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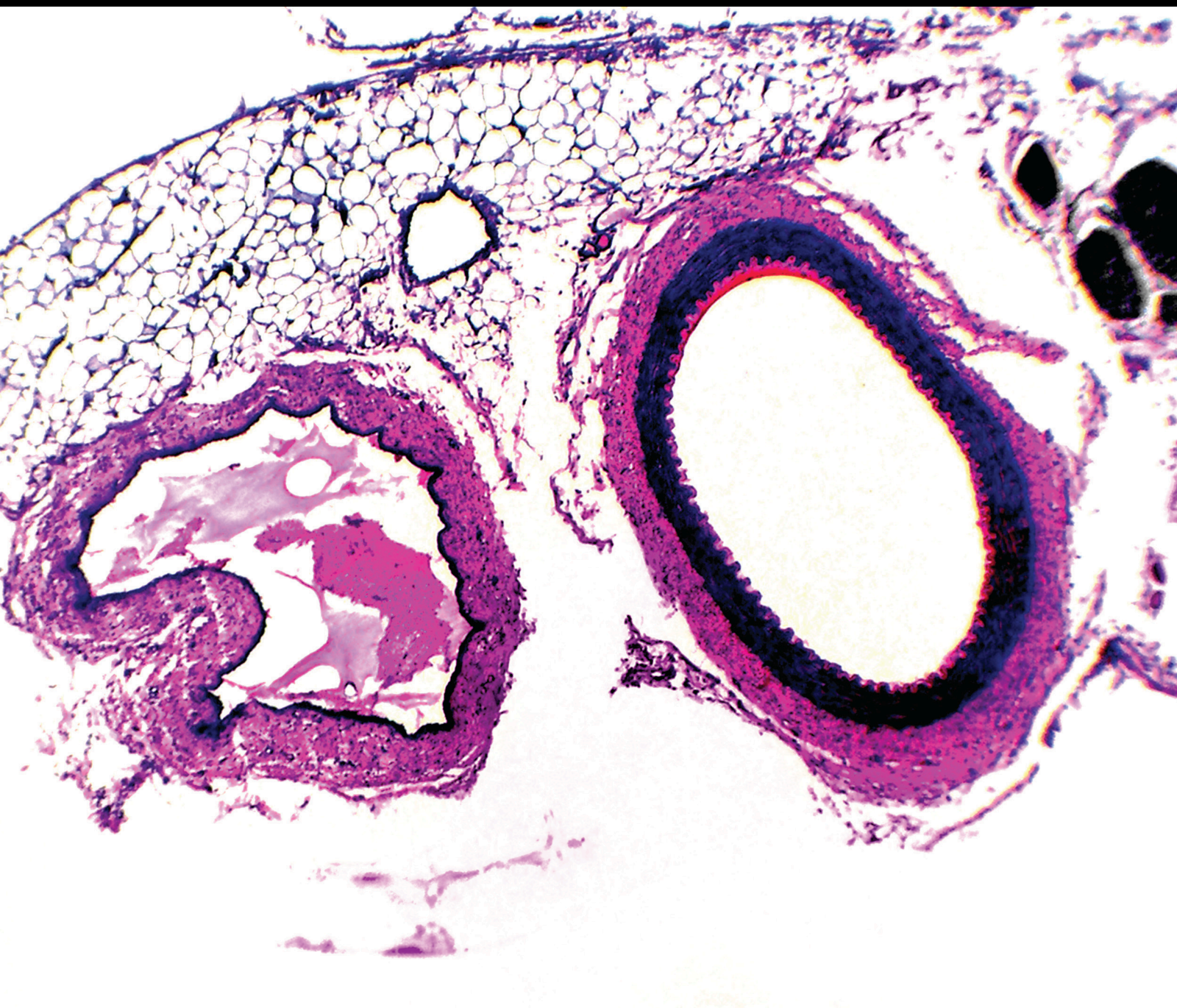
New Biomarkers of Hypertension and Related Vascular Disorders

Lead Guest Editor: Hao Peng
Guest Editors: Chao Li and Changwei Li



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Editorial

New Biomarkers of Hypertension and Related Vascular Disorders

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As a very common risk factor of cardiovascular disease (CVD) and premature death, hypertension is preventable, and extensively controlling blood pressure to targets set forth by the current guideline is key to prevent and manage the debilitating disorder and CVD. In recent decades, the advancement of multiomics studies including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and gut microbiome has uncovered many potential biomarkers for hypertension and its related cardiovascular complications including myocardial infarction, heart failure, and stroke [1–3]. Due to limited budget, most omics studies adopted a case-control study design which precludes causal inference. A better understanding of the causality and molecular mechanisms underlying the associations between the reported biomarkers and hypertension will undoubtedly promote clinical translation of the findings from omics studies. Therefore, follow-up studies targeting at those reported biomarkers are urgently needed. The aim of this special issue is to present recent progress in these exciting fields. A brief summary of all accepted papers is provided as follows.

The paper written by Dr. X. Peng et al. reported a longitudinal association between dynamic fasting glucose and risk of incident stroke in 12,321 Chinese adults. They found that a higher variance in addition to a higher level of fasting glucose predicted a higher risk of incident stroke, independent of lifestyle behaviors and metabolic factors. These results suggest that high variability of fasting glucose may be a risk factor for stroke. The homeostasis of glucose metabolism should be maintained for community members, diabetic patients in particular.

In the paper written by Dr. M.-C. Chen et al., the authors examined whether serum angiopoietin-like protein 3 (ANGPTL3) levels at baseline could predict adverse cardiovascular events in 90 patients with coronary artery disease (CAD). After a median follow-up of 54 months, 33 patients developed major adverse cardiovascular events, and these patients had higher ANGPTL3 levels at baseline compared with those who were free of adverse cardiovascular events during the follow-up. Cox regression models revealed that a higher baseline level of ANGPTL3 was significantly associated with an increased risk of developing adverse cardiovascular events for CAD patients. ANGPTL3 may be a predictor for major adverse cardiovascular events in CAD patients, but the efficacy and effectiveness of screening ANGPTL3 for CAD patients warrant further evaluation.

Leveraging data from 285 ischemic stroke patients receiving intravenous r-tPA thrombolysis within 4.5 hours of stroke onset, Dr. Y.-L. Liu and colleagues found that higher neutrophil-to-lymphocyte ratio at admission predicted risks of hemorrhagic transformation after r-tPA thrombolysis in acute ischemic stroke patients. Neutrophil-to-lymphocyte ratio, a parameter that can be easily estimated from blood cell count analysis, could be a useful marker for predicting hemorrhagic transformation in acute ischemic stroke patients after intravenous r-tPA thrombolysis.

The paper written by Dr. J. Zhang and colleagues reported a modification effect of platelet count on the association between homocysteine and blood pressure in more than 30,000 hypertensive patients. The association between homocysteine and blood pressure was much stronger in participants having low platelet count, compared with those having high platelet count. These results indicate that platelet count may be useful in the identification of individuals who are major beneficiaries of reducing-homocysteine treatments, but the clinical application still have a long way to go.

Dr. H. Xie et al. conducted a nested case-control study to examine the association between plasma urotensin II and the risks of incident hypertension by exploring 723 participants who developed hypertension and 1,096 participants who remained free of hypertension after about 5 years of follow-up. In contrast to prior case-control studies, they did not observe a significant association between urotensin II and hypertension. The causal effect of urotensin II on hypertension is still unspecified.

Dr. X. Jia et al. conducted an animal study to test the effect of resveratrol on pregnant hypertension reduction using mice models of high-salt diet-induced hypertension. They found that resveratrol supplementation could decrease blood pressure, serum urea, serum creatinine, and urinary protein in hypertensive pregnant mice. Resveratrol could be a promising candidate of an effective blood pressure lowering agent for pregnancy-induced hypertension, but further evidence is needed from clinical trials in humans.

Learning the new biomarkers of hypertension may help clinicians and researchers to understand the underlying mechanisms and provide additional motivation for undertaking the difficult challenge to reduce hypertension and its related cardiovascular disorders.

Conflicts of Interest

The editors declare that they have no conflicts of interest.

Acknowledgments

We are deeply appreciative of the authors and editors who made this special issue possible and thank all staff members for their support and assistance. We hope this collection of articles would be useful to the scientific community. This work was supported by a project of the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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Research Article

Longitudinal Average Glucose Levels and Variance and Risk of Stroke: A Chinese Cohort Study

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Background. Diabetes is a known independent risk factor for stroke. However, whether higher glucose levels (126–139.9 mg/dl) can increase the risk of stroke in people without diabetes is still unknown. Moreover, as a fluctuating parameter, long-term glucose levels may also be related to the risk of stroke outcome. It is important to explore the correlation between long-term average blood glucose, as well as its variability, and stroke. *Methods*. We used 40,975 clinical measurements of glucose levels and 367 measurements of glycated hemoglobin A1c levels from 12,321 participants without stroke to examine the relationship between glucose levels and the risk of stroke. Participants were from the Weitang Geriatric Diseases study, including 5,707 men and 6,614 women whose mean age at baseline was 60.8 years; 1,011 participants had diabetes, and 11,310 did not. We estimated the long-term average blood glucose level based on the multilevel Bayesian model and fit in Cox regression models, stratified according to diabetes status. *Results*. Over a median follow-up period of 5 years, stroke developed in 279 of the 12,321 participants (244 without diabetes and 35 with). For people with an average glucose level of 126–139.9 mg per deciliter, compared with 90–99.9 mg per deciliter, the adjusted hazard ratio (HR) for total stroke was 1.78 (95% confidence interval (CI), 1.16–2.75), and the HR for levels higher than 140 mg per deciliter was 1.89 (95% CI, 1.09–3.29). Among those without diabetes whose glucose level was higher than 140 mg per deciliter, compared with 90–99.9 mg per deciliter, the adjusted HRs for total stroke and fatal stroke were 3.66 (95% CI, 1.47–9.08) and 5 (95% CI, 1.77–14.15), respectively. For a glucose standard deviation level higher than 13.83 mg per deciliter, compared with that lower than 5.91 mg per deciliter, the adjusted HR for total stroke was 2.31 (95% CI, 1.19–4.48). *Conclusions*. Our results suggest that higher average glucose levels (126–139.9 mg/dl) and variance may be risk factors for stroke, even among people without diabetes diagnosis.

1. Introduction

Stroke is the third leading cause of death in the world, seriously threatening human health and placing economic and medical burden on society and families [1]. Diabetes mellitus (DM) is a known risk factor for stroke, and hyperglycemia can cause various microvascular and macrovascular diseases [1, 2]. However, the development of diagnostic criteria for diabetes is based on the risk of developing eye and kidney disease rather than cardiovascular disease [3]. Some studies have shown that high fasting plasma glucose (FPG) levels were significantly associated with a subsequent risk of cardiovascular disease in individuals without diabetes [4–6], so it is very likely that blood glucose levels below the diagnostic criteria will also cause cardiovascular disease or that there is no threshold for FPG and cardiovascular disease risk [7]. Some studies have found that the risk of cardiovascular disease is directly proportional to the concentration of FPG [3], while other studies found that the relationship between the two is J-shaped [8].

Moreover, people's blood glucose levels fluctuate, and one blood glucose measurement cannot accurately represent long-term average FPG levels. Therefore, it is important to explore the correlation between long-term average blood glucose and the risk of stroke, rather than random FPG measurements. In addition, some studies have found that the rate of blood glucose variability is related to the risk of kidney disease, retinopathy, and cardiovascular disease because FPG fluctuation may cause a series of injury responses, such as reactive oxygen production, inflammation, and endothelial dysfunction [9]. Therefore, it is also important to investigate the association between blood glucose variability and stroke.

In previous studies, the relationship between blood glucose and the risk of cardiovascular disease was measured by only one or a few blood glucose value measurements, which may lead to bias and the misinterpretation of the correlation [6, 10, 11]. Therefore, in our study, we estimated long-term average blood glucose based on a multilevel Bayesian model.

2. Research Design and Methods

2.1. Study Population. The Weitang Geriatric Diseases study was a community-based survey conducted in Weitang, an urban metropolis located in Suzhou in eastern China. This study initially included 12,413 randomly selected members of a preventive medical examination. All members were stroke-free and had no previous stroke history. From 2011 to 2012, participants aged 45 years old or older at the time of enrollment were included. Participants were invited to return at 1-year intervals for the purpose of collecting FPG data and identifying incident cases of stroke and other chronic diseases by conducting annual physical examinations. The sample for the current study was limited to 12,321 participants who had at least one follow-up visit and had at least one measurement of glucose or glycated hemoglobin A1c (HbA1c). The study procedures were approved by the Institutional Review Board of Soochow University, and all the participants provided their written informed consent.

2.2. Stroke Outcomes Assessment. The outcome was the first occurrence of stroke, either nonfatal or fatal. The incidence date of stroke was recorded when stroke was diagnosed in the hospital. All potential stroke cases were identified by the stroke code (tenth versions of International Classification of Diseases, I63), death certificates, and questionnaires. Two physicians who were experienced neurologists and blinded to the FBG concentrations reviewed the medical records and judged the cases annually. In cases of disagreement, a third physician was consulted to make the final decision. Study participants were assessed for stroke every year with the use of a regional health information system. Stroke-free participants continued with scheduled follow-up visits. Fatal cases were confirmed by medical records, autopsy reports, or death certificates with stroke as the primary cause. Nonfatal stroke was defined as the sudden onset of a focal neurological deficit and the demonstration of acute primary intraparenchymal hemorrhage on either brain computed tomography or magnetic resonance imaging, which was available for all suspected nonfatal stroke cases.

2.3. Assessment of FPG. Participants were classified as having diabetes (with previous hospital diagnosis certificate) or not having diabetes (including undiagnosed diabetes) at enrollment according to their previous medical history and a personal statement. FPG or glycated hemoglobin levels for each participant were collected via at least one measurement during the follow-up years. Fasting plasma samples were collected in the morning after an 8 h overnight fast and were transfused into vacuum tubes containing EDTA. Plasma was separated from blood immediately and stored at 4°C. Blood glucose concentrations were measured 4 h after blood sample collection. The collection was divided into two samples used to test FPG and HbA1c. Blood glucose was determined by Glucose Oxidase method (GOD-POD) using Roche Cobas 501 automatic biochemical analyzer, and HbA1c was analyzed by high-performance liquid chromatography method with TOSOH HLC-723G7 automatic glycohemoglobin analyzer. Once nondiabetic participants were diagnosed with diabetes during the follow-up period, they would be divided into diabetic groups. For those whose FPG exceeded 126 mg/dl, as well as those who were not diagnosed by a a physician, their group status remained unchanged as undiagnosed diabetes. Average glucose levels were categorized into 6 ranges (80–89.9, 90–99.9, 100–109.9, 110–125.9, 126–139.9, and >140 mg/dL).

We selected an analytical strategy using a hierarchical Bayesian framework [12] to develop a measure of average glucose levels in the prior 5 years based on clinical laboratory measures of glucose and HbA1c for all time frames in which at least one measurement of glucose or HbA1c was available. We transformed the calculated HbA1c values to daily average glucose values with this formula: daily average glucose = $(28.7 \times \text{HbA1c}) - 46.7$, while stabilizing estimates for individuals with relatively sparse observations by borrowing information across participants with more measurements, and accounting for the relative variability in glucose and HbA1c measures by creating a combined estimate. Let X_{ij} represent the jth glucose measures for the ith participant, and let Y_{ij} represent the jth measure of glucose in the time period of interest estimated from HbA1c for participant i. We assume the hierarchical model $X_{ij} \sim N(\mu_i, \sigma_X^2), Y_{ij} \sim N(\mu_i, \sigma_Y^2), \mu_i \sim N(\theta, \tau^2)$, where μ_i represents the average daily glucose level for the ith individual in the time period of interest. Then an empirical Bayes estimation formula was used to calculate μ from X and Y. This model assumes that given a participant's average daily glucose level, measured glucose and glucose estimated from HbA1c are independent and vary randomly around an individual's daily average with variance σ_X^2 and σ_Y^2, respectively, and that, in the population of interest, average daily glucose levels vary across individuals with population mean θ and variance τ^2. In this framework, θ means prior or population mean average glucose which is the data based on clinical parameters of large population.

2.4. Assessment of Covariates. The baseline assessment, which was performed after 8 h of overnight fasting, included a physical examination; clinical evaluations such as blood chemistry analyses and blood pressure measurement; questionnaires on personal and family medical history, smoking habits, and drinking habits; and an exercise test. Briefly, body mass index (BMI) was calculated from measured weight and height (kg/m^2); alcohol consumption was quantified as drinks per week (drinks/week); and smoking status included never, former smoker, or current smoker. We defined current smoking as smoking more than one cigarette per day for more than one year, and former smoking as stopping smoking for at least one year since enrollment and having smoking history in the past. Exercise level was assessed with questions about types of physical activity (sedentary or active) and the number of times exercise was performed in a week. Those who exercised 3 or more days per week were categorized as having regular exercise. Blood pressure was determined by averaging two measurements on the right arm while the participant was seated, with a 5-minute rest period between each measurement. Hypertension was defined as either systolic/diastolic blood pressure of 140/90 mmHg or higher or a history of hypertension. Hypercholesterolemia was defined as serum total cholesterol of 240 mg/dL or higher. Serum samples were analyzed in the laboratory of the 3rd People's Hospital of Xiangcheng District, Suzhou.

2.5. Statistical Analysis. The average blood glucose of the participants estimated by the Bayesian method was divided into six groups according to the average blood sugar concentration. The Cox regression model was used to assess the relationship between blood glucose and the risk of stroke. First, hazard ratios (HRs) and the 95% confidence interval (CI) for the relationship between FPG and the risk of stroke in all participants with and without diabetes were calculated by Cox regression model. There are three Cox regression models. The independent variable in model 1 is average blood glucose, and model 2 adjusts for age and gender. Model 3 includes the factors used in model 2 in addition to education, smoking behavior, drinking behavior, regular exercise, BMI, systolic blood pressure, and total cholesterol. Second, we predicted the linear trend between blood glucose levels and risk of stroke in different groups. Glucose levels were incorporated in models with the use of natural cubic splines with 4 knots (at the 5th, 35th, 65th, and 95th percentiles) to allow for a nonlinear association between glycemia and risk of stroke as measured by the log hazard [13]. Third, we calculated the degree of blood glucose variability (standard deviation, SD) in participants with no less than twice follow-up data based on the Bayesian method and determined the quartiles according to the division of the standard deviation into four groups, repeating the above two steps to explore the risk of stroke. All P values were 2-tailed, and a significance level of 0.05 was used. Statistical analysis was conducted using SAS statistical software, version 9.4 (SAS Institute Inc., Cary, NC).

3. Results

The baseline characteristics of the 12,321 study participants are presented in Table 1. Upon enrollment, the average age of study participants was 60.8 years for participants without diabetes and 61.3 years for participants with diabetes, and the average BMI was 23.5 kg/m^2 and 24.8 kg/m^2 for each group, respectively. The average fasting glucose values were 102.5 mg/dL and 154 mg/dL in those without and with diabetes, respectively. In the normal group, 24.9% were current smokers, and 67.1% had never been smokers; among the DM group, the corresponding figures were 23.2% and 68.6%, respectively. There are 40,975 values available for fasting glucose levels and 367 values available for HbA1c levels.

Over a median follow-up period of 5 years, stroke developed in 279 of the 12,321 participants (2.26%), including 244 of the 11,310 participants who did not have diabetes at the end of follow-up (2.2%) and 35 of the 1,011 participants who had stroke at the end of follow-up (3.5%). A total of 133 participants had probable or possible nonfatal stroke at the end of follow-up, and 146 had fatal stroke.

Associations between average glucose levels and the development of stroke are shown in Table 2. Among participants, an increased risk of total stroke with increasing glucose levels was significant ($P = 0.0009$) for model 3, and restricted cubic spline regression models showed that lower limits of 95% CI for HR are more than 1.0 when average glucose level was higher than 110 mg per deciliter (Figure 1). For an average glucose level of 126–139.9 mg per deciliter compared with 90–99.9 mg per deciliter, the adjusted HR for total stroke was 1.78 (95% CI, 1.16–2.75), and the HR for more than 140 mg per deciliter was 1.89 (95% CI, 1.09–3.29). Those with higher levels of glucose had an increased risk of nonfatal stroke ($P = 0.0067$). For an average glucose level of 126–139.9 mg per deciliter compared with 90–99.9 mg per deciliter, the adjusted hazard ratio for nonfatal stroke was 2.16 (95% CI, 1.19–3.93). The association of glycemia with risk of stroke was similar across subgroups stratified according to sex and history of hypertension ($P < 0.05$ for trend for total stroke stratified by sex; $P < 0.05$ for trend for total stroke in people with hypertension and for fatal stroke in people without hypertension; Supplementary Table 1).

We also assessed the association between stroke outcomes and average glucose levels among participants without diabetes (Table 3). For an average glucose level higher than 140 mg per deciliter compared with 90–99.9 mg per deciliter, the adjusted HRs for total stroke and fatal stroke were 3.66 (95% CI, 1.47–9.08) and 5 (95% CI, 1.77–14.15), respectively.

Associations between glucose variability and the development of stroke are presented in Table 4. Among participants, an increased risk of nonfatal stroke with increasing glucose SD was significant ($P = 0.0198$) for model 3. For a glucose SD level higher than 13.83 mg per deciliter, compared with less than 5.91 mg per deciliter, the adjusted HR for total stroke was 2.31 (95% CI, 1.19–4.48).

Table 1: Baseline characteristics of the study participants*.

	Total ($N = 12,321$)	Participants without diabetes ($N = 11,310$)	Participants with diabetes ($N = 1,011$)	P value
Age	60.8 ± 10.2	60.8 ± 10.3	61.3 ± 9.6	0.1173
Female sex	6,614 (53.7)	6,073 (53.7)	541 (53.51)	0.9103
BMI	23.6 ± 3.1	23.5 ± 3.1	24.8 ± 3.3	<.0001
Systolic blood pressure (mmHg)	141 ± 20.4	140.4 ± 20.2	148.2 ± 20.5	<0.0001
Diastolic blood pressure (mmHg)	84.1 ± 12.3	83.8 ± 12.3	86.7 ± 12.4	<0.0001
Fasting plasma glucose (mg/dL)	106.7 ± 21.9	102.5 ± 11.5	154 ± 43.8	<0.0001
Total cholesterol (mg/dL)	185.1 ± 34.3	184.7 ± 33.8	189.7 ± 39	<0.0001
LDL-C (mg/dL)	100.6 ± 26.7	100.3 ± 26.5	103.9 ± 28.3	<0.0001
HDL-C (mg/dL)	57.9 ± 15.6	58.3 ± 15.6	53.8 ± 14.6	<0.0001
Triglycerides (mg/dL)	120.3 ± 85.8	117.5 ± 81.5	152.3 ± 119.7	<0.0001
Education level (no formal education)	6,096 (49.5)	5,605 (49.6)	491 (48.6)	0.3049
Smoke				0.4424
Never smoked	8,284 (67.2)	7,590 (67.1)	694 (68.6)	
Current smoker	3,056 (24.8)	2,822 (24.9)	234 (23.2)	
Former smoker	981 (8)	898 (7.9)	83 (8.2)	
Alcohol drinking	2,801 (22.7)	2,578 (22.8)	223 (22.1)	0.5924
Regular exercise	5,332 (43.3)	4,849 (42.9)	483 (47.8)	0.0026
Hypertension	6,339 (51.4)	5,625 (49.7)	714 (70.6)	<0.0001
High cholesterol	3,712 (30.1)	3,350 (29.6)	362 (35.8)	<0.0001

*Continuous variables are expressed as mean ± SD or as median (interquartile range). Categorical variables are expressed as frequency (percentage).

Table 2: Risk of incident stroke associated with average glucose level ($N = 12,321$).

	Average glucose level (mg/dl)						P for trend
	80–89.9	90–99.9	100–109.9	110–125.9	126–139.9	140-	
Total stroke							
Events, n (%)	5 (2.07)	97 (1.90)	105 (2.18)	30 (2.94)	27 (3.55)	15 (4.01)	
Model 1	0.73 (0.32–1.68)	1	1.12 (0.85–1.48)	1.46 (0.97–2.20)	1.99 (1.30–3.05)	2.31 (1.34–3.98)	0.0007
Model 2	0.70 (0.31–1.61)	1	1.18 (0.9–1.55)	1.40 (0.92–2.11)	1.98 (1.29–3.03)	2.18 (1.26–3.77)	<0.0001
Model 3	0.74 (0.32–1.69)	1	1.11 (0.84–1.46)	1.29 (0.85–1.95)	1.78 (1.16–2.75)	1.89 (1.09–3.29)	0.0009
Nonfatal stroke							
Events, n (%)	3 (1.24)	42 (0.82)	49 (1.02)	17 (1.67)	15 (1.97)	7 (1.87)	
Model 1	1.03 (0.31–3.22)	1	1.24 (0.82–1.86)	1.73 (0.98–3.04)	2.38 (1.32–4.29)	2.23 (1.01–4.97)	0.0008
Model 2	1.01 (0.31–3.28)	1	1.25 (0.83–1.99)	1.75 (1.00–3.09)	2.41 (1.33–4.34)	2.19 (0.98–4.88)	0.0009
Model 3	1.06 (0.33–3.42)	1	1.17 (0.77–1.78)	1.60 (0.90–2.82)	2.16 (1.19–3.93)	1.87 (0.83–4.22)	0.0067
Fatal stroke							
Events, n (%)	2 (0.83)	55 (1.08)	56 (1.16)	13 (1.27)	12 (1.58)	8 (2.14)	
Model 1	0.53 (0.13–2.16)	1	1.02 (0.7–1.48)	1.22 (0.66–2.23)	1.64 (0.88–3.06)	2.4 (1.14–5.04)	0.0096
Model 2	0.42 (0.1–1.74)	1	1.09 (0.75–1.58)	1.05 (0.57–1.94)	1.5 (0.8–2.8)	2.13 (1.01–4.5)	0.0245
Model 3	0.43 (0.11–1.79)	1	1.02 (0.7–1.49)	0.98 (0.53–1.81)	1.34 (0.71–2.54)	1.85 (0.86–3.96)	0.0871

Model 2, adjusted for age, sex; Model 3, adjusted for age, sex, education, smoke, drink, sport, BMI, systolic blood pressure, total cholesterol.

4. Discussion

In this prospective cohort study including 12,321 Chinese adults, we found that higher FPG levels increased the risk of stroke after controlling for other potential confounders. However, an association between elevations in FPG in the nondiabetic range and long-term risk of stroke outcomes was not found in our study. Furthermore, variation in FPG measurements was strongly correlated with stroke outcomes, which indicates that therapies should be evaluated further to minimize glucose fluctuation in diabetic patients to prevent stroke outcomes.

Diabetes is an established independent risk factor for stroke [14]. Previous studies have demonstrated that a high risk of myocardial infarction and other macrovascular diseases can be predicted by long-term glycemia [5, 8]. However, the stroke risk related to glycemia below the current diabetic threshold remains controversial. Some prior studies have reported a positive association between elevated FPG levels and stroke [15], while many others showed opposite conclusions [16]. A study of 47,865 men aged 34–54 years from the Aerobics Center Longitudinal Study found that, in FPG levels of 110 mg/dL or higher, each 10-unit increment of FPG was associated with a 6% higher risk of total stroke events. However, they had just one baseline measurement of FPG, and the cohort population may have reduced the possibility that many confounders had influence. In a prospective cohort study of 3,246 British women

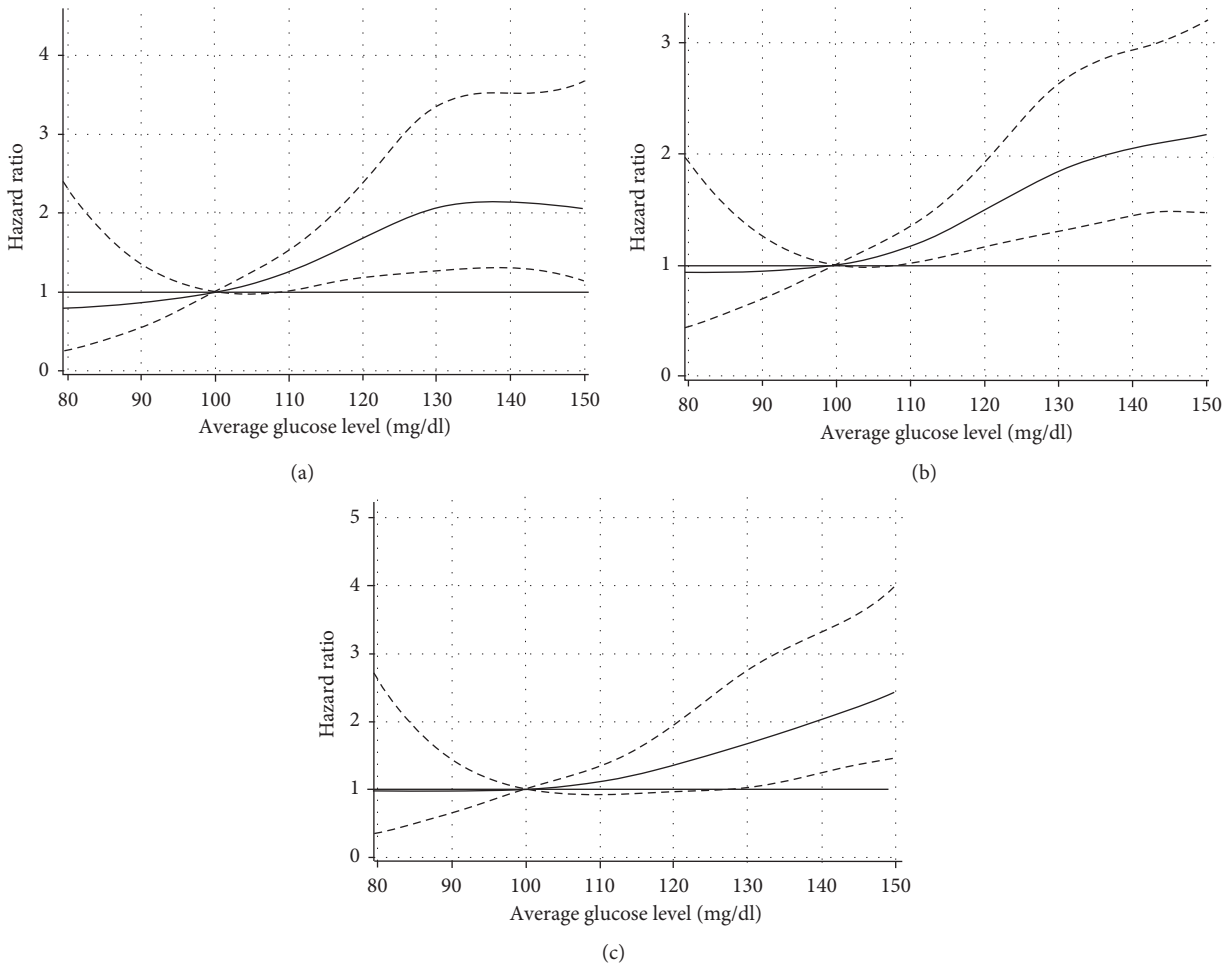

Figure 1: Risk of incident stroke associated with the average glucose level during the preceding 5 years.

Table 3: Risk of incident stroke associated with average glucose level over the preceding 5 years among participants without diabete ($N = 11{,}310$).

	Average glucose level (mg/dl)						P for trend
	80–89.9	90–99.9	100–109.9	110–125.9	126–139.9	140-	
Total stroke							
Events, n (%)	5 (2.07)	96 (1.88)	104 (2.18)	27 (3.21)	7 (2.33)	5 (8.20)	
Model 1	0.74 (0.3–1.83)	1	1.11 (0.84–1.47)	1.53 (1–2.35)	1.27 (0.59–2.74)	4.24 (1.72–10.44)	0.0050
Model 2	0.68 (0.28–1.67)	1	1.17 (0.88–1.54)	1.49 (0.97–2.3)	1.25 (0.58–2.69)	4.07 (1.65–10.05)	0.0045
Model 3	0.7 (0.28–1.73)	1	1.1 (0.83–1.46)	1.39 (0.9–2.14)	1.15 (0.53–2.5)	3.66 (1.47–9.08)	0.0205
Nonfatal stroke							
Events, n (%)	3 (1.24)	42 (0.82)	49 (1.03)	15 (1.78)	4 (1.33)	1 (1.64)	
Model 1	0.84 (0.26–2.72)	1	1.25 (0.83–1.88)	1.78 (0.99–3.21)	1.44 (0.52–4.03)	1.56 (0.21–11.33)	0.0586
Model 2	0.88 (0.27–2.85)	1	1.30 (0.86–1.96)	1.84 (1.02–3.33)	1.46 (0.52–4.07)	1.61 (0.22–11.70)	0.0475
Model 3	0.90 (0.28–2.91)	1	1.24 (0.82–1.88)	1.72 (0.95–3.13)	1.38 (0.49–3.87)	1.53 (0.21–11.22)	0.0912
Fatal stroke							
Events, n (%)	2 (0.83)	54 (0.83)	55 (1.15)	12 (1.43)	3 (1.00)	4 (6.56)	
Model 1	0.54 (0.13–2.23)	1	1.02 (0.7–1.49)	1.3 (0.69–2.43)	1.09 (0.34–3.51)	7.19 (2.59–19.98)	0.0345
Model 2	0.43 (0.11–1.78)	1	1.09 (0.75–1.59)	1.14 (0.6–2.14)	0.99 (0.31–3.19)	5.92 (2.12–16.59)	0.0481
Model 3	0.45 (0.11–1.85)	1	1.01 (0.69–1.49)	1.06 (0.56–2)	0.88 (0.27–2.85)	5 (1.77–14.15)	0.1295

Model 2, adjusted for age, sex; Model 3, adjusted for age, sex, education, smoke, drink, sport, BMI, systolic blood pressure, total cholesterol.

aged 60–79 years old, there was no evidence of linear or nonlinear associations between either fasting glucose or HbA1c and incident stroke, suggesting that in 60–79-year-old women [17], insulin resistance, rather than chronic hyperglycemia, is a more important risk factor for stroke. In our study, average glucose levels over 126–139.9 mg/dl may

Table 4: Risk of incident stroke associated with variance ($N = 11{,}310$).

	Standard deviation				P for trend
	Quartile 1 (<5.91)	Quartile 1 (5.91 to 8.9)	Quartile 1 (8.91 to 13.83)	Quartile 1 (>13.83)	
Total stroke					
Events, *n* (%)	42 (1.68)	48 (1.92)	40 (1.60)	75 (3.00)	
HR	1	1.25 (0.85–1.84)	1.72 (1.15–2.59)	1.83 (1.07–3.11)	0.001
Nonfatal stroke					
Events, *n* (%)	16 (0.64)	33 (1.32)	26 (1.04)	40 (1.6)	
HR	1	1.47 (0.87–2.47)	1.98 (1.14–3.43)	1.71 (0.79–3.72)	0.008
Fatal stroke					
Events, *n* (%)	26 (1.04)	15 (0.60)	14 (0.56)	35 (1.40)	
HR	1	1.13 (0.66–1.95)	1.34 (0.74–2.45)	2.31 (1.19–4.48)	0.0198
Participants without diabetes					
Total stroke					
Events, *n* (%)	42 (1.7)	47 (1.9)	39 (1.63)	46 (2.56)	
HR	1	1.33 (0.89–2.00)	1.11 (0.52–2.36)	3.49 (1.43–8.55)	0.0246
Nonfatal stroke					
Events, *n* (%)	26 (1.05)	32 (1.3)	25 (1.04)	23 (1.28)	
HR	1	1.56 (0.90–2.70)	1.25 (0.46–3.41)	1.42 (0.2–10.23)	0.2053
Fatal stroke					
Events, *n* (%)	16 (0.65)	15 (0.60)	14 (0.58)	23 (1.28)	
HR	1	1.24 (0.70–2.17)	0.90 (0.28–2.85)	6.38 (2.55–15.95)	0.016

Adjusted for age, sex, education, smoke, drink, sport, BMI, systolic blood pressure, total cholesterol.

be risk factors for stroke according to all the participants; however, for undiagnosed group, FPG had little if any association with the risk of stroke, which only clearly increased at FPG ≥140 mg/dl after adjusting for classic risk factors. These inconsistent findings may be due to differences in study populations, length of follow-up, stroke outcome definitions (such as fatal, nonfatal, or a combination), confounder selection, or a combination of these factors.

In addition, blood glucose variation may also be related to the risk of stroke. A study in Taiwan reported that variation in FPG measurements can predict stroke in type 2 diabetes patients [18], showing that the coefficient of variation of FPG (FPG-CV) is a glucose variation measure that pinpoints the association between oscillating plasma glucose and stroke, independent of HbA1c. However, glycemic variation as an independent predictor of stroke among participants without diabetes remains incompletely defined. We investigated the standard deviation (SD) of long-term FPG variability during the 5-year follow-up and observed a novel predictor representing glucose instability (SD), indicating higher risk of the development of fatal stroke outcomes. Thus, the evaluation of the effects of long-term variation in blood glucose levels may be highly significant for stroke prevention.

The major hazards of hyperglycemia were specifically due to the irreversible damage caused by high glucose levels. Hyperglycemia can cause related diseases by damaging vascular endothelial cells in the central nervous system [19]. At least 4 major pathways are involved in hyperglycemia-induced vascular damage. Deleterious metabolic events are thought to be triggered by a single process: the overproduction of superoxide by the mitochondrial electron-transport chain indicates that the activation of oxidative stress by hyperglycemia plays a major role in the pathogenesis of diabetic complications [20]. This overproduction can result in endothelial dysfunction and contribute to vascular damage. Evaluated glucose levels have now been seen as a time-varying phenomenon. However, most previous studies merely used a single measure of FPG to evaluate the association between hyperglycemia and stroke outcomes, failing to take into account the long-term effects in FPG concentrations over time and neglecting repeated obtained measurements of random fasting blood glucose and glycated hemoglobin. Recent studies have reported that glucose fluctuations during glucose swings exhibited a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia [21, 22]. Some studies reported that acute glucose fluctuations can enhance brain microvascular endothelial barrier dysfunction, which may implicate cerebral microvasculature [23]. Acute fluctuations also influence endothelial function, even in nondiabetic subjects and play a significant role in the risk of several chronicle complications [24–27]. The findings indicate that future therapy for DM should not only target the chronic sustained hyperglycemia index, such as fasting glucose levels and HbA1c, but also control glycemic variability in clinical practice. Future studies should pay attention to the verification of whether the risk of stroke outcomes can be controlled, or even reduced, after stabilizing glucose levels.

The strengths of our study include its prospective community-based design and the selection of an analytical strategy called the hierarchical Bayesian framework to assess the average FPG, making full use of all measured blood glucose values to estimate the average blood glucose load during follow-up as accurately as possible and develop a time-varying estimate of glucose levels. This analytical approach enabled us to incorporate clinically obtained measurements of random fasting blood glucose and HbA1c in a single composite estimate of glucose exposure and combine information on glucose levels from these measures, while

stabilizing estimates for individuals with relatively sparse observations, by borrowing information across participants, and accounting for the relative variability in glucose and HbA1c measures to create our combined estimate.

Several limitations should be acknowledged. First, the possibility of confounding by unmeasured or unknown factors cannot be excluded. Some participants may have reached the criteria for diabetes but were allocated into the undiagnosed diabetes group because they did not present clinical symptoms after enrollment, which may be a cause of bias. However, considering that DM is a chronic disease, once the participants were diagnosed during a five-year follow-up, they were assigned to a DM group. Second, the assessment of glucose variability is complex, and only analyzing the long-term FPG levels may overlook the potential effects of postprandial blood glucose levels and some other factors, which may result in a lack of representation [28, 29]. Third, we were unable to consider stroke subtypes and failed to analyze the possibility of differences in outcomes between several stroke subtypes. Additionally, although we have recorded participants' medication status when they enrolled, there was no detailed information on the antihypertensive or lipid-lowering drug, which may be impact factors associated with blood glucose and stroke risk.

In conclusion, we observed that higher FPG levels are associated with an increased risk of stroke incident. Moreover, the variability in long-term FPG is closely correlated with the risk of fatal stroke outcomes. Our findings have important public health value and are highly significant for guiding clinical treatment for diabetes.

Data Availability

Data are available in a public, open-access repository (http://charls.pku.edu.cn/zh-CN/page/data/2011-charls-wave1; http://charls.pku.edu.cn/zh-CN/page/data/2013-charls-wave2; http://charls.pku.edu.cn/zh-CN/page/data/2015-charls-wave4).

Disclosure

The funders of this study had no role in its design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the paper for publication.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xuenan Peng and Jinzhuo Ge contributed equally to this work.

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Supplementary Materials

Figure S1: number of times of participants blood glucose measurements. Figure S2: number of times of participants glycated hemoglobin A1c measurements. Supplementary Table 1: risk of incident stroke associated with average glucose level stratification by sex or hypertension. (*Supplementary Materials*)

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Research Article

High-Serum Angiopoietin-Like Protein 3 Levels Associated with Cardiovascular Outcome in Patients with Coronary Artery Disease

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Background. Angiopoietin-like protein 3 (ANGPTL3) plays a pivotal role in lipid metabolism and angiogenesis, and there is growing interest regarding the association between ANGPTL3 and coronary artery disease (CAD). This study aims to investigate whether ANGPTL3 levels can be used to predict the future occurrence of major adverse cardiovascular events (MACEs) in patients with CAD. *Methods*. Overall, 90 patients with CAD were enrolled between January and December 2012. The study's primary endpoint was incidence of MACEs. Patient follow-up was completed on June 30, 2017. *Results*. Following a median follow-up period of 54 months, 33 MACEs had occurred. Patients reporting MACEs had lower statin use ($P = 0.022$) and higher serum C-reactive protein ($P < 0.001$) and serum ANGPTL3 ($P < 0.001$) levels than those without MACEs. Kaplan–Meier analysis revealed higher cumulative incidence of CV events in the high ANGPTL3 group (median ANGPTL3 level ≥ 222.37 ng/mL) than in the low ANGPTL3 group (log-rank $P = 0.046$). Multivariable Cox regression analysis demonstrated that ANGPTL3 levels were independently associated with MACEs in patients with CAD (hazard ratio: 1.003; 95% confidence interval: 1.000–1.005; $P = 0.026$) after adjusted for age, gender, and body mass index, classical risk factors, and potential confounders. *Conclusions*. Serum ANGPTL3 levels could serve as a biomarker for future occurrence of MACEs in patients with CAD.

1. Introduction

Coronary artery disease (CAD), a significant health problem and global burden, is a leading cause of disability and death worldwide [1]. In developing countries, CAD-related deaths were estimated to be as high as 17.5 million in 2005 and are further expected to increase by 137% in males and 120% in females by 2020 [2]. Patients with CAD are typically asymptomatic initially, and major adverse cardiovascular events (MACEs) are more likely to occur in those presenting with severe CAD and significant clinical conditions, including myocardial infarction (MI), cardiac arrest, stroke, or death from cardiovascular (CV) events [3]. Therefore, it is important to identify biomarkers that may be early indicators of CAD or MACEs and further strengthening preventive strategies.

Angiopoietin-like protein 3 (ANGPTL3) is a 70 kDa 460-amino acid long secretory glycoprotein primarily expressed in the human liver [4]. It can be detected in systemic circulation and has been implicated in angiogenesis and atherogenesis; currently, it is regarded as an endocrine signaling factor [5–8]. ANGPTL3 can regulate serum lipid levels by acting on lipoprotein lipase- (LPL-) and endothelial lipase- (EL-) mediated triglyceride (TG) and phospholipid hydrolysis [9]. An inherited disorder of familial combined hypolipidemia with complete ANGPTL3 deficiency was

associated with protection from CAD due to absence of coronary atherosclerotic plaque [10, 11]. ANGPTL3 plays important roles in lipid and lipoprotein trafficking and metabolism, affecting lipid and glucose metabolism homeostasis [12, 13]. Moreover, ANGPTL3 has demonstrated a positive correlation with CV risk assessment parameters of carotid and femoral artery intima-media thickness in healthy human subjects after adjusting for classical risk factors [14]. In a previous study, we have shown a positive association between serum ANGPTL3 levels and aortic augmentation index values in patients with CAD [15]. Dewey et al. have reported that patients with heterozygous, loss-of-function (LOF) ANGPTL3 variants had significantly lower serum TG, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels than those without these variants [16]. Furthermore, in dyslipidemic mice treated with an ANGPTL3-inhibiting human monoclonal antibody further decreased in the atherosclerotic lesion area than the control group [17].

Although evidence is accumulating of an association between ANGPTL3 and CAD, the association between serum ANGPTL3 levels and long-term CV outcomes in patients with CAD has rarely been reported [11, 16, 18]. Therefore, we conducted this study to determine the association between serum ANGPTL3 levels and MACEs in patients with CAD.

2. Methods

2.1. Patients. Overall, 90 participants with CAD visiting the CV outpatient department of Buddhist Tzu Chi General Hospital, Hualien, Taiwan, were recruited between January and December 2012. This study was approved by the Protection of Human Subjects Institutional Review Board of Tzu Chi University and Hospital. After reviewing patients' medical records, those with >50% stenosis in any segment following coronary angiography were identified as having CAD. Using standard mercury sphygmomanometers with appropriate cuff sizes, morning blood pressure levels were measured by trained staff on the right arm of all study participants after a minimum 10 min rest. Systolic and diastolic blood pressure were measured thrice at 5 min intervals and averaged for analysis. We defined hypertension according to the Eighth Joint National Committee (JNC 8) guidelines (SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or receiving any antihypertensive drugs in the past 2 weeks). Patients were diagnosed with diabetes mellitus (DM) if fasting plasma glucose levels were ≥126 mg/dL or were undergoing oral hypoglycemic medications or insulin therapy [19]. All participants were asked to provide a signed informed consent form before the investigation. Only patients from the CV outpatient department with a CAD history were included. Participants with acute infections, acute MI, or pulmonary edema during blood sampling and those who refused to provide informed consent were excluded.

2.2. Anthropometric Analysis. Patient weight and height were measured in light clothing without shoes (adjusted to nearest 0.5 kg and 0.5 cm, respectively), and body mass index (BMI) was calculated using Quetelet's formula (weight (kg)/height (m^2)) [16, 20, 21].

2.3. Biochemical Investigations. After an 8 h overnight fasting, approximately 5 mL blood was sampled from all participants and immediately centrifuged at 3000 g for 10 min. Serum blood urea nitrogen (BUN), creatinine, fasting glucose, TG, total cholesterol (TCH), HDL-C, LDL-C, and C-reactive protein (CRP) levels were determined using an autoanalyzer (COBAS Integra 800, Roche Diagnostics, Basel, Switzerland) [16, 20, 21]. Serum ANGPTL3 (R&D Systems, Inc., Minneapolis, MN) levels were quantified using the commercial enzyme-linked immunosorbent assay [16]. The intra-assay and interassay coefficients of variation in the measurement for ANGPTL3 were 4.1% and 6.7%, respectively. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.

2.4. CV Event Monitoring. This study's primary endpoint was the incidence of MACEs, including cardiac death, cardiac arrest, MI, stroke, nonfatal stroke or other arterial thrombotic events, and hospitalization from CV conditions, such as unstable or progressive angina and heart failure. Follow-up time (months) was estimated after the last hospital outpatient or inpatient record was reviewed or the last telephone interview was conducted (June 30, 2017). Moreover, event time (months) was estimated when the first MACE occurred. Patient follow-up was conducted by a study nurse who was blinded for participants' baseline measurements and study protocol.

2.5. Statistical Analysis. Data were coded and analyzed using the Statistical Package for Social Sciences (SPSS) version 19.0 (SPSS Inc., Chicago, IL, USA) software. Variable distribution pattern was analyzed with the Kolmogorov–Smirnov test. Normally distributed variables were expressed as mean ± standard deviation, and patient comparisons were performed using Student's independent *t*-test (two-tailed). Data not normally distributed were expressed as median and interquartile range, with patient differences compared using the Mann–Whitney *U* test (TG, fasting glucose, BUN, creatinine, CRP, and ANGPTL3). Data expressed as the number of patients were analyzed using the chi-squared test. Kaplan–Meier survival curves with a log-rank test were used to estimate event-free survival during follow-up based on median ANGPTL3 levels. Cox regression models were used to examine factors associated with CV events. A P value of <0.05 was considered significant.

3. Results

Demographic, clinical, and biochemical characteristics of the 90 patients with CAD are shown in Table 1. Overall, 44 (48.9%) and 70 (77.8%) patients had DM and hypertension, respectively. The high ANGPTL3 group (median ANGPTL3

Table 1: Clinical variables of the 90 coronary artery disease patients according to the serum median of angiopoietin-like protein 3 levels.

Variables	All participants ($n = 90$)	Low ANGPL3 group ($n = 45$)	High ANGPL3 group ($n = 45$)	P value
Age (years)	65.51 ± 9.02	67.04 ± 10.04	63.98 ± 7.69	0.107
Height (cm)	161.14 ± 8.18	161.80 ± 7.61	160.49 ± 8.75	0.456
Body weight (kg)	68.61 ± 12.26	69.07 ± 12.46	68.15 ± 12.18	0.724
Body mass index (kg/m^2)	26.31 ± 3.52	26.29 ± 3.76	26.33 ± 3.30	0.960
Systolic blood pressure (mmHg)	131.08 ± 16.66	129.78 ± 16.65	132.38 ± 16.76	0.462
Diastolic blood pressure (mmHg)	71.99 ± 9.46	73.27 ± 9.62	70.71 ± 9.23	0.202
Total cholesterol (mg/dL)	163.60 ± 32.20	160.09 ± 28.86	167.11 ± 35.21	0.304
Triglycerides (mg/dL)	127.50 (88.75–181.00)	127.00 (91.00–155.50)	128.00 (88.50–201.00)	0.634
HDL-C (mg/dL)	43.81 ± 11.43	43.36 ± 9.25	43.27 ± 13.35	0.758
LDL-C (mg/dL)	95.76 ± 26.61	92.64 ± 25.56	98.87 ± 27.55	0.270
Fasting glucose (mg/dL)	111.00 (95.75–150.50)	111.00 (94.00–167.50)	111.00 (98.50–134.00)	0.865
Blood urea nitrogen (mg/dL)	16.00 (13.00–19.00)	16.00 (14.00–19.00)	16.00 (13.00–19.00)	0.509
Creatinine (mg/dL)	1.10 (0.90–1.30)	1.10 (0.90–1.25)	1.10 (0.90–1.30)	0.687
eGRF (mL/min)	68.53 ± 18.17	68.71 ± 19.77	69.02 ± 18.59	0.936
ANGPTL3 (ng/mL)	222.37 (152.57–320.12)	152.94 (93.10–197.90)	318.40 (278.94–463.04)	<0.001*
C-reactive protein (mg/dL)	0.20 (0.14–0.26)	0.17 (0.13–0.20)	0.26 (0.18–0.39)	<0.001*
Female (%)	23 (25.6)	9 (20.0)	14 (31.1)	0.227
Diabetes (%)	44 (48.9)	22 (48.9)	22 (48.9)	1.000
Hypertension (%)	70 (77.8)	37 (82.2)	33 (73.2)	0.310
ACE inhibitor use	28 (31.1)	15 (33.3)	13 (28.9)	0.649
ARB use	36 (40.0)	18 (40.0)	18 (40.0)	1.000
β-blocker use	54 (60.0)	29 (64.4)	25 (55.6)	0.389
CCB use	30 (33.3)	17 (37.8)	13 (28.9)	0.371
Statin use	67 (74.4)	33 (73.3)	34 (75.6)	0.809
Fibrate use	16 (17.8)	10 (22.2)	6 (13.3)	0.270
One-vessel CAD	37 (41.1)	23 (51.1)	14 (31.1)	0.151
Two-vessel CAD	30 (33.3)	12 (26.7)	18 (40.0)	
Three-vessel CAD	23 (25.6)	10 (22.2)	13 (28.9)	

Values for continuous variables are given as means ± standard deviation and compared by Student's t-test; variables not normally distributed are given as medians and interquartile range and compared by Mann–Whitney U test; values are presented as number (%), and analysis was performed using the chi-square test. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ANGPTL3, angiopoietin-like protein 3; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; CCB, calcium-channel blocker; and CAD, coronary artery disease. *$P < 0.05$ was considered statistically significant.

level > 222.37 ng/mL) showed significantly higher serum CRP levels than the low ANGPTL3 group (median ANGPTL3 level ≤ 222.37 ng/mL; $P < 0.001$). Patients reported the use of the angiotensin-converting enzyme inhibitor (ACEi; $n = 28$; 31.1%), angiotensin-receptor blockers (ARB; $n = 36$; 40.0%), β-blockers ($n = 54$; 60.0%), calcium-channel blockers (CCB; $n = 30$; 33.3%), statins ($n = 67$; 74.4%), and fibrate ($n = 16$; 17.8%). No significant differences were found between ANGPTL3 groups considering age, sex, BMI, BP, DM, or hypertension comorbidities or ACEi, ARB, β-blockers, CCB, statins, or fibrate use.

After a median follow-up of 54 months, 33 CV events were reported. Patients with CV events had higher ANGPTL3 ($P < 0.001$), CRP ($P < 0.001$) levels, and severity of baseline CAD ($P < 0.001$) and lower statin use ($P = 0.022$) than those without CV events. No significant differences in age, sex, DM, or hypertension comorbidities or ACEi, ARB, β-blockers, CCB, or fibrate use were observed between patients with and without CV events (Table 2).

Kaplan–Meier analysis revealed higher cumulative incidence of CV events in the high than in the low ANGPTL3 group (log-rank $P = 0.046$; Figure 1). In patients with CAD, the unadjusted and Cox regression analysis of ANGPTL3 levels with other factors associated with CV events is presented in Table 3. In CAD patients, ANGPTL3 remained a significant predictor of the increased risk for CV events (unadjusted hazard ratio (HR) per increase of ANGPTL3 by 1 ng/mL: 1.003, 95% confidence interval (CI): 1.002–1.004; $P < 0.001$). ANGPTL3 remained significantly associated with an increased risk for CV events following adjustment for age, gender, and BMI (adjusted HR 1.003, 95% CI: 1.002–1.005; $P < 0.001$) as well as following additional adjustment for DM, hypertension, fasting glucose, TCH, TG, LDL-C, eGFR, statin used, serum CRP level, and severity of baseline CAD (adjusted HR 1.003, 95% CI: 1.000–1.005; $P = 0.026$).

4. Discussion

This study reveals higher fasting ANGPTL3 levels that developed MACEs in patients with CAD during follow-up, and serum ANGPTL3 levels are independently associated with an increased risk of MACEs in these patients.

Previous studies have reported that inflammation and dyslipidemia are pivotal contributors to initiation and progression of coronary atherosclerosis [22–24]. Systemic inflammatory status is positively associated with severity of CAD, and CRP is a well-established biomarker of inflammation [22, 25]. The present study confirms that patients with CAD having high-serum ANGPTL3 levels have significantly higher CRP values than those with low ANGPTL3

Table 2: Clinical variables of the 90 coronary artery disease patients with or without the cardiovascular event.

Variables	Participants without cardiovascular events ($n = 57$)	Participants with cardiovascular events ($n = 33$)	P value
Age (years)	65.70 ± 9.29	65.18 ± 8.68	0.794
Height (cm)	161.11 ± 8.70	161.21 ± 7.31	0.953
Body weight (kg)	68.74 ± 12.32	68.38 ± 12.34	0.896
Body mass index (kg/m^2)	26.39 ± 3.61	26.18 ± 3.41	0.793
Systolic blood pressure (mmHg)	128.82 ± 15.85	134.97 ± 17.55	0.092
Diastolic blood pressure (mmHg)	71.16 ± 9.54	73.42 ± 9.30	0.276
Total cholesterol (mg/dL)	159.65 ± 30.44	170.42 ± 34.45	0.127
Triglycerides (mg/dL)	111.00 (87.50–153.00)	150.00 (90.50–208.00)	0.117
HDL-C (mg/dL)	42.72 ± 9.81	45.70 ± 13.75	0.236
LDL-C (mg/dL)	93.93 ± 26.58	98.91 ± 26.77	0.395
Fasting glucose (mg/dL)	107.00 (96.50–132.50)	111.00 (95.00–181.50)	0.533
Blood urea nitrogen (mg/dL)	16.00 (14.00–19.00)	15.00 (12.00–19.00)	0.176
Creatinine (mg/dL)	1.10 (0.90–1.30)	1.00 (0.90–1.25)	0.187
eGFR (mL/min)	66.36 ± 17.04	72.27 ± 19.69	0.138
ANGPTL3 (ng/mL)	206.67 (110.39–274.38)	318.40 (195.56–490.61)	<0.001*
C-reactive protein (mg/dL)	0.16 (0.12–0.21)	0.26 (0.21–0.55)	<0.001*
Female (%)	15 (26.3)	8 (24.2)	0.828
Diabetes (%)	25 (43.9)	19 (57.6)	0.210
Hypertension (%)	43 (75.4)	23 (81.8)	0.483
ACE inhibitor use	21 (36.8)	7 (21.2)	0.123
ARB use	21 (36.8)	15 (45.5)	0.422
β-blocker use	33 (57.9)	21 (63.6)	0.592
CCB use	17 (29.8)	13 (39.4)	0.353
Statin use	47 (82.5)	20 (60.6)	0.022*
Fibrate use	8 (14.0)	8 (24.2)	0.222
One-vessel CAD	33 (57.9)	4 (12.1)	<0.001*
Two-vessel CAD	14 (24.6)	16 (48.5)	
Three-vessel CAD	10 (17.5)	13 (39.4)	

Values for continuous variables are given as means ± standard deviation and compared by Student's t-test; variables not normally distributed are given as medians and interquartile range and compared by Mann–Whitney U test; values are presented as number (%), and analysis was performed using the chi-square test. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; ANGPTL3, angiopoietin-like protein 3; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; CCB, calcium-channel blocker; and CAD, coronary artery disease. *$P < 0.05$ was considered statistically significant.

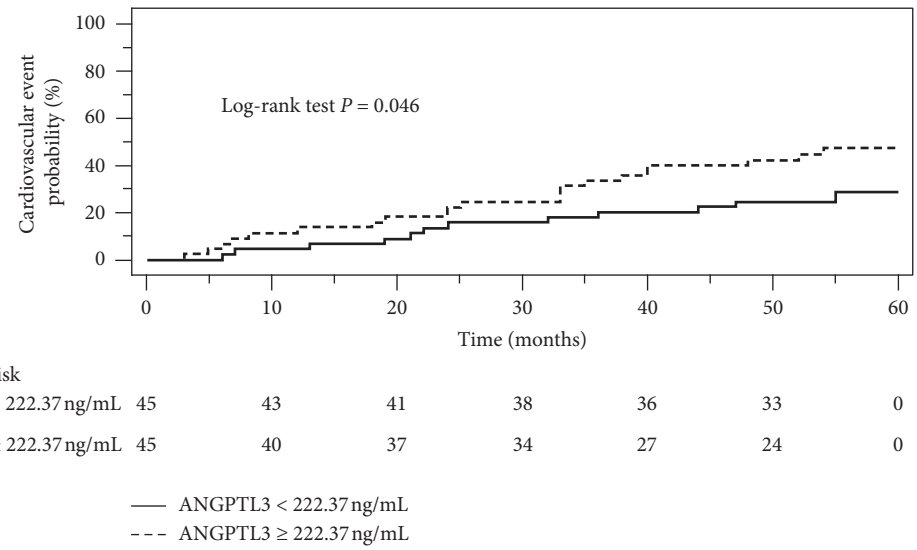

Figure 1: Kaplan–Meier analysis of cardiovascular events in 90 patients with coronary artery disease according to median serum angiopoietin-like protein 3 (ANGPTL3) levels.

Table 3: Hazard ratio for cardiovascular events by multivariable Cox regression of angiopoietin-like protein 3 levels among the 90 patients with coronary artery disease.

ANGPTL3 (ng/mL)	Unadjusted		Model 1		Model 2		Model 3	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Per 1 ng/mL ANGPTL3 increase	1.003 (1.002–1.004)	<0.001*	1.003 (1.002–1.005)	<0.001*	1.004 (1.003–1.006)	<0.001*	1.003 (1.000–1.005)	0.026*

Model 1 is adjusted for age, gender, and body mass index. Model 2 is adjusted for Model 1 variables and for diabetes mellitus, hypertension, fasting glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol, and estimated glomerular filtration rate. Model 3 is adjusted for Model 2 variables and for C-reactive protein, statin used, and severity of coronary artery disease. *$P < 0.05$ was considered statistically significant after Cox regression analysis. ANGPTL3, angiopoietin-like protein 3; HR, hazard ratio; and CI, confidence interval.

levels. Patients with CAD who developed new MACEs had significantly higher CRP levels than those without MACEs during the follow-up period. Although no direct evidence of ANGPTL3-induced inflammation exists, certain studies have indicated that other ANGPTL family members, such as ANGPTL2, promote chronic adipose tissue inflammation and plasma CRP positively correlating with plasma ANGPTL4 in patients with metabolic syndrome and type 2 diabetes [26, 27]. Further studies are necessary to investigate the precise mechanisms of ANGPTL3 and inflammation in humans.

ANGPTLs are important modulators of lipoprotein metabolism and potential targets for CV disease risk regulation [17]. Animal studies have shown that deletion of *Angptl3* can reduce atherosclerosis development in apolipoprotein *E* knockout mice [7]. Higher circulating ANGPTL3 levels were observed in patients with CAD compared with healthy controls [11]. In the study by Stitzielet al., three individuals with complete ANGPTL3 deficiency due to heterozygous ANGPTL3 LOF mutations demonstrated no evidence of coronary atherosclerotic plaque compared with matched first-degree relative controls without ANGPTL3 LOF mutations [11]. Whole-exome sequencing analysis of 58,335 participants from the DiscovEHR study and 130,483 participants from four human genetic cohorts (including Duke Catheterization Genetics cohort, Copenhagen General Population Studies, the University of Pennsylvania Medicine BioBank, and the Taiwan Metabochip consortium) revealed that heterozygous ANGPTL3 LOF carriers with approximately 50% lower serum ANGPTL3 levels than noncarriers had a 39% lower probability of CAD [17]. A recent study has shown that patients with the lowest circulating ANGPTL3 levels (mimicking pharmacological inhibition of ANGPTL3) had a 35% reduced risk of MI compared with those with highest levels [11]. In our previous study, circulating ANGPTL3 levels positively correlated with aortic augmentation index values (a marker of arterial stiffness significantly associated with CAD degree) among patients with CAD, even after adjusting for confounding factors [16]. The present study corroborates that patients with CAD who developed MACEs have significantly higher ANGPTL3 levels than patients without MACEs. These findings indicate that elevated serum ANGPTL3 level is an independent risk factor for CV events in populations with established CAD and suggest that including ANGPTL3 in a CV risk model may increase the predictive power for early detection of MACEs.

In multivariable Cox regression analysis, elevated ANGPTL3 levels independently increased the risk of MACEs in patients with CAD. The mechanism underlying the induction of adverse CV event by ANGPTL3 among patients with CAD is likely to be multifactorial. Dyslipidemia is the major contributor to CV diseases [12]. The ANGPTL3 deficiency-related hypolipidemic phenotype is driven by enhanced lipoprotein turnover resulting in impaired energy substrate distribution in tissues [9]. Studies in mice and humans have shown that ANGPTL3 acts as a potent inhibitor of LPL, clearing TG-rich lipoproteins from circulation, particularly in the postprandial state [28]. Additionally, ANGPTL3 is an endogenous inhibitor of EL which might regulate HDL-C particles and affect glucose homeostasis [29, 30]. LOF variants in *ANGPTL3* have been associated with decreased plasma TG, LDL-C, and HDL-C levels via loss of LPL and EL inhibition [17, 30]. Furthermore, a study in Ldlr-deficient mice revealed that ANGPTL3 modulates serum LDL-C clearance independently of the LDL receptor [31]. Alternatively, decreased LDL-C levels may be the result of lower LDL precursor and hepatic VLDL particle secretion rates, suggesting that ANGPTL3 may effectively reduce serum LDL-C levels in patients with homozygous familial hypercholesterolemia with a complete LDL receptor-mediated LDL-C uptake deficiency [31]. In the DiscovEHR study, ANGPTL3 LOF mutation carriers had significantly lower circulating TG, LDL-C, and HDL-C (27%, 9%, and 4%, respectively) levels than noncarriers [17]. Moreover, the genetic and therapeutic antagonism of *Angptl3* in mice and *ANGPTL3* in humans has been associated with decreased levels of all major lipid fractions, thereby providing protection from atherosclerotic CV disease [17].

Atherosclerosis of the coronary artery is associated with endothelial dysfunction, adipocyte metabolism dysregulation, and various inflammatory processes [32]. ANGPTL3 has potential atherogenic properties and could directly promote atherosclerosis in humans [15]. ANGPTL3 acts as proangiogenic and could induce angiogenesis *in vivo* via binding of the C-terminal fibrinogen-like domain to the integrin $\alpha_v\beta_3$ receptor on vascular endothelial cells, affecting blood vessel formation via the induction of integrin-$\alpha_v\beta_3$-dependent endothelial cell migration and adhesion [5]. ANGPTL3

induced angiogenesis with a magnitude comparable to vascular endothelial growth factor-A, which promotes intimal thickening and induces atherosclerosis [33]. Additionally, the association between *ANGPTL3* polymorphisms and coronary plaque is independent of lipids and other confounding variables in MI survivors [34]. Positively associated with plasma ANGPTL3 level and intima-media thickness of the human carotid and femoral arteries is independent of lipids and other classical risk factors, including age, BP, and plasma lipid and glucose levels [15]. All these studies indicate that ANGPTL3 is significantly associated with atherosclerosis and is independent of plasma lipid levels.

The present study has some limitations. First, a limited number of MACE patients, all recruited at a single center, were included. Additionally, lifestyle habits known to influence the occurrence of MACEs, including smoking, alcohol consumption, physical inactivity, and unhealthy diet, were not evaluated and could restrict the study's predictive power. Second, although several medications commonly used by patients with CAD may influence the underlying inflammatory and atherosclerotic status, the present study demonstrated that ACEi, ARB, β-blockers, CCB, and fibrates have no impact on circulating ANGPTL3 levels or on new MACE development. However, statin use was significantly associated with a lower occurrence of new MACEs in patients with CAD [35, 36]. Further studies are necessary to clarify the impact of the above medications on serum ANGPTL3 levels and new MACE development in the CAD population. Finally, although we propose an explanation for the mechanism underlying serum ANGPTL3-induced MACEs in patients with CAD, further studies are required before a direct causal relationship can be established between circulating ANGPTL3 levels and development of MACEs in this patient population.

5. Conclusion

The present study shows that elevated ANGPTL3 levels represent an independent risk factor for CV events in patients with CAD, with an increased predictive value for MACEs.

Data Availability

The data underlying this study are available from the corresponding author on reasonable request.

Disclosure

The funding source had no role in the conception and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

J. H. Wang and B. G. Hsu conceived and designed the experiments. C. J. Lee and J. H. Wang performed the experiments. C. J. Lee and B. G. Hsu analyzed the data. M. C. Chen and B. G. Hsu wrote the manuscript. All of the authors reviewed and approved the final version of this paper. Ming-Chun Chen and Bang-Gee Hsu contributed equally to this study.

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Research Article

High Neutrophil-to-Lymphocyte Ratio Predicts Hemorrhagic Transformation in Acute Ischemic Stroke Patients Treated with Intravenous Thrombolysis

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Background. The relationship between the neutrophil-to-lymphocyte ratio (NLR) and hemorrhagic transformation (HT) in acute ischemic stroke (AIS) treated with intravenous thrombolysis (IVT) remains unclear. This study assessed whether high NLR is associated with HT in this population. *Methods*. Data were prospectively collected for continuous patients with AIS treated with IVT and retrospectively analyzed. Clinical variables included age, sex, vascular risk factors, National Institutes of Health Stroke Scale (NIHSS) score, onset-to-treatment time, and initial hematologic and neuroimaging findings. HT was confirmed by imaging performed within 3 days after IVT. Symptomatic HT (sHT) was defined as NIHSS score increased by 4 points compared with that on admission according to previously published criteria. The NLR value was based on the blood examination before IVT, and high NLR was defined as ≥75th percentile. *Results*. The study included 285 patients (201 (70.5%) males, the mean age was 62.3 years (range 29–89)). Seventy-two (25.3%) patients presented with HT, including three (1.1%) with sHT. The median NLR was 2.700 (1.820–4.255, interquartile range). Seventy-one (24.9%) patients had a high NLR (≥4.255) on admission. Univariate analysis indicated that patients with HT had higher NIHSS scores ($P < 0.001$), systolic blood pressure (SBP), platelet counts, lymphocyte counts, and NLR ($P < 0.05$), as well as a greater prevalence of high NLR than those without HT (37.5% vs. 20.7% and $P = 0.004$). Patients with HT were more likely to have hypertension and AF. As lymphocyte counts and high NLR were highly correlated, we used two logistic regression models. In model 1 (with high NLR), NIHSS score on admission (odds ratio (OR) = 1.110, 95% confidence interval (CI) = 1.015–1.044, and $P = 0.001$), AF (OR = 3.986, 95% CI = 2.095–7.585, and $P < 0.001$), and high NLR (OR = 2.078, 95% CI = 1.078–4.003, $P = 0.029$, sensitivity 0.375, and specificity 0.793) were significant predictors of HT. In model 2 (with lymphocyte counts), NIHSS score on admission (OR = 1.111, 95% CI = 1.050–1.175, and $P < 0.001$), AF (OR = 3.853, 95% CI = 2.048–7.248, and $P < 0.001$), and lymphocyte counts (OR = 0.522, 95% CI = 0.333–0.819, and $P = 0.005$) were significantly associated with HT. *Conclusions*. High NLR could be a useful marker for predicting HT in AIS patients after IVT.

1. Introduction

Intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (r-tPA) is an effective treatment for acute ischemic stroke (AIS) when administered within the hyperacute period [1, 2]. Hemorrhagic transformation (HT) is common in AIS with an incidence ranging from 8.5% to 40% [3–5], and symptomatic HT (sHT) is a risk factor for poor prognosis after AIS [6]. IVT has been reported to increase the incidence of HT markedly [7]. Atrial fibrillation (AF) [8, 9], National Institutes of Health Stroke Scale (NIHSS) score [10], blood glucose level [11], leukoaraiosis

[12], dual antiplatelet agent treatment before IVT [13], and systolic blood pressure variability [14] are the predictors of HT after IVT.

In recent years, researchers have attempted to identify convenient serum biomarkers to help predict AIS outcomes. Several studies reported that a high neutrophil-to-lymphocyte ratio (NLR) was predictive of HT in AIS patients [15, 16]. However, there were limited data on the relationship between NLR and HT in AIS patients treated with IVT. The present study was performed to assess whether high NLR is associated with HT in AIS patients after IVT.

2. Methods

2.1. Patients. AIS patients treated with IVT after admission to Dongguan People's Hospital between 1 January 2016 and 31 May 2019 were continuously recruited. The inclusion criteria were as follows: (1) age >18 years, (2) AIS confirmed by magnetic resonance imaging (MRI), and (3) onset of stroke symptoms within 4.5 hours and treated with r-tPA. The exclusion criteria were as follows: (1) hemorrhagic lesions detected on initial computed tomography (CT), (2) temporary or permanent contraindications for MRI scan, (3) no acute lesion on diffusion-weighted imaging (DWI), and (4) additional endovascular therapy after IVT. This study was approved by the hospital ethics committee (approval number: KYKT2018-002). The consent of each subject was obtained in accordance with the Declaration of Helsinki.

2.2. Data Collection. NIHSS score, onset-to-treatment time (OTT), and blood pressure on admission were collected, as well as demographic data including age, sex, and history of hypertension, diabetes mellitus, smoking, AF, antiplatelet therapy, oral anticoagulant therapy, and previous stroke. Initial counts for white blood cells, neutrophils, lymphocytes, and platelets before IVT were also collected, and NLR was calculated accordingly.

2.3. MRI Analysis. As MRI is more sensitive than CT for detecting HT in AIS [17], we used MRI to confirm and categorize HT. A brain MRI scan was performed for each participant using a 3.0T system (Skyra, Siemens Medical Solutions, Erlangen, Germany) within 3 days after IVT. The sequences of MRI included T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), DWI, and susceptibility-weighted imaging (SWI).

The parameters of each sequence were shown as follows: axial SE T1: time of repetition (TR) = 1500 ms, time of echo (TE) = 11 ms, field of view (FOV) = 220 mm, slice thickness/gap = 4 mm/1.2 mm, and time of acquisition = 1 min 26 s; Turbo spin echo (TSE) T2: TR = 4720 ms, TE = 96 ms, FOV = 220 mm, slice thickness/gap = 4 mm/1.2 mm, and time of acquisition = 1 min 50 s; DWI: TR = 4640 ms, TE = 67 ms, FOV = 230 mm, slice thickness/gap = 4 mm/1.2 mm, spin echo planar imaging (EPI) factor = 91, and acquisition time = 1 min 44 s; SWI: TR = 27 ms, TE = 20 ms, FOV = 220 mm, slice thickness/gap = 3 mm/0.6 mm, and time of acquisition = 2 min 28 s.

HT was defined as the secondary hemorrhage within or away from the infarction area, which appeared as hypointensive lesions on SWI [18, 19] and DWI [20]. Calcification was distinguished by CT combined with SWI. As chronic infarction lesions and corresponding old hemorrhage can be detected by T1-weighted imaging (T1WI) and T2-weighted imaging (T2WI), we used these sequences to differentiate acute HT from old hemorrhage.

(1) sHT was defined as NIHSS score increase by ≥ 4 points compared with that on admission [21].

(2) When HT was positive on MRI, the images were categorized into hemorrhagic infarct (HI) and parenchymatous hemorrhage (PH) according to the ECASS II criteria as follows: HI1, small petechiae along the margins of the infarct; HI2, confluent petechiae within the infarcted area but no space effect; PH1, blood clots in ≤30% of the infarcted area with some slight space-occupying effect; and PH2, blood clots in >30% of the infarcted area with substantial space-occupying effect.

(3) Small vessel disease (SVD) burden was rated on brain MRI by the presence of lacunes, white matter hyperintensities, cerebral microbleeds, and perivascular spaces. The presence of each SVD feature was summed as an "SVD score" (range 0–4) [22].

Two neurologists (D.H.Q. and J.F.Q.) experienced in neuroimaging and trained by a neuroradiologist (M.Q.L.) evaluated the imaging findings for the presence of HT independently, blinded to the patients' clinical information. After observing the images individually, the two observers reviewed all the images to achieve final interobserver consensus.

2.4. Statistical Analysis. Statistical analyses were conducted using SPSS for Windows (v.20.0, IBM Corp., Armonk, NY, USA). Continuous variables with a normal distribution are reported as mean ± SD, and nonnormally distributed variables as median and interquartile range (IQR). All subjects were divided into two groups based on the presence of HT. Variables were compared using t-tests, Mann–Whitney U tests, Pearson χ^2 tests, or Fisher's exact tests, as appropriate. Variables with $P < 0.05$ in the univariate analysis were included in further binary multivariate logistic regressions. Statistical significance was defined as $P < 0.05$ (two-sided).

3. Results

During the study period, 306 consecutive patients received IVT with r-tPA within 4.5 hours of stroke onset. In the present study, 21 patients were excluded for the following reasons: additional endovascular therapy after IVT ($n = 2$), permanent or temporary contraindication for MRI ($n = 3$), and no acute lesion found on DWI ($n = 16$). A total of 285 patients were ultimately included.

The average age of the 285 patients was 62.3 ± 12.0 years, and 201 (70.5%) patients were male. The median interval between stroke onset and MRI scanning was 42 (range,

13–65) hours. Antiplatelet agents (aspirin 100 mg/day or clopidogrel 75 mg/day) were prescribed 24 hours after IVT when PH (7 patients, 2.4%) was excluded, and no anticoagulants were prescribed during the acute phase. Among the 72 (25.3%) patients with HT confirmed by MRI, 44 (15.4%) presented with HI1, 21 (7.4%) with HI2, 4 (1.4%) with PH1, and 3 (1.1%) with PH2. Three (1.1%) patients had sHT. No remote HTs were found in our study. The mean OTT was 200.4 ± 55.9 minutes, and the median NIHSS score on admission was 7 (4–10, IQR). The median NLR was 2.700 (1.820–4.255, IQR). High NLR was defined as an NLR value ≥4.255 (75th percentile). Seventy-one (24.9%) patients had a high NLR before IVT. The demographic and clinical characteristics of this study are shown in Table 1.

3.1. Univariable Analysis. Compared with those without HT, patients with HT had significantly higher NIHSS scores ($P < 0.001$), systolic blood pressure (SBP), platelet counts, lymphocyte counts, and NLR ($P < 0.05$). They also had a greater prevalence of high NLR than those without HT (37.5% vs. 20.7% and $P = 0.004$). Patients with HT were also more likely to have a history of hypertension and AF. The univariable analysis results are shown in Table 2.

3.2. Multivariate Logistic Regressions. Variables that were significantly different between the two groups in the univariable analysis were entered into subsequent logistic regression model. Since lymphocyte counts and high NLR were highly correlated ($r = -0.499$), we used two separate logistic regression models. Besides, hypertension and SBP on admission were also highly correlated ($r = 0.412$); therefore, SBP on admission was not included in the regression models to avoid the risk of multicollinearity. In model 1 (with high NLR), NIHSS score on admission (odds ratio (OR) = 1.110, 95% confidence interval (CI) = 1.015–1.044, and $P = 0.001$), AF (OR = 3.986, 95% CI = 2.095–7.585, and $P < 0.001$), and high NLR (OR = 2.078, 95% CI = 1.078–4.003, $P = 0.029$, sensitivity 0.375, and specificity 0.793) were significant predictors of HT. In model 2 (with lymphocyte counts), NIHSS score on admission (OR = 1.111, 95% CI = 1.050–1.175, and $P < 0.001$), AF (OR = 3.853, 95% CI = 2.048–7.248, and $P < 0.001$), and lymphocyte counts (OR = 0.522, 95% CI = 0.333–0.819, and $P = 0.005$) were significantly related with HT. Platelet counts and hypertension were not significantly associated with HT in either model. The multivariate logistic regression results for HT risk factors are shown in Table 3.

Table 1: Demographic and clinical characteristics of the study sample.

Characteristics	Mean (SD)/median (IQR)/*n* (%) (*n* = 285)
Age (years)	62.3 ± 12.0
Men (*n*, %)	201 (70.5%)
Hypertension (*n*, %)	211 (74.0%)
Diabetes mellitus (*n*, %)	79 (27.7%)
Smokers/ex-smokers (*n*, %)	102 (35.8%)
Atrial fibrillation (*n*, %)	67 (23.5%)
Previous stroke (*n*, %)	52 (18.2%)
PAT (*n*, %)	23 (8.1%)
POAT (*n*, %)	8 (2.8%)
Time of poststroke antiplatelet therapy	
Before MRI scan (*n*, %)	212 (74.4%)
After MRI scan (*n*, %)	66 (23.2%)
No antiplatelet therapy	7 (2.4%)
OTT (minutes)	200.4 ± 55.9
NIHSS score on admission	7 (4–10)*
Platelet counts (10^9/L)	214.0 ± 55.9
WBC counts (10^9/L)	8.4 ± 2.9
Neutrophil counts (10^9/L)	5.8 ± 2.8
Lymphocyte counts (10^9/L)	1.9 ± 0.9
NLR	2.7 (1.8–4.3)*
High NLR (*n*, %)	71 (24.9%)
Uric acid (mmol/L)	390.1 ± 108.6
BG on admission (mmol/L)	7.5 ± 3.2
SBP on admission (mmHg)	157.4 ± 24.8
DUB on admission (mmHg)	91.4 ± 16.5
SVD burden	1 (0–2)*
Hemorrhagic transformation (*n*, %)	72 (25.3%)
HI1	44 (15.4%)
HI2	21 (7.4%)
PH1	4 (1.4)
PH2	3 (1.1%)
sHT (*n*, %)	3 (1.1%)

BG = blood glucose; DBP = diastolic blood pressure; HI = hemorrhagic infarct; NIHSS=National Institutes of Health Stroke Scale; NLR=neutrophil-to-lymphocyte ratio; OTT = onset-to-treatment time; PAT = previous antiplatelet therapy; PH = parenchymatous hemorrhage; POAT = previous oral anticoagulant therapy; SBP = systolic blood pressure; sHT = symptomatic hemorrhagic transformation; SVD = small vessel disease; WBC = white blood cell *median (25Q–75Q).

4. Discussion

In our study, high NLR (≥4.255) was significantly associated with HT in AIS patients treated with IVT, which was in accordance with two previous studies [15, 16]. The mechanism of HT remains uncertain. The disruption of blood-brain barrier (BBB) and focal inflammation of the infarcted lesion have been reported to be correlated with HT [23]. In accordance with an existing report, neutrophils play a role in BBB in AIS [24]. Increased neutrophils can result in enhanced expression of matrix metalloproteinase-9 [25], which has been linked to BBB damage and HT in AIS patients [26–28]. Lymphocytes play important roles in inflammation [29, 30]. However, the precise effects depend on the subtype of lymphocytes. Some are neuroprotective [31, 32], while others exacerbate inflammation [33, 34]. A high NLR value represents high neutrophil counts and/or low lymphocyte counts. NLR is considered a good marker that simultaneously reflects the negative effects of neutrophils and positive effects of lymphocytes in stroke patients [35, 36]. High NLR was found to predict poor outcomes of AIS patients [37–39]. In our study, both lymphocyte counts and high NLR were significantly associated with HT in logistic regression analyses. However, absolute lymphocyte counts vary among individuals, even in healthy subjects. Thus, NLR may be a more stable and suitable marker than absolute lymphocyte counts for predicting HT. Neutrophil

Table 2: Comparisons of clinicial and laboratory variables in IVT patients with and without HT.

Variable	With HT ($N = 72$)	Without HT ($N = 213$)	$t/X^2/z$ value	P value
Age[a] (years)	64.0 ± 12.3	61.8 ± 11.9	−1.354	0.177
Men[b]	51 (70.8%)	150 (70.4%)	0.004	0.947
NIHSS score on admission[c]	10 (6–14.8)	6 (4–9)	−5.429	<0.001
Hypertension[b]	46 (63.9%)	165 (77.5%)	5.159	0.023
Diabetes[b]	19 (26.4%)	60 (28.2%)	0.085	0.770
Smokers[b]	25 (34.7%)	77 (36.2%)	0.048	0.827
AF[b]	35 (48.6%)	32 (15.0%)	33.759	<0.001
Previous stroke[b]	13 (18.1%)	39 (18.3%)	0.002	0.961
PAT[b]	3 (4.2%)	20 (9.4%)	1.979	0.213
POAT[b]	3 (4.2%)	5 (2.3%)	0.653	0.421
Antiplatelet therapy prescribed before MRI scan[b]	45 (62.5%)	167 (78.4%)	2.315	0.128
Uric acid[a] (mmol/L)	369.2 ± 108.8	396.9 ± 107.9	1.844	0.066
OTT[a] (minutes)	195.3 ± 52.6	202.2 ± 56.9	0.898	0.37
Platelet counts[a] (10^9/L)	201.3 ± 43.2	218.3 ± 59.0	2.246	0.025
WBC counts[a] (10^9/L)	8.6 ± 3.2	8.4 ± 2.8	−0.586	0.558
Neutrophil counts (10^9/L)	6.3 ± 3.3	5.6 ± 2.6	−1.759	0.08
Lymphocyte counts (10^9/L)	1.6 ± 0.7	2.1 ± 0.9	4.349	<0.001
High NLR[c]	27 (37.5%)	44 (20.7%)	8.16	0.004
BG on admission[a] (mmol/L)	7.6 ± 3.4	7.5 ± 3.2	−0.280	0.779
SBP on admission[a] (mmHg)	152.0 ± 24.1	159.2 ± 24.8	2.135	0.035
DBP on admission[a] (mmHg)	90.1 ± 15.0	91.8 ± 17.0	0.754	0.452
SVD burden[c]	0 (0–1)	1 (0–2)	−1.434	0.152

[a]Mean (SD), t-test; [b]n (%), chi-square test; [c]Mann-Whitney U test. AF = atrial fibrillation; BG = blood glucose; DBP = diastolic blood pressure; HTh = e-morrhagic transformation; NIHSS = National Institutes of Health Stroke Scale; NLR = neutrophil-to-lymphocyte ratio; OTT = onset-to-treatment time; PAT = previous antiplatelet therapy; POAT = previous oral anticoagulant therapy; SBP = systolic blood pressure; SVD = small vessel disease; WBC = white blood cell.

Table 3: Multivariate logistic regression of risk factors for hemorrhagic transformation.

Variable	β	OR (95% CI)	P value
Model 1 (with high NLR entered)			
NIHSS score on admission	0.100	1.110 (1.015–1.044)	0.001
Hypertension	−0.399	0.671 (0.352–1.280)	0.226
AF	1.383	3.986 (2.095–7.585)	<0.001
Platelet counts	−0.006	0.995 (0.988–1.001)	0.078
High NLR	0.731	2.078 (1.078–4.003)	0.029
Model 2 (with lymphocyte counts entered)			
NIHSS score on admission	0.105	1.111 (1.050–1.175)	<0.001
Hypertension	−0.440	0.644 (0.336–1.237)	0.181
AF	1.349	3.853 (2.048–7.248)	<0.001
Platelet counts	−0.004	0.996 (0.990–1.003)	0.240
Lymphocyte counts	0.650	0.522 (0.333–0.819)	0.005

AF = atrial fibrillation; NIHSS = National Institutes of Health Stroke Scale; NLR = neutrophil-to-lymphocyte ratio.

counts were not significant in the univariable analysis. However, since neutrophil counts were highly related with NLR ($r = 0.676$), our findings did not contradict previous studies. NLR is easily evaluated with a routine blood test, making it an economic and effective marker, even in regional hospitals.

There were several advantages to our study. First, to the best of our knowledge, it was one of the few that focused on the association between high NLR and HT in IVT-treated AIS patients. Second, all the participants had relatively complete data of neuroimaging including SWI. However, our results should be considered in the context of several limitations. First, repeated MR scanning was not performed, which might have led to underestimation of HT in the subacute phase. Second, we lacked dynamic NLR data, which would be considered a more effective predictor. Third, the numbers of cases with PH1, PH2, or sHT were too small to perform further analyses of these severe HT subtypes.

5. Conclusion

High NLR was a useful predictor of HT in AIS patients after IVT. Further prospective studies with larger sample sizes, repeated MR scans, and dynamic NLR are warranted.

Data Availability

The data used to support the findings of the study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Y. L. L. and Y. K. C. participated in the conception and design of the study, the analysis of clinical data, and critical revision of the manuscript for scientific validity. D. H. Q., J. F. Q., and M. Q. L. analyzed the imaging data. J. K. L., H. P. Y., and P. S. X. helped to acquire raw data. All authors have read and approved the final manuscript.

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Research Article

Modification of Platelet Count on the Association between Homocysteine and Blood Pressure: A Moderation Analysis in Chinese Hypertensive Patients

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Background. Platelet consumption followed by homocysteine-induced endothelial injury suggests a crosstalk between platelet activation and homocysteine on hypertension. Platelet count has been found to modify the effect of folic acid on vascular health. However, whether platelet count could modify the contribution of homocysteine to blood pressure (BP) remains unclear. *Methods*. Leveraging a community-based cross-sectional survey in 30,369 Chinese hypertensive patients (mean age 62 years, 52% female), we examined the moderation of platelet count on the association between serum homocysteine and BP by constructing hierarchical multiple regression models, adjusting for conventional risk factors. If adding the interaction term of homocysteine and platelet count could explain more variance in BP and the interaction is significant, then we believe that moderation is occurring. *Results*. The association between serum homocysteine and diastolic BP was significantly stronger ($\beta = 0.092$ vs. 0.035, $P = 0.004$) in participants with low platelet count ($<210 \times 10^9$/L) than in those with high platelet count ($\geq 210 \times 10^9$/L). Adding the interaction term of homocysteine and platelet count additionally explained 0.05% of the variance in diastolic BP ($P = 0.0001$), and the interaction was significant ($\beta = -0.021$, $P < 0.001$). Excluding participants receiving antihypertensive medications did not change our results. *Conclusions*. The association between homocysteine and BP was significantly stronger in participants with low *vs*. high platelet count and was partially moderated by platelet count. These results indicate that platelet count may be useful in the identification of individuals who are most beneficial to reducing-homocysteine treatments but this usefullness still needs further investigation.

1. Introduction

Essential hypertension is highly prevalent all over the world and a leading modifiable risk factor for cardiovascular disease (CVD) [1, 2]. Better understanding the underlined mechanisms of hypertension is likely to improve prevention and management of this debilitating disorder and reduce related disease burden. Homocysteine (Hcy) has been involved in the pathogenesis of hypertension by producing endothelial injury [3, 4], increasing oxidative stress [5], stimulating the proliferation of vascular smooth muscle cells (VSMCs) [6], and altering the elastic properties of the vascular wall [7]. Elevated Hcy and its related genetic variants have been associated with blood pressure [8–12], hypertension [13–15], and related vascular complications, e.g., atherosclerosis [16, 17], coronary heart disease [18, 19], and stroke [20, 21], in humans. In the process of Hcy-induced hypertension, the endothelial injury resulting from Hcy promotes platelet consumption and adherence which stimulates the proliferation of VSMCs through releasing mitogenic factors [22], thereby contributing to atherosclerosis, arterial stiffness, and high blood pressure [23, 24] Furthermore, platelet activation has been associated with Hcy [25] but also hypertension [26] in population studies. As such,

platelet response may play a potential role in enhancing the contribution of Hcy to the risk and severity of hypertension. In support of this hypothesis, a recent trial found that platelet count interacted with Hcy and modified the effect of folic acid on risk of stroke [27]. To date, however, the moderation or interaction between Hcy and platelet count on blood pressure has been poorly studied but deciphering their crosstalk is likely to provide novel insights into mechanisms of hypertension and may also identify novel therapeutic targets for this debilitating disorder and related conditions. The aim of this study was to examine the moderation and interaction of platelet count on the association between homocysteine and blood pressure in more than 30 thousand Chinese adults with essential hypertension.

2. Methods

2.1. Participants. Hyperhomocysteinemia (HHcy) has been considered extremely and highly prevalent in Chinese population in the last decades due to lack of mandatory folic acid fortification of grain products, insufficient consumption of folate-containing foods, a low rate of folic acid supplementation, and a high prevalence of the methylenetetrahydrofolate reductase (*MTHFR*) C677T gene mutation (25% in Chinese vs. 10% to 12% in the general U.S. population) [28] After decades of efforts, e.g., health education and dietary promotion, the prevalence of HHcy is supposed to be declined in this population, hypertensive patients in particular. To estimate the prevalence of HHcy among Chinese hypertensive patients, we conducted a community-based cross-sectional survey in 21 communities residing in Taicang, a traditional but economically developed county in China. There is an effective system of registry and management of chronic diseases, including hypertension, diabetes, and CVD, covering all of the adult residents in this county. More than 70,000 living hypertensive patients were registered in this system by the end of 2016. Of them, 30,379 patients agreed to participate in our cross-sectional clinical examination operated in 2017 and gave written informed consent. After excluding participants with missing data on either serum Hcy or platelet count ($N = 100$), a total of 30,369 hypertensive patients were finally included in the current analysis. Hypertension defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or use of antihypertensive medications in the last 2 weeks was used in our study [29].

2.2. Assessment of Homocysteine. Overnight fasting venous blood samples were collected for each participant and shipped to the core laboratory of the Center for Disease Prevention and Control of Taicang within four hours after venipuncture. All laboratory tests were performed at this laboratory on the same day. Serum total Hcy was determined by chemistry enzymatic assay (Axis-Shield Diagnostics Ltd., Dundee, UK) on the Cobas c501 analyzer (Roche Diagnostics, Indianapolis, IN). The intra- and interassay coefficients of variation were less than 7.5% and 4.5%, respectively.

2.3. Measurement of Blood Pressure. Blood pressure was measured three times by trained staff using a standard mercury sphygmomanometer and a cuff of appropriate size according to a standard protocol [29], after the participants had been resting for at least 5 min in a relaxed, sitting position. The first and fifth Korotkoff sounds were recorded as systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively. The mean of the three measurements was used in statistical analyses.

2.4. Measurement of Other Risk Factors. Blood cell analysis, including platelet count, plateletcrit, platelet distribution width, and mean platelet volume, was obtained using a BC-3200 hematology analyzer (Mindray Medical, Shenzhen, China). Fasting glucose, blood lipids including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), and creatinine were measured by standard laboratory methods [30]. Diabetes was defined as fasting glucose over 7.0 mmol/L and/or receiving hypoglycemic medications in the last 2 weeks [31]. Body weight (kg) and height (cm) were measured when participants wore light clothes and no shoes by trained staff. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters (kg/m^2).

2.5. Statistical Analysis. Given the extreme sample of only hypertensive patients included in our study, *z*-transformation was applied to standardize the distribution of SBP and DBP with a mean of 0 and a standard deviation of 1. The generated *z*-scores were used in all downstream analyses. To examine whether platelet count modifies the association between Hcy and blood pressure, we first constructed a robust linear regression model in which z-transformed SBP/DBP was the dependent variable and log-transformed Hcy (log-Hcy) was the independent variable, adjusting for age, sex, receiving antihypertensive medication (y/*n*), fasting glucose, LDL-C, HDL-C, and creatinine in participants with low and high platelet count (less than vs. over $210 \times 10_9$/L), due to that platelet count less than $210 \times 10_9$/L has been identified to modify the efficacy of folic acid in preventing stroke in Chinese hypertensive patients [27]. The robust regression model was used here to account for the influence of extreme values on the model fitting. The modification of platelet count levels on the association between Hcy and blood pressure was tested by comparing the regression coefficients of the linear models fitted in participants with low *vs*. high platelet count. To further examine whether and to what extent does platelet count moderate the association between Hcy and blood pressure, we constructed the following hierarchical multiple regression models:

(i) Model 1: the regression of blood pressure on log-Hcy and platelet count, adjusting for covariates listed above

(ii) Model 2: add the interaction term of log-Hcy × -platelet count as a predictor to model 1

To facilitate comparison, all variables were centered to a distribution with a mean of 0 and a standard variation of 1. If the R^2 of Model 2 is significantly improved compared with Model 1 and the interaction effect is significant, then we believe that moderation is occurring. Moderation analysis was performed using *R* package "medmod" [32].

2.6. Sensitivity Analysis. To examine whether medications affect our results, we repeated the analysis after excluding participants receiving antihypertensive or antidiabetic medications. We additionally examined the moderation of other markers of platelet consumption including plateletcrit, platelet distribution width, and mean platelet volume on the association between Hcy and blood pressure. All statistical analyses were conducted using SAS statistical software (version 9.4; Cary, North Carolina, USA) unless otherwise noted. A two-tailed *P* value of less than 0.05 was considered statistically significant.

3. Results

This study included 30,369 hypertensive patients (mean age 62 years, 52% female) including 4,160 (14%) patients not receiving antihypertensive drugs. Among these, 61%, 43%, and 22% individuals suffered HHcy defined as a serum Hcy level over 10, 12, and 15 μmol/L, respectively. Table 1 presents the clinical characteristics of these hypertensive patients according to platelet count. Participants with low platelet count were more likely to be elder, male, and have lower levels of SBP, DBP, BMI, blood lipids, and serum Hcy, but a higher level of creatinine than those with high platelet count (all $P < 0.001$). Higher levels of platelet consumption as indicated by higher MPV and PDW and lower PCT occurred in participants with low platelet count compared with those with high platelet count (all $P < 0.05$).

3.1. Association between Hcy and Blood Pressure according to Platelet Count. Table 2 shows the associations of serum Hcy with SBP and DBP in hypertensive patients with low *vs*. high platelet count, independent of age, sex, BMI, fasting glucose, lipids, kidney function, and antihypertensive medication. In participants with low platelet count, higher serum Hcy was significantly associated with higher SBP (bottom-line significant with $\beta = 0.026$, $P = 0.079$) and DBP ($\beta = 0.092$, $P < 0.001$), whereas these associations were not significant in participants with high platelet count. And the magnitudes of the associations of serum Hcy with SBP ($P = 0.017$) and DBP ($P = 0.004$) in hypertensive patients with low platelet count were much stronger than that in those with high platelet count. These results indicated a possible modification of platelet count on the association between serum Hcy and blood pressure.

3.2. Moderation of Platelet Count on the Association between Hcy and Blood Pressure. The results of moderation analysis further demonstrated the modification of platelet count on the association between Hcy and DBP. As shown in Table 3, after further adjusting for the interaction term of Hcy and platelet count, the magnitude of the association between serum Hcy and DBP remained unchanged and significant but the R^2 of the regression model significantly increased by 0.0005 ($P = 0.0001$). Furthermore, the interaction between Hcy and platelet count was significantly associated with DBP ($\beta = -0.021$, $P < 0.001$). These results indicated that the association between Hcy and DBP may be partially moderated by platelet count and seemed to be stronger in participants with low platelet count. We failed to identify statistically but bottom-line significant moderation of platelet count on the association between Hcy and SBP ($\beta = -0.011$, $P = 0.057$).

3.3. Results of Sensitivity Analysis. After excluding participants receiving antihypertensive drugs ($N = 26{,}209$), the association between Hcy and blood pressure was significant in patients with low platelet count but not significantly stronger than that in patients with high platelet count (Supplementary Table S1). Although we failed to observe a statistically significant moderation of platelet count on the association between Hcy and blood pressure in this small subsample of hypertensive patients, the inconsistent results in patients with low *vs*. high platelet count may support our findings in total participants. Excluding participants receiving antidiabetic medications did not change our results (Supplementary Table S2). In addition to platelet count, plateletcrit also significantly moderated the association between Hcy and blood pressure (all $P < 0.05$, Supplementary Table S3). We did not find significant moderation of platelet distribution width or volume on the association between Hcy and blood pressure (Supplementary Tables S4 and S5).

4. Discussion

A recent clinical trial including 10,789 Chinese hypertensive adults found that platelet count modified the effect of folic acid supplementation on the risk of incident stroke [27]. This secondary analysis of China Stroke Primary Prevention Trial (CSPPT) introduced for the first time a probable moderating effect of platelet count on the contribution of Hcy to risks of stroke and other vascular disorders, such as hypertension, but it may be prone to uncover a false-positive finding. In order to replicate this finding of interest, our study examined the moderation of platelet count on the association between Hcy and blood pressure, leveraging a large sample of hypertensive patients with available data on Hcy. The results showed that the association between Hcy and blood pressure, DBP in particular, was partially moderated by platelet count and was significantly stronger in participants with low platelet count than that in those with high platelet count, regardless of antihypertensive medications and metabolic risk factors. Platelet consumption may be involved in the progression of hypertension through mechanisms beyond metabolic risk factors.

In line with our study, the identified association between Hcy and blood pressure was also demonstrated by many previous studies. For example, basic experiments found that injection of Hcy resulted in blood pressure elevation in rats

Table 1: Clinical characteristics of study participants according to platelet count.

Characteristics	Low platelet count ($<210\times10^9$/L)	High platelet count ($\geq210\times10^9$/L)	P
No. of participants	21,980	8,389	
Age (years)	63.3 ± 7.1	61.7 ± 7.2	<0.001
Sex, men (%)	11,087 (50.44)	3,516 (41.91)	<0.001
Antihypertensive medication, *n* (%)	18,897 (85.97)	7,312 (87.16)	0.007
Body mass index (kg/m$^{2)}$	24.71 ± 3.36	25.08 ± 3.31	<0.001
Fasting glucose (mmol/L)	5.92 ± 1.48	5.94 ± 1.46	0.252
Total cholesterol (mmol/L)	4.82 ± 1.01	5.10 ± 1.04	<0.001
Triglycerides (mmol/L)	1.80 ± 1.40	1.96 ± 1.43	<0.001
LDL-C (mmol/L)	2.78 ± 0.81	3.06 ± 0.84	<0.001
HDL-C (mmol/L)	1.49 ± 0.48	1.47 ± 0.45	<0.001
Creatinine (mmol/L)	73.37 ± 36.96	69.57 ± 30.47	<0.001
Systolic blood pressure (mmHg)	143.8 ± 17.6	144.6 ± 17.5	<0.001
Diastolic blood pressure (mmHg)	83.0 ± 10.5	84.0 ± 10.7	<0.001
Log-Hcy	2.40 ± 0.49	2.41 ± 0.48	0.037
Platelet consumption			
Mean platelet volume (fL)	10.10 ± 1.75	8.87 ± 1.45	<0.001
Platelet crit (%)	0.1559 ± 0.0350	0.2192 ± 0.0450	<0.001
Platelet distribution width (%)	16.36 ± 0.93	15.90 ± 1.08	<0.001

All results are expressed in mean ± SD unless otherwise noted. LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; Log-Hcy: log-transformed homocysteine.

Table 2: The association between serum homocysteine and blood pressure according to platelet count.

Subgroups	*z*-transformed SBP			*z*-transformed DBP		
	β^* (SE)	P^*	$P^\ddagger$ for difference in β	$\beta^\ddagger$ (SE)	$P^\ddagger$	$P^\ddagger$ for difference in β
Total participants	0.017 (0.013)	0.181	—	0.078 (0.012)	<0.001	—
Subgroup by quartiles of platelet count						
Q1 ($<146\times\times10^9$/L)	−0.010 (0.025)	0.683	0.249	0.108 (0.024)	<0.001	0.003
Q2 ($146–178\times10^9$/lL)	0.053 (0.026)	0.044		0.098 (0.025)	<0.001	
Q3 ($179–213\times10^9$/lL)	0.044 (0.026)	0.091		0.058 (0.025)	0.019	
Q4 ($\geq214\times10^9$/L)	−0.027 (0.026)	0.297		0.039 (0.025)	0.119	
Subgroup by platelet count referring to CSPPT						
Platelet count $<210\times10^9$/L	0.026 (0.015)	0.079	0.017	0.092 (0.014)	<0.001	0.004
Platelet count $\geq210\times10^9$/L	−0.017 (0.025)	0.502		0.035 (0.024)	0.144	

*The increase of *z*-transformed SBP (β) per unit increment of log-transformed homocysteine and its significance test (P); ‡the increase of *z*-transformed DBP (β) per unit increment of log-transformed homocysteine and its significance test (P); ‡the significance test of the difference in the regression coefficients between the two subgroups.

Table 3: Moderation of platelet count on the association between serum homocysteine and blood pressure.

Independent variables	Model 1			Model 2		
	β (SE)	P	R^2	β (SE)	P	R^2
z-transformed SBP						
z-transformed log-Hcy	0.007 (0.006)	0.240	0.0478	0.007 (0.006)	0.271	
z-transformed platelet count	0.017 (0.006)	0.004		0.017 (0.006)	0.005	0.0479
Interaction term	—	—		−0.011 (0.006)	0.057	
Moderation tests			$\Delta R^2 = 0.0001$, $F = 2.9585$, $P = 0.085$			
z-transformed DBP						
z-transformed log-Hcy	0.036 (0.006)	<0.001	0.0975	0.035 (0.006)	<0.001	
z-transformed platelet count	0.019 (0.006)	0.001		0.019 (0.006)	0.001	0.0980
Interaction term	—	—		−0.021 (0.005)	<0.001	
Moderation tests			$\Delta R^2 = 0.0005$, $F = 14.734$, $P = 0.0001$			

Log-Hcy: log-transformed homocysteine; model 1: the regression of blood pressure on z-transformed log-Hcy and z-transformed platelet count, adjusting for age, sex, body mass index, fasting glucose, lipids, and creatinine; model 2: further adjusting for the interaction term of z-transformed log-Hcy and z-transformed platelet count.

[33]. Genome-wide association studies revealed that several variants in the *MTHFR*, a gene regulating Hcy synthesis [34], were associated with blood pressure in humans, regardless of hypertensive status and antihypertensive medications [8, 9, 35]. The positive association between circulating Hcy and blood pressure has also been found in diverse populations by many large-scale epidemiological studies [36], including the Third National Health and Nutrition Examination Survey (NHANES) [37]. The association between Hcy and blood pressure has been mostly studied in general populations comprising normal and hypertensive individuals, and the role of Hcy in the progression of hypertension is not well studied. Our study included only hypertensive individuals and found a significant association between Hcy and blood pressure in this population and therefore may provide an initial evidence for the contributing role of Hcy in the progression of hypertension.

In addition to the association between Hcy and blood pressure, the other important goal of our study is to examine the moderation effect of platelet count on this association. In line with the results from the CSPPT [27], we found that the association between Hcy and blood pressure was significantly moderated by platelet count, where this association was significantly stronger for participants with low platelet count. Although the underlined mechanisms are not clear, some probable mechanisms shared by Hcy and platelet involved in vascular health may explain, at least partly, the moderating effect of platelet count on the association between Hcy and blood pressure. As an illustration, as a fundamental pathogenesis of hypertension, endothelial dysfunction could be resulted by Hcy [38] and can stimulate aggregation and adherence of platelet [39]. Platelet consumption and activation consequently stimulate the proliferation of VSMCs by releasing mitogenic factors [22]. Further, Hcy per se could also induce the proliferation of VSMCs 6 probably through interaction with platelet, indicated by an Hcy-dependent expression of the platelet-derived growth factor [40], thereby contributing to atherosclerosis, arterial stiffness, and high blood pressure [23, 24]. Together with these basic studies, our findings may suggest that the contribution of Hcy to atherosclerosis and the progression of hypertension may be boosted in individuals with a status of high active platelet consumption. In further support of this speculation, we found that higher Hcy (after log-transformation) was significantly associated with higher levels of platelet count ($\beta = 1.28$, $P = 0.045$), mean platelet volume ($\beta = 0.059$, $P = 0.005$), and plateletcrit ($\beta = 0.002$, $P = 0.002$) and a lower level of platelet distribution width ($\beta = -0.029$, $P = 0.013$) in our study. We also found that plateletcrit, a complementary analysis of platelet count and measures the percentage of platelets in the blood [41], significantly moderated the association between Hcy and blood pressure in our study. The more active is platelet consumed, the fewer platelets are in the circulation. Although we failed to identify a statistically significant moderation for the other two markers of platelet consumption activation, platelet distribution width and mean platelet volume [42, 43], the identified statistically significant moderation of platelet count and plateletcrit on the association between Hcy and blood pressure, together with prior consistent findings, may shed light on a biological moderation effect of platelet consumption on modifying vascular toxicity of Hcy. The vascular toxicity of Hcy, indicated by a higher blood pressure here, seems relatively greater in individuals who do not have reduced platelet production. Of note, we found that the magnitude of the association between Hcy and DBP seemed higher than that between Hcy and SBP. This phenomenon is also observed in prior population studies [44]. Although some mechanisms through which Hcy leads to DBP dysfunction have been indicated, e.g., reduced the production of vasodilators such as endogenous nitric oxide [45], reduced arterial wall compliance through impairing vascular matrix metalloproteinase activity [46], and increased endothelial-myocyte uncoupling [47], the mechanisms underlining this interesting phenomenon are still not very clear and warranted further investigation because DBP was considered associated with blood flow in the brain.

This study reserves the first to examine the moderation of platelet count on the association between Hcy and blood pressure in populations. The strengths of our study included the large sample size, comprehensive assessment, and adjustment for multiple confounding factors including metabolic factors, stratification analysis by using antihypertensive medications to eliminate its bias, and the careful moderation analysis by constructing hierarchical multiple regression models. Some limitations of our study also should be acknowledged. First, the cross-sectional study design prevents us from exploring the causal or temporal relationship between Hcy, platelet count, and blood pressure elevation. Second, only Chinese hypertensive adults were included in our study. Given the higher prevalence of HHcy in Chinese than European ancestry populations [28], the generalizability of our findings to other ethnic groups, nonhypertensive adults, or younger populations is concerned. Third, to facilitate data interpretation, we used the same cutoff value of platelet count as used in the CSPPT [27]. In fact, it is still unclear about the reference level of platelet count that could be used in clinical settings to identify individuals who are sensitive to Hcy or folic acid supplementation. However, our study may suggest that platelet count possesses a potential to identify individuals who are the most appropriate for the treatment of folic acid or vitamin B12. Fourth, we did not obtain data on organ damage of hypertension, e.g., CVD events and chronic kidney disease, although serum creatinine was adjusted for in our statistical analysis. Whether and to what extent these organic dysfunctions influence our results is not clear. Fifth, although blood pressure was measured three times, the level of blood pressure under analysis was obtained on only 1 occasion. Inaccuracy may still exist and may affect our data interpretation. Sixth, we did not obtain data on the use of B12 or folate supplements which directly influence Hcy levels.

Data Availability

All information analyzed is deidentified, and the anonymous data will be shared by a request to the corresponding author at penghao@suda.edu.cn.

Additional Points

Perspectives. The moderation effect of platelet count on the contribution of Hcy to vascular disorders suggested by the CSPPT [27] was successfully demonstrated by our study in a large sample of Chinese hypertensive adults. The association between serum Hcy and blood pressure was significantly stronger in participants with low *vs*. high platelet count and was partially moderated by platelet count. These results indicate that platelet count may be useful in the identification of individuals who are most beneficial to reducing-homocysteine treatments but this potential usefullness still needs further investigation.

Disclosure

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Jianan Zhang, Jing Li, and Shi Chen contributed equally to this work and should be considered as co-first authors.

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Supplementary Materials

To further examine the robustness of our results, we performed a series of sensitivity analyses. Specifically, to examine whether antihypertensive medications affect our results, we repeated the analyses in participants receiving antihypertensive medications or not. As shown in Supplementary Table S1, after excluding participants receiving antihypertensive drugs ($N = 26{,}209$), the association between Hcy and blood pressure was significant in patients with low platelet count but not significantly stronger than that in patients with high platelet count. Although we failed to observe a statistically significant moderation of platelet count on the association between Hcy and blood pressure in this small subsample of hypertensive patients, the inconsistent results in patients with low vs. high platelet count may support our findings in total participants. To examine whether receiving antidiabetic medications affects our results, we repeated the analyses in participants receiving antidiabetic medications or not. As shown in Supplementary Table S2, excluding participants receiving antidiabetic medications did not change our results. The association between Hcy and blood pressure was significantly stronger in participants with low platelet counts. To examine whether other index of platelet consumption could modify the association between Hcy and blood pressure, we additionally examined the association between Hcy and blood pressure in subgroups by plateletcrit (Supplementary Table S3), platelet distribution width (Supplementary Table S4), and mean platelet volume (Supplementary Table S5). We found that plateletcrit also significantly moderated the association between Hcy and blood pressure (all $P < 0.05$). We did not find significant moderation of platelet distribution width or volume on the association between Hcy and blood pressure. (*Supplementary Materials*)

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Research Article

Association between Plasma Urotensin II and Risk of Hypertension: Findings from a Prospective Study

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Up to date, human urotensin II (UII) is the most potent vasoconstrictor in mammalian animals. To explore whether UII played an important role in the development of hypertension, we conducted a prospective study in Changshu city, China. The baseline investigation was carried out in 2007, and the first follow-up investigation was conducted in 2013. From the participants, we randomly obtained 2000 normotensive subjects aged 40 years and older without any severe disease at baseline and examined plasma UII and endothelin-1 (ET-1) with their blood samples at baseline. Logistic models were used to analyze the association between baseline UII, baseline ET-1, and newly occurring hypertension. In 1,819 subjects with complete data, 723 subjects developed into hypertensive in about five years. After adjusting some potential confounders, the odds ratio (95% confidence interval) for risk of hypertension comparing the highest with the lowest quartile of baseline UII was 0.888 (0.689–1.144). The role of UII in the development of hypertension was not found in the current study; therefore, further research studies should be conducted to explore the relationship between UII and hypertension.

1. Introduction

Hypertension, the leading cause of morbidity and mortality of cardiovascular diseases (CVD), has become a global public health challenge [1]. Although a lot of studies have been conducted to explore the risk factors of hypertension, the etiology of hypertension is still obscure.

Urotensin II (UII) is a cyclic peptide initially isolated from the urophysis of the goby fish based on its potent vasoconstrictor effect [2], and then it was cloned from humans. UII is considered as the most potent vasoconstrictor, and its vasoactive activity is even more potent than endothelin-1 (ET-1) [3, 4]. Because of the inconsistent results of the published studies [5–8], the association between UII and hypertension has not been confirmed yet. Furthermore, most of these studies are case-control studies, which are weak to explore the causal relationship between UII and hypertension.

Moreover, some experimental researchers have found that UII, angiotensin II (Ang II), and ET-1 may have a potential interaction in modulating the cardiovascular effects [9–11]. And a case-control study also found that increased plasma levels of UII and ET-1 in patients with coronary heart disease (CHD) and UII and ET-1 were positively correlated [12]. All these studies indicated that UII may play a role in the development of hypertension. So we conducted the prospective study to verify the effect of UII in the development of hypertension.

2. Materials and Methods

2.1. Study Populations. A large-scale cohort on CVD has been built in rural communities of Changshu city, Jiangsu province, China since 2007. From 2007 to 2008, we conducted a baseline investigation. A total of 20,343 participants aged 18 years and older were enrolled in the study. The first follow-up investigation was held in 2013. For the current study, we randomly selected 2000 normotensive subjects aged 40 years and older according to the baseline characteristics and examined the plasma UII and ET-1 levels with their baseline blood samples. The chosen individuals have also been required no coronary heart disease, stroke, chronic kidney diseases, tumors, chronic obstructive pulmonary diseases, or peripheral artery diseases. Finally, 1819 subjects with complete data were included in the current study. The detailed description of methods for study participant recruitment and baseline data collection has previously been done [13].

This study was approved by the Soochow University Institutional Ethics Review Board and was following the guidelines of the Declaration of Helsinki. All participants provided written informed consent.

2.2. Data Collection. The data on demographic characteristics, lifestyle risk factors, family history of CVDs, and medical history were obtained using a standard questionnaire administered by the trained staff. Cigarette smoking was defined as ever having smoked at least 100 cigarettes. The information regarding the amount and type of alcohol consumed during the past years was collected, and alcohol consumption was defined as drinking any alcoholic beverage at least 12 times during the past year. All the subjects underwent three BP measurements in a sitting position with a 30 s interval after 5 minutes of rest, with electronic BP monitors (Omron HEM-770A, OMRON Healthcare Inc., Dalian, China). The mean of the three BP measurements was calculated for analysis. Waist circumference (WC) was measured at the level of 1 cm above the umbilicus. Body weight and height were measured with each subject wearing light clothing and without shoes. The body mass index (BMI) was calculated as weight in kilograms divided by square of the height in meters.

2.3. Follow-Up and Outcome Assessment. All participants were first followed up in 2013. Each participant was interviewed face to face by trained staff with a standard questionnaire. The investigated content was the same as that of the baseline investigation, also including histories of diseases and medication during follow-up. If a specific disease mentioned in the questionnaire was reported during the follow-up period, hospital records and experienced physicians also needed to be provided. Death data were confirmed by obtaining death certificates from the local civil registry or the attended hospital. The method and equipment for three BP measurements were the same as those used in the baseline survey. Hypertension was defined as systolic BP $\geq$ 140 mmHg and/or diastolic BP $\geq$ 90 mmHg and/or current use of antihypertensive medications.

2.4. Measurements. In the baseline investigation, blood samples were obtained in the morning by venipuncture after a requested overnight fasting period (at least 8 hours) and sampled in EDTA tubes and immediately spun at 3000 rpm for 15 minutes. Plasma samples were frozen at −80°C until laboratory testing, and measurements were performed by laboratory technicians who were blinded to the characteristics of the study patients. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and fasting plasma glucose (FPG) were analyzed enzymatically on a Hitachi 7020 automatic biochemical analyzer using commercial reagents (Kangxiang Medical Appliance, Shanghai, P.R China).

Plasma UII and ET-1 measurements were performed with each blood sample at baseline with enzyme-linked immunosorbent assay (ELISA) using standard kits (UII (human)-EIA kit, Phoenix Biotech, USA; ET-1 kit, R&D Systems China Co, Ltd) according to the manufacturer's instructions. Intra- and interassay coefficients of variation were both less than 4% and 8%.

2.5. Statistical Analysis. Due to skewed distribution, the concentration of ET-1 and other quantitative characteristics at baseline were described with the median and interquartile range (P_{25}–P_{75}). Independent Kruskal–Wallis rank tests were used to compare differences in these variables among four quartile groups according to the baseline UII level. Qualitative characteristics were presented as percentages and compared with the $\chi 2$ test. The characteristics were also presented with median (P_{25}–P_{75}) or absolute number (percentages) by the status of hypertension at the follow-up endpoint and were compared with $\chi 2$ test or Wilcoxon rank test. Four multiple logistic regression models were performed to analyze the association between baseline UII and the risk of hypertension with different combinations of confounder variables adjusted. The confounder variables including age, sex, systolic blood pressure, drinking, smoking, family history of hypertension, BMI, FPG, TC, and ET-1 were put into the four models from less to more. Participants were divided into four quartile groups according to the baseline UII levels and also were divided into two groups by the upper quartile of the baseline UII levels. The odds ratio (OR) and 95% confidence interval (CI) were calculated for the risk of hypertension associated with the baseline UII levels. A two-tailed P value less than 0.05 was considered statistically significant. All statistical analyses were conducted using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. General Characteristics of Participants at Baseline. In the current population, there were 732 males and 1087 females. The average age at baseline was 51.6. There was no difference in levels of most characteristics except ET-1 at baseline

among the quartile groups according to UII levels. The general characteristics of the participants at baseline are presented in Table 1 in detail.

3.2. General Characteristics at Baseline by Hypertension Status at the Follow-Up Point. Among the 1,819 subjects with normal blood pressure at baseline, 723 new cases of hypertension were found at the first follow-up point. Levels of ET-1 at baseline were significantly different between the new cases of hypertension and the normotensive participants, while levels of UII were not. The other general characteristics at baseline were compared between the new cases of hypertension and the normotensive participants (see Table 2).

3.3. Association between UII Level at Baseline and Risk of Hypertension. The participants were divided into four groups according to the quartiles of baseline UII levels to examine the prediction of UII at baseline on incident hypertension. ORs for risk of hypertension comparing the second quartile, the third quartile, and the highest quartile with the lowest quartile of baseline UII were not statistically significant in four models in which different combinations of confounding variables were adjusted. The confounding variables included age, sex, systolic blood pressure, drinking, smoking, family history of hypertension, BMI, FPG, TC, and ET-1. The detailed results are shown in Table 3.

We also pooled the lower three quartile groups as a reference and calculated ORs for risk of hypertension of the highest quartile of baseline UII. However, the ORs were not significant in the four logistic models. The results are listed in Table 4.

3.4. Analysis of the Association between Risk of Hypertension and UII Level at Baseline by Strata of ET-1 Levels. Furthermore, the subjects were divided into two groups by the 75th percentile ($P_{75} = 1.56$ pg/ml) of ET-1 levels. Then, we analyzed the association between the risk of hypertension and baseline UII in the subgroups. With the lower levels ($<P_{75}$) of UII as a reference, the ORs and 95% CI of the higher levels of UII ($\geq P_{75}$) for hypertension were calculated in both the subgroups, and none of the ORs were significant with different combinations of confounder variables adjusted in the four logistic models. The detailed results are listed in Table 5.

4. Discussion

To our knowledge, this is the first prospective study to explore the association between plasma UII and the risk of hypertension. Our results showed that plasma UII was not associated with the risk of hypertension. Moreover, we did not find the effect of ET-1 on the association between plasma UII and the risk of hypertension. These indicated that plasma UII might not play a role in the development of hypertension. Our current study has a certain significance to demonstrate the association between plasma UII and the risk of hypertension with a large-scale prospective study. Some published studies showed that hypertension was associated with UII and indicated that it had an important significance in the treatment or prevention of hypertension. However, most of these studies were case-control studies, and the association was not verified in our prospective study. So further research is still needed to verify the association.

UII is a vasoconstrictor in rodent and primate animals. The vasoconstrictor activity depends on the race of animals, the position of vessels, and the status of vascular endothelium. The vasocontractile effect of UII acts by binding to the UII receptor (UT receptor). Ames et al. [14] found that UII existed widely in cardiovascular tissues and cloned the UT receptor, GPR14. UT receptor mRNA is widely expressed in human cardiovascular tissues, including cardiac myocytes, vascular smooth muscle cells, and endothelial cells. However, in rats, UT receptor mRNA was also expressed in motor neurons of the spinal cord, smooth muscle cells of the bladder, and cardiomyocytes [15]. The distribution patterns of the UII and UT receptor mRNA in man are not similar [16]. Maguire et al. [17] found that UII was approximately 50 times more potent than ET-1 in human arteries, while there was less than a ten-fold increase in potency of UII compared to ET-1 in human veins. MacLean et al. [18] found that UII was about four-fold more potent vasoconstrictor than ET-1 in rat main pulmonary arteries. And the response was increased by endothelial factors, vascular tone, and the onset of pulmonary hypertension, while inhibited by nitric oxide synthase. This evidence uncovers contractile responses to UII in human pulmonary arteries. Bottrill et al. [19] declared that UII was a potent endothelium-dependent vasodilator. However, most of these results were from animal studies or vitro studies and few population-based studies could verify these results.

Some clinical studies demonstrated that increased plasma UII levels were associated with the severity of carotid atherosclerosis and the severity of coronary artery lesions, such as in patients with essential hypertension [20] or patients with coronary heart disease [12]. Studies on the association between UII and hypertension were conducted mostly with the case-control study design. Some showed that levels of UII in plasma were higher in hypertensive than that in the control group [5, 6, 21–23]. Although these results indicated that UII might be a risk factor of hypertension, the relationship is not very certain for uncertain temporal relationships. In addition to this, several case-control studies showed that there was no significant difference in levels of plasma UII between normotensive and hypertensive patients [7, 8]. Even lower levels of plasma UII were found in hypertensive than in normotensive [24]. Zhou and Tian [25] compared levels of plasma UII among four groups with different levels of blood pressure, normal blood pressure, hypertension stage I, hypertension stage II, and hypertension stage III. They found no significant difference between the two groups of normal blood pressure and hypertension stage I. While higher levels in the group of hypertension stage II and hypertension stage III than in the normal group. The conclusion can be given that UII may drop out of the occurrence and development of hypertension.

Table 1: General characteristics of the 1819 participants by quartiles of levels of urotensin II at baseline.

	Quartiles of plasma urotensin II				P value
	Quartile 1 ($n = 453$)	Quartile 2 ($n = 456$)	Quartile 3 ($n = 455$)	Quartile 4 ($n = 455$)	
Age, year	52 (48–55)	52 (47–55)	52 (48–55)	51 (17–55)	0.386
Drinking, n (%)	103 (22.7)	96 (21.1)	88 (19.3)	91 (20.0)	0.612
Smoking, n (%)	135 (29.8)	139 (30.5)	143 (31.4)	148 (32.5)	0.827
Family history of HTN, n (%)	187 (41.3)	223 (48.9)	201 (44.2)	208 (45.7)	0.135
Body mass index (kg/m^2)	22.5 (20.4–25.0)	22.8 (20.2–25.1)	22.4 (20.6–24.1)	22.5 (20.5–24.6)	0.699
Waist circumference (cm)	80 (74–87)	80 (73–86)	80 (73–85)	79 (72–85)	0.100
Systolic blood pressure (mmHg)	122 (115–130)	121 (113–130)	120 (112–128)	121 (113–129)	0.268
Diastolic blood pressure (mmHg)	77 (72–82)	77 (71–82)	75 (69–81)	76 (71–82)	0.210
Fasting plasma glucose (mmol/L)	4.9 (4.6–5.2)	4.9 (4.5–5.2)	4.8 (4.4–5.2)	4.8 (4.4–5.2)	0.043
Total cholesterol (mmol/L)	4.5 (4.0–5.1)	4.5 (3.9–5.1)	4.5 (4.0–5.2)	4.5 (3.9–5.1)	0.830
Triglycerides (mmol/L)	1.2 (0.9–1.9)	1.3 (1.1–1.5)	1.3 (0.9–1.9)	1.3 (1.0–1.5)	0.225
HDL-cholesterol (mmol/L)	1.3 (1.1–1.5)	1.3 (1.1–1.5)	1.3 (1.1–1.5)	1.3 (1.1–1.5)	0.052
LDL-cholesterol (mmol/L)	2.5 (2.0–3.0)	2.4 (2.0–2.9)	2.5 (2.0–3.0)	2.5 (2.1–3.0)	0.389
Endothelin-1 (pg/ml)	1.21 (0.86–1.68)	1.09 (0.80–1.54)	1.04 (0.73–1.48)	1.17 (0.81–1.57)	<0.0001

Numerical variables were expressed as median (P_{25}–P_{75}); HTN: hypertension; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Table 2: Comparison of baseline characteristics between the new cases of hypertension and the normotensive participants.

	Normotensive ($n = 1096$)	Hypertensive ($n = 723$)	P value
Age, year	51 (47–54)	53 (49–56)	<0.0001
Drinking, n (%)	218 (19.9)	160 (22.1)	0.249
Smoking, n (%)	351 (32.0)	214 (29.6)	0.274
Family history of HTN, n (%)	465 (42.4)	354 (49.0)	0.006
Body mass index (kg/m^2)	21.9 (20.0–24.1)	23.4 (21.1–25.7)	0.699
Waist circumference (cm)	76 (70–82)	80 (73–86)	0.100
Systolic blood pressure (mmHg)	116 (109–126)	128 (121–134)	<0.0001
Diastolic blood pressure (mmHg)	74 (68–79)	81 (76–85)	<0.0001
Fasting plasma glucose (mmol/L)	4.8 (4.4–5.1)	4.9 (4.6–5.3)	0.043
Total cholesterol (mmol/L)	4.4 (3.9–5.0)	4.6 (4.1–5.3)	0.830
Triglycerides (mmol/L)	1.2 (0.9–1.7)	1.4 (1.0–1.5)	0.225
HDL-cholesterol (mmol/L)	1.3 (1.1–1.5)	1.3 (1.1–1.5)	0.052
LDL-cholesterol (mmol/L)	2.4 (2.0–3.0)	2.5 (2.1–3.0)	0.389
Endothelin-1 (pg/ml)	1.12 (0.78–1.57)	1.15 (0.80–1.53)	<0.0001
Urotensin II (pg/ml)	83.37 (65.91–109.45)	80.63 (64.11–105.62)	0.203

Numerical variables were expressed as median (P_{25}–P_{75}); HTN, hypertension; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3: Odds ratios for risk of hypertension comparing the three higher quartiles with the lowest quartile of baseline urotensin II levels.

	Quartile of plasma urotensin II				P value for trend
	Quartile 1 ($n = 453$)	Quartile 2 ($n = 456$)	Quartile 3 ($n = 455$)	Quartile 4 ($n = 455$)	
Number of new cases of HTN (%)	188 (41.5)	190 (41.7)	173 (38.0)	172 (37.8)	
Model 1[a]	1.000	1.008 (0.772–1.317)	0.848 (0.647–1.110)	0.870 (0.665–1.140)	0.573
Model 2[b]	1.000	1.119 (0.829–1.511)	1.046 (0.774–1.414)	0.930 (0.688–1.256)	0.910
Model 3[c]	1.000	1.096 (0.811–1.481)	1.037 (0.766–1.403)	0.923 (0.683–1.248)	0.948
Model 4[d]	1.000	0.993 (0.753–1.310)	0.860 (0.650–1.140)	0.879 (0.664–1.163)	0.775

[a]Adjusted for age and sex; [b]adjusted for age, sex, and systolic blood pressure at baseline; [c]adjusted for age, sex, systolic blood pressure, drinking, smoking, and family history of hypertension; [d]adjusted for age, sex, systolic blood pressure, drinking, smoking, family history of hypertension, body mass index, fasting plasma glucose, total cholesterol, and endothelin-1; HTN, hypertension.

As far as we know, there has been no other prospective cohort study on the association between UII and hypertension. We conducted the population-based study with about 2000 normotensive subjects and 5-year follow-up and found that levels of plasma UII at baseline were not associated with the risk of hypertension. Our result firstly exposed the association of UII and hypertension under the condition of clear temporal relationship.

Affolter et al. [26] conducted an intervention study with intravenous UII and saline placebo on ten healthy male volunteers and found no effect of intravenous UII infusion on systemic hemodynamics or arterial stiffness. They

Table 4: Odds ratios of the risk of hypertension associated with baseline urotensin II levels in two categories.

	Odds ratio (95% confidence interval)	
	Quartiles 1–3 ($n = 1364$)	Quartile 4 ($n = 455$)
Number of new cases of HTN (%)	551 (40.4)	172 (37.8)
Model 1[a]	1.000	0.917 (0.747–1.094)
Model 2[b]	1.000	0.883 (0.689–1.131)
Model 3[c]	1.000	0.885 (0.690–1.135)
Model 4[d]	1.000	0.888 (0.689–1.144)

[a]Adjusted for age and sex; [b]adjusted for age, sex, and systolic blood pressure at baseline; [c]adjusted for age, sex, systolic blood pressure, drinking, smoking, and family history of hypertension; [d]adjusted for age, sex, systolic blood pressure, drinking, smoking, family history of hypertension, body mass index, fasting plasma glucose, total cholesterol, and endothelin-1; HTN, hypertension.

Table 5: Odds ratios of risk of hypertension associated with baseline urotensin II levels by strata of endothelin-1 levels.

	Endothelin-1 <1.56 pg/ml ($n = 1357$)		Endothelin-1 ≥1.56 pg/ml ($n = 452$)	
	Urotensin II < P_{75}	Urotensin II ≥ P_{75}	Urotensin II < P_{75}	Urotensin II ≥ P_{75}
Model 1[a]	1.000	0.914 (0.707–1.180)	1.000	0.928 (0.595–1.449)
Model 2[b]	1.000	0.847 (0.635–1.129)	1.000	1.012 (0.609–1.684)
Model 3[c]	1.000	0.846 (0.634–1.129)	1.000	1.027 (0.615–1.712)
Model 4[d]	1.000	0.866 (0.647–1.159)	1.000	0.987 (0.587–1.660)

[a]Adjusted for age and sex; [b]adjusted for age, sex, and systolic blood pressure at baseline; [c]adjusted for age, sex, systolic blood pressure, drinking, smoking, and family history of hypertension; [d]adjusted for age, sex, systolic blood pressure, drinking, smoking, family history of hypertension, body mass index, fasting plasma glucose, and total cholesterol.

concluded that UII was unlikely to have a physiological role in the short-term regulation of vascular tone or blood pressure in man. Debiec and colleagues [27] studied the UII system in genetic control of blood pressure and renal function. No difference in renal expression of the UII system between normotensive and hypertensive subjects was found. This result suggested that UII system genes were unlikely to play a major role in the genetic control of human blood pressure or renal function. To some extent, the above two studies supported the results of our current study.

The association might be due to the condition of the endothelium and the modulation of the action of UII by endothelium-derived vasorelaxant factors such as nitric oxide [28]. Therefore, it is conceivable that the vasoconstricting effect of UII is brought to play in endothelial dysfunction. The results from subgroup analysis also indicated that there was no association between baseline UII level and risk of hypertension in different subgroups with different ET-1 levels. The subjects of our study were all with normal blood pressure at baseline, and endothelial dysfunction was unlikely to happen in these normotensive individuals. So our results indicated that UII may not participate in the development of hypertension.

The potential limitations of our study should be considered. Firstly, our current study is an observational study, which cannot confirm the causal relationship very powerfully. Further studies should be conducted, such as Mendelian randomization studies. Secondly, the vasodilatory actions of UII depend on the condition of the endothelium. However, we only examined levels of ET-1 in plasma to evaluate endothelial function in our study. Thirdly, levels of UII were examined only once at baseline in our current study. In consideration of the dynamic development of endothelial dysfunction and hypertension, two or more tests on UII and ET-1 at different time points should be performed, which may be better to explore the association between dynamic changes of levels of UII and ET-1 with the development of hypertension.

In summary, our study is the first one to explore the relationship between UII and risk of hypertension with a prospective cohort study and a large sample size. Our results indicate that UII is unlikely to play a role in the development of hypertension. Further confirmatory studies with UII system genetic expression based on population are required.

Data Availability

The data used to support the findings of this study were supplied by Mingzhi Zhang under license and so cannot be made freely available. Requests for access to these data should be made to Mingzhi Zhang, zhangmingzhi@suda.edu.cn.

Disclosure

Huijian Xie, Xinya Wang, and Yan He contributed equally to this work and should be considered as co-first authors. All authors have agreed to both be personally accountable for the author's contributions and to ensure questions related to the accuracy or integrity of any part of the work.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Z. M. designed the study, conducted investigation, performed data analysis, and revised the manuscript; X. H. performed laboratory testing, data analysis, and drafted the manuscript; W. X carried out the investigation and drafted the manuscript; H. Y., Q. X., and J. Y. carried out the investigation; H. D. and Z. S. carried out the investigation and

substantively revised the manuscript. All authors reviewed the paper and approved the submitted version.

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Research Article

Resveratrol Supplementation Prevents Hypertension in Hypertensive Pregnant Rats by Increasing Sodium Excretion and Serum Nitric Oxide Level

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Background. Pregnancy-induced hypertension (PIH) remains a major cause of morbidity and mortality in pregnancy worldwide. This study was designed to study the blood pressure-lowering effect of resveratrol (RES) in a salt-induced hypertensive pregnant rat model. *Methods*. Forty female Sprague Dawley (SD) rats were randomized into 4 groups: Normal Preg (0.9% salt diet), Normal Preg + RES (0.9% salt diet plus daily oral RES for 4 weeks), Salt Preg (8% salt diet), and Salt Preg + RES (8% salt diet plus daily oral RES for 4 weeks). Noninvasive blood pressure was recorded on gestational days 7 and 14. On the gestational day 19, foetuses were weighed, and blood and urine samples were harvested for electrolytes and biochemical assays. *Results*. RES significantly reduced SBP, DBP, and MAP on gestational days 7 and 14 in the Salt Preg + RES group compared to the Salt Preg group (all $P < 0.05$). Compared to the Salt Preg group, the foetal weight, serum NO level, urinary sodium, and 24 hour urine volume were significantly increased in the Salt Preg + RES group (all $P < 0.05$). On the contrary, the levels of serum urea, serum creatinine, and urinary protein were significantly decreased in the Salt Preg + RES group compared to the Salt Preg group (all $P < 0.05$). *Conclusions*. RES decreases blood pressure in a hypertensive pregnant rat model. Increasing sodium excretion and serum nitric oxide level might be, at least part of, the underlying mechanisms.

1. Introduction

Pregnancy-induced hypertension (PIH) complicates 6–10% of pregnancies [1]. It remains a major cause of morbidity and mortality in pregnancy worldwide, with the increased risk of renal failure, pulmonary edema, and stroke for mothers, as well as intrauterine growth restriction (IUGR), prematurity, and death for fetus [2].

It has been reported that endothelial dysfunction, oxidative stress, inflammatory responses, the renin-angiotensin system (RAS) activation, defective synthesis of nitric oxide (NO), and dysregulation of hydrogen sulfide (H_2S) producing enzymes contribute to maternal hypertension [3–6]. However, the underlying mechanisms involved in PIH are still not completely understood.

Resveratrol (3,5,4-trihydroxystilbene, RES) is a natural polyphenolic compound found in various plants species, including grapes, berries, and peanuts [7]. Numerous studies have demonstrated the diverse biologic effects of RES, such as antioxidative, anti-inflammatory, antiviral, and anti-platelet aggregation activities [8–11]. Other studies reported it could modulate the cell functions, signal transduction, and gene expression [12]. Some recent studies demonstrated that RES could increase sodium excretion [13] and the release of NO from endothelial cells [14], as well as protect against the development of general hypertension in rat models [15–17]. However, the effect of RES on NO synthesis and sodium excretion in PIH is still unclear.

Therefore, the present research was designed to study the effect of RES on blood pressure regulation in a hypertensive

pregnant rat model. Our results suggested that RES might be a potential blood pressure lowering (BPL) agent for PIH treatment.

2. Methods

2.1. Animals. A total of 20 male Sprague Dawley (SD) rats weighing between 190 and 210 g 9- to 10-week old and 40 female SD rats weighing between 180 and 200 g 9- to 10-week old were purchased from the Animal Center of Xi'an Jiaotong University (Xi'an, China). Rats were kept in sterile cages under standard laboratory conditions. All animals were allowed free access to tap water and standard rodent diet. All experiments were performed in accordance with the "National Institutes of Health Guidelines on the Use of Laboratory Animals" and approved by the Ethics Committee, Xi'an Jiaotong University Health Science Center.

2.2. Reagents. Resveratrol was purchased from Sigma Chemical (USA). Dimethyl sulfoxide (DMSO) and RPMI-1640 were from Xi'an Sobeo Pharmtech Co., Ltd. (Xi'an, China). The RES was dissolved in DMSO and then diluted to 5 mg/mL in RPMI-1640.

2.3. Experimental Design. Forty female rats were randomized into four groups (10 rats/group)s: Normal Preg: the rats were fed with normal salt chow (0.9% NaCl) for 10 weeks; Normal Preg + RES: rats were fed with the same feeding regimens as the Normal Preg group, plus daily oral RES (250 mg/kg/day, by intragastric gavage) for 4 weeks (from the 7th week till the 10th week); Salt Preg: the rats were fed with high-salt chow (8% NaCl) [18] for 10 weeks; Salt Preg + RES: rats were fed with the same feeding regimens as the Salt Preg group, plus daily oral RES (250 mg/kg/day, by intragastric gavage) for 4 weeks (from the 7^{th} week till the 10^{th} week). We choose high salt intake to induce the PIH model due to its noninvasiveness.

For these female rats, the oestrous cycle was monitored by the vaginal smear method from the 7th week [19]. Each female rat on the proestrous phase was separately mated with male rat overnight. Mating was confirmed by the presence of sperm in a vaginal smear on the next day, and this day was considered as day 1 of pregnancy.

2.4. Noninvasive Blood Pressure Measurement. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) (mmHg) were measured before mating and on gestational days 7 and 14 using a tail-cuff blood pressure instrument (Kent Scientific, Torrington, CT, USA).

2.5. Euthanasia and Foetal Weight Measurement. On the gestational day 19, the rats were euthanized by ether and received laparotomy. The blood samples were collected from the suprahepatic segment of inferior vena cava using a cannula for analysis, and the foetuses were isolated and weighed.

2.6. Serum and Urine Samples Collection. Blood samples were centrifuged at 4000 r/min at 4C for 3 min, and serum was taken and stored at −20°C for measurement of the serum electrolytes, urea, creatinine, and NO levels.

Twelve-hour urine samples were individually collected in a metabolic cage on the gestational day 18 and preserved with toluene. Urine samples were used for measurement of urinary electrolytes and protein levels, as well as urine volume.

2.7. Measurement of Serum Electrolytes, Urea, Creatinine, and NO Levels. Serum levels of sodium, potassium, urea, creatinine, and NO were measured by an automated chemistry analyzer (Shenzhen Kaguwi Imp & Exp Co., Ltd., Shenzhen, China) using commercial diagnostic kits following the manufacturer's instructions (Jilin Painuo Biological Technology, Ltd., Jilin, China).

2.8. Measurement of Urinary Electrolytes and Protein Levels. Urinary levels of sodium and potassium were measured using the same method described above for serum electrolytes. Urinary protein concentration was determined by Bradford's method (Bio-Rad protein assay, Kidlington, UK) [20].

2.9. Statistical Analysis. Data were presented as mean ± SD. Statistical differences were determined by using SPSS 24.0 software. As a first step, analysis of variance of factorial design was performed to check for any interaction between salt concentration and RES on blood pressure, foetal weight, and other biochemical indexes. If an interaction was ruled out, the pooled analysis remained the primary analysis. If an interaction could not be ruled out, then the effect of RES in different salt concentration subgroups would be considered by the *t*-test or the nonparametric test. Differences were considered statistically significant if the P value was <0.05.

3. Results

3.1. Blood Pressure. SBP, DBP, and MAP were measured before mating and on gestational days 7 and 14 using a noninvasive tail-cuff method. An interaction existed between salt concentration and RES with respect to SBP, DBP, and MAP (all $P < 0.001$). In the 10th week, compared to the Salt Preg + RES group, all three parameters were slightly increased in the Salt Preg group. However, there was no statistical difference ($P > 0.05$, data not shown). On gestational days 7 and 14, compared to the Salt Preg group, all three parameters were obviously decreased in the Salt Preg + RES group (all $P < 0.001$), but there was no statistical difference in the three parameters between group Normal Preg + RES and group Normal Preg (all $P > 0.05$). It was declared that RES has an antihypertensive effect with high concentration of salt, and it may not have antihypertensive effect if it is used for pregnancy hypertension with normal salt intake (Table 1).

Table 1: Effect of RES on blood pressure in hypertensive pregnant rats.

Blood pressure		Normal Preg	Normal Preg + RES	Salt Preg	Salt Preg + RES	Salt* RES[1]		Normal Preg + RES vs. Normal Preg		Salt Preg + RES vs. Salt Preg	
						F	P	t	P	t	P
SBP (mmHg)	Gestational day 7	117.3 ± 3.7	120.4 ± 2.9	148.5 ± 4.9	130.1 ± 3.2	95.907	<0.001	−2.046	0.056	11.281	<0.001
	Gestational day 14	99.5 ± 4.0	97.5 ± 24.2	151.8 ± 5.1	128.5 ± 3.7	61.129	<0.001	1.122	0.277	11.684	<0.001
DBP (mmHg)	Gestational day 7	85.7 ± 3.7	86.9 ± 3.3	116.5 ± 2.6	107.5 ± 3.2	25.189	<0.001	−0.748	0.464	6.972	<0.001
	Gestational day 14	60.4 ± 4.5	57.9 ± 4.7	113.8 ± 3.4	96.9 ± 3.8	30.174	<0.001	1.237	0.232	10.516	<0.001
MAP (mmHg)	Gestational day 7	92.5 ± 4.2	94.4 ± 5.4	130.0 ± 4.9	111.4 ± 4.5	46.344	<0.001	−0.886	0.388	8.840	<0.001
	Gestational day 14	74.3 ± 4.0	77.3 ± 4.2	118.7 ± 4.6	99.4 ± 2.3	82.780	<0.001	−1.652	0.116	11.850	<0.001

Salt* RES[1], interaction between salt and RES.

3.2. Foetal Weight Measurement. On the gestational day 19, the foetuses were isolated and weighed. The interaction existed between salt concentration and RES with respect to foetal weight ($P < 0.05$). Compared to the Salt Preg group, the foetal weight was increased in the Salt Preg + RES group ($P < 0.05$), and there was no statistical difference in foetal weight between the Normal Preg + RES group and Normal Preg group ($P > 0.05$) (Figure 1).

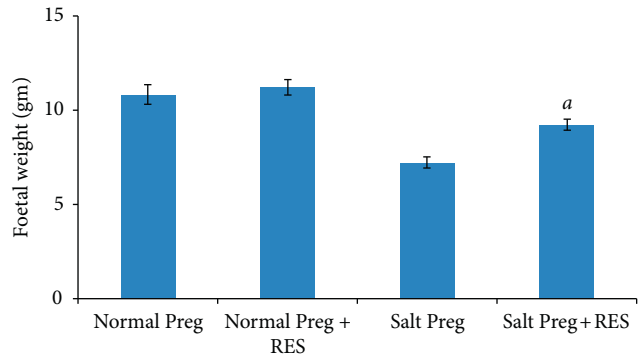

Figure 1: Foetal weight in hypertensive pregnant rats following RES supplementation. [a]$P < 0.05$ vs. Salt Preg.

3.3. Measurement of Serum Electrolytes, Urea, Creatinine, and NO Levels. The interaction existed between salt concentration and RES with respect to serum sodium, potassium, urea, creatinine, and NO levels (all $P < 0.05$). There was no statistical difference in serum sodium and potassium between the Salt Preg + RES group and Salt Preg group or between the Normal Preg + RES group and Normal Preg group (all $P > 0.05$) (Figures 2(a) and 2(b)). The levels of serum urea and serum creatinine were significantly higher in the Salt Preg group compared to the Salt Preg + RES group (all $P < 0.05$), and there was no statistical difference in serum urea and serum creatinine between the Normal Preg + RES group and Normal Preg group ($P > 0.05$) (Figures 2(c) and 2(d)). For the level of serum NO, it was significantly lower in the Salt Preg group than the Salt Preg + RES group ($P < 0.05$), and there was no statistical difference in serum NO between the Normal Preg + RES group and Normal Preg group ($P > 0.05$) (Figure 3).

3.4. Measurement of Urinary Electrolytes and Protein Levels. The interaction existed between salt concentration and RES with respect to urinary sodium, potassium, and protein levels (all $P < 0.05$). There was no statistical difference in urinary potassium between the Salt Preg + RES group and Salt Preg group or between Normal Preg + RES group and Normal Preg group (all $P > 0.05$). However, the urinary sodium was significantly increased in the Salt Preg + RES group compared to the Salt Preg group ($P < 0.05$), and there was no statistical difference in urinary sodium between the Normal Preg + RES group and Normal Preg group ($P > 0.05$) (Figures 4(a) and 4(b)). For the urinary protein level, it was obviously higher ($P < 0.05$) in the Salt Preg group than the Salt Preg + RES group, and there was no statistical difference in urinary protein between the Normal Preg + RES group and Normal Preg group ($P > 0.05$) (Figure 4(c)).

3.5. Twenty-Four-Hour Urine Volume. The interaction existed between salt concentration and RES with respect to 24-hour urine volume ($P < 0.05$). The 24-hour urine volume was significantly higher in the Salt Preg + RES group compared to the Salt Preg group, and there was no statistical difference in 24-hour urine volume between the Normal Preg + RES group and Normal Preg group ($P > 0.05$) (Figure 4(d)).

4. Discussion

The effect of RES on blood pressure regulation has been previously studied. A recent systematic review concluded that RES appears to have antihypertensive effects, depending on the dose and duration of treatment, and one of the

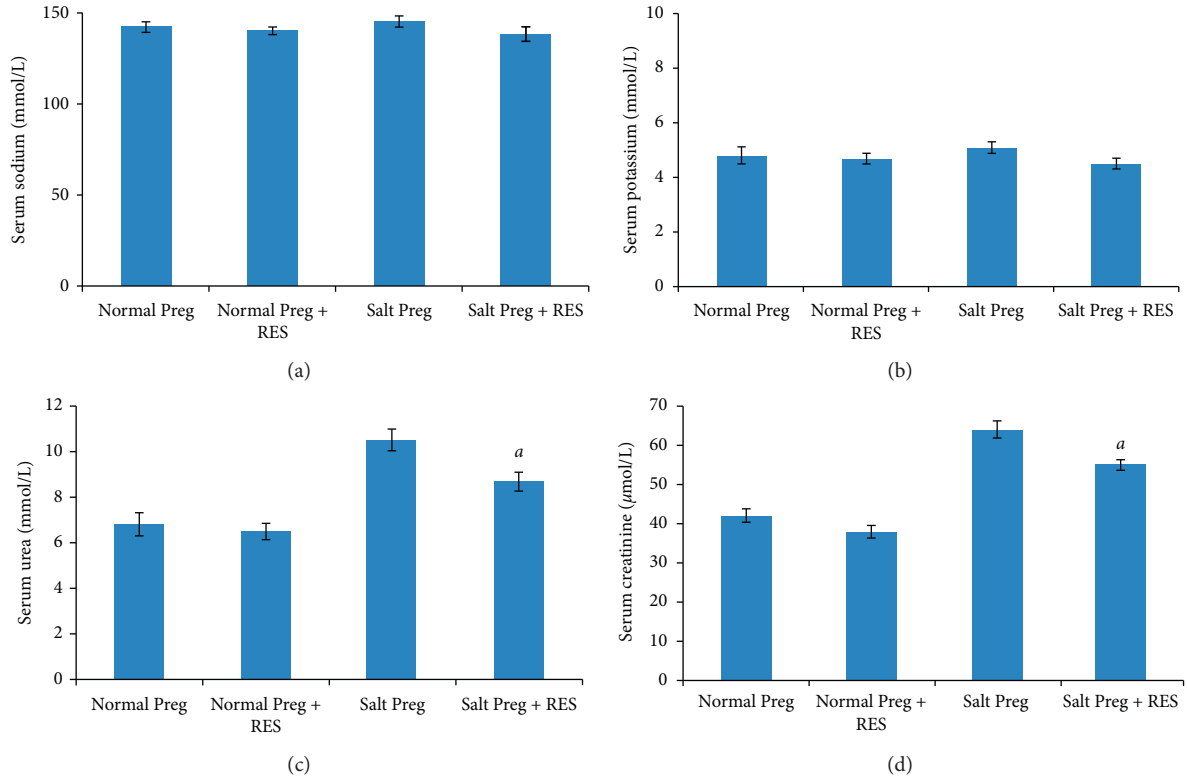

Figure 2: Serum levels of sodium (a), potassium (b), urea (c), and creatinine (d) in hypertensive pregnant rats following RES supplementation. $^{a}P < 0.05$ vs. Salt Preg.

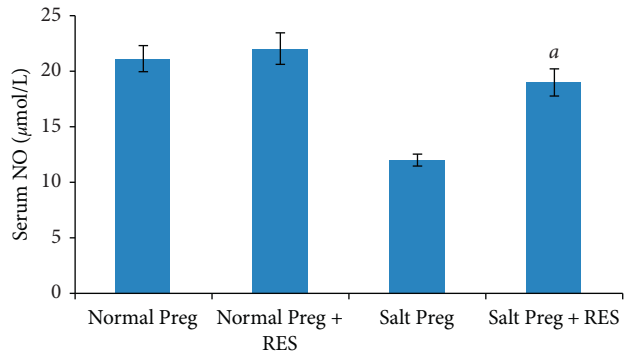

Figure 3: Serum level of NO in hypertensive pregnant rats following RES supplementation. $^{a}P < 0.05$ vs. Salt Preg.

important blood pressure-reducing mechanisms of RES is to increase the level of NO [21]. However, the effect of RES on PIH remains unclear. In the present study, we find that oral RES supplementation could decrease the blood pressure of high-salt diet-induced hypertensive pregnant rats compared to control animals without RES supplementation (Table 1).

Increased salt intake is an important factor in elevating the BP in human [22]. Sodium retention causes expansion of extracellular volume (ECV) and total vascular volume, which may lead to hypertension. Thus, reducing the amount of sodium in the body became an important therapeutic strategy for decreasing BP. For example, diuretics exert their hypotensive effects via increasing sodium excretion [23]. In our study, we used high-salt diet to induce hypertension in pregnant rats. Our results demonstrated that RES supplementation could significantly increase the excretion of sodium in urine without affecting the serum sodium level in a hypertensive pregnant rat model. Consistent with this, the 24 h urine volume was also significantly increased in the hypertensive pregnant rats treated with RES supplementation compared to control rats without RES treatment (all $P < 0.05$). These findings are consistent with a published report showing RES infusion increases sodium excretion while not altering the glomerular filtration rate (GFR), suggesting it may have a direct effect on renal tubular sodium handling [24].

Preeclampsia (PE) complicates about 3–5% of all pregnancies and is simply defined by the new onset of hypertension and proteinuria occurring from 20 weeks of gestation [25]. Studies had demonstrated that kidney function injury frequently occurred in PE patients even before proteinuria detection [26]. The protective effect of RES on kidney functions had been previously studied. Kitada et al. showed that, in diabetic db/db mice, RES treatment for 8 weeks (0.3% diet) resulted in decreased urinary albumin excretion [27]. Qiao et al. showed that treatment of STZ-induced diabetic SD rats with RES (20 mg/kg/day) for 4 weeks resulted in reduced serum glucose and creatinine levels [28]. Consistent with these results, we observed that the serum urea and creatinine levels, as well as the urinary protein level, were obviously increased in a rat model of pregnant hypertension.

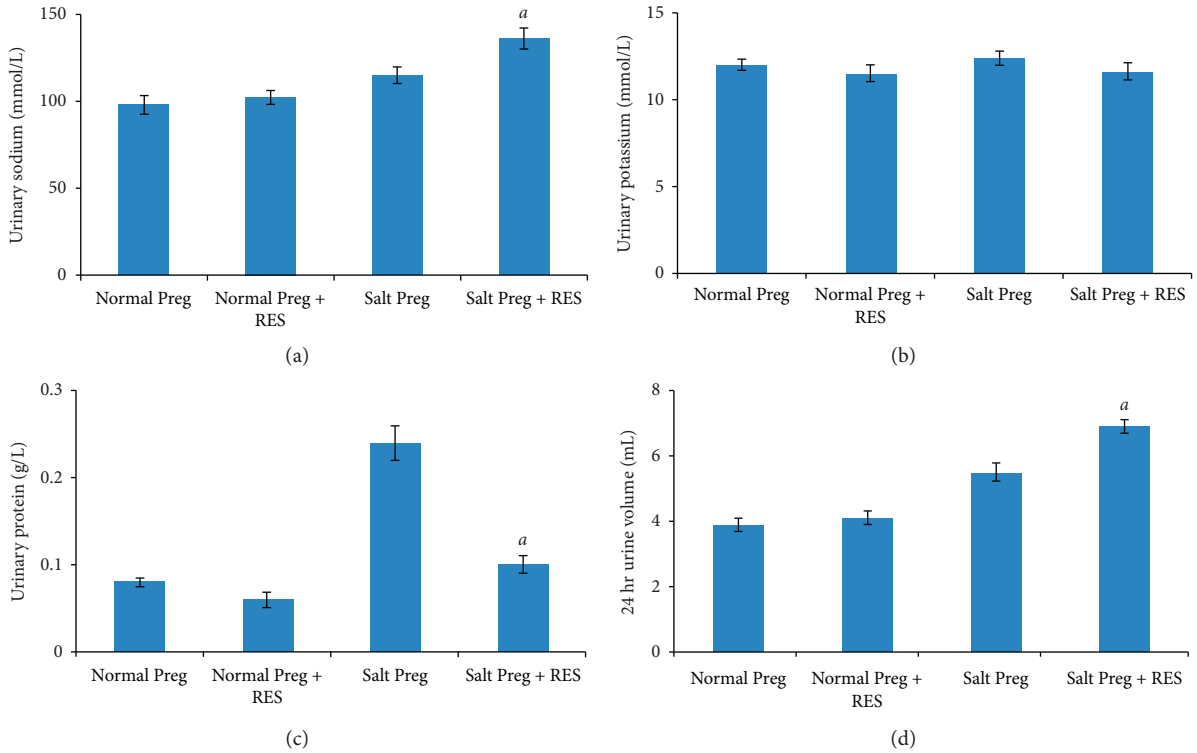

Figure 4: Urinary levels of sodium (a), potassium (b), and protein (c), as well as 24 h urine volume in hypertensive pregnant rats following RES supplementation. $^{a}P < 0.05$ vs. Salt Preg.

However, RES supplementation significantly reduced these parameters compared to control pregnant rats (all $P < 0.05$).

Another suggested mechanism of PIH is defective NO synthesis, which could lead to endothelial dysfunction [29]. NO-mediated activation of cGK leads to relaxation of vascular smooth muscle cells, which could cause vasodilatation and subsequent blood pressure reduction [30]. Previous studies had showed that RES could increase the production of NO in endothelial cells by upregulating the expression of endothelial NO synthase (eNOS), stimulating eNOS enzymatic activity, and preventing eNOS uncoupling [31]. In a recent study, Xu et al. found RES could also stimulate NO release in rat platelets [32]. In this study, the level of serum NO was significantly decreased in the pregnant hypertensive rats, and as expected, RES supplementation significantly increased it to a level comparable to nonhypertensive pregnant control rats. Previous studies had demonstrated that NO is also a potent natriuretic agent, playing a major role in inhibiting epithelial sodium channel (ENaC) activity in the collecting duct [33], which hinted that the different antihypertensive mechanisms of RES might result in a synergistic effect.

Having low birth weight is associated with fetal and neonatal morbidity and mortality [34]. PIH had been identified to be closely associated with low birthweight [35]. The weight of the foetal rat on the 19th day of gestation varied slightly in different reports. In our study, the foetal weights in normal salt intake groups are comparable to that reported by Arikawe et al.[18]. However, foetal weight was obviously lower in the hypertensive pregnant rats than the control pregnant rats, and RES supplementation partly reversed this trend (all $P < 0.05$). These results are in line with a previous study showing subcutaneous maternal RES treatment increases uterine artery blood flow in the pregnant ewe and increases fetal growth [36]. We hypothesized that the effect of increasing foetal weight by RES may be related to its upregulation on the serum NO level.

5. Conclusion

Taken together, our present study has provided the first evidence that increasing sodium excretion and serum nitric oxide level might be, at least part of, the underlying mechanisms by which RES decreases blood pressure in a hypertensive pregnant rat model. RES could be a promising candidate in the development of an effective BPL agent for PIH treatment.

However, the mechanism of PIH in humans is complex, and high salt intake or defective NO synthesis is only part of the risk factors. Therefore, further studies are needed to identify the detailed mechanisms by which RES regulated blood pressure in pregnancy for its possible application in the treatment of PIH.

Data Availability

All data generated or analysed during this study are included in this published article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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