent one data set at a time. In conclusion, these results suggest that hyperglycemia may be a useful predictor of sICH, poor clinical outcome and all-cause mortality in stroke patients who received intravenous tissue plasminogen activator.

Conclusion

High blood glucose level is an important clinical consideration for prognosis in patients with AIS who receive intravenous thrombolytic therapy. Our meta-analysis showed that high blood glucose levels were closely associated with sICH, poor clinical outcomes, and mortality following intravenous thrombolysis.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding authors.

Author contributions

JHZ, YW and JZ contributed to conception and design of the study. YW organized the database. GJ performed the statistical analysis. YW wrote the first draft of the manuscript. YW, GJ and JZ wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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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.

Publisher's note

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Relationship between plasma glutamate and cardiovascular disease risk in Chinese patients with type 2 diabetes mellitus by gender

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Objectives: This study aimed to assess the association between plasma glutamate (Glu) and the risk of cardiovascular disease (CVD) in patients with type 2 diabetes mellitus (T2DM) and whether this association differs by gender.

Material and methods: We retrieved clinical information on 1032 consecutive patients with T2DM from a same tertiary care center from May 2015 to August 2016. Glu was quantified by liquid chromatography-tandem mass spectrometry analysis. Glu was converted into a categorical variable based on the median concentration in the whole population, while logistic regression was used to obtain the odds ratio (OR) and 95% confidence interval (CI), and the correlation between Glu and various biochemical indices was analyzed.

Results: We found that Glu was positively associated with the risk of CVD in patients with T2DM. This correlation was more significant in women. In T2DM patients, the higher the age, body mass index (BMI), weight and systolic blood pressure (SBP), the lower the glycosylated hemoglobin (HbA1C) concentration and the higher the Glu. In female patients, the correlation between age, weight, BMI, SBP, and plasma Triglycerides (TG), and Glu was also statistically significant.

Conclusion: In conclusion, female T2DM patients with high levels of Glu have a higher risk of developing CVD.

KEYWORDS

cardiovascular disease, metabolomics, glutamate, type 2 diabetes mellitus, gender

1 Introduction

Cardiovascular disease (CVD) is one of the most serious complications of type 2 diabetes mellitus (T2DM), accounting for more than 20% of all-cause deaths in T2DM patients in China (1). In turn, diabetes mellitus patients are also at high risk for CVD (2). Notably, many studies have found that the burden of diabetes varies by gender. For example, 51% of women in Europe have been reported to die from CVD compared to 42% of men (3), with a significant difference between the sexes in this regard. One possible speculation is that men and women may differ in terms of CVD pathology and predictors.

With the development of metabolomics, we have been able to explore the role of a range of metabolites in diseases, including CVD, from a new perspective (4). Metabolomics, the comprehensive analysis of small molecule metabolites in cells, tissues, or whole organisms, has undergone rapid technological advances in the last two decades (5).

Glu homeostasis is essential for various functions such as insulin fraction, gluconeogenesis and glutathione synthesis (6). The role of Glu in metabolic disorders and certain diseases has been explored in several epidemiological studies. The early Framingham Heart Study revealed a positive correlation between Glu levels and insulin resistance in the general population (7).

Previous studies have shown that more than 75% of patients with high Glu levels, especially those with T2DM, die from the cardiovascular-related disease, a figure twice as high as that of Non-diabetes mellitus (8). A German cross-sectional study found an association between Glu levels and higher CVD (9). A case-control study in China also suggests this idea (10).Although several studies have described the relationship between glutamate and CVD, it is still lacking whether there is some association between CVD and Glu in patients with type 2 diabetes.

In this study, we established a cross-sectional study in a Chinese population with the aim of exploring the relationship between plasma Glu and CVD risk in patients with type 2 diabetes and to examine whether this relationship differs by gender.

2 Methods

2.1 Study populations

The First Affiliated Hospital of Liaoning Medical University in Jinzhou, Liaoning Province, a tertiary care center, established a metabolomics laboratory in 2013 to provide metabolomics testing to outpatients and inpatients or individuals who agreed to pay for a physical examination.

Inclusion criteria were 1. Diagnosed with T2DM according to the 1999 World Health Organization criteria (11). 2. On diabetes medication. The exclusion criteria were: 1. Under the age of 18 years. 2. Living in the hospital's service area as a local resident for less than six months prior to the start of the study. 3. Diabetes secondary to other diseases. 4. Having a mental illness that makes it difficult to cooperate with health screening. Among consecutive patients aged 18 years or older with complete data on height, weight, and blood pressure, 1032 cases were diagnosed with T2DM, of whom those suffering from stroke or myocardial infarction, or both, we designated them as the case group, and the rest of the T2DM patients we designated as the control group.

The Clinical Research Committee of the First Affiliated Hospital of Liaoning Medical University approved the ethical nature of this study, and informed consent was waived due to the retrospective nature of this study, following the Declaration of Helsinki.

2.2 Data collection and definitions

Retrieved data for these cases included demographic and anthropometric information, as well as current clinical parameters, medications, and complications of diabetes. Clinical parameters included glycated hemoglobin, blood pressure and lipids. Diabetic complications included coronary heart disease, cerebrovascular disease, diabetic retinopathy, and diabetic nephropathy. We documented detailed medication use, including oral antidiabetic drugs (OADs) and insulin, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and other antihypertensives, statins, and other lipid-lowering drugs.

CVD was defined as a history of coronary heart disease or stroke. Coronary heart disease was defined as a history of angina pectoris, abnormal ECG or stress test, myocardial infarction, angina pectoris, coronary artery bypass grafting, or angioplasty; stroke was defined as non-fatal subarachnoid hemorrhage, cerebral hemorrhage or other unspecified intracranial hemorrhage and ischemic stroke. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

2.3 Laboratory tests

Capillary whole blood was collected after fasting for at least 8 hours and preserved as dried blood spots for metabolomic analysis. Metabolites in dried blood spots were determined by direct infusion mass spectrometry using an AB Sciex 4000 QTrap system (AB Sciex, Framingham, MA, USA). High-purity water and acetonitrile from Thermo Fisher (Waltham, MA, USA) were used as diluent and mobile phases. 1-Butanol and acetyl chloride from Sigma-Aldrich (StLouis, MO, USA) were used to obtain samples. Isotopically labeled internal standard samples of 12 amino acids (NSK-A) were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA), while standard samples of amino acids were purchased from Chrom Systems (Grafelfing, Germany).

2.4 Statistical analysis

For missing CVD, case deletion was performed. And for missing other biochemical indices, multiple interpolation was used to fill in the missing values. Normality was tested by Q-Q plots or P-P plots. The quantitative data for normal distributions were expressed as mean ± standard deviation (SD), and the data that did not obey the normal distribution were expressed as the

median interquartile range (IQR). The continuous variable was judged by student's t-test or Wilcox-W test when appropriate.

Categorical data were expressed as n (%), and X^2 tests (or Fisher test, if applicable) were used to compare the differences in categorical variables between the CVD and non-CVD groups.

Binary logistic regression models were used to obtain the odd ratio (OR) and 95% confidence interval (CI). A multivariable model was used to adjust for the confounding effects of other variables. The unadjusted OR was first obtained, and then a multivariable analysis was performed to include confounding factors including age, BMI, smoking, alcohol consumption, systolic blood pressure (SBP), glycated hemoglobin (HbA1C), high-density lipoprotein cholesterol(HDL-C), low-density lipoprotein cholesterol (LDL-C), Triglyceride(TG), and medication use to obtain a structured adjusted OR.

To explore a possible nonlinear association between Glu and CVD, we used the median as the cutoff point in logistic regressions. The Glu was divided into two segments according to the median, and the relationship between Glu and CVD in different gender groups was analyzed in the T2DM patient population to obtain the corresponding OR and 95% CI.

Pearson or Spearman correlations were used to calculate the correlation coefficients within Glu and clinical biochemical parameters, namely age, SBP, DBP, BMI, height, weight, TG, HbA1, HDL-C, and LDL-C.

IBM SPSS Statistics was used for statistical analysis, and linear plots for correlation analysis were drawn using RStudio 4.0.5. All P values were two-tailed, and $P < 0.05$ was considered a statistically significant difference.

3 Results

3.1 Characteristics of the study population

Table 1 demonstrates the basic characteristics of the all people as well as the different gender groups. In all people, patients with CVD were older, had higher SBP, higher glutamate concentrations,

TABLE 1 Clinical and biochemical characteristics of participants according to the occurrence of CVD.

Variables	All people (N=1032)			Women (N=483)			Men (N=549)		
	CVD	Non-CVD	P-Value	CVD	Non-CVD	P-Value	CVD	Non-CVD	P-Value
Numbers	350 (33.9%)	682 (66.1%)		160 (33.1%)	323 (66.9%)		190 (34.6%)	359 (65.4%)	
Smoking	104 (10.1%)	227 (22.0%)	0.245	10 (2.1%)	23 (4.8%)	0.721	94 (17.1%)	204 (37.2%)	0.100
Drinking	88 (8.5%)	202 (19.6%)	0.130	11 (2.3%)	4 (0.8%)	0.589	84 (16.0%)	191 (34.8%)	0.045
Age (years)	64.78 ± 11	53.37 ± 13.52	<0.001	65.21 ± 9.79	56.56 ± 12.42	<0.001	64.42 ± 11.94	50.49 ± 13.83	<0.001
Weight (kg)	69.326 ± 11.8	70.862 ± 13.8	0.062	63.94 ± 10.12	64.48 ± 11.69	0.617	73.86 ± 11.20	76.60 ± 13.02	0.010
Height (cm)	166 (160, 172)	167 (160, 173)	0.177	160 (156, 163)	160 (158, 164)	0.118	171.34 ± 5.98	172.38 ± 5.66	0.045
BMI (kg/m2)	25.16 ± 3.74	25.36 ± 3.91	0.423	25.17 ± 3.89	24.96 ± 3.98	0.59	25.15 ± 3.61	25.722 ± 3.82	0.090
SBP,mmHg	145.79 ± 25.45	137.65 ± 22.73	<0.001	145.12 ± 27.02	139.94 ± 24.71	0.036	146.35 ± 20.62	135.60 ± 20.62	<0.001
DBP,mmHg	83.21 ± 15.48	82.07 ± 12.37	0.231	82.29 ± 15.33	80.74 ± 12.28	0.268	83.99 ± 15.60	83.26 ± 12.38	0.577
Glu,μmol/L	103.63 ± 33.34	105.85 ± 39.43	0.012	109.17 ± 37.11	104.20 ± 40.48	0.193	108.20 ± 34.02	101.21 ± 32.76	0.019
HbA1c,%	9.28 ± 2.38	9.78 ± 2.35	0.001	9.38 ± 2.50	9.75 ± 2.42	0.097	9.21 ± 2.28	9.82 ± 2.28	0.003
TG,mmol/L	1.65 (1.13,2.22)	1.73 (1.15,2.48)	0.065	1.71 (1.14,2.36)	1.73 (1.20, 2.47)	0.528	1.58 (1.10,2.16)	1.70 (1.13,2.50)	0.060
HDL-C,mmol/L	1.02 (0.83,1.26)	1.01 (0.85,1.24)	0.999	1.10 (0.91,1.28)	1.06 (0.87, 1.26)	0.413	0.94 (0.80,1.20)	0.96 (0.83,1.23)	0.495
LDL-C,mmol/L	2.77 (2.13,3.31)	2.84 (2.32,3.44)	0.022	2.87 (2.34,3.40)	2.92 (2.31, 3.49)	0.376	2.71 (2.06,3.19)	2.78 (2.33,3.35)	0.020
Insulin	237 (23.0%)	535 (51.8%)	<0.001	115 (23.8%)	245 (50.7%)	0.345	122 (22.2%)	290 (52.8%)	<0.001
Other hypoglycemic drugs	182 (17.6%)	387 (37.5%)	0.147	92 (19.0%)	179 (37.1%)	0.664	90 (16.4%)	208 (37.9%)	0.018
Statins	184 (17.8%)	186 (18.0%)	<0.001	88(18.2%)	88(18.2%)	<0.001	96(17.5%)	98(17.9%)	<0.001
Other lipid-lowering drugs	5 (0.5%)	18 (1.7%)	0.212	3(0.6%)	4(0.8%)	0.582	2(0.4%)	14(2.6%)	0.059

(Continued)

TABLE 1 Continued

Variables	All people (N=1032)			Women (N=483)			Men (N=549)		
	CVD	Non-CVD	P-Value	CVD	Non-CVD	P-Value	CVD	Non-CVD	P-Value
ACEIs	68 (6.6%)	67 (6.5%)	<0.001	30 (6.2%)	34 (7.0%)	0.012	38 (6.9%)	33 (6.0%)	<0.001
ARBs	64 (6.2%)	70 (6.8%)	<0.001	34 (7.0%)	45 (9.3%)	0.041	30 (5.5%)	25 (4.6%)	0.001
Other antihypertensives	178 (17.2%)	131 12.7%)	<0.001	77 (15.9%)	75 (15.5%)	<0.001	101 (18.4%)	56 (10.2%)	<0.001

BMI, body mass index; SBP, systolic blood pressure, DBP, diastolic blood pressure; Glu, glutamate;HbA1c, glycated hemoglobin; TG, Triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ACEIs,angiotensin-converting enzyme inhibitors; ARBs angiotensin receptor blockers.
Data are mean ± standard deviation, median (IQR), or n (%).
P values were derived from the t-test for normally distributed variables, Mann-Whitney U test for skewed distributions, Chi-square test for categorical variables. $P < 0.05$ was defined as statistically significant.

lower HbA1C, lower LDL-C concentrations, less use of insulin, statins, ARBs, and more use of ACEI drugs than those without CVD. In women, patients with CVD were older than those without CVD, had higher systolic blood pressure, used statins more often, and other antihypertensive drugs, used ACEI and ARB drugs less often, and whether they smoked and drank alcohol, used insulin, other hypoglycemic drugs, other lipid-lowering drugs, weight, height, BMI, DBP, Glu, TG, HDL-C, or LDL-C were no significant difference. In men, patients with CVD were older, heavier, and lower in height, had higher Glu, lower HbA1C, lower LDL-C, used ACEI and other antihypertensive drugs more often, used insulin, other hypoglycemic drugs, statins less often, had alcohol habits, smoked or not, used or not other lipid-lowering drugs, BMI, diastolic blood pressure, TG, HDL-C, and LDL-C than patients without CVD. TG, HDL-C were not significantly different.

3.2 Association of Glu with CVD risk in patients with T2DM

In all patients, after standardization of Glu, unadjusted OR=1.176, p=0.012 was obtained using univariable model binary logistic regression. Glu was positively associated with the risk of CVD prevalence (OR=1.176,95% CI:1.036,1.334).

Age, BMI, smoking, alcohol consumption, SBP, HbA1C, HDL-C, LDL-C, TG, and medication use were included as confounders in a multivariable model for analysis, and this correlation was found to be no longer statistically significant, yielding an adjusted OR=1.003, p=0.105. The adjusted positive correlation was attenuated (OR=1.003, 95% CI:0.999,1.008). In the male group, using univariable model, an unadjusted OR=1.006, p=0.021; adjusted OR=1.005, p=0.139. In the female group, using univariable model, an unadjusted OR=1.003, p=0.194; adjusted OR=1.003, p=0.318. The relationship between Glu and CVD was not statistically significantly different in the multivariable model in both men and women. The results are shown in Table 2.

3.3 Interaction between Glu and gender

Logistic regression was performed by converting Glu to categorical variables according to the median. Higher Glu increased the risk of CVD in patients with T2DM (OR: 1.657, 95% CI, 1.277-2.150, P<0.001, adjusted OR: 1.539, 95% CI, 1.156-2.050, P=0.003). In the male group, the unadjusted OR= 1.580 (95% CI, 1.107-2.253), P=0.012, and the adjusted OR= 1.552 (95% CI, 0.989-2.433), P=0.056, with no significant correlation between Glu and risk of CVD. In the female group, the unadjusted OR= 1.747, (95% CI 1.191-2.563), P=0.004, and the adjusted OR = 1.591, (95% CI 1.024-2.471), P=0.039, higher Glu was associated with increased risk values for CVD in patients with T2DM. The results are shown in Table 3.

3.4 Correlation of Glu with clinical biochemical parameters

Correlations between Glu and various biochemical parameters were calculated using Pearson correlation coefficients. Among all patients with T2DM, the relationship between them is shown in Figure 1.The older the age, the higher the BMI, the higher the SBP, the heavier the weight, the lower the HbA1C, and the higher the Glu. In the female group, the relationship between them is shown in Figure 2, the older the age, the higher the BMI, the higher the SBP and the heavier the weight, the higher the Glu. Correlations with other biochemical indicators are plotted in the Supplementary Material.

4 Discussion

In this study, we found that plasma Glu levels above the population median concentration increased the risk of CVD in

TABLE 2 Relationship between Glu and CVD in patients with type 2 diabetes.

Glu (μmol/L)	OR	95%CI	*P* Value
Univariable model			
All people	1.176	1.036-1.334	0.012
Men	1.006	1.001-1.012	0.021
Women	1.003	0.998-1.008	0.194
Multivariable model[a]			
All people	1.003	0.999-1.008	0.105
Men	1.005	0.998-1.012	0.139
Women	1.003	0.997-1.008	0.318

Glu, glutamate; OR: odds ratio; CI, confidence interval.
[a]Model adjusted for age, BMI, smoking, alcohol consumption, SBP, HbA1C, HDL-C, LDL-C, TG, and medication use.

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TABLE 3 Association between dichotomized Glu and CVD risk in patients with type 2 diabetes.

Glu≥97.9vsGlu<97.9 (μmol/L)	OR	95%CI	*P* Value
Univariable model			
All people	1.657	1.277-2.150	<0.001
Men	1.580	1.107-2.253	0.012
Women	1.747	1.191-2.563	0.004
Multivariable model[a]			
All people	1.539	1.156-2.050	0.003
Men	1.552	0.989-2.433	0.056
Women	1.591	1.024-2.471	0.039

Glu, glutamate; OR: odds ratio; CI, confidence interval.
[a]Model adjusted for age, BMI, smoking, alcohol consumption, SBP, HbA1C, HDL-C, LDL-C, TG, and medication use.

patients with type 2 diabetes, and this relationship remained significant in women with type 2 diabetes.

Some data analyzing the correlation between Glu receptor genes and angiogenic genes suggest that Glu receptors have anti-vascular effects, that the expression of genes controlling the production of this receptor is negatively correlated with angiogenic genes, and that changes in Glu receptor activity may affect the formation of microvascular networks (12). Glu ionotropic receptors are present in the center and periphery, and peripheral ones are also present in pancreatic islet cells (13). It was demonstrated that the activation of the receptor by elevated Glu and its dual action on islet cells may exacerbate the destruction of vascular endothelial cells, leading to an increased likelihood of CVD in diabetic patients. It has also been shown that such receptors are also present in the heart and blood vessels (14). Activation of the receptor promotes calcium inward flow, which in the cardiovascular system determines excitation-contraction coupling, and increasing Glu may lead to excessive activation of these

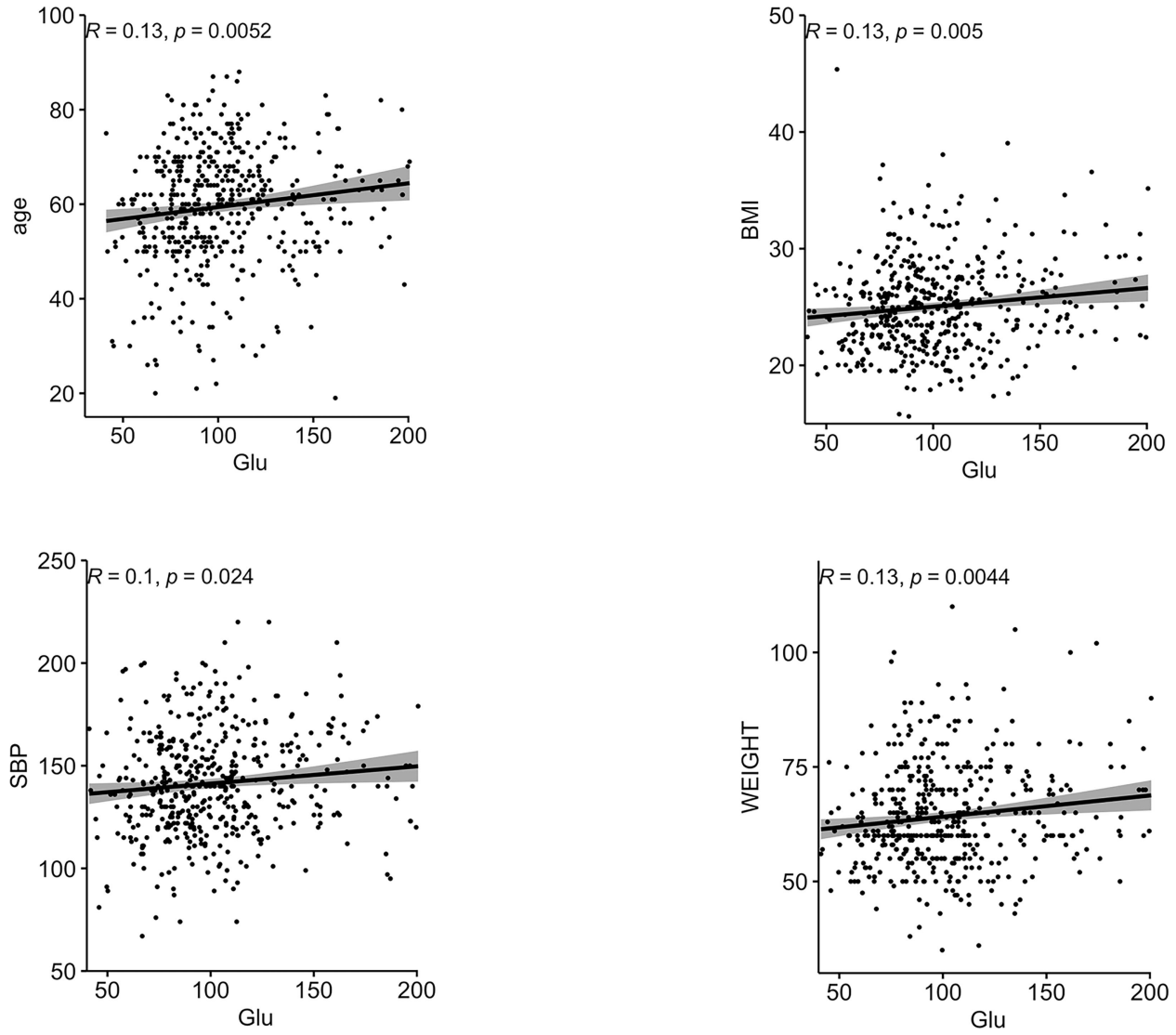

FIGURE 1
Correlation between Glu and each biochemical index in all people. BMI, body mass index; HbA1c, glycated hemoglobin; SBP, systolic blood pressure; Glu, glutamate.

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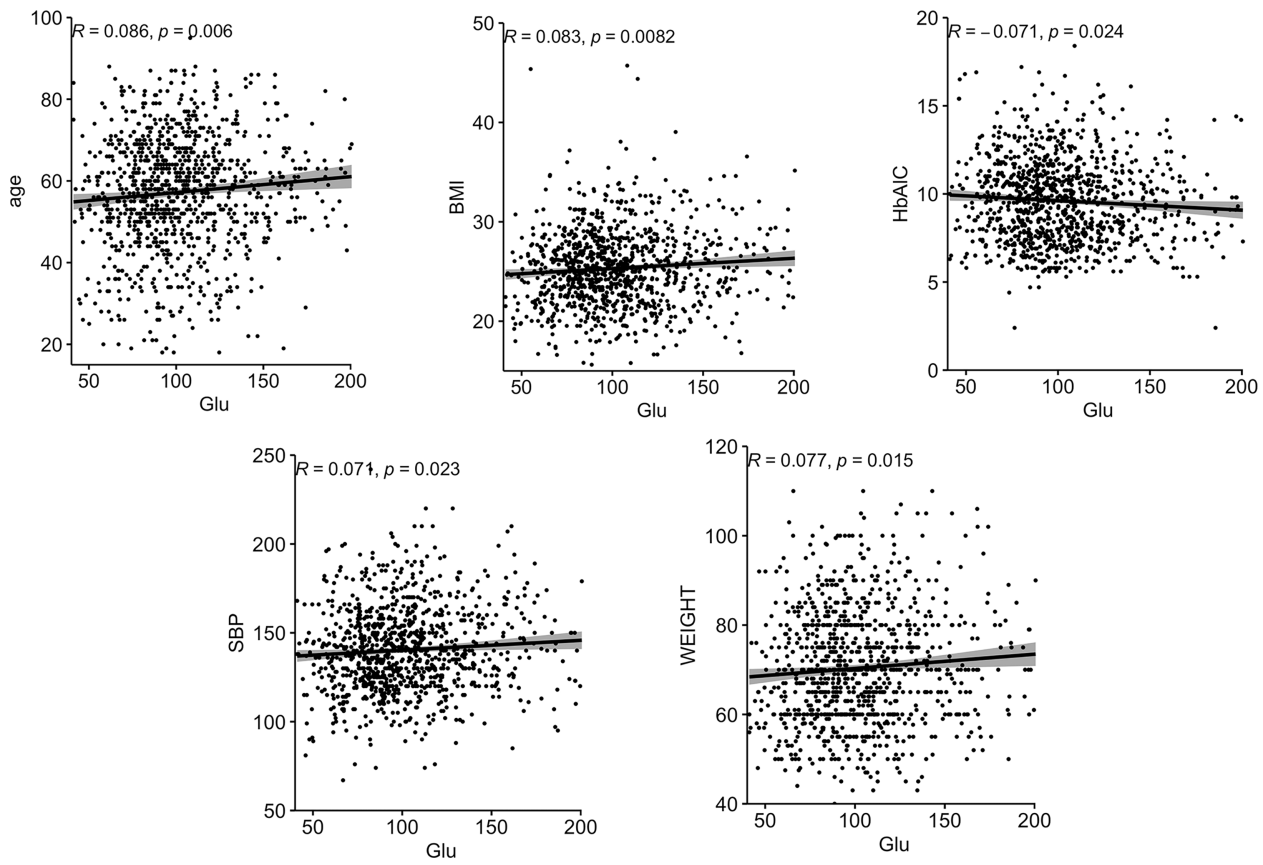

FIGURE 2
Correlation between Glu and various biochemical indicators in the female group. BMI, body mass index; SBP, systolic blood pressure; Glu, glutamate.

receptors, resulting in intracellular calcium overload in cardiac myocytes, which in turn leads to apoptosis, which may be one of the pathogenic mechanisms (15). Also some studies on glutamate proved that it can stimulate the release of glucagon in pancreatic alpha cells (16) and increase the transamination of pyruvate to alanine, a powerful promoter of the abundant gluconeogenesis in obese patients (17).Glutamate is also known as a direct precursor of alpha-ketoglutarate, an intermediate in the Krebs cycle, which acts as an anabolic and anti-catabolic source of energy for many cell types (18). In our study, higher levels of Glu in plasma increased the risk of CVD in women with type 2 diabetes, but not in men. Although women's estrogen levels are higher than men's, which is important for maintaining vascular health and promoting blood vessel growth (19), the decline in estrogen levels in women after menopause greatly weakens this protective effect, and the average age of female participants in this study is higher than the age at which women develop menopause. Therefore, we hypothesized that in the presence of elevated Glu, which tend to destroy the vascular endothelium, women with T2DM are more susceptible to its negative effects, thus increasing the risk of CVD.

When Glu was used as a continuous variable, no significant relationship was found with CVD in T2DM, while when Glu was transformed into a categorical variable according to median concentration, higher levels of plasma Glu were positively associated with CVD in all T2DM patients and in female T2DM patients. Once Glu is elevated to a certain threshold, it will cause cardiovascular abnormalities in the body. For example, it has been found that glutamate has a positive correlation with BMI, waist circumference, glucose, insulin, insulin resistance index, systolic blood pressure, diastolic blood pressure and triglycerides, and a negative correlation with HDL-C (7).

Our study has important implications for public health. The diabetes epidemic poses a major health and economic threat to the world, including China. Early management of its important co-morbidities with lifestyle and medical interventions is essential. Our findings provide new predictive ideas for myocardial infarction and ischemic stroke prevention in T2DM patients, especially for female patients. This provides a new direction for further research into the possible role of Glu in T2DM patients suffering from CVD.

There are some limitations of this study. First, due to the nature of retrospective cross-sectional studies, these findings cannot determine the causal relationship between Glu and diabetic CVD, and a larger study in a population is needed. Also, this study was limited to Chinese patients and is not representative of other countries and ethnicities. In addition, the exact level of Glu above which it significantly increases the risk of CVD in female patients with T2DM needs to be studied in more depth. Finally, although some of the confounding factors were screened and taken into account in this study, the effect of other potential confounding factors on the results cannot yet be completely eliminated.

In conclusion, we found that higher plasma concentrations of Glu were associated with an increased risk of CVD in patients with T2DM and that this association remained significant in female patients, but not in male patients. Therefore, this result is derived from a cross-sectional study, so further prospective studies and mechanistic studies are needed to further validate this conclusion.

Data availability statement

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

Author contributions

R-TL wrote the article. YL and B-WW analyzed the data for this article. X-QG and J-XZ collected and collated the data. FL reviewed the relevant literature. Z-ZF and X-YZ conceived the project and designed the experiments. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1095550/full#supplementary-material

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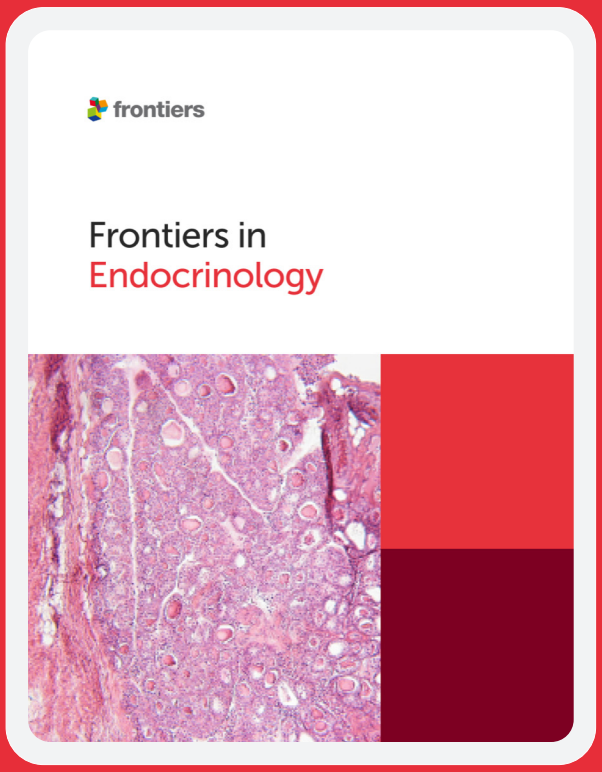

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