Cardiology Research and Practice

Endothelial Injury and Repair in Coronary Heart Disease 2021

Lead Guest Editor: Zhen Yang
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Contents

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Review Article

Flow-Mediated Dilatation in the Assessment of Coronary Heart Disease: A Meta-Analysis

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Endothelial dysfunction may contribute to the increased morbidity and mortality associated with coronary heart disease (CHD). Flow-mediated dilatation (FMD) is the most popular noninvasive method for vascular endothelial function evaluation. This meta-analysis aimed to investigate the association between FMD and CHD. We searched the publications listed in the PubMed, Web of Science, Scopus, and Embase databases. Stata 14 software was used to analyze the data. Standardized mean difference (SMD) was used to calculate FMD levels, and the effect sizes were expressed with a 95% confidence interval (CI). I^2 statistics were used to evaluate statistical heterogeneity. In this meta-analysis, 9 studies enrolled a total number of 943 participants, including 534 (56.63%) patients with CHD and 409 controls (43.37%). We found that patients with CHD showed a significantly lower FMD than the controls (SMD −0.706%; 95% CI: −0.985, −0.427; $P = 0.001$) with high heterogeneity. In addition, funnel plot analysis suggested asymmetry that could be evidence of publication bias. But sensitivity analyses show that there were no influential studies. This meta-analysis provides evidence that patients with CHD show a significantly lower FMD than controls and highlights the literature on FMD as a hallmark in CHD diseases.

1. Introduction

The vascular endothelium plays an essential role in various pathological and physiological processes, for instance, regulating vascular homeostasis, vasoconstriction and dilation, thrombosis, fibrinolysis, inhibition of inflammation, and smooth muscle cell function [1]. An imbalance between the magnitude of injury and the repair capacity of the endothelium is a key factor in the occurrence and development of various cardiovascular diseases, such as myocardial infarction, stroke, and hypertension [2]. Accumulating evidence has confirmed that endothelial dysfunction is considered an important pathological feature of early atherosclerosis [3]. Endothelial function deteriorates during the natural history of cardiovascular diseases, suggesting that it may be a potential biomarker in the context of this disease and warrants further investigation [4]. Therefore, early detection of vascular endothelial dysfunction, timely intervention, and guided treatment are significant for maintaining cardiovascular health and reducing morbidity and mortality associated with cardiovascular diseases and medical costs [5].

Currently, invasive and noninvasive methods are commonly used to evaluate vascular function. Invasive tests require the injection of acetylcholine into human coronary arteries, but their clinical application is limited because they are invasive, time-consuming, and expensive, and they are not recommended for healthy or asymptomatic patients [5]. The noninvasive endothelial function detection method first appeared in the 1990s. With the continuous development of basic and clinical research, the medical industry has paid

increasing attention to examining endothelial function in cardiovascular diseases, and noninvasive vascular endothelial function detection has become a research hotspot [6]. At present, brachial artery flow-mediated dilatation (FMD) is the most popular method for evaluating vascular endothelial function evaluation [7]. It has the advantages of less or no trauma, relatively low cost, simple operation, good repeatability, and wide application and is more acceptable [7]. Therefore, FMD detection has been widely adopted in cardiovascular research in clinical trials.

Many large prospective cohort studies have accepted FMD as an adjunctive marker of coronary heart disease (CHD). Several studies found that a decreased FMD could increase the risk of CHD [8, 9]. However, some of these studies have been summarized in qualitative reviews. Therefore, we conducted a meta-analysis that aims to describe FMD as a potential biomarker for CHD.

2. Methods

2.1. Search Strategy. This meta-analysis followed the guidelines of the preferred reporting items. We searched the publications listed in the electronic databases MEDLINE (source PubMed, January 1, 2001, to January 1, 2021) and Embase (January 1, 2001, to January 1, 2021) using the following text and keywords in combination both as MeSH terms and text word: "flow-mediated dilatation" and "coronary artery disease" or "coronary heart disease" or "coronary vascular disease."

2.2. Study Inclusion/Exclusion Criteria. Inclusion criteria of this study were as follows: patients with coronary heart disease; FMD brachial artery in CHD and control was detected by high-resolution ultrasound methods, and the brachial artery FMD was calculated as [(reactive hyperemia diameter of the brachial artery-baseline diameter)/baseline diameter × 100%]; and the enrolled studies were prospectively or retrospectively designed. Exclusion criteria were as follows: patients with infections, tumors, and immune disease; incomplete FMD data; literature that was duplicated and poorly designed; and reviews.

2.3. Search and Screening. Two investigators independently screened the titles and abstracts of the studies and excluded those that did not meet the inclusion criteria. After screening the full text, the studies that met the requirements were selected according to the inclusion and exclusion criteria. If two authors had objections to the extracted literature, a third investigator was invited to arrive at the final conclusion. Finally, the articles were written according to the systematic reviews and meta-analyses guidelines.

2.4. Data Analysis and Statistical Methods. Stata 14 software was used to analyze the data. Standardized mean difference (SMD) was used to calculate FMD levels, and the effect sizes were expressed with a 95% confidence interval (CI). Chi-square Cochran's Q test and the I^2 statistic were used to assess the heterogeneity between studies. In detail, if there was no heterogeneity ($I^2 < 50\%$, $P > 0.05$), the fixed-effects model was used for analysis. If heterogeneity existed ($I^2 \geq 50\%$, $P \leq 0.05$), the random-effects model was used for analysis. Sensitivity analysis was conducted to determine the stability and credibility of the results. Funnel plots and shear complement methods were used to detect publication biases.

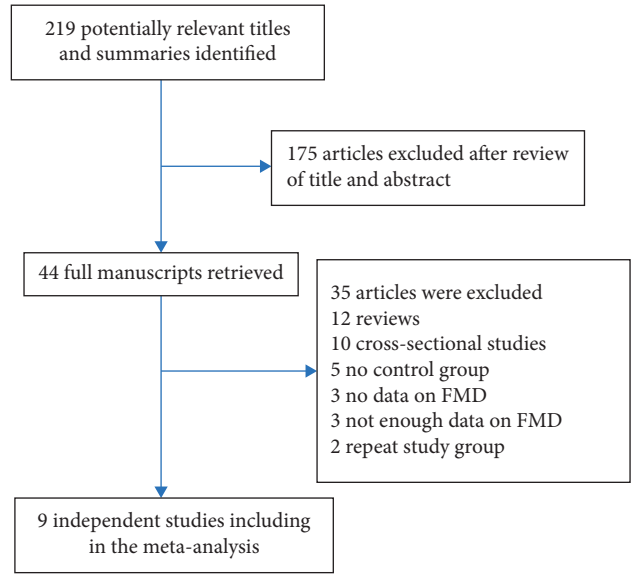

Figure 1: Flowchart of the selection of studies included in meta-analysis.

3. Results

3.1. Literature Results. The results of the literature search are shown in Figure 1. After reviewing the titles and abstracts, we retrieved 219 unique citations and eliminated 175. Of the 44 full manuscripts retrieved, 35 were excluded because 12 were reviews, 10 were cross-sectional studies, 5 had no controls, 3 had no data on FMD, 3 did not have enough data on FMD, and 2 were repeat studies. Ultimately, 9 articles were included in the meta-analysis [10–18].

3.2. Study Characteristics. All included studies were observational and were prospectively designed. As shown in Table 1, 9 studies used ultrasound-based FMD to determine the endothelial function in this meta-analysis. A total of 943 participants were included: 534 patients with coronary heart disease (56.63%) and 409 controls (43.37%). Most controls were healthy. The study population is mainly European and Asian. The mean age for the entire study population was 58 years, and males represent 75.59% of them. Most patients in the control group were healthy (Table 1).

3.3. Endothelial Function Assessment in Cardiovascular Diseases. In 9 studies, heterogeneity was $I^2 = 71.2\%$ and $P = 0.001$, suggesting heterogeneity was statistically significant among these studies. Therefore, a random-effects

Table 1: Clinical data of patients with CHD and controls in included studies.

Study	Country	CHD	Control	Pop (*n*)	Man (%)	Age (mean) (years)		Hypertension (%)		Diabetes (%)		CAD (%)		Smoking (%)	
						CHD	Control	CHD	Control	CHD	Control	CHD	Control	CHD	Control
Maciej K (10)	Poland	CAD	No CAD	65	0	50.3	50.2	66	61	44	15	25	24	75	39
Kyoung HP (11)	Korea	CAD	No CAD	413	76.5	59.4	58.1	56.9	52.9	26.9	22.2	13.8	15	37.7	25.5
Rui H (12)	China	CAD	Healthy	135	70.3	62.4	54.9	62.5	34.8	20.3	8.7	NA	NA	37.3	20.2
Yoshifumi O (13)	Japan	CAD	Healthy	33	NA	61.3	64.7	NA	NA	NA	NA	NA	NA	56	40
Sergey K (14)	Russia	CAD	Healthy	78	100	43	43	4	17	NA	NA	80	NA	86	52
Stefan A (15)	Sweden	CAD	Healthy	33	NA	57	53	NA	NA	NA	NA	NA	NA	48	12
Ibrahim A (16)	Turkey	CAD	Healthy	56	100	52.5	49.69	NA	NA	36	0	NA	NA	77	86
Anil N (17)	Canada	CAD	Healthy	20	100	56	38	10	0	20	0	NA	NA	30	0
Micheal S (18)	Israel	CAD	Healthy	110	83	60	55	48	36	24	6	NA	NA	17	14

CHD: coronary heart disease. CAD: coronary artery disease.

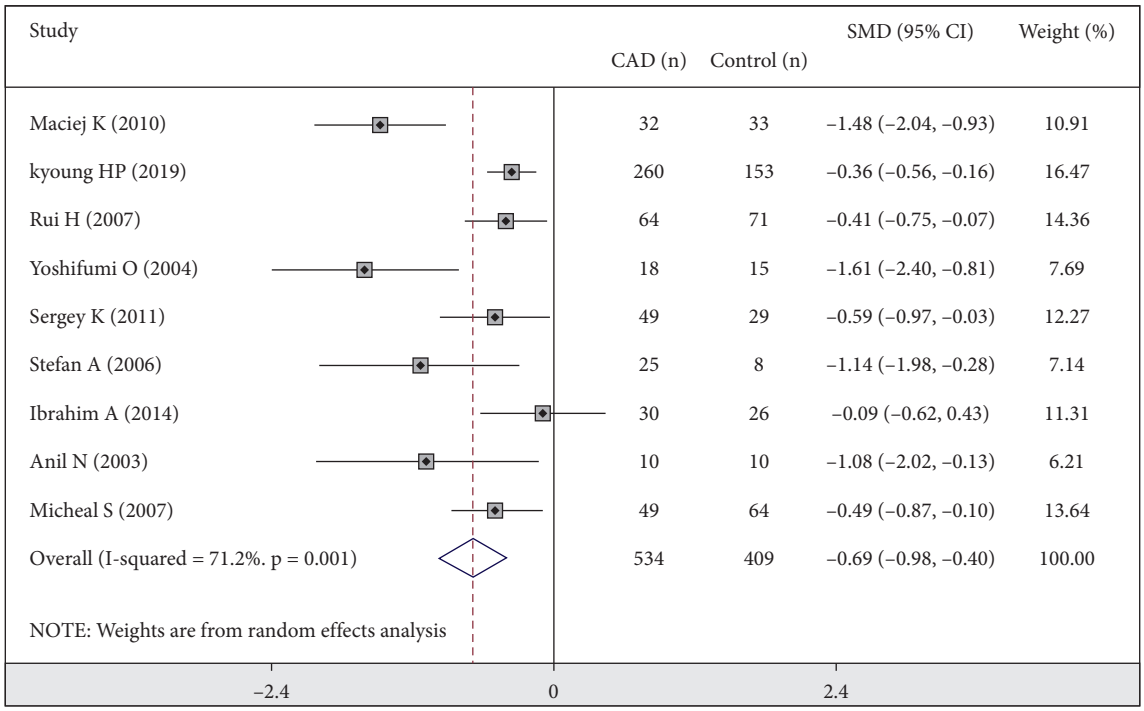

Figure 2: SMD with 95 % CI of FMD in CHD and control.

model was used to analyze the data in this meta-analysis. As shown in Figure 2, we found that the 534 coronary heart disease patients showed a significantly lower FMD than the 409 controls (MD −0.690%; 95% CI: −0.981, −0.398; $P = 0.001$), suggesting that endothelial dysfunction correlates with coronary heart diseases.

3.4. Publication Bias. Publication bias was assessed using a funnel plot analysis. As shown in Figure 3, funnel plot analysis confirmed the existence of significant publication bias, suggesting that publication bias was present in this meta-analysis. Thus, we used the trim and fill method to correct the funnel asymmetry caused by publication bias, and we obtained a similar SMD, indicating that although there was a certain publication bias, the results were stable and reliable. As shown in Figure 4, a sensitivity analysis was performed by culling the included studies one by one, and the SMD was reweighed to evaluate the stability of the results. Similarly, there was a significant difference in FMD between patients with CHD and control, indicating stable results.

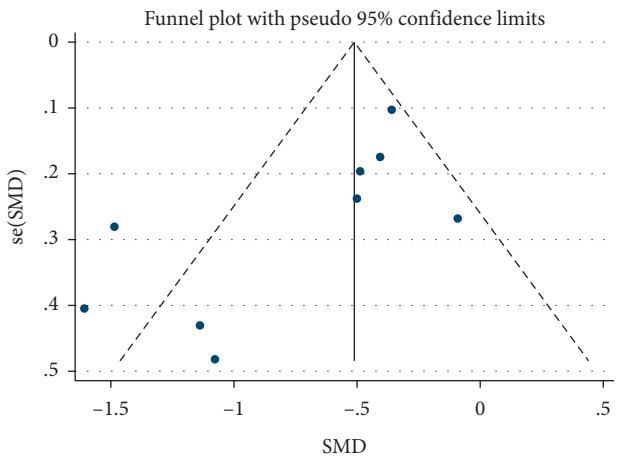

Figure 3: Funnel plot for analyzing publication bias.

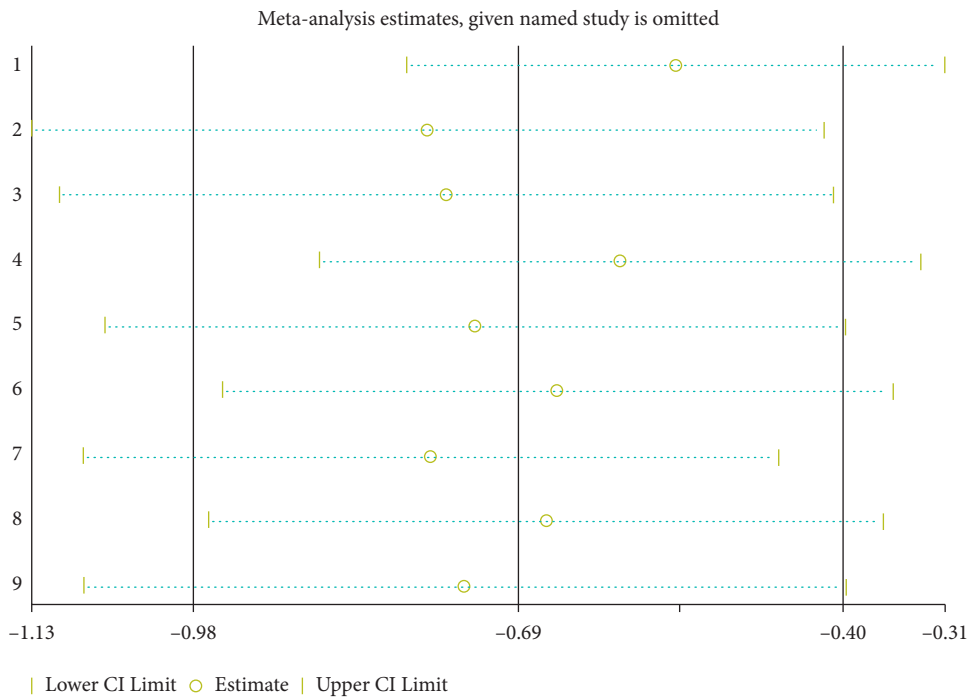

Figure 4: Sensitivity analysis of the meta-analysis.

4. Discussion

Endothelial cell damage and dysfunction play an important role in developing the process [3]. Studies have shown that arteriosclerosis is related to CHD and can increase the risk of cardiovascular disease [19, 20]. In recent clinical studies, FMD, an accepted method for noninvasive assessment of systemic endothelial function, has been extensively examined [21]. Accumulating evidence indicates that FMD is a hallmark of CHD [10–18]. However, few reviews have summarized the relationship between endothelial function and CHDs. In this study, we systematically reviewed the scientific literature and assessed the differences in endothelial function between the CHD and control groups.

The present meta-analysis results consistently showed that CHDs might be associated with endothelial dysfunction, as evaluated by ultrasound-based FMD of the brachial artery. In particular, we demonstrated that 534 patients with CHD showed a significantly lower FMD than the 409 controls (SMD −0.690%; 95% CI: −0.981, −0.398; $P = 0.001$), indicating that patients with CHD show a reduced vasodilatory response and endothelial function. Therefore, evaluation of FMD can be a useful noninvasive diagnostic tool for CHD risk assessment.

Many cardiovascular risk factors affect endothelial function, such as race, age, estrogen, hypertension, diabetes, and smoking [22]. Although most patients with CHD have a history of smoking, the relationship between FMD and CHD

appears to be more complex, and smoking habits may not fully explain the decline in FMD of CHD in this clinical setting.

The funnel plot analysis confirmed the existence of significant publication bias. However, we obtained a similar SMD by the trim and fill method, and there was no obvious difference compared with previous results, possibly because of low power due to the small number of studies [23]. In addition, we obtained the stability of the results in this meta-analysis, which was evaluated by sensitivity analysis. Therefore, we conclude that the results were stable and reliable.

Accumulating evidence indicates that early endothelial dysfunction is a reversible disorder, and several interventions, such as exercise and bilirubin or other medicine, can increase endothelial function and reduce CVD risk [24, 25]. If FMD is taken to be an early marker for CHD, vascular endothelial dysfunction will be evaluated early. Timely intervention and guided treatment can reverse endothelial dysfunction and reduce the morbidity and mortality associated with CHD.

Some potential limitations of our study must be discussed. First, there was a publication bias in this meta-analysis. However, the combined SMD was consistent with the uncut and complemented conclusions, indicating that although there was a certain publication bias, the results were stable and reliable. Second, heterogeneity among the studies was generally significant, and it was not possible to conclusively ascertain sources of heterogeneity. Finally, the small number of studies and participants limited the accuracy of the results. Further efforts will be made to conduct further studies in the future.

5. Conclusion

In conclusion, this meta-analysis provides evidence that patients with CHD show a significantly lower FMD than controls and highlights the literature on FMD as a hallmark in CHD diseases. Although limited in number, these studies provide important evidence that FMD should be considered a worthy biomarker for assessment in future research.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Zhe Deng and Dehong Liu designed the study. Xiaoyong Xiao, Jingjing Wang, and Xiang Li analyzed the data, plotted the figures, and wrote the manuscript. Xiaohua Xiao was responsible for funding acquisition. All authors read and approved the final manuscript. Xiaoyong Xiao, Xian Li, Xiaohua Xiao, and Jingjing Wang contributed equally to this work.

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Research Article

Relationship of the Circulating Endothelial Progenitor Cells to the Severity of a Coronary Artery Lesion in Unstable Angina

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The number and function of circulating endothelial progenitor cells (EPCs) decreased in stable coronary artery disease. Nevertheless, there were few studies that explored the variation of EPC and the relationship with the severity of coronary artery lesions in unstable angina (UA). Therefore, this leaves an area for the investigation of the difference in the number and activity of circulating EPCs and the relationship with the Gensini score in unstable angina. Fluorescence-activated cell sorter analysis, as well as DiI-acLDL and lectin fluorescent staining measure the number of circulating EPCs. The transwell chamber assay and MTT were evaluated by the migration and proliferation of circulating EPCs. In addition, the flow-mediated dilation (FMD), Gensini score, and IL-6 levels in plasma were determined. We found that UA patients had the higher number and lower function of circulating EPCs. With the increase in severity of coronary artery lesions, the migration and proliferation of EPCs were decreased. Moreover, the function of the circulating EPCs was negatively associated with severity of coronary artery lesions in unstable angina. In addition, UA patients presented elevated IL-6, which was negatively correlated with the function of circulating EPCs and FMD and positively correlated with the severity of coronary artery lesions evaluated by the Gensini score. These findings revealed the decline in the function of circulating EPCs was associated with the severity of coronary artery disease, which may be related to systemic inflammation.

1. Introduction

Vascular endothelial injury and endothelial dysfunction are the initiating factors of coronary atherosclerosis. Clinical studies have shown that the combined action of risk factors such as hypercholesterolemia and hypertension leads to endothelial injury and repair imbalance, promotes the development of atherosclerosis, leads to coronary artery stenosis and occlusion, and finally leads to heart disease with myocardial ischemia, hypoxia, and necrosis [1–3]. The Gensini score is a very effective method to evaluate the severity of coronary artery disease. Studies have confirmed that the Gensini score has widely been used to explore the correlation between the severity of coronary artery lesions and some clinical indexes [4–6].

Endothelial progenitor cells (EPCs) are pluripotent stem cells derived from the bone marrow. After entering the blood, they eventually differentiate into mature vascular endothelial cells and participate in the process of angiogenesis and repair after endothelial injury [7, 8]. When the vascular endothelium is injured, EPCs are mobilized and released from the bone marrow to peripheral circulation to accelerate vascular re-endothelialization through chemotaxis, adhesion, migration, and proliferation, which plays a key role in the repair of vascular endothelium injury. A large number of studies have confirmed that cardiovascular risk factors and patients with coronary heart disease can lead to different degrees of vascular endothelial injury, and the effect on peripheral circulation EPCs is inconsistent [9]. The more

risk factors, the more obvious the decline in the number and function of EPCs, suggesting that the lower function of repairing endothelial injury [10]. In patients with coronary heart disease, the mobilization of peripheral EPC was also inconsistent. In patients with stable angina pectoris, the number and function of EPC decreased, while in patients with unstable angina pectoris, the mobilization of peripheral EPC increased [11–13]. However, there are few studies on the relationship between peripheral circulation EPC function and coronary artery severity in patients with unstable angina pectoris.

Inflammatory response is the key to the occurrence of atherosclerosis. When endothelial progenitor cells are inflamed, in order to reduce the damage caused by them, the body will trigger induced cells to promote the apoptosis of endothelial progenitor cells, leading to the migration of T cells, monocytes, platelets, and the proliferation of smooth muscle cells and then leading to the formation of atherosclerosis [14]. A large number of studies have shown that interleukin-6 (IL-6) plays an important role in regulating the adhesion, migration, and proliferation of endothelial progenitor cells and mediating the repair of vascular injury [14]. Therefore, we hypothesized that the function of EPCs in patients with unstable angina pectoris is related to the degree of coronary artery disease, which may be caused by the increase in IL-6. In order to test this hypothesis, this study detected the function of circulating EPCs and endothelial function evaluated by flow-mediated dilatation (FMD) of unstable angina pectoris, compared the changes of the different Gensini scores, analyzed its correlation with the Gensini score, and discussed the possible mechanism.

2. Methods

2.1. Characteristics of Subjects and Method. In this study, we enrolled 60 UA patients and 20 controls and their age was >18 years. Inclusion criteria were as follows: UA group: (1) the patients had typical angina attack within 1 month before admission; (2) the ECG scan showed ST-segment descending; (3) the left main artery, left anterior descending artery, left circumflex artery, and right coronary artery were the main blood vessels, and at least two orthogonal projection post urography showed that the diameter of the main blood vessels was narrowed ≥50%. Control group: the normal coronary artery was confirmed by selective coronary angiography. Exclusion criteria were as follows: (1) taking statins and other drugs that may affect circulation of EPC for more than 2 weeks before operation; (2) acute myocardial infarction, tumor, other heart diseases such as chronic cardiac insufficiency, infectious diseases, and liver and kidney dysfunctions; (3) the patient who previously received coronary intervention or coronary artery bypass surgery. The protocol was approved by the ethical committee of our hospital. The basic characteristics of enrolled patients are shown in Table 1.

The degree of the coronary artery lesion and the grouping degree of coronary artery stenosis were quantitatively evaluated by the percentage of reduction of a coronary artery diameter with reference to an angiography catheter or finger guide tube and QCA system software. The Gensini integral method was used to evaluate the degree of coronary artery disease. The quantitative score is as follows: stenosis ≤25% is 1 point, 25%~50% is 2 points, 51%~75% is 4 points, 76%~90% is 8 points, 91%~99% is 16 points, and 100% is 32 points. For multiple stenoses of a single vessel, the narrowest place was used as the score. Different coronary arteries should also be multiplied by corresponding coefficients, which are left main artery lesions, respectively ×5. The lesion of the left anterior descending branch is the proximal ×2.5 middle section ×1.5 far section ×1. The diagonal branch lesion is $D1 \times 1$, $D2 \times 0.5$, and the lesion of the left circumflex branch was proximal ×2.5. Both distal and posterior descending branches ×1 posterior branch ×0.5, the lesions of the right coronary artery were proximal, middle, distal, and posterior descending branches ×1. The score of coronary artery stenosis was the sum of the scores of each branch.

According to the Gensini score, the coronary heart disease group was divided into three subgroups: low-risk group: Gensini score ≤30; medium-risk group: Gensini score 31~59; severe-risk group: Gensini score ≥60.

Table 1: Clinical and biochemical characteristics of the two groups.

Characteristics	Control ($n = 20$)	UA ($n = 60$)
Age (years)	63.3 ± 9.3	66.9 ± 10.2
BMI (kg/cm^2)	24.7 ± 3.2	24.9 ± 3.3
Heart rate (beats/min)	74.0 ± 12.8	71.6 ± 11.4
Diastolic BP (mmHg)	68.6 ± 5.7	73.9 ± 8.2
Systolic BP (mmHg)	128.7 ± 20.9	132.8 ± 18.4
ALT (mmol/L)	29.6 ± 11.9	30.9 ± 15.5
AST (mmol/L)	31.0 ± 20.7	27.8 ± 14.4
GLU (mmol/L)	8.5 ± 4.5	7.5 ± 3.5
FMD (%)	8.7 ± 1.0	7.0 ± 1.2*
IL-6 (pg/ml)	6.7 ± 2.5	11.1 ± 3.1*

AST, aspartate aminotransferase; ALT, alanine transaminase; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; Cr, serum creatinine; CRP, C-reactive protein; GLU, glucose; FMD, flow-mediated dilation; IL-6, interleukin-6. Note. Data are given as the mean ± SD. *$P < 0.05$ vs. control.

2.2. Cell Culture Test to Evaluate the Number of Circulating EPCs. Blood samples were collected from enrolled patients. The isolation and culture of EPCs are described. The number of circulating EPCs was evaluated by the ratio of CD34+ KDR+ cells per 100 peripheral blood mononuclear cells as previously described. The number of cultured EPCs was also evaluated by DiI-acLDL/lectin double-positive cells/200 and counted manually by two independent observers blinded to the study.

2.3. EPC Migration and Proliferation Test. EPC proliferation was determined by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. After 7 days of culture, EPCs were digested with 0.25% trypsin and then transferred to a serum-free medium in a 96-well plate (200 ul/well). After 24 hours of culture, EPCs were supplemented with 10 μl

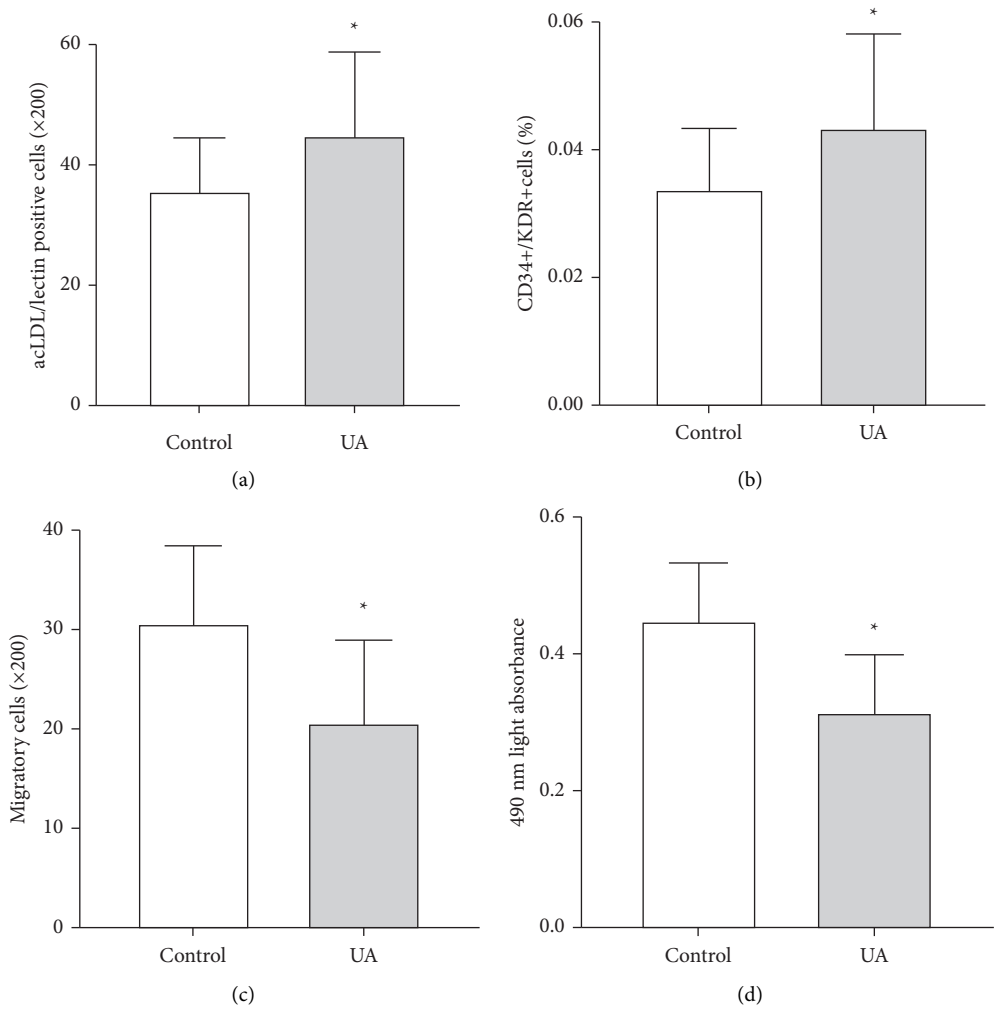

Figure 1: The number and function of circulating EPCs between the two groups. The number of circulating EPCs was detected by determining the number of CD34+/KDR+ cells per 100 peripheral blood mononuclear cells and then by examining the numbers of DiI-acLDL/lectin double-positive cells (a), (b) The results of migration and proliferation assays (c), (d) Statistical significance was evaluated using Student's t-test or analysis of variance. $^{*}P < 0.05$

MTT (5 g/L; Fluka, Sigma-Aldrich, St. Louis, Missouri, USA) and incubated for 4 hours. Then, the supernatant was discarded, and 200 μl dimethyl sulfoxide was added by shaking it for 10 min before measuring the optical density at 490 nm.

EPC migration is carried out by using a modified Boyden chamber. $2 * 10^4$ EPCs were placed in the upper chamber of the modified Boyden chamber. The incubator was placed in a 24-well culture dish containing EBM and human recombinant VEGF (50 ng/ml). After incubation at 370°C for 24 hours, the lower side of the filter was cleaned with PBS and then fixed with 4% paraformaldehyde. Cell nuclei were stained with DAPI for quantification. Then, the cells that migrated to the lower chamber were manually counted by two independent experimenters in three random microscope fields.

2.4. Measurement of Plasma Levels of IL-6. Plasma levels of IL-6 were measured by the high sensitivity enzyme-linked immunosorbent assay (R & D Systems, Wiesbaden, Germany) as previously described [15].

2.5. Evaluating FMD. In brief, brachial artery FMD was measured by high-resolution ultrasonography using a 5–12 MHz linear transducer on an HDI 5000 system (Washington, USA) as previously described [16].

2.6. Statistical Analysis. The statistical software was SPSS VII.0 (SPSS Corporation, Chicago, Illinois, USA). All the data were presented as the mean SD. Statistical significance was evaluated using Student's t-test or analysis of variance. Univariate correlations were calculated using Pearson's coefficient (r). $P < 0.05$ was considered statistically significant.

3. Result

3.1. Baseline Characteristics. Baseline characteristics and laboratory findings of the control and UA groups are listed in Table 1. There was no significant difference in age, BMI, blood pressure, heart rate, AST, ALT, and GLU found in the

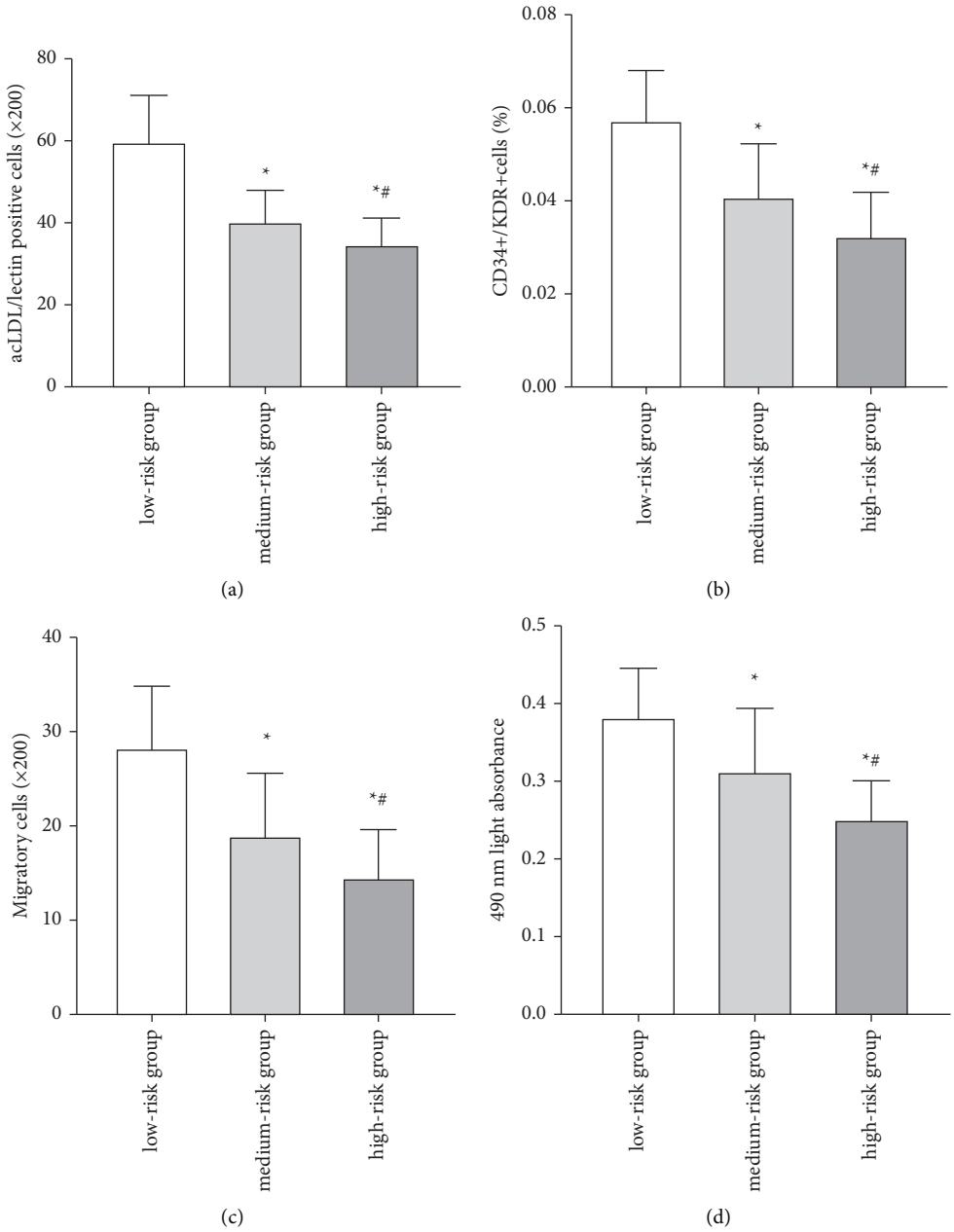

Figure 2: The number and function of circulating EPCs between three subgroups. As the Gensini score increased, the number of circulating EPCs was decreased (a), (b) and the migration and proliferation of circulating EPCs were also declined. (c), (d) Statistical significance was evaluated using Student's *t*-test or analysis of variance.

two groups ($P > 0.05$). The FMD in UA patients was lower than that in the control group ($P < 0.05$). However, the plasma IL-6 level in the UA group was significantly higher than that in the control group ($P < 0.05$).

3.2. EPC Number and Function between UA Patients and Control. The circulating EPC number (a-b) decreased in patients with UA compared with control (Figures 1(a) and 1(b), $P < 0.05$). Compared with the control group, the migration and proliferation of circulating EPCs decreased in UA patients (Figures 1(c) and 1(d), $P < 0.05$).

3.3. EPC Number and Function between Three Subgroups. As the Gensini score increased, the number of circulating of EPCs was decreased (Figures 2(a) and 2(b), $P < 0.05$), and the migration and proliferation of circulating of EPCs were also declined (Figures 2(c) and 2(d), $P < 0.05$).

3.4. Correlation between EPC Function and FMD with Severity of Coronary Artery Lesions. As shown in Figures 3(a) and 3(b), FMD was positively correlated with the migration and proliferation of circulating EPCs (Figures 3(a) and 3(b), $P < 0.05$). In contrast, the Gensini score was negatively

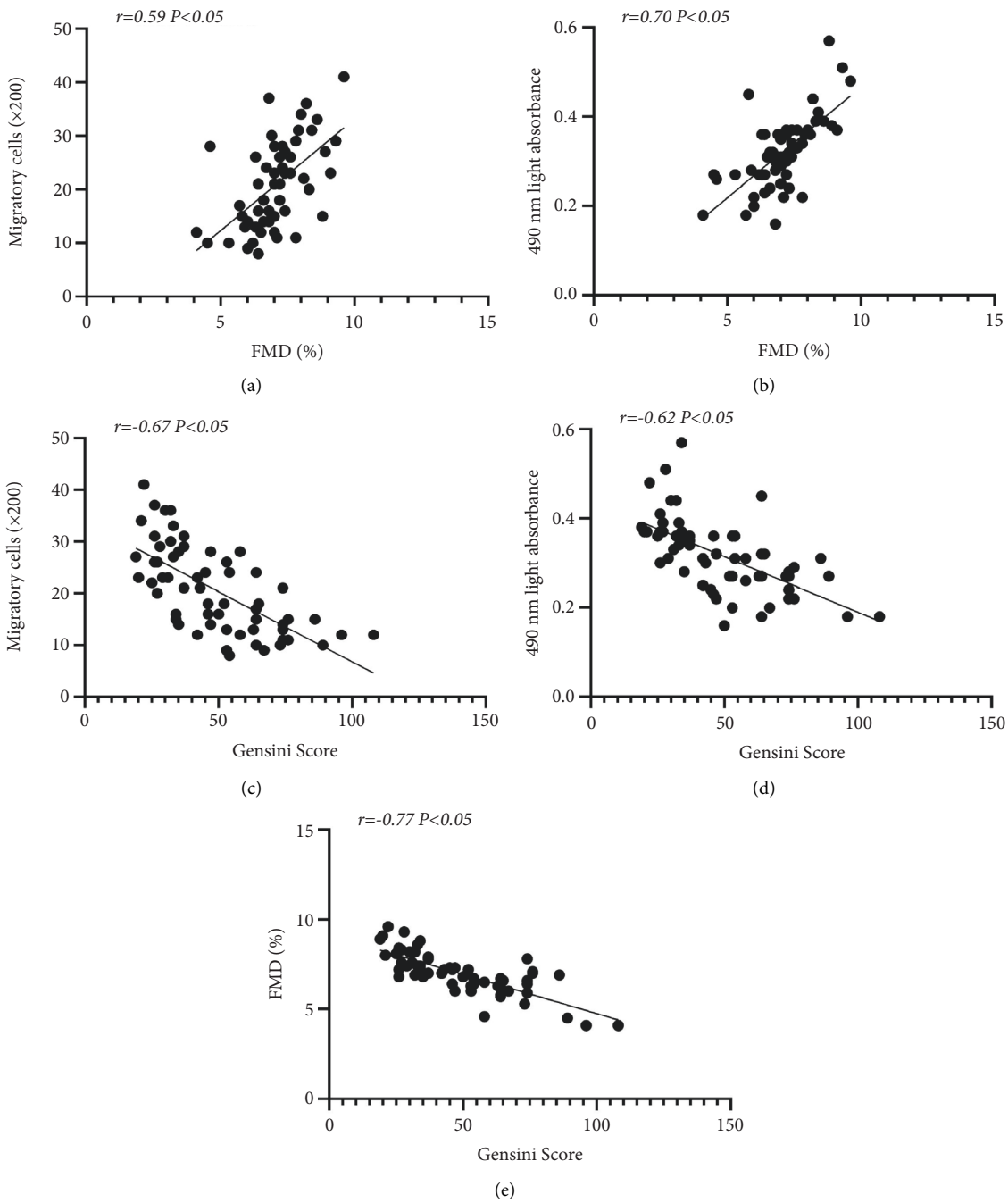

Figure 3: Correlation between FMD and the Gensini score and EPC function. FMD was positively correlated with EPC migration (a) and proliferation (b) In addition, the Genisini score was inversely related to EPC migration (c) and proliferation (d) And the Genisini score was inversely related to FMD (e) Univariate correlations were calculated using Pearson's coefficient (r).

correlated with FMD and migration and proliferation of circulating EPCs (Figures 3(c)–3(e), $P < 0.05$).

3.5. Correlation between EPC Function and FMD with Severity of Coronary Artery Lesions and IL-6. As shown in Figures 4(a) and 4(b), the migration and proliferation of circulating EPCs were negatively correlated with IL-6 (Figures 4(a) and 4(b), $P < 0.05$). Moreover, FMD was also inversely related to IL-6 (Figure 4(c), $P < 0.05$). In contrast, the Gensini score was positively correlated with IL-6 (Figure 4(d), $P < 0.05$).

4. Discussion

The present study demonstrated that UA patients had the higher plasma IL-6 level and the number of circulating EPCs. In contrast, UA patients had lower FMD and the migration and proliferation of EPCs than those in the control group. With the increase in the Gensini score, the

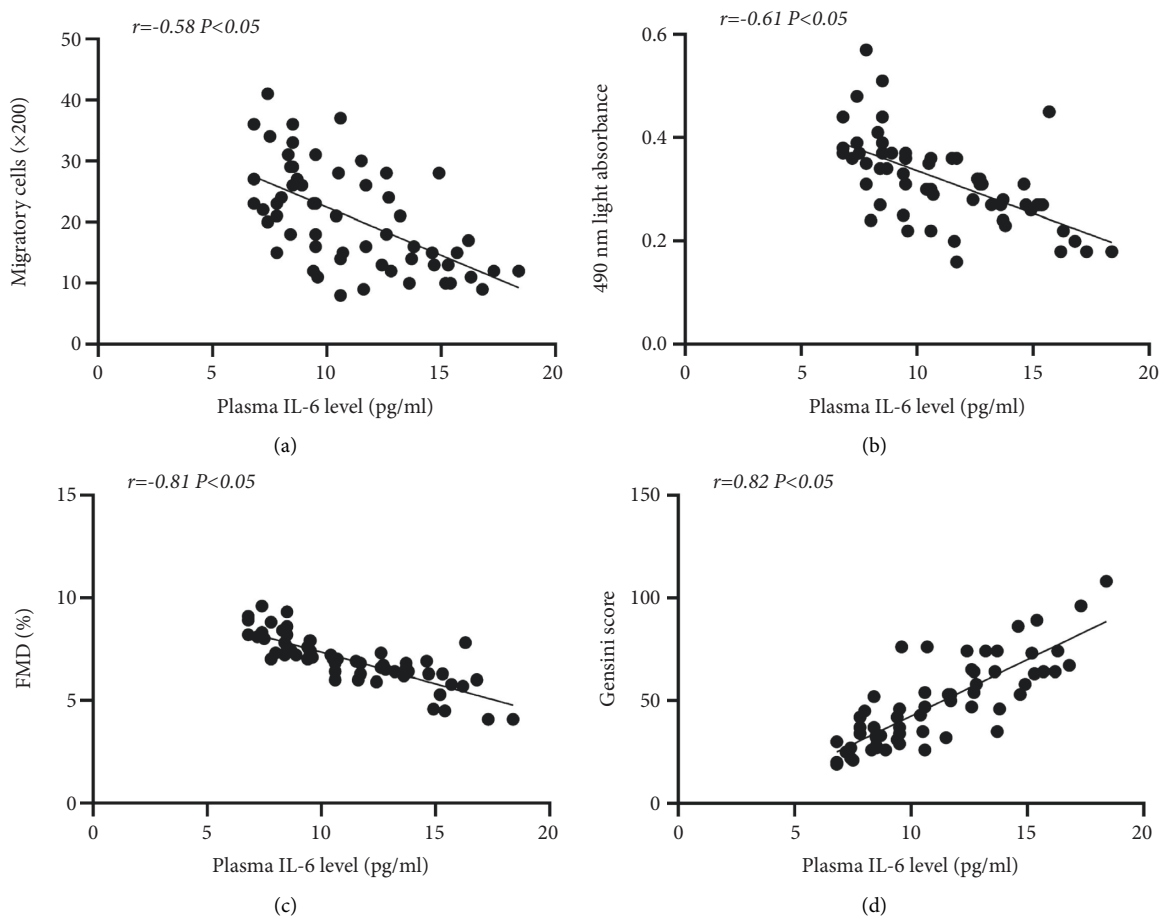

Figure 4: Correlation between the function of circulating EPCs or FMD or the Gensini score and IL-6. EPC migration (a) proliferation (b) and FMD (c) were inversely related to IL-6. In addition, the Gensini score was positively correlated with IL-6 (d) Univariate correlations were calculated using Pearson's coefficient (r).

migration and proliferation of endothelial progenitor cells decreased. In addition, the function of the circulating EPCs was positively associated with FMD. And severity of coronary artery lesions in unstable angina was negatively associated with FMD and the EPC migration and proliferation. Moreover, UA patients presented elevated IL-6, which was negatively correlated with the function of circulating EPCs and FMD and positively correlated with the severity of coronary artery lesions evaluated by the Gensini score. These findings revealed the decline in the function of circulating EPCs was consistent with endothelial dysfunction, and the mitigated endothelial repairability was associated with the severity of coronary artery disease, which may be related to systemic inflammation.

Several lines of evidence obtained in clinical research have demonstrated that the circulating EPC level was declined in the accumulation of cardiovascular risk factors, which was significantly related to endothelial dysfunction [16–18]. However, peripheral circulating EPC mobilization is inconsistent in patients with coronary heart disease. Previous investigations showed that the number of circulating EPCs was decreased in stable CAD but enhanced in acute myocardial infarction [18, 19]. Similarly, the EPC level was significantly higher in unstable angina patients compared with stable angina patients [20], suggesting that necrosis due to infarction is not requisite for their peripheral mobilization. However, there are very few reports concerning the function of circulating EPCs in UA. Our results showed that the migration and proliferation of EPCs in UA patients were lower than those in control. Interestingly, our research revealed that FMD was significantly reduced in UA patients and positively correlated with EPC function; it suggested that although EPC numbers were increased in these patients with UA, vascular endothelial function was decreased.

Circulating EPCs are essential for repairing endothelial injury in coronary artery disease [15]. With the aggravation of coronary artery lesions, the EPC function decreased gradually, and endothelial function injury aggravated gradually, suggesting the severity of coronary artery lesions may be associated with a decline in EPC functions. Increasing evidence suggests that the Gensini score is a common method for estimating the severity of coronary artery lesions of STEMI, NSTEMI, unstable angina, and anginal syndrome [21, 22]. It has a wide range of clinical

applications. In the present study, we found that circulating EPC activity was significantly negatively correlated with the Gensini score. The results showed that subdued endogenous endothelial repair capacity accelerated the progression of pathogenesis in UA patients. Therefore, the impairment of endothelial progenitor cells may contribute to evaluating the degree of coronary artery lesions in UA patients.

IL-6 has been shown to be involved in inflammatory processes and in the development and progression of atherosclerosis [23]. Previous studies have demonstrated that the level of IL-6 in patients with ACS is significantly higher than that in patients with stable angina pectoris [23]. Here, we similarly observed the higher plasma IL-6 level in unstable angina relative to those in the control group. In addition, several studies have demonstrated that a correlation exists between IL-6 levels and the severity of coronary artery lesions in CAD [24, 25]. Similarly, in the present study, we demonstrated that positive association between IL-6 levels and the severity of coronary artery lesions were evaluated by the Gensini score in unstable angina. Indeed, we also reported a negative relation between IL-6 levels and the function of circulating EPCs and FMD. These results suggested that IL-6 may be the potential mechanism underlying hypofunction of circulating EPCs, which may be a potential biomarker for evaluation of coronary artery lesions of unstable angina.

The findings presented in this study have important clinical implications. Firstly, the impairment of circulating EPC function and endothelial dysfunction in patients with unstable angina pectoris are negatively correlated with the Gensini score, indicating that circulating EPC and FMD may be an important biomarker for evaluation of severity of coronary artery lesions in unstable angina. Secondly, IL-6 may be partly responsible for the hypofunction of circulating EPCs and severity of unstable angina patients. Anti-inflammation treatment may be beneficial to unstable angina patients [26].

Some limitations should be acknowledged. The enrolled patients were not enough. Furthermore, a large-sample study needs to be carried out to explore the potential predictive value of circulating EPCs in UA. Moreover, our study is designed to eliminate the influence of IL-6, but other clinical indexes, such as ox-LDL, ac-LDL, and LP (a), also have an effect on circulating EPC.

5. Conclusion

In conclusion, this study confirmed the relationship between circulating endothelial progenitor cells and the severity of coronary artery disease in unstable angina pectoris, which may be related to the IL-6 level.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Xiaoan Yang and Lixiang Liu designed the study. Cheng Xiao Liu performed the experiments. Hanxiong Liu and Xi Li analyzed the data, plotted the figures, wrote the manuscript, and performed the experiments. All authors read and approved the final manuscript. Cheng Xiao, Lixiang Liu, and Xi Li have contributed equally to this work.

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Review Article

Clinical Status of Cardiac Rehabilitation Manners and Models

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Cardiac rehabilitation, which combines cardiology and preventive medicine, is an important part of treatment for cardiovascular diseases. Systematically, cardiac rehabilitation, including simultaneously inhibiting endothelial injury and promoting endothelial repair, is beneficial for physical and mental recovery and reduces the risks of recurrence and death in patients with cardiovascular diseases. Cardiac rehabilitation has developed rapidly in the last 50 years. A preliminary system for cardiac rehabilitation has been developed in China. The present article mainly focuses on the progress of cardiac rehabilitation from the aspects of goals, measures, and modes of research in the current scenario.

1. Introduction

Cardiac rehabilitation has a history of more than 200 years and has rapidly developed for nearly half a century. At present, approximately 54.7% of countries worldwide, mainly including the middle and high-income countries, have already started cardiac rehabilitation [1]. In recent years, its role and position in cardiovascular medicine in China has been gradually accepted. Modern cardiac rehabilitation integrates several disciplines, including cardiovascular medicine, sports medicine, nutrition, psychology, behavioral medicine, and preventive medicine. The aim of cardiac rehabilitation is to achieve recovery as soon as possible, reduce the recurrence and mortality of cardiovascular diseases, and reduce medical and healthcare expenditure. Cardiac rehabilitation has been included in the clinical medical care quality evaluation system in the United States; medical insurance in Germany also requires patients to undergo cardiac rehabilitation [1]. Cardiac rehabilitation has been listed as a Class I recommendation for the prevention and treatment of cardiovascular diseases by several European and American cardiology academic organizations [2]. The research progress in cardiac rehabilitation is briefly described in this review.

2. Means of Cardiac Rehabilitation

In 2007, the American Association of Cardiovascular and Pulmonary Rehabilitation/American Heart Association (AACVPR/AHA) defined cardiac rehabilitation as a comprehensive and coordinated long-term plan that includes medical care evaluation, exercise prescription, correction of cardiovascular risk factors, education, consultation, and behavioral intervention [3]. At the beginning of the 21st century, Professor Hu Dayi [3] concretized the means of cardiac rehabilitation into five prescriptions: medication, exercise, psychological, nutrition, and smoking cessation. The clinical function of cardiac rehabilitation mainly lies in the prevention and treatment of the risk factors of cardiovascular diseases.

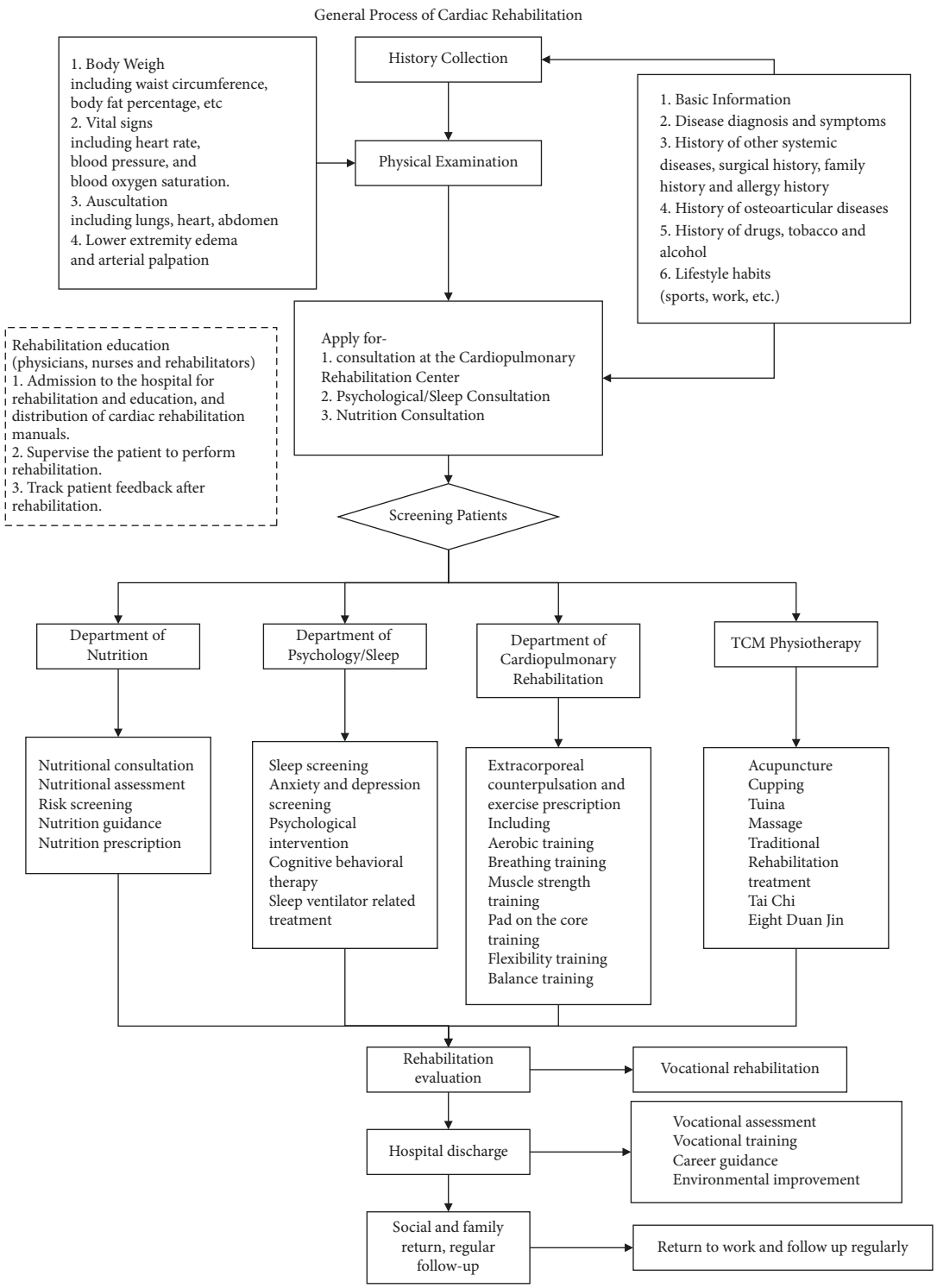

Figure 1: General process of cardiac rehabilitation. The flowchart summarized the standard procedure of cardiac rehabilitation for patients in China.

2.1. Medication Prescription. Medication and surgery (including interventional therapy) are important modalities in the treatment of cardiovascular diseases. Both can ameliorate and stabilize most diseases within a short period and provide an important foundation for further long-term cardiac rehabilitation. Most patients under cardiac rehabilitation have chronic cardiovascular diseases and require long-term medication, which is another reason that medication prescription plays a basic role in cardiac rehabilitation. Certain guidelines should be followed during prescription. Currently, drugs with sufficient evidence for treatment of cardiovascular diseases include antiplatelet

drugs, beta blockers, angiotensin-converting enzyme inhibitors (ACEI), angiotensin II receptor blockers (ARB), and statins [4]. Prescriptions are not invariable but should be dynamically adjusted under the guidance of specialists to achieve the best therapeutic effect. Overall, the general process of cardiac rehabilitation requires "individual therapy" which depends on multiple factors, including medical history, demographic information, and lifestyle. After screening the patients, detailed management of the patients who need cardiac rehabilitation includes nutritional prescription, psychological intervention, extracorporeal counterpulsation, and traditional Chinese medicine (TCM). A detailed description of the process of cardiac rehabilitation is shown in Figure 1.

2.2. Exercise Prescription. Exercise prescription plays a key role in various methods of cardiac rehabilitation and is also the determining factor of treatment success or failure. It involves various aspects of exercise: mode, frequency, intensity, and venue. At present, the major modes of exercise are aerobic training, dynamic and static resistance training, flexibility training, and balance training, among others [4].

Individualized exercise prescription guarantees exercise safety and effectiveness but is a difficult aspect of cardiac rehabilitation. However, there are no clear guidelines on creating exercise prescriptions. Presently, exploring various exercise modalities is the main direction that scholars focus on [5].

While the pathophysiological effects of exercise on the human body are still under continuous investigation, its benefits have been confirmed. Furthermore, most scholars believe that more advantages are seen with high-intensity intermittent exercise [6]. Experiments and clinical studies have proven that exercise provides benefits by increasing the active substances of vascular endothelial cells. These substances regulate blood vessels, enhance vagus nerve tension, and lower the level of plasma norepinephrine, among other mechanisms [4]. This in turn improves cardiac function, regulates blood lipid levels, lowers blood pressure, improves coronary artery and cerebral ischemia, and reduces the incidence of arrhythmia, among other benefits.

Exercise enhances cardiac function since it is conducive to improved myocardial remodeling [7]. The possible mechanisms responsible include reversal of the metabolic decoupling process and reduction of glucose uptake in patients with metabolic syndrome [8]. Angiogenesis in exercising muscles is mediated by the effect of vascular endothelial growth factor and platelet-derived growth factor on β-adrenergic receptors [2]. These processes are stimulated by insulin-like growth factor-1, which is expressed in proportion to exercise. In addition, animal models have proven that insulin-like growth factor-1 reverses adrenergic-related myocardial remodeling [9]. Recent studies have shown that exercise-related post-transcriptional genes can reduce myocardial remodeling through mRNA regulation of the interactions among metabolism, contraction, and epigenetic genes [10]. The increase in metabolic demand during exercise leads to an increase in mitochondrial division and changes in energy pathways within organelles [11]. The increase in mitochondrial content in muscles promotes the preferential oxidation of fat rather than carbohydrate, thus reducing the production of lactic acid and enhancing organ function [2].

Numerous studies have proven that exercise increases patients' 6-minute walking distance, prolongs the exercise time in cardiopulmonary exercise test, and significantly reduces the readmission rate, incidence of cardiovascular events, and mortality rate [2]. Another study has shown that exercise significantly improves exercise duration, peak oxygen consumption, and ventilator threshold [12]. In patients with heart failure with preserved ejection fraction, exercise significantly increased peak oxygen consumption, peak left ventricular ejection fraction, peak stroke volume, and peak cardiac output. This effect can be attributed to the enhanced oxidation efficiency of peripheral skeletal muscles, which in turn lead to improved ventricular-vascular coupling [2]. The 2016 European Society of Cardiology (ESC) Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure recommend that patients with chronic heart failure should actively carry out exercise-based cardiac rehabilitation [13].

Exercise can regulate nerve function, inhibit sympathetic tension, and excite parasympathetic nerves through reduction of aldosterone secretion and inhibition of sympathetic nerve excitability [14]. In addition, exercise inhibits the secretion of norepinephrine and endothelin-1 and improves endothelial function through the interactions between plasma adrenomedullin and atrial/brain natriuretic peptide, the latter being closely related to aerobic consumption [15]. Autonomic nervous system dysfunction may result in coronary artery contraction, increased myocardial oxygen consumption, and fatal cardiovascular events. All of these play a significant pathophysiological role in the early stages of myocardial infarction, essential hypertension, and chronic heart failure [16, 17]. On the other hand, hyperfunction of the sympathetic nervous system is a trigger for arrhythmia and sudden death. High-intensity exercise can effectively achieve autonomic nerve rebalancing in patients with chronic heart failure [17]. A large number of studies have shown that exercise reduces the incidence of myocardial infarction. Moreover, the same studies report improvements in oxygen carrying capacity, endothelial function, and health-related quality of life with high-intensity exercise. Patients with coronary heart disease, especially those with left ventricular dysfunction, are at high exercise risk. However, the risk stratification of exercise and prevention strategies require further study [2]. Previous studies have also shown that different types of exercise have positive effects on endothelial cell structure and endothelium-dependent relaxation (EDR) function. Exercise intervention can cause changes in endothelial-derived vasoactive substances, reduce the level of oxidative stress and inflammatory response, and also lead to a gradual increase in arterial fluid shear stress, which is quite beneficial to the structural remodeling of vascular endothelial cells [18, 19]. Exercise further increases the level of myocardial enzymes secreting angiogenesis-promoting follistatin-like protein 1

(FSTL1), which promotes endothelial cell proliferation and angiogenesis [20]. In recent years, studies have also shown a potential link between the fluid shear force in the arterial tube and the morphological structure of mitochondria in endothelial cells. Trials have shown that all types of exercise are beneficial for correcting endothelial dysfunction, which can reduce mortality and morbidity in patients, and the effects of different types of exercise interventions are not significantly different [21, 22].

2.3. Psychological Prescription (including Sleep Management). Psychological factors share a close relationship with the development, progression, and outcome of diseases. Poor mental state is not only a causative factor in cardiovascular diseases but also affects disease outcomes through negative feedback. A survey showed that among patients in the cardiology department, the detection rate of mental and psychological disorders in outpatients and inpatients was 55% and 50%, respectively. Moreover, almost all patients undergoing stent implantation had different degrees of psychological problems [18]. Exploring effective psychological intervention measures with concurrent integration into cardiac rehabilitation plans to improve rehabilitation quality is the current research focus of scholars. Rozanski et al. [4] pointed out that the development and progression of cardiovascular diseases are related to anxiety, depression, certain personality traits, social isolation, and chronic life stress. The psychological changes in spouses, other relatives, and friends after cardiovascular events also impact the patients.

Psychological intervention aims to relieve tension, improve treatment compliance, and boost self-confidence. However, repetition is required to avoid the disadvantages of the simple biomedical model. Depressive symptoms were found to decrease from 17% to 6% after cardiac rehabilitation training. In addition, the mortality rate of patients with depression who completed rehabilitation treatment was 73% lower than those who did not [2]. Sleep quality is another factor closely related to mental state. A meta-analysis found that among 173,301 participants, in comparison to those who slept 7-8 h a day, those who received 6-7 h of sleep a day had an increased risk of hypertension by 7% while those who received less than 6 h sleep a day had an increased risk of hypertension by 35%. Furthermore, patients in the chronic sleep deprivation state of passive sleep insufficiency may show blood pressure elevation in the short term and cardiovascular risk factors such as systemic inflammatory response and impaired glucose tolerance in the long term [4].

2.4. Smoking Cessation Prescription. Smoking is an independent risk factor for cardiovascular diseases. Several studies have outlined the significant causative effect of smoking on coronary heart disease, atherosclerotic peripheral vascular diseases, and stroke. Smoking cessation can reduce the risk of cardiovascular disease morbidity and mortality, and its long-term benefits are at least equivalent to those of commonly used secondary preventive drugs for coronary heart disease such as aspirin and statins. Therefore, medical professionals must explain the negative effects of smoking and the benefits of smoking cessation to the patients and their families. The optimal treatment plan for tobacco dependence is a comprehensive method combining drug therapy, psychotherapy, and behavioral therapy.

2.5. Nutrition Prescription. Existing evidence-based medicine has proven that excessive intake of energy, saturated fat, and cholesterol and insufficient intake of vegetables and fruits increase the risk for cardiovascular diseases. Conversely, the intake of a scientific and reasonable diet was found to reduce this risk. Considering this, the purpose of nutrition prescription is to guide patients in forming healthy eating habits.

The implementation of nutrition prescription is crucial to effective cardiac rehabilitation. Rehabilitation evaluation is an important starting point in the rehabilitation process. Current disease condition, exercise ability, nutrition, and psychological status of patients can be comprehended through rehabilitation evaluation. This enables the formulation of individualized, safe, and accurate rehabilitation plans. In America and developed European countries, the cardiopulmonary exercise test is considered the "gold standard" for clinical evaluation of cardiopulmonary function and formulation of exercise prescription [5]. All patients under cardiac rehabilitation must undergo pre-rehabilitation evaluation, rehabilitation process evaluation, and rehabilitation stage evaluation. Lastly, timely corrections must be made in prescription deviations.

3. Modes of Cardiac Rehabilitation

The current focus of research is to explore cardiac rehabilitation modalities that offer easy facilitation and high patient compliance while ensuring safe and effective rehabilitation. However, rehabilitation should be conducted under the guidance of cardiologists regardless of the chosen modality.

Cardiac rehabilitation can be classified into three modes according to location of implementation: inpatient rehabilitation (stage I rehabilitation), early outpatient rehabilitation or clinic rehabilitation (stage II rehabilitation), and long-term outpatient rehabilitation (stage III rehabilitation). These modes correspond to high-risk, medium-risk, and low-risk patients, respectively, with respect to disease condition.

Stage I rehabilitation refers to cardiac rehabilitation under the guidance and supervision of medical staff in the hospital. The goals of this mode include shortening the length of hospital stay and promoting the recovery of patients' daily life and exercise ability while avoiding the adverse effects caused by bed rest (such as reduced exercise tolerance and thromboembolic complications). In addition, stage I also involves reminding patients of smoking cessation and providing comprehensive and complete information in preparation for stage II rehabilitation. It is generally

implemented in high-risk patients. Stage II rehabilitation is generally carried out in the first 6 months after discharge. Patients continue to undergo rehabilitation treatment under the guidance and supervision of medical staff at the outpatient clinic. At the onset of disease stabilization, self-monitored exercises may be carried out at home, and remote network and electronic information technology can be used to supervise the entire process of their cardiac rehabilitation. Generally, it is adopted for medium-risk patients. Stage III rehabilitation involves long-term rehabilitation at home. Patients undergo in-hospital evaluation, and rehabilitation exercise is carried out at home under the guidance of rehabilitation personnel and self-management to cultivate a healthy lifestyle. It is generally utilized in low-risk patients [19–22].

Selection of rehabilitation mode is influenced by factors such as income, education level, and the establishment level of tiered medical services. The United States implements hospital-based cardiac rehabilitation mode, with stage I rehabilitation being paid by medical insurance. Europe implements the mode of rehabilitation center combined with community, while Taiwan in China and Japan adopt the mode of family-driven rehabilitation clinic. In the Chinese mainland, the hospital cardiac rehabilitation mode, the family cardiac rehabilitation mode, or a mixture of both has begun to play a role in community cardiac rehabilitation [4]. Most of the cardiovascular treatment levels in China's tertiary hospitals have been in line with international standards. Among them, stage I rehabilitation after interventional surgery, that is, the hospital cardiac rehabilitation model, has been widely used in clinical practice with good results. The family cardiac rehabilitation model lacks a unified management model. It is generally guided by indirect forms such as self-help education and rehabilitation manuals, telephone supervision, and return visits, and it is difficult to grasp the relevant risk factors outside the hospital [4].

Another difficulty in cardiac rehabilitation is improving patient compliance [23, 24]. Studies have reported that only 15%–50% of patients under cardiac rehabilitation could continue rehabilitation for 6 months after the end of treatment, and even fewer patients could continue for 12 months [25]. At 6 months after the onset of the cardiovascular event, approximately 50% of smokers had quit smoking, and less than 50% of obese patients could comply to dietary recommendations [26]. AHA encourages nurses or non-medical care personnel to take the roles of the main supervisors and managers of family and community rehabilitation to increase the participation rate [18]. A prospective, multi-center, controlled study showed that strengthening follow-up after rehabilitation could achieve positive results, with high feasibility and satisfaction [27]. The rapid development of the Internet has seen application of more technologies in cardiac rehabilitation with beneficial results. Lunde et al. [28] conducted a single-group pre-test and post-test control study lasting 12 weeks, wherein patients utilized an application to help guide changes or maintain a healthy lifestyle. The results of this study show that the follow-up intervention based on this technology not only resulted in patient benefit but also stimulated their motivation for rehabilitation with high satisfaction. In another study, the use of a mobile application to monitor patients taking ticagrelor post-myocardial infarction showed significantly improved medication compliance [29]. A study by Gabelhousej et al. [30] showed that the hospital-centered, community-based combined cardiac rehabilitation mode integrating mobile technology had a similar effect as traditional cardiac rehabilitation mode but had the advantage of convenience. Research has also explored the application of virtual reality (VR) in the field of cardiac rehabilitation [31]. Strong evidence has led to the assumption that technologies such as artificial intelligence and the Internet will play a significant role in the field of cardiac rehabilitation in future.

4. Conclusions

Presently, cardiac rehabilitation has been internationally recognized as a safe, effective, and cost-effective method for treating patients with cardiovascular diseases. Research on cardiac rehabilitation has shifted from the demonstration of various mechanisms and principles to the exploration of various patient-centered rehabilitation methods and modes. However, there is still a lack of an individualized and standardized cardiac rehabilitation mode with high patient compliance. In addition, the large-scale promotion of modes such as telerehabilitation, community rehabilitation, and family rehabilitation still requires further research and demonstration. The application of modern technologies such as the Internet and artificial intelligence in cardiac rehabilitation will actively promote the development of cardiac rehabilitation.

Data Availability

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

Conflicts of Interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Authors' Contributions

Wei Wei, Jingjie Zhao, and Lingzhang Meng contributed equally to this work. Lingzhang Meng and Lihua Yang designed and composed the manuscript. Wei Wei and Jingjie Zhao retrieved the referenced articles and helped abstract the main concept for this manuscript. Xi Wang, Hongdi Wei, Keji Nong, Jiahao Li, Zechen Wang, Jiajia Shen, and Siyuan He helped organize the references and contributed to compose the flowchart in the manuscript.

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Review Article

The Beneficial Role of Nrf2 in the Endothelial Dysfunction of Atherosclerosis

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Cardiovascular disease (CVD) is a serious public health issue in China, accounting for more than 40% of all mortality, and it is the leading cause of death worldwide. Atherosclerosis is the pathological basis for much CVD, including coronary heart disease, acute myocardial infarction, and stroke. Endothelial dysfunction is an initiating and exacerbating factor in atherosclerosis. Recent research has linked oxidative stress and mitochondrial damage to endothelial dysfunction. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor with antioxidant effects that is strongly connected to several CVDs. However, the mechanism by which Nrf2 reduces CVD is unknown. Research indicates that Nrf2 improves endothelial function by resisting oxidative stress and mitochondrial damage, thereby delaying atherosclerosis. This article examines the mechanisms and potential targets of Nrf2 affecting endothelial cell function to improve atherosclerosis and to provide ideas for the development of new CVD treatments.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death and premature death in China, posing a significant public health risk. In 2019, CVD caused 18.6 million deaths worldwide and roughly 58% of all cardiovascular deaths in Asia [1]. The morbidity and mortality of CVD will only increase with the increasing elderly population [2]. At present, the treatment of CVD relies mainly on coronary revascularization and oral antiplatelet aggregation drugs. Coronary revascularization is an invasive operation with a risk of surgical complications, while long-term antiplatelet aggregation drug use has a risk of bleeding. Because many people cannot tolerate surgery or long-term antiplatelet drug therapy [3–5], clinicians hope to develop new methods for preventing and treating CVD.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor with antioxidant effects strongly related to several CVDs [6]. Nrf2 regulates the biosynthesis, utilization, and regeneration of glutathione, thioredoxin, and NADPH, as well as the production of reactive oxygen species via the mitochondria and NADPH oxidase, to maintain cellular redox homeostasis [7]. Nrf2 can lower the risk of atherosclerosis-related chronic diseases by improving endothelial function [8–10]. This article examines the mechanisms and potential targets of Nrf2 affecting endothelial cell dysfunction and delaying atherosclerosis, starting from the pathophysiological basis of CVDs to provide ideas for the development of new therapeutic methods for CVDs.

2. Endothelial Cells

The blood vessel wall forms a selective barrier to molecular transport between blood and tissue, and endothelial cells form the inner lining of blood vessels, controlling the exchange of substances between blood and tissues. Endothelial cells form continuous thin monolayers that maintain vascular homeostasis by interacting with cells in the vessel wall and lumen [11]. They control vascular tone by releasing vasodilatory factors, such as nitric oxide (NO) and

contractile factors; regulate blood flow and coagulation by producing factors that regulate platelet activity, the coagulation cascade, and the fibrinolytic system; and secrete adhesion molecules and cellular cytokines to coordinate the inflammatory response [12].

3. Endothelial Cells and Atherosclerosis

Atherosclerosis is a chronic disease process involving lipid accumulation [13]. It begins with endothelial cell dysfunction and is arbitrated by a cascade of intracellular and intercellular responses [14]. Endothelial cell dysfunction results in the infiltration of low-density lipoprotein (LDL) particles and their subsequent oxidation to oxidized LDL (oxLDL) [15]. Increased chemokine secretion by endothelial cells and increased adhesion protein expression on their surface allow them to recruit inflammatory cells, particularly monocytes, to the arterial intima. Monocytes differentiate into macrophages, which subsequently phagocytose lipids into foam cells, which undergo necrosis and apoptosis, forming the lipid core of progressive atherosclerotic lesions [16]. Injured endothelial cells secrete growth factors to activate smooth muscle cells (SMCs) in the arterial media, migrate into the intima through fenestrations in the inner elastic membrane, and phagocytose lipids mediated by lipoprotein lipase receptors on the surface to form SMC-derived foam cells. In the late stages of atherosclerosis, SMCs secrete extracellular matrix (collagen and elastin), forming fibrous caps that increase the instability of atherosclerotic plaques [17]. Communication between endothelial cells and other vascular cell populations in this atherogenic environment stimulates the release of proinflammatory cues, increasing the native inflammatory response and promoting atheromatous plaque development [18]. Reduced collagen synthesis and increased degradation due to inflammation cause progressive thinning of the fibrous cap, resulting in plaque rupture, thrombosis, and vascular occlusion [14]. The disturbance of vascular endothelial structure and function is a key link in the occurrence and development of atherosclerotic vascular diseases [19–21].

4. Nrf2

In 1994, researchers discovered Nrf2 in a study of beta-globin gene regulation. Nrf2, also known as NFE2L2, helps to regulate the cellular oxidative stress response. Nrf2 is classified in the Cap-n-Collar family of basic leucine zipper proteins, with 7 functional domains involved in the regulation of their stability or transcriptional activity [22]. Under basal conditions, Nrf2 binds to the Keap1/Cu13 ubiquitin ligase complex with a half-life of 10–30 minutes and is in a low activity state. When exposed to oxidative stress or other stimuli, the cysteine residues in Kelch-like epichlorohydrin-associated protein 1 (Keap1) are modified, decreasing its activity and inhibiting its binding to Nrf2. After release from the complex, Nrf2 enters the nucleus and forms a heterodimer with the small protein Maf (Nrf2-Maf) [7, 23]. Heterodimers connect to antioxidant response elements (AREs) in the initiation domain in a sequence-specific manner [24], promoting antioxidant enzyme transcription. These antioxidant genes have important anti-tumor, anti-inflammatory, antiapoptotic, antioxidant, and tissue protection effects [25, 26].

5. Role of Nrf2 in Atherosclerosis-Associated Endothelial Dysfunction

In addition to regulating vascular tone and permeability, healthy endothelial cells maintain hemostasis and coagulation, transport oxygen and nutrients to tissues, coordinate inflammatory and immune responses, and induce angiogenesis [27, 28]. Endothelial injury is a complex pathological process involving increased endothelial cell activation and endothelial dysfunction. When endothelial injury occurs, endothelial cells within the vascular lumen transform into a proinflammatory, proadhesive, procoagulant phenotype, in a process called endothelial cell activation. This is immediately followed by decreased endothelial NO bioavailability, altered vascular tone, and endothelial cell transformation to other phenotypes, collectively referred to as endothelial dysfunction [29–31]. Many studies have shown that Nrf2 has anti-inflammatory, proangiogenic, antioxidative damage, and mitochondrial protection roles in atherosclerosis-related endothelial cell dysfunction [32–35]. However, the mechanism of Nrf2 in endothelial cell oxidative stress and mitochondrial damage is unclear. Therefore, the role of Nrf2 in oxidative stress and mitochondrial injury in endothelial cells will be elaborated.

6. Nrf2 Improves Endothelial Dysfunction by Inhibiting Oxidative Stress

6.1. Oxidative Stress and Endothelial Dysfunction. Oxidative stress is important in mediating cytokine production and secretion, linking ROS to endothelial dysfunction [31, 36]. NO is the main reason that endothelial cells maintain vascular homeostasis. The production of NO in vivo uses l-arginine as a substrate, instigated by NO synthase (NOS), and generates l-citrulline and NO [37]. Several factors affect the vascular distribution of NO ultimately resulting in reduced NO release, including impaired endothelial cell membrane receptors paired with NO agonists or reduced endothelial diffusivity, physiological changes, inappropriate use of l-arginine, reduced enzymes responsible for converting or changing cGMP levels, reduced substrates for synthesizing NO, or significant degradation of NO [38]. Endothelial dysfunction is associated with decreased NO bioavailability as a result of decreased NO production and increased consumption. Superoxide anions react with NO to generate peroxynitrite (ONOO-), which promotes protein tyrosine nitration in vivo, affects protein structure and function, and further impairs endothelial function [39, 40]. Guzik et al. [41] studied the role of superoxide production due to NAD(P) H oxidase in human atherosclerosis in relation to NO-mediated vasodilation. Xu et al. [42] found that berberine protects against the human coronary artery endothelial cell disorder induced by Kawasaki disease by impairing oxidation and endoplasmic reticulum stress.

6.2. Nrf2 Improves Endothelial Dysfunction by Inhibiting Oxidative Stress. A growing body of evidence suggests that the Nrf2-driven antioxidative pathway has vascular protective effects in CVDs such as atherosclerosis, hypertension, diabetes, myocardial infarction, and heart failure [43, 44]. Oxidative damage induced by ROS or lipid peroxidase aggravates endothelial cell damage, which in turn activates the transcription factor Nrf2 in endothelial cells, and stimulated Nrf2 exerts its protective capacity by inducing downstream gene transcription [45]. This pathway involves more than 200 genes with antioxidative capabilities by increasing the ability of cells to combat oxidative stress and promote cell survival. These genes include antioxidant proteins that maintain intracellular glutathione homeostasis and decrease intracellular reactive oxygen levels, phase II detoxification enzymes, such as glutathione S-transferase (GST) and NADPH quinone oxidoreductase 1 (NQO1), which are mainly involved in decomposing toxic substances and promoting the metabolism and elimination of toxic substances and transporters, including multidrug resistance-related proteins implicated in the control of endogenous and exogenous substance output and uptake [44, 46, 47]. Chen et al. [8] discovered that expressing Nrf2 in human aortic endothelial cells increased ARE-driven transcriptional activity and increased intracellular HO-1 protein levels to protect endothelial cells from tumor necrosis factor (TNF)-α-mediated cytotoxicity. Ginsenoside Rg3 upregulates the Nrf2-ARE pathway by activating AKT and improves endothelial dysfunction caused by oxidative stress [48]. Similarly, paeoniflorin inhibits the tert-butyl hydroperoxide-induced overproduction of intracellular ROS and apoptosis in human umbilical vein endothelial cells via the Nrf2/HO-1 signal transduction pathway [49]. Blood flow through the vessel wall causes mechanotransduction, mainly including shear stress and tensile stress, which contributes to the maintenance of endothelial function and homeostasis. Shear stress is regarded as the most important element influencing the development of atherosclerosis [50, 51]. High unidirectional shear stress promotes endothelial Nrf2 signaling, whereas arterial regions exposed to low oscillatory shear stress are prone to atherosclerosis, in part due to reduced endothelial nitric oxide synthase expression and the attenuated antioxidant and anti-inflammatory properties of Nrf2 activation [52].

7. Nrf2 Suppresses Endothelial Dysfunction by Improving Mitochondrial Function

7.1. Mitochondrial Function and Endothelial Cell Dysfunction. Mitochondrial dysfunction contributes to increased oxidative stress in atherosclerosis, promoting inflammatory responses and lesion formation [53]. Mitochondria can also produce ROS (mtROS) under basal conditions in complexes I and III of the mitochondrial electron transport chain. Simultaneously, limited ROS production has important signaling functions, which has attracted much attention. However, various pathological stressors can induce an abnormal increase in mtROS production, which in turn leads to impaired NO synthesis in endothelial cells and the production of inflammatory cytokines, which favor atherosclerosis. Therefore, targeting mtROS may be an effective way to avoid endothelial damage and atherosclerosis [54].

Endothelial cells have a low mitochondrial content, but mitochondrial dynamics are critical to maintaining endothelial cell homeostasis under normal conditions. Several studies show that altered mitochondrial dynamics are linked to increased mtROS production and are implicated in endothelial damage and various vascular diseases [55]. Moreover, mitochondrial dynamics include both fusion and fission, and proteins involved in mitochondrial dynamics contribute to guanosine triphosphatase (GTPase) function [56], including various proteins. Mitochondrial fusion proteins 1 (MFN1) and 2 (MFN2) are proteins that regulate mitochondrial outer membrane fusion with the *N*-terminal GTPase structural domain and *C*-terminus to induce mitochondrial fusion protein oligomerization. MFN2 is also associated with mitochondrial autophagy. Optic dystrophin 1 is a protein that controls mitochondrial inner membrane fusion, ensuring the consistency of mitochondrial inner membrane structure, while also participating in mitochondrial cristae remodeling. Drp1, a member of the GTPase family that is found in the cytoplasm and is involved in fission of the mitochondrial outer membrane, is the protein that regulates mitochondrial fission [57, 58]. DRP1-mediated mitochondrial fission has been linked to endothelial dysfunction, including endothelium-dependent diastolic dysfunction, reduced microvessels, and decreased wound healing and angiogenic capacity [59–61]. Regulating Drp1 phosphorylation, inhibiting mitochondrial fission, and restoring mitochondrial morphology can protect mitochondrial function in vascular endothelial cells [62]. Protein disulfide isomerase A1 (PDIA1) is a thiol reductase of the mitochondrial fission protein Drp1. In endothelial cells, depletion of PDIA1 induces the thiolation of Drp1 at Cys644, promotes mitochondrial fragmentation and increased ROS, and impairs endothelial cell function and angiogenesis [61]. Several studies have shown that reduced MFN1 and MFN2 expression increases human umbilical vein endothelial cell injury and promotes the development of atherosclerosis [63, 64]. Retinol-binding protein 4 (RBP4) incubation inhibited mitochondrial MFN1 protein expression in human aortic endothelial cells, increased mitochondrial superoxide production, and aggravated mitochondrial damage [65].

Mitophagy is a defensive process by which the body selectively removes damaged mitochondria and is a fundamental mechanism of mitochondrial homeostasis. Mitophagy promotes mitochondrial turnover and prevents the accumulation of dysfunctional organelles. A moderate amount of mitophagy can prevent endothelial cell damage and avoid further CVD development [66].

7.2. Nrf2 Suppresses Endothelial Dysfunction by Improving Mitochondrial Function. There is growing evidence that Nrf2 is closely linked to mitochondrial functions, including mitochondrial antioxidant defense, mitochondrial dynamics, mitochondrial autophagy, biogenesis, and mitochondria-related intermediary metabolism [67–70]. Mitoquinone

(MitoQ) is a mitochondrial-targeting antioxidant. Yang et al. [71] discovered that MitoQ intervention increased Nrf2 and HO-1 expression in high glucose-induced brain microvascular endothelial cells, while improving the mitochondrial membrane potential and decreasing mtROS generation. There is evidence that mtROS is necessary for Nrf2 activation and that the Nrf2-Keap1 complex binds directly to the outer mitochondrial membrane protein PGAM5, sensing ROS from mitochondria [72]. Nrf2 regulates mtROS homeostasis via the ARE-mediated activation of antioxidant enzymes in mitochondria, and a reduction in Nrf2/ARE activity leads to increased oxidative stress and mitochondrial dysfunction in blood vessels, resulting in endothelial damage [73]. Intracellular chloride channel 1 (CLIC1) is an oxidative stress sensor in endothelial cells. CLIC1 overexpression inhibits Nrf2 nuclear translocation, contributing to the hydrogen peroxide-induced activation of mitochondrial fission in human umbilical vein endothelial cell functional impairment [74]. Zhu et al. [34] found that Nrf2 activation inhibits Drp1-mediated mitochondrial fission, improving endothelial dysfunction. As a potent antioxidant in mitochondria, coenzyme Q10 exerts beneficial effects on mouse glomerular endothelial cells by restoring the Nrf2/ARE signaling pathway and promoting mitophagy [75].

8. Nrf2 and Atherosclerosis

Atherosclerosis is a chronic systemic disease characterized by lipid metabolism disorders, vascular endothelial damage, lipid deposition in the vascular wall, mononuclear-macrophage hyperplasia, and atherosclerotic plaque formation. Nrf2 depletion in macrophages leads to increased foam cell formation, increases the inflammatory phenotype, and aggravates atherosclerosis [76]. *Z*-Lig, a natural benzoquinone derivative, acts as an Nrf2 inducer and protects vascular endothelial cells from atherosclerosis caused by a high-fat diet. It reduces lipid peroxidation and increases antioxidant enzyme activity in Ldlr–/–mice [77]. Nrf2 is essential for lowering serum total cholesterol and reducing atherosclerotic plaques in an apolipoprotein *E* (ApoE) knockout animal model [78]. Nrf2 knockdown significantly increased the oxLDL-induced elevation of ROS levels, increasing the risk of CVD [79]. Nevertheless, there is some evidence that Nrf2 also exacerbates atherosclerosis. Barajas et al. [78] found that ApoE (–/–) Nrf2 (–/–) mice had reduced atherosclerotic lesions, while the occurrence of atherosclerosis was not affected in ApoE(–/–) Nrf2(–/+) mice. This is consistent with the report of Barajas et al. [78, 80]. In addition, the Nrf2 signaling pathway promotes inflammasome activation and contributes to atherosclerosis progression [81]. Further research should examine the complex role of Nrf2 in atherosclerosis to give new perspectives on the future therapeutic direction of Nrf2 in atherosclerosis.

9. Conclusion

Atherosclerosis is a disease that imposes a heavy burden on families, society, and the nation. Vascular endothelial injury is its main driver. In this review, we explain how the Nrf2 pathway protects endothelial cells from oxidative stress and mitochondrial dysfunction. In addition, the Nrf2-mediated transcription of antioxidant enzymes reduces endothelial cell damage, which in turn improves atherosclerosis. In this context, we believe that the Nrf2-ARE pathway could be an effective therapeutic target to reduce the occurrence and development of these diseases. However, the opposite effect seen in Nrf2-deficient animals casts doubt on whether Nrf2 activation can ameliorate atherosclerosis. Therefore, additional studies are needed to explore novel therapeutics for atherosclerosis targeting the Nrf2 signaling pathway.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Zixia Huang and Mingyue Wu contributed equally to this study.

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Review Article

Programmed Cell Death of Endothelial Cells in Myocardial Infarction and Its Potential Therapeutic Strategy

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Cardiovascular disease, especially coronary artery disease and stroke, kills around one-third of the world's population, and myocardial infarction, a primary symptom of coronary heart disease, is a major worldwide health problem. Cardiovascular disease research has historically focused on promoting angiogenesis following myocardial damage. Myocardial vascular repair is crucial for improving myocardial infarction prognosis. Endothelial cells, the largest population of nonmyocytes within myocardial tissue, play an important role in angiogenesis. In recent years, different types of programmed cell death such as apoptosis, necroptosis, pyroptosis, ferroptosis, and autophagy have been described and found to be linked with cardiovascular diseases such as myocardial infarction, heart failure, and myocarditis. This will have important implications for reforming the treatment strategy of cardiovascular diseases. Different types of cell death of endothelial cells in myocardial infarction have been proposed, the roles and mechanisms of endothelial cell death in myocardial infarction are summarized in this review, and endothelial cell death inhibition as a therapeutic technique for treating myocardial infarction might be advantageous to human health.

1. Introduction

Myocardial infarction (MI) is defined as the death of myocardial cells due to insufficient myocardial perfusion, which can be caused by coronary occlusion or reduced blood flow, or by an acute supply-demand mismatch without coronary occlusion [1]. MI and subsequent heart failure are the leading causes of death in patients with coronary heart disease [2]. Perfusion of cardiac microvessels that is sustained and efficient is a significant element in avoiding cell death and extending the lifespan of injured myocardium following MI [3]. As the most nonmuscle cells in the heart, endothelial cells (ECs) play a crucial role in ischemic heart disease. The results of genetic lineage tracing revealed that newly formed capillaries after MI were generated by pre-existing ECs in the infarct marginal zone [4], and this process is facilitated by the clonal proliferation of resident ECs [3], and other cell sources have no bearing on angiogenesis.

Programmed cell death (PCD) was first used to describe cell death during insect development, and a similar process was subsequently found in humans and referred to as apoptosis. Programmed cell death is the process of removing unwanted, infected, or damaged cells and plays an important role in maintaining homeostasis of the body and host defense against pathogens. Unlike nonprogrammed cell lysis, programmed cell death is controlled by distinct signaling pathways, and these signaling pathways are cross-linked. Apoptosis, necroptosis, pyroptosis, ferroptosis, autophagy, and other kinds of PCD have all been found thus far. PCD is significant in the etiology of atherosclerosis [5], cardiovascular disease [6], and neurological disease [7]. The types of PCD of ECs in MI have been documented, but no comprehensive review has been published to date. The mechanism of distinct forms of ECs death is discussed in this review, as well as its importance in the potential therapy of MI.

2. Apoptosis

2.1. Overview of Apoptosis. Apoptosis, which is divided into two stages: the formation of apoptotic vesicles, and the phagocytosis and degradation of apoptotic vesicles by other cells, is a biological phenomenon first proposed by Kerr et al. [8], morphological changes such as chromatin coalescence and margination, followed by nuclear rupture, plasma membrane, or nuclear membrane wrapping broken DNA and organelles to form apoptotic vesicles, which can be quickly phagocytosed and therefore are mostly present in the cytoplasm of phagocytosed cells. Apoptosis is involved in the regulation of cell numbers in a variety of tissues, both physiologically and pathologically [8]. The intrinsic (also known as mitochondrial or Bcl-2-controlled) pathway and the extrinsic (also called death receptor) pathway both initiate apoptosis [9]. The nematode Caenorhabditis elegans expresses the human Bcl-2 gene, which decreases apoptosis [10], and ced-9 inhibits the function of ced-3 or ced-4, which has comparable effects to Bcl-2 [11]. The Bcl-2 homology 3 (BH3), Bcl-2-associated *X* protein (BAX), and Bcl-2 antagonist/killer (BAK), three functionally and physically separate subgroups of the Bcl-2 protein family, interact in the outer mitochondrial membrane and are seen as apoptosis switches. BAX and BAK produce oligomers that permeate the outer mitochondrial membrane when enough BH3-only proteins are triggered by various intracellular damages that surpass the apoptotic threshold, resulting in mitochondrial outer membrane permeabilization (MOMP) [12], which releases apoptotic factors into the cytoplasm, particularly cytochrome c [13, 14], and then, activation of caspase-9 on apoptosis activating factor 1 (APAF1) is thus promoted [15], APAF1 is homologous to ced-4 [13], and subsequent activation of caspase-3 and caspase-7 initiates the caspase cascade event, resulting in apoptosis [15, 16]. MOMP also promotes the production of second mitochondrial activator of caspases (SMAC) and HTRA serine peptidase 2 (HTR2), which both inhibit X-linked inhibitor of apoptosis (XIAP) [17] that inhibits the effector caspases [18] (Figure 1).

Another mitochondrial change is the opening of the mitochondrial permeability transition pore (MPTP), which allows nonselective passage of ≤1.5 kD molecules and causes apoptosis when the mitochondrial matrix is under high osmotic pressure [19]. Outer membrane proteins (voltage-dependent anion channel, VDAC), intermembrane proteins, at least one inner membrane protein (the adenine nucleotide translocator, ANT), and at least one matrix protein (cyclophilin D) are known to be involved in the permeability transition [20–22]. It was found that mitochondria-targeted PT inducers do trigger apoptosis in thymocytes, which is also precluded by mitochondria-specific PT inhibitors such as bongkrekic acid (ligand of ANT) [23, 24].

Apoptosis can also be triggered by the death receptor pathway (Figure 1). Tumor necrosis factor receptor superfamily, such as TNFR1, Fas, CAR1, DR4, and DR5, are related to intracellular death structural domains via their ligands [25], encouraging the development of the death-inducing signaling complex (DISC) [26], activating caspase-8, and its downstream effector caspases (caspase-3 and caspase-7) [27]. Caspase-8 also cleaves Bid, a BH3-only protein, into tBid, a pro-apoptotic form that accumulates in mitochondria and stimulates cytochrome c release, causing apoptosis via the intrinsic pathway [28].

2.2. Therapeutic Strategies of Inhibiting Endothelial Cells Apoptosis and Improving the Myocardial Infarction. Apoptosis was shown to be 8-9 times higher in nonmyocytes than in myocytes in myocardial biopsies from individuals with ischemic cardiomyopathy, such as myocardial infarction, and 25% of these nonmyocytes were endothelial cells [29]. Increased amounts of mitochondrial reactive oxygen species accompany cardiac ischemia-reperfusion damage, which can lead to ECs apoptosis [30]. In the mini-swine model of AMI reperfusion, Fas and Bax overexpression or Bcl-2 low expression in the infarct region and marginal tissues plays a crucial role in accelerating ECs apoptosis at Day 7 [31].

Long noncoding RNAs (lncRNAs) are important regulators of cardiac remodeling. By suppressing the miR-26b-5p/Mfn1 pathway-mediated apoptosis of cardiac microvascular endothelial cells (CMECs) following MI, the lncRNA Malat1 plays a crucial role in microcirculation repair [32]. In ECs, the long noncoding ribonucleic acid-cardiac apoptosis-related (lncRNA-CARL) can reduce the expression of Bax and PHB2, decrease caspase-3 activity, and raise the amount of the anti-apoptotic protein Bcl-2 [33]. Knocking down the lncRNA KCNQ1OT1 (KCNQ1OT1) promotes CMECs proliferation in a mouse model of AMI, while inhibiting apoptosis and lowering inflammatory factor levels [34].

Extracellular vesicles (EVs) are naturally secreted nanovesicles that play a key role in stem cell-mediated cardioprotection, and exosome production from induced pluripotent stem cells (iPSCs)-EV improves the angiogenic and anti-apoptotic properties of cardiac endothelial cells when compared to iPSCs [35]. Exosomal EV-C-MSCsN1ICD from cardiac mesenchymal stem cells reduces ECs and cardiomyocyte apoptosis mediated by oxidative stress and ischemia injury [36]. Remote ischemic conditioning (RIC)-EV inhibits ECs apoptosis by Hsp70 [37]. In vitro, miRNA-21-loaded EVs successfully transfer miR21 to recipient cells and lower the amount of the pro-apoptotic protein PDCD4; in vivo, they prevent apoptosis in ECs in the ischemia marginal zone [38]. MiR-21 prevents CMECs damage following AMI via the PTEN/VEGF pathway, according to another research [39]. MiR-24 was found to be highly expressed in cardiac ECs and was increased considerably following MI. In mice, blocking miR-24 inhibited ECs apoptosis and reduced the size of MI [40]. MiR-17-5p downregulation raises the amount of the anti-apoptotic protein Bcl-2 and lowers the level of apoptotic protein (bax/caspase 3/caspase 9) in the heart, maintaining cardiac function after an AMI by slowing apoptosis and healing vascular damage [41]. Cdip1 silencing by exosomal miRNA-21-5p-targeting cardiac telocytes prevents CMECs apoptosis and increases angiogenesis after MI [42]. MiR-124 induces ECs apoptosis via the P38/MAPK and PI3K/AKT pathways

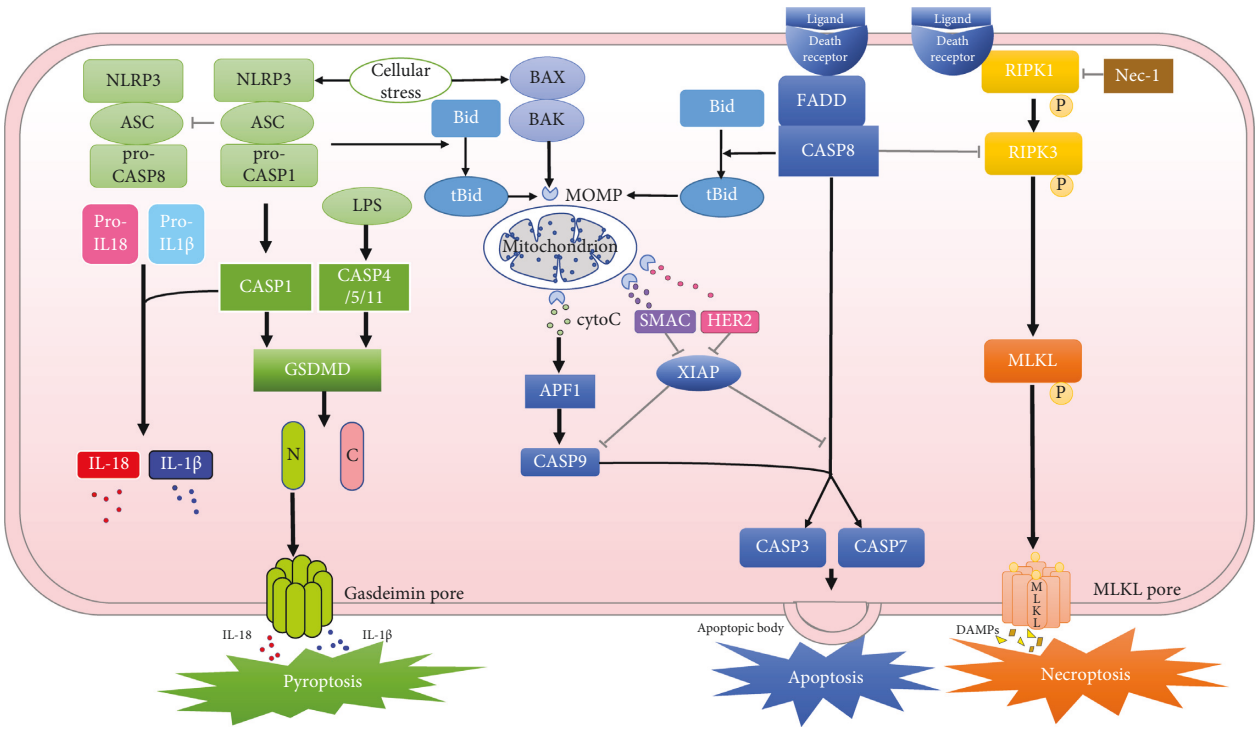

Figure 1: Molecular mechanisms of pyroptosis, apoptosis, and necroptosis and their network interactions. The intrinsic pathway of apoptosis (blue) is induced by the triggering of MOMP by BAX and BAK leading to cytochrome C activation and further activation of caspase-9 on APF1. Death receptor-mediated apoptosis requires the formation of a pro-apoptotic caspase-8 dimer, and both caspase-8 and caspase-9 promote the downstream executioner caspase-3 and caspase-7 activation, which induces apoptosis. Activation of death receptors similarly triggers necroptosis (orange). Activated RIPK3 phosphorylates and activates the executor of necroptosis, MLKL, which forms pores in the cell membrane, leading to cell lysis and the release of DAMPs. Cellular stress leads to inflammasome formation and activation of caspase-1 and cleavage of GSDMD, and cytokines such as IL-18 and IL-1β are released through the membrane pores formed by GSDMD-N and trigger pyroptosis (green).

and may be a contributing factor in vascular endothelial damage in AMI [43]. By targeting granzyme B, MiR-518a-5p can reduce hypoxia/reoxygenation (H/R)-induced apoptosis and cell damage in human umbilical vein endothelial cells (HUVECs) [44].

Alpha-antitrypsin (AAT) overexpression reduces vascular endothelial cell death and increases cell proliferation under H/R conditions via decreasing the Rac1/PAK/p38 signaling pathway and antioxidative stress [45]. Fibroblast growth factor (FGF) is expressed in ECs, and basic FGF inhibits CMECs apoptosis and promotes angiogenesis via HIF-1, reducing myocardial injury [46]. CXCR7 activation has a protective impact on hypoxic ECs, reducing apoptosis, and promoting angiogenesis [47]. Under hypoxic circumstances, foxo3-mediated autophagy enhances CMECs apoptosis, which is a key pathophysiological cause of MI [48].

In addition, clinical trials have shown that the circulating apoptotic markers soluble TNF receptor 1 (sTNFR1) and sTNFR2 were found to be associated with myocardial infarct size and left ventricular insufficiency in patients with ST-segment elevation myocardial infarction (STEMI), suggesting that apoptosis may be a key determinant of the extent of I/R injury [49]. Perindopril is a third-generation angiotensin-converting enzyme inhibitor, which significantly reduces ECs apoptosis and improves abnormal ECs function in patients with MI [50]. Perindopril also reduces the pro-apoptotic effect of serum on ECs and promotes ECs renewal in patients with acute coronary syndrome [51]. The above studies suggest that therapeutic strategies targeting apoptosis have a wide range of clinical applications in myocardial infarction.

3. Necroptosis

3.1. Overview of Necroptosis. Necroptosis is a form of programmed death activated when apoptosis is inhibited, which is independent of caspase family activation pathways and can cause inflammatory responses. This process involves the activation and phosphorylation of receptor-interacting serine-threonine kinase 3 (RIPK3), which activates the pseudokinase mixed lineage kinase domain-like protein (MLKL), the final effector in necroptosis [52, 53] (Figure 1). This process promotes the release of damage-associated molecular patterns (DAMPs), which trigger inflammatory responses and the activation of pyroptosis [54, 55], as well as the efflux of pathogen-associated molecular patterns (PAMPs) [56] in the context of cellular infection, which triggers an inflammatory response. Multiple innate immune signaling pathways, including those mediated by activation of TLRs, death receptors, and RIG-I-like receptors, can cause necroptosis [57]. RIPK1's kinase activity is important in death receptor-induced necroptosis [58–60], and RIPK1

facilitates canonical necroptosis signaling when cell surface TNF binds to TNF receptor 1 (TNFR1) transduces and autophosphorylates RIPK1. Direct inhibitors of RIPK1, necrostatins, can block this pathway [57]. Intervention in the cell membrane repair process involving the ESCRT-III protein complex appears to interrupt or even prevent cell death in the early stages of MLKL activation [61]. Caspase-8 suppresses necroptosis mediated by RIPK3 and MLKL as well as acting as the starting caspase of exogenous apoptosis. The cleavage of RIPK1 by caspase-8 is a critical step in the decomposition of the death-inducing complex. TNF-induced aberrant cell death can be reduced by Asp325 of RIPK1 [62].

3.2. Therapeutic Strategies of Inhibiting Endothelial Cells Necroptosis and Improving the Myocardial Infarction. In CMECs, RIPK3 levels and mPTP opening rates were considerably elevated after H/R damage. Ischemia/reperfusion (I/R) injury can also activate RIPK3; however, melatonin reduces mPTP opening and prevents CMECs necroptosis after cardiac I/R injury by inhibiting the RIPK3-PGAM5-CypD signaling pathway [63]. Baicalin, a compound derived from scutellaria baicalensis, inhibits the production of the proteins RIP1, RIP3, and p-MLKL, preventing necroptosis in CMECs following H/R injury [64]. Overexpression of the sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) protects I/R-treated CMECs by preventing aberrant mPTP opening and suppressing the necroptosis signaling pathway [65]. These findings imply that inhibiting necroptosis could be a useful therapeutic method for treating endothelial damage following MI.

4. Pyroptosis

4.1. Overview of Pyroptosis. The caspase-1-mediated canonical route and the caspase-4/5/11-mediated non-canonical pathway may both initiate pyroptosis [66, 67]. The caspase-1 pathway is vital in innate immunity, and the inflammasome complex signals the downstream adaptor ASC or NLRC4 to activate the caspase-1 molecule when particular stimulatory signals are detected [68], such as bacteria, viruses, and toxins [69]. The AIM2/ASC inflammasome is the most well-known, as it identifies cytoplasmic double-stranded DNA linked to AIM2; the NLRP3/ASC inflammasome is triggered by diverse membrane-damaging chemicals; the Pyrin/ASC inflammasome indirectly senses bacterial toxins inactivating host Rho GTPases; and the NAIP/NLRC4 acts as a receptor and signal amplifier for direct recognition of flagellin and type III secretion system on bacteria, respectively. The caspase-4/5/11 pathway is activated when lipopolysaccharide (LPS) is detected in the cytoplasm. The roles of caspase-4 and caspase-5 in humans are similar. Murine caspase-11 is located adjacent to caspase-1 on chromosome position, a receptor for cytoplasmic lipopolysaccharide, and a homolog of human caspase-4. Caspase-11 deletion protects mice from endotoxic shock as compared to caspase-1 deletion [70]. Caspase-4/5/11 bind directly to cytoplasmic LPS to initiate pyroptosis, and CARD on caspases mediates this binding [71]. Furthermore, Pannexin-1 channels, purinergic P2X7 pore [72], and high mobility group protein B1 (HMGB1) [73] are required for caspase-11-mediated pyroptosis and endotoxin shock [72], a mechanism that seems to be independent of TLR4 [74]. Heparin inhibits cytoplasmic transport of LPS and caspase-11 activation by reducing HMGB1-LPS interactions and glycocalyx breakdown in macrophages [75]. GSDMD is cleaved into the GSDMD-N-terminal and GSDMD-C-terminal when caspase-1 and caspase-4/5/11 are activated. The N-terminus of GSDMD is carried to the plasma membrane, where it homo-oligomerizes to form a 10- to 15-nm pore [76, 77]. This pore promotes rapid cell enlargement and membrane rupture, allowing IL-18, IL-1β, and HMGB1 to be released [73, 78] (Figure 1). Recent research has found that cell membrane rupture is not required for cytokine release and that IL-1β may be released from live cells, indicating that GSDMD might have a role in cytokine release without triggering pyroptosis [79–81]. GSDMD pores provide reasonable channels for the release of IL-1β (4.5 nm in diameter) and IL-18 (5.0 nm in diameter) [82]. In both mouse and human models, IL-1β secretion through GSDMD membrane pores has been observed [81, 83, 84], and GSDMD loss lowers IL-1β secretion [85].

4.2. Therapeutic Strategies of Inhibiting Endothelial Cells Pyroptosis and Improving the Myocardial Infarction. After myocardial infarction, ASC speck formation was observed in mouse cardiac ECs, suggesting that endothelial NLRP3 inflammasomes may play a role in MI. NLRP3 inflammasomes are involved in IL-1β release and pyroptosis in endothelial cells and cardiac fibroblasts [86]. Bean1 overexpression resulted in a large rise in NLRP3 and IL-1β in CMECs following MI damage [87], suggesting that Bean1 may serve as a therapeutic target for NLRP3-mediated vascular injury in the treatment of MI. Ventricular remodeling is a critical link in the evolution of MI into heart failure, and it is suggested that NLRP3 inflammasome also plays a significant role in the process of ventricular remodeling following MI [88]. Furthermore, by lowering vascular endothelial cell pyroptosis, chitosan hydrogel increases the efficiency of bone marrow mesenchymal stem cells in the treatment of MI [89]. The above evidence suggests that means of inhibiting ECs pyroptosis need to be discovered and applied in the treatment of MI.

5. Ferroptosis

5.1. Overview of Ferroptosis. Oxidative stress and lipid peroxidation are key determinants of cell fate, and iron-dependent lethal lipid peroxidation drives ferroptosis [90]. Glutathione peroxidase 4 (GPX4) is a selenoprotein that was discovered to cause nonapoptotic cell death due to lipid peroxidation long before the concept of ferroptosis was proposed [91]. Ferroptosis is thought to be aided by the system xc^--GSH-GPX4 pathway [90], with glutathione (GSH) depletion and reduced GPX4 activity affecting the metabolic process of lipid oxides, boosting the generation of

reactive oxygen species (ROS) by Fe^{2+}, and so inducing ferroptosis. Phospholipid hydroperoxides (PLOOHs) are major executors of ferroptosis, and GPX4 acts as a neutralizing enzyme for PLOOH, converting PLOOH to PLOH [92]. Erastin and RSL3 act as inhibitors of system xc^- and GPX4 to trigger ferroptosis, RSL3 directly inactivates GPX4, and erastin inhibits cystine input, reducing cysteine in glutathione GSH synthesis, indirectly reducing GPX4 production, and causing the accumulation of PLOOHs, which will lead to lipid excess. Oxidation products and an increase in oxidatively changed proteins further compromise cell membrane integrity, resulting in cell death. Acyl-CoA synthetase long-chain family member 4 (ACSL4) is a key driver of ferroptosis. Polyunsaturated fatty acids (PUFA) are the precursors of PLOOHs. ACSL4 catalyzes the linking of free PUFA with coenzyme A to generate PUFA-CoA, which is then esterified under the catalysis of LPCAT3 and forms PUFA-PL with PL. PUFA-PL is prone to peroxidation in the condition of rich iron and ROS. This accumulation of lipid peroxides in the cell membrane eventually destroys the integrity of the membrane, resulting in ferroptosis. ACSL4 depletion or inhibition drastically transforms the long-chain PUFA tails in phospholipids to short-chain or monounsaturated fatty acid (MUFA) tails [93, 94], determining ferroptosis sensitivity [95]. Ferritin heavy chain 1 (FTH1) is involved in ferritin phagocytosis and ferroptosis, and the cargo receptor NCOA4 detects and binds to FTH1 and transports iron-bound ferritin to autophagosome, a selective autophagy form, completing ferritin breakdown, and iron release [96, 97].

5.2. Therapeutic Strategies of Inhibiting Endothelial Cells Ferroptosis and Improving the Myocardial Infarction. Ferroptosis has been linked to the development of cardiovascular diseases [98], tumors [99], metabolic diseases [100], neurological diseases [101], and inflammatory and immunological diseases [102] in recent investigations, and ferroptosis has also been detected in cardiomyocytes from mice with MI [103]. However, ferroptosis has rarely been reported in endothelial cells of MI. Using biological information technology, Yifan et al. [104] screened the differential expression of ferroptosis-related genes in circulating endothelial cells of patients with AMI. These genes included C-X-C motif ligand 2 (CXCL2), FTH1, toll-like receptor 4 (TLR4), JUN (AP-1 transcription factor subunit), endothelial PAS domain protein 1 (EPAS1), DNA damage-inducible transcript 3 (DDIT3), Jun dimerization protein 2 (JDP2), activating transcription factor 3 (ATF3), were considerably overexpressed in the AMI group, showing that ferroptosis is involved in the control of metabolism in the circulating EC of AMI patients. This lays the groundwork for future research into the pathophysiology of ferroptosis in AMI patients.

6. Autophagy

6.1. Overview of Autophagy. Autophagy is a highly conserved adaptive mechanism that helps keep cells in a state of homeostasis. Macroautophagy, microautophagy, and chaperone-mediated autophagy [105] are three subtypes of autophagy that all entail the breakdown of cellular contents in lysosomes; however, the methods are distinct. The most common kind is macroautophagy. Macroautophagy was formerly thought to be a nonselective process, but it has now been found to selectively degrade intracellular microorganisms, damaged lysosomes, and mitochondria [106, 107]. Under cellular stress circumstances such as food restriction, oxidative stress, protein aggregation, and hypoxia, autophagy is triggered, and stress signaling pathways are mainly focused on the ULK1 complex, which is made up of four proteins, ATG101, ATG13, ULK1, and FIP200 [108]. PI3KC3 complex I, which is sorted by class III PI3K, vacuolar protein 34 (VPS34), Beclin 1, ATG14, activating molecules in Beclin 1-regulated autophagy protein 1 (AMBRA1), and a general vesicle transporter (p115), initiates phagophore nucleation [109], then comes phagophore expansion, which requires the binding of membrane-resident phosphatidylethanolamine by Ub-like Atg8 family proteins such microtubule-associated protein light chain 3 (LC3) protein and γ-aminobutyric acid receptor-associated protein (GABARAPs) [109]. Finally, autophagosomes are formed when a portion of the cytoplasm is phagocytosed to form double-membrane vesicles known as autophagosomes. After autophagosome maturation, the autophagosome outer membrane combines with lysosomes to produce autophagolysosomes, and the contents of the autophagosome are degraded and recycled [110].

6.2. Therapeutic Strategies of Inhibiting Endothelial Cells Autophagy and Improving the Myocardial Infarction. Autophagy sheds light on new therapeutic targets in the myocardial infarction. VEGF-A, ROS, GRP78/Bip, and LC3-II/LC3-I expression levels in the vascular ECs of MI mice were considerably enhanced, and VEGF-A promotes angiogenesis after AMI via the ROS-ER-autophagy axis [111]. In the injured myocardium of zebrafish, autophagy was also discovered, and metformin increased autophagic flux; caused endocardial, epicardial, and endothelial regeneration; and accelerated MI cell proliferation. When autophagic flow was stopped, however, all of these effects were delayed [112]. Angiogenic factor 1 (AGGF1) is an angiogenic factor that activates JNK in endothelial cells, causing autophagy and the formation of the Becn1-Vps34-Atg14 complex, and therapy with Aggf1 protein can greatly enhance heart function after MI [113]. Moreover, transplanted mesenchymal stem cells (MSCs) generate apoptotic bodies (Abs), which stimulate lysosomal activity and enhance the expression of transcription factor EB (TFEB), a lysosome master gene for biogenesis and autophagy, in a MI model. Following a myocardial infarction, elevated TFEB boosted the expression of autophagy-related genes in ECs, boosting angiogenesis, and cardiac function recovery [114]. To attenuate I/R-induced cardiac damage, exosomal LINC00174 derived from vascular ECs suppressed p53-mediated autophagy [115]. AMI patients exhibited considerably greater levels of sTREM-1 and significantly lower levels of Sirt6, compared to healthy controls, and Sirt6-induced autophagy was observed to restrict TREM-1-mediated pyroptosis in ECs treated with oxidatively modified low-density lipoprotein (ox-LDL)

[116]. The development of drugs that enhance autophagic flow would be a potential therapeutic strategy to improve the prognosis of MI.

7. Cross-Talk between Apoptosis, Necroptosis, Pyroptosis, Ferroptosis, and Autophagy

It was speculated that inhibition of caspase-8 and its adapter FADD would prevent apoptosis induced by the death receptor pathway, leading to an increase in cell numbers. However, this was not the case, and the study found that necroptosis was activated after caspase 8 [117] and FADD [118] were inhibited, which is the first time that the possibility of functional intertwining of different programmed cell deaths was identified [119, 120]. Necroptosis may be a means to kill damaged cells that have evaded apoptosis. Caspase-8 not only plays an important role in apoptosis and necroptosis, but also is able to regulate pyroptosis. Procaspase-8 inactivation also leads to ASC spot production, which promotes caspase-1 activation and subsequent GSDMD lysis and pyroptosis [121]. In the later stages of apoptosis, a decrease in intracellular K^+ concentration leads to the activation of NLRP3 inflammasome and the onset of pyroptosis [122]. Notably, cells lacking caspase 1 or GSDMD still die due to NLRC4 activation, and this death is morphologically characteristic of apoptosis [123] and involves caspase 8, which can bind to ASC or NLRC4 [124, 125]. This suggests that in the absence of GSDMD, sustained inflammasome activation, although not inducing pyroptosis, leads to apoptotic forms of cell death. Alternatively, inflammasome activation can trigger apoptosis via caspase-1 or caspase-8-mediated processing of the BH3-only protein BID into a pro-apoptotic tBID, followed by activation of BAX/BAK and induction of MOMP via the intrinsic pathway [126, 127] (Figure 1). We know that both active MLKL and GSDMD may lead to membrane damage and that MLKL-dependent K^+ spillover leads to NLRP3 activation [128]. Ferroptosis is a ferritin-degrading autophagy-dependent process [129], and it has been reported that knockdown or knockout of ATG5 and ATG7 can prevent iron death induced by the ferroptosis-inducer erastin by reducing intracellular iron levels and lipid peroxidation [130]. In addition, autophagy can reduce intracellular GSH levels [131], so the use of autophagy inhibitors can prevent the occurrence of GSH depletion-dependent ferroptosis [132]. This indicates that autophagy plays an important role in ferroptosis.

8. Conclusions

As summarized herein, apoptosis, necroptosis, pyroptosis, ferroptosis, and autophagy are all seen in endothelial cells following myocardial infarction. The majority of current research focuses on a single kind of cell death, despite the fact that many types of cell death have interconnected effects [133]. Cross-talk between different modes of endothelial cell death in myocardial infarction will be an area of ongoing interest, and it is unclear whether cell death is a direct cause or a key factor in the development of disease, or a consequence of disease-induced injury, and the extent to which therapeutic strategies targeting cell death can alleviate disease remains to be explored. With a better understanding of the different cell death modalities, more research focusing on the role of endothelial cell death in the pathogenesis of myocardial infarction is needed in the future, thus providing more possibilities for the treatment of myocardial infarction.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Mingyue Wu and Zixia Huang contributed equally to the paper.

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Review Article

Quality of Evidence Supporting the Effects of Tai Chi Exercise on Essential Hypertension: An Overview of Systematic Reviews and Meta-Analyses

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Objectives. Tai Chi (TC) is a potential complementary treatment for essential hypertension (EH). This overview systematically summarizes and evaluates the existing evidence of TC in the therapy of EH. *Methods*. Systematic reviews (SRs)/meta-analyses (MAs) on TC interventions for EH were comprehensively searched in seven databases. Methodological quality, risk of bias, reporting quality, and quality of evidence were assessed by means of the Assessment of Multiple Systematic Reviews 2 (AMSTAR-2), the Risk of Bias in Systematic (ROBIS) scale, the list of Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA), as well as the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system. *Results*. Twelve published SRs/MAs were included in our study. According to the results of the AMSTAR-2, ROBIS, PRISMA, and GRADE assessment, only 1 SR/MA was assessed as high quality and only 1 SR/MA was assessed as low risk of bias. Only 2 SRs/MAs have been fully reported on the checklist. In addition to that, the quality of evidence was assessed for a total of 69 outcome indicators extracted from the SRs/MAs included in this overview, and only 3 items were assessed as high quality. *Conclusions*. TC may be an effective and safe complementary treatment for EH. However, this conclusion must be approached with caution, as the quality of the evidence provided by the SRs/MAs is usually low.

1. Introduction

Cardiovascular disease accounts for one-third of global deaths and remains a growing public health concern [1]. As one of the most common diseases in the world, hypertension (HT) is the most important risk factor for cardiovascular disease [2]. HT affects more than 1.39 billion people worldwide and is estimated to cause 9.4 million deaths each year, making HT one of the most serious chronic problems threatening public health [3]. HT is divided into essential hypertension (EH) and secondary HT, of which the former is the most common (about 90%), but its pathogenesis is still unclear [4]. Although the treatment of HT has been continuously explored in the past few decades, HT drug therapy is still used as the main means of treatment of EH [5]. The likelihood that patients with EH will be able to maintain their blood pressure in the normal range with antihypertensive medications alone remains low [6]. As a result, many patients have to take several antihypertensive drugs at the same time to control their blood pressure. However, this practice can impose an increased financial burden and may have unforeseen side effects [7]. Therefore, there is an urgent need for better management of HT.

The Canadian Hypertension Education Program (CHEP), the Eighth Joint National Committee (JNC-8), and the American Heart Association (AHA) have recommended aerobic exercises for people with HT [8], and Traditional Chinese Exercise (TCE) has gained worldwide popularity

due to its beneficial effects on improving the physical and mental health of patients with chronic diseases [9]. Tai Chi (TC) is a traditional Chinese physical and mental exercise with moderate movement intensity. Originated in China thousands of years ago, TC combines Chinese philosophy with martial arts and healing arts [10]. A growing number of studies have shown that TC can modulate pressure receptors in the carotid sinus and aortic arch, and play a part in dilating coronary arteries and lowering blood pressure [11, 12].

Many systematic reviews/meta-analyses (SRs/MAs) have been conducted to evaluate the potential therapeutic benefits of TC for patients with EH. However, the conclusions are inconsistent due to the defects of the quality and the method of the preliminary research. The overview of systematic reviews is a novel tool for solving specific and key issues related to policies and practices [13]. The purpose is to synthesize the evidence from multiple SRs/MAs into a useable document that can be employed to guide healthcare professionals and decision-makers. To this end, our research is to use a systematic overview to critically evaluate the scientific quality of related SRs/MAs in the TC treatment of EH.

2. Methods

2.1. Research Methods. The SRs/MAs overview was based on the guidelines specified in Cochrane Handbook [14] and relevant methodologies for a high-quality overview [15].

2.2. Development of Inclusion and Exclusion Criteria

2.2.1. Literature Inclusion Criteria

(a) Type of research: This overview included SRs/MAs of randomized controlled trials (RCTs) on the effects of TC exercise on EH.

(b) Type of participants: The subjects were patients diagnosed with EH by any international [16] or national [17] standard regardless of their gender, age, or race.

(c) Type of intervention: The intervention for the control group was antihypertensive drugs (AHD), other exercises (OE), no treatment (NT), routine care (RC), or health education (HE), and the intervention for the experimental group was TC exercise or TC combined with the treatments received by the control group.

(d) Types of outcomes: Outcomes assessed in this overview included systolic/diastolic blood pressure (S/DBP), total cholesterol (TCL), high-density lipoprotein (HDL), triglycerides (TG), low-density lipoprotein (LDL), quality of life (QOL), and body mass index (BMI).

2.2.2. Exclusion Criteria. Repeated publications, other overviews, network meta-analyses, narrative reviews, dissertations, and conference abstracts were excluded.

2.3. Data Sources and Search Strategy. Seven electronic databases were searched by 2 researchers (HS-S and D-W) from their respective inception times to January 1, 2022, including the Cochrane Library, PubMed, EMBASE, China Biomedicine (CBM), Wanfang Database, CNKI, and Chongqing VIP. The literature search was carried out using a combination of MeSH terms and free words, and MeSH terms include "Tai Chi," "Hypertension," "Systematic Review," and "Meta-Analysis," and adjustment was made according to different databases. The search strategy of the PubMed database is shown in Table 1.

2.4. Literature Screening and Data Extraction. Two researchers (HS-S and ZX-W) independently screened the retrieved literature studies. Then, the researchers removed the duplicate publications, read the publication titles and abstracts, and finally read the full text to assess their eligibility. All SRs/MAs were read by two independent researchers (D-W and CD-D), and the following data were extracted from the SRs/MAs: first author, publication year, country, number of RCTs included, interventions for experimental and control groups, included RCT quality assessment tools, and main conclusion. The disagreement between the two researchers was resolved through discussion.

2.5. SRs/MAs Quality Estimate. Two researchers (PL-L and HS-S) independently assessed the methodological and evidence quality of the included MAs.

2.5.1. Estimate of Methodological Quality. The methodological quality of the included SRs/MAs was assessed by the Assessment System for Evaluating Methodological Quality 2 (AMSTAR-2) [18]. Seven (2, 4, 7, 9, 11, 13, and 15) of the 16 items in the tool were critical areas.

2.5.2. Estimate of Risk of Bias. The Risk of Bias In Systematic Review (ROBIS) [19] scale was used in this overview to evaluate the risk of bias for the inclusion of SRs/MAs. The scale was divided into three stages to assess the overall risk of bias of the included SRs/MAs.

2.5.3. Estimate of Reporting Quality. The quality of each SR/MA report of the included SRs/MAs was evaluated by the list of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [20], which consisted of 27 items focusing on reporting methods and results that were incorporated into SRs/MAs.

2.5.4. Assessment of Quality of Evidence. The quality of evidence for each SR/MA outcome was evaluated by means of the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) [21], according to which, five aspects will lead to the degradation of evidence quality, including limitations, inconsistencies, indirectness, imprecision, and publication bias.

Table 1: Search strategy for the PubMed database.

Query	Search term
#1	"Tai Ji" [Mesh]
#2	"Tai-ji" OR "Tai Chi" OR "Chi, Tai" OR "Tai Ji Quan" OR "Ji Quan, Tai" OR "Quan, Tai Ji" OR "Taiji" OR "Taijiquan" OR "T'ai Chi" OR "Tai Chi Chuan" OR "Tai ji"
#3	#1 OR #2
#4	"Hypertension" [Mesh]
#5	"Blood Pressure, High" OR "Blood Pressures, High" OR "High Blood Pressure" OR "High Blood Pressures" OR "Hypertension"
#6	#4 OR #5
#7	Meta-Analysis as Topic [Mesh]
#8	"Systematic review" OR "meta-analysis" OR "meta analysis" OR "meta-analyses" OR "Review, Systematic"
#9	#7 OR #8
#10	#3 AND #6 AND #9

3. Results

3.1. Results on Literature Search and Selection. Through our search strategy, a total of 138 articles were identified. After removing 37 duplicate articles, the researchers screened the remaining 101 articles by reading the titles and abstracts. Subsequently, 16 articles were obtained. After reading the full text, 1 article [22] was not about SRs/MAs in TC, and 2 SRs/MAs [23, 24] were not about people with EH. In addition, there was an article [25] on an RCT of the efficacy of TC in EH. Finally, a total of 12 SRs/MAs [26–37] were finally included in this overview. The process of study selection is shown in Figure 1.

3.2. Description of Included SRs/MAs. The characteristics included in the overview are shown in Table 2. These SRs/MAs were all published between 2011 and 2021, 7 [26–32] of which were in English, and the remaining 5 [33–37] were in Chinese, and all were written by Chinese authors. The SRs/MAs included in this overview contained a total of 58 RCTs, of which 42 (72.4%) RCTs are overlapping (Table 3). The number of RCTs was between 5 and 28, and the sample size was between 402 and 2,937. In terms of quality evaluation scales, 9 SRs/MAs [26–33, 37] used the Cochrane risk of bias standard, and 3 SRs/MAs [34–36] used the Jadad Scale.

3.3. Results on SRs/MAs Quality Assessment

3.3.1. Methodological Quality Assessment. The evaluation details of the included MAs on the AMSTAR-2 are shown in Table 4. Only 1 SR/MA [26] was rated as high quality, and the quality of the remaining SRs/MAs [27–37] was rated very low since more than one critical area was missing. Methodological quality limitations come from the following items: Item 2 (only 3 SRs/MAs registered the study protocol), Item 7 (only 1 SR/MA [26] provided a literature exclusion list), and Item 13 (when interpreting the evaluation results, only 4 SRs/MAs [26, 28, 29, 36] considered the risk of bias in the main study).

3.3.2. Risk of Bias of the Included SRs/MAs. Regarding the results of the ROBIS assessment, Phase 1 assessed the relevance of the study topic and Domain 1, with all MAs rated as low risk of bias in both items. In Domain 2, 6 SRs/MAs [26–28, 30, 32, 33] were assessed as low risk. In Domain 3, 7 SRs/MAs [26, 27, 29, 30, 32, 34, 37] were assessed as low risk of bias and 3 SRs/MAs [26, 29, 34] were assessed as low risk of bias in Domain 4. In Phase 3, only 1 SR/MA [26] had a low risk of bias. The evaluation details of the included SRs/MAs on the ROBIS scale are shown in Table 5.

3.3.3. Report Quality of the Included SRs/MAs. The results of the PRISMA assessment are shown in Table 6. Twenty-one of the 27 items had a "yes" response rate of over 70%, indicating the inclusion of relatively complete reporting of SRs/MAs. Nevertheless, there are reporting deficiencies on some items. The reports of Item 5 (protocol and registration) and Item 8 (search) were incomplete (the "yes" response rate was less than 50%).

3.3.4. Evidence Quality of the Included SRs/MAs. The 12 SRs/MAs included 69 outcome indicators related to the effectiveness of TC for EH. By means of GRADE evaluation, 3 were rated as high quality of evidence, 15 moderate, 29 low, and 22 very low for all the outcome indicators. Inconsistency ($n = 45$) and publication bias ($n = 45$) were the most common downgrading factors, followed by the risk of bias ($n = 36$), imprecision ($n = 21$), and indirectness ($n = 0$). GRADE specific assessment details are shown in Table 7.

3.4. Summary Results of the Included SRs/MAs. The result indicators extracted from the included studies are listed in Table 7.

3.4.1. Blood Pressure. Fifteen SBP-related outcomes were reported in 11 SRs/MAs [26–29, 31–37], all of which indicated that TC was effective in reducing SBP in EH patients. Of the 11 SRs/MAs [26–29, 31–37] that reported 16 outcomes related to DBP, only 1 [33] outcome showed that TC was ineffective in reducing DBP compared with HE/NT and the rest showed that TC was effective in reducing DBP.

3.4.2. Outcomes Related to Lipid Metabolism. Three SRs/MAs [26, 27, 31] reported the effect of TC on TCL, and the results indicated that TC could effectively reduce TCL in EH

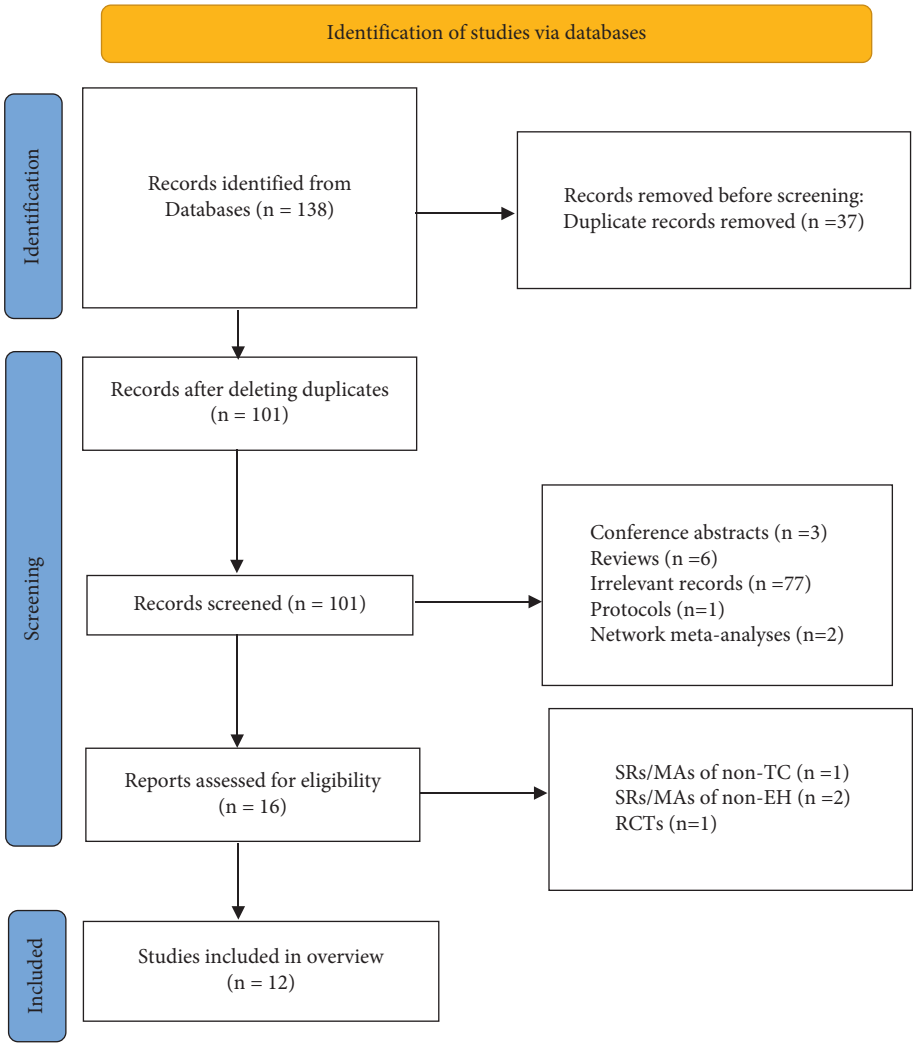

Figure 1: The flowchart of the screening process.

Table 2: Characteristics of the included SRs/MAs.

Author, year (country)	Trials (subjects)	Intervention group	Control group	Quality assessment	Main results
Zhong et al., 2020 (China) [26]	28 (2,937)	TC, TC+Control Group	HE, AHD, NT, OE	Cochrane Criteria	TC may be recommended as an adjunctive treatment for HT, especially in patients under the age of 50.
Liang et al., 2020 (China) [27]	15 (1,543)	TC, TC+Control Group	HE, AHD, NT, OE	Cochrane Criteria	TC reduces blood pressure, TCL, TG, LDL, and blood glucose, and significantly improves QOL in adult patients with EH.
Wang et al., 2013 (China) [28]	18 (1,371)	TC, TC+Control Group	AHD, RC	Cochrane Criteria	There is some encouraging evidence that TC reduces blood pressure in hypertensive patients, but the evidence remains weak due to the poor methodological quality of the included studies.
Pan et al., 2021 (China) [29]	24 (2,095)	TC, TC+Control Group	AHD, NT, OE	Cochrane Criteria	The results showed that TC exercise could effectively reduce SBP, DBP and QOL in hypertensive patients. Therefore, it should be promoted as a safe and effective adjuvant therapy for hypertension.
Song et al., 2021 (China) [30]	10 (1,177)	TC, TC+Control Group	HE, AHD, NT	Cochrane Criteria	TC can be an effective therapy for improving the QOL of patients with EH.
Guan et al., 2020 (China) [31]	13 (1,461)	TC	HE, AHD	Cochrane Criteria	Compared with the control group intervention, TC is an effective physical exercise intervention in patients with EH.

Table 2: Continued.

Author, year (country)	Trials (subjects)	Intervention group	Control group	Quality assessment	Main results
Lian et al., 2017 (China) [32]	20 (1,641)	TC, TC+Control Group	HE, AHD, NT, OE	Cochrane Criteria	The results of this study show that TC can reduce SBP, DBP, BMI, and WC.
Cai et al., 2016 (China) [33]	8 (881)	TC, TC+Control Group	AHD, NT	Cochrane Criteria	The results of this meta-analyses suggest that TC exercise can reduce SBP in patients with EH. At the same time, on the basis of conventional western medicine treatment, TC exercise can significantly lower blood pressure than the group using western medicine alone, with better curative effect and fewer side effects.
Jin et al., 2018 (China) [34]	19 (1,545)	TC	HE, AHD, NT	Jadad Scale	TC can reduce SBP and DBP to different degrees in middle-aged and elderly patients with EH. It is one of the effective methods for non-drug treatment of hypertension, and can provide a basis for the choice of clinical treatment of hypertension.
Li et al., 2011 (China) [35]	5 (402)	TC, TC+Control Group	AHD, NT, OE	Jadad Scale	TC exercise is effective in treating EH, both in reducing SBP and DBP.
Zhang et al., 2017 (China) [36]	6 (629)	TC	AHD	Jadad Scale	This study shows that TC exercise can effectively improve blood pressure in patients with EH.
Zhang et al., 2019 (China) [37]	15 (732)	TC, TC+Control Group	AHD, NT	Cochrane Criteria	The results show that TC can effectively reduce SBP and DBP in patients with EH, especially in patients under 65 years old.

patients. Four outcome indicators in three SRs/MAs [26, 27, 31] reported the effect of TC on TG, 3 outcome indicators [26, 27, 31] showed that TC could effectively reduce TG, and 1 outcome indicator [26] showed that TC was ineffective in reducing TG in EH patients compared with HE/NT. Three SRs/MAs [26, 27, 31] reported that TC was ineffective in improving HDL in EH patients. In addition, three SRs/MAs [26, 27, 31] reported that TC could reduce LDL in EH patients.

3.4.3. Other Outcome Measures. Three SRs/MAs [27, 29, 30] reported that TC exercise could improve QOL in EH patients. Three SRs/MAs [29, 31, 32] reported the efficacy of TC exercise on BMI, and 2 SRs/MAs [29, 32] showed that TC could reduce BMI in EH patients. Two SRs/MAs [28, 35] reported the superiority of TC exercise in terms of efficacy in treating EH patients. The results of two SRs/MAs [31, 32] indicated that TC could reduce WC in EH patients. In addition to this, one SR/MA [27] showed that TC exercise reduced blood glucose levels in EH patients.

3.4.4. Adverse Event. The five SRs/MAs [26, 28, 29, 31, 32] described TC as having a good safety profile.

4. Discussion

HT is an important risk factor for a variety of cardiovascular and cerebrovascular diseases. With the development of science and technology, people gradually realize the important role of TC in healthcare as well as the prevention and treatment of cardiovascular and cerebrovascular diseases [38]. In recent years, multiple SRs/MAs have been performed to elucidate the potential efficacy and safety of TC on EH. Therefore, we conducted this overview to synthesize multiple published SRs/MAs to assess their methodological quality and level of evidence.

4.1. Summary of the Main Findings. This overview included 12 SRs/MAs on the impact of TC on EH. All SRs/MAs were based on RCTs and published from 2011 to 2021. Among them, 9 (9/12, 75%) SRs/MAs were published in the past five years, indicating that the improvement effect of TC on EH has attracted increasing attention over the period. We performed an extraction analysis for all the original RCTs covered by the SRs/MAs included in this overview, and we found differences in the inclusion of RCTs across these SRs/MAs. The reasons are as follows: (1) the search date and the number of RCTs included in the earliest published SRs/MAs were limited; (2) the focus on the outcomes was different in the included SRs/MAs, e.g., Song 2021 [30] focused on the quality of life of EH patients; (3) the age limit of the included population varied greatly, e.g., Jin 2018 [34] focused on middle-aged and elderly patients with EH; (4) The trial period of RCTs was different, e.g., Liang 2020 [27] limited the trial period of RCTs to more than one month.

Based on the results of the AMSTAR-2 evaluation in this overview, the methodological quality of only 1 included SR/MA was rated high and that of the remaining SRs/MAs was rated very low, especially in Item 2 (Protocol registration, 3/12, 25%), Item 7 (Exclusion list, 1/12, 8.3%), and Item 13 (RoB account, 4/12, 33.3%). Only 3 SRs/MAs contained initial research protocol registrations, which could lead to greater than expected adjustments to the research process, increasing the risk of bias and impacting the rigor and credibility of the final MAs results [39]. Only 1 SR/MA provided a complete exclusion of the reasons for each study, which may affect the reliability of the results and assessment of publication bias. The provision of a list of exclusion

Table 3: This overview contains the distribution table of RCTs contained in SRs/MAs.

RCT ID	Zhong et al., 2020 (China) [26]	Liang et al., 2020 (China) [27]	Wang et al., 2013 (China) [28]	Pan et al., 2021 (China) [29]	Song et al., 2021 (China) [30]	Guan et al., 2020 (China) [31]	Lian et al., 2017 (China) [32]	Cai et al., 2016 (China) [33]	Jin et al., 2018 (China) [34]	Li et al., 2011 (China) [35]	Zhang et al., 2017 (China) [36]	Zhang et al., 2019 (China) [37]	No. of times included
Sun, 2015	✓	✓		✓	✓	✓	✓	✓	✓				8
Luo, 2006	✓	✓	✓	✓			✓	✓		✓		✓	8
Zhou, 2007	✓	✓	✓	✓			✓		✓			✓	7
Zheng, 2015	✓	✓		✓	✓		✓	✓	✓				7
Qi, 2015	✓	✓		✓			✓		✓			✓	6
TSAI, 2003	✓			✓			✓	✓	✓			✓	6
Han, 2010	✓		✓	✓	✓		✓		✓				6
Mao, 2006	✓		✓				✓		✓		✓	✓	6
Xie, 2014	✓			✓			✓		✓			✓	5
Sun, 2014	✓	✓					✓		✓		✓		5
Xiao, 2018	✓	✓		✓	✓							✓	5
Xu, 2016b	✓	✓			✓	✓	✓						5
Ma, 2018	✓	✓		✓	✓	✓							5
Wang, 2011b			✓	✓					✓		✓	✓	5
Lo, 2012			✓			✓		✓	✓			✓	5
Shi, 2017	✓	✓		✓								✓	4
Shou, 2018	✓	✓		✓	✓								4
Sun, 2010	✓			✓			✓			✓			4
Liu, 2018	✓	✓		✓	✓								4
Chen, 2013	✓			✓			✓				✓		4
Tang, 2009		✓	✓						✓			✓	4
Chen, 2006			✓				✓		✓	✓			4
Lee, 2004			✓			✓		✓				✓	4
Jin, 2016	✓	✓					✓						3
Wei, 2015	✓			✓			✓						3
Chan, 2018		✓		✓		✓							3
Young, 1999				✓		✓			✓				3
Pan, 2015						✓			✓			✓	3
Wolf, 2006						✓		✓	✓				3
Zhang, 2017	✓			✓									2
Zhou, 2015	✓						✓						2
Chen, 2011a	✓		✓										2
Wang, 2019	✓			✓									2
Wang, 2011a			✓				✓						2

Table 3: Continued.

RCT ID	Zhong et al., 2020 (China) [26]	Liang et al., 2020 (China) [27]	Wang et al., 2013 (China) [28]	Pan et al., 2021 (China) [29]	Song et al., 2021 (China) [30]	Guan et al., 2020 (China) [31]	Lian et al., 2017 (China) [32]	Cai et al., 2016 (China) [33]	Jin et al., 2018 (China) [34]	Li et al., 2011 (China) [35]	Zhang et al., 2017 (China) [36]	Zhang et al., 2019 (China) [37]	No. of times included
He, 2011			✓						✓				2
Wang, 2007a			✓							✓			2
Wang, 2007b			✓							✓			2
Kim, 2016				✓								✓	2
Kim, 2014				✓								✓	2
Tang, 2008				✓			✓						2
Thomas, 2005						✓		✓					2
Lu, 2015									✓		✓		2
Lin, 2019	✓												1
Liu, 2017	✓												1
Chen, 2011b			✓										1
Xu, 2016a							✓						1
Li, 2016	✓												1
Pan, 2014	✓												1
Wei, 2003			✓										1
Yi, 1990			✓										1
Song, 2011			✓										1
Gou, 2017					✓								1
Li, 2018					✓								1
Lee, 2017						✓							1
Nguyen, 2012						✓							1
Hsu, 2015						✓							1
Jing, 2015									✓				1
Zheng, 2014											✓		1
Total studies included	28	15	18	24	10	13	20	8	19	5	6	15	

Table 4: Result of the AMSTAR-2 assessments.

Author, year (country)	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Quality
Zhong, et al., 2020 (China) [26]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	H
Liang et al., 2020 (China) [27]	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	VL
Wang et al., 2013 (China) [28]	Y	PY	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N	Y	VL
Pan et al., 2021 (China) [29]	Y	PY	Y	PY	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	VL
Song et al., 2021 (China) [30]	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	Y	VL
Guan et al., 2020 (China) [31]	Y	PY	Y	PY	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	VL
Ziyu Lian, 2017 (China) [32]	Y	PY	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	VL
Cai et al., 2016 (China) [33]	Y	PY	Y	Y	N	N	N	Y	Y	Y	Y	N	N	N	N	N	VL
Chengji Jin, 2018 (China) [34]	Y	PY	Y	PY	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	N	VL
Li et al., 2011 (China) [35]	Y	PY	Y	PY	N	N	N	Y	Y	Y	Y	Y	N	N	N	N	VL
Zhang et al., 2017 (China) [36]	Y	PY	Y	PY	N	N	N	Y	Y	Y	Y	Y	Y	Y	N	Y	VL
Zhang et al., 2019 (China) [37]	Y	PY	Y	PY	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	N	VL

Note: Y, yes; PY, partial yes; N, no; VL, very low; H, high. Critical areas are marked in red.

Table 5: Results of the ROBIS assessments.

Author, year (country)	Phase 1	Phase 2				Phase 3
	Assessing relevance	Domain 1: Study eligibility criteria	Domain 2: Identification and selection of studies	Domain 3: Collection and study appraisal	Domain 4: Synthesis and findings	Risk of bias in the review
Dongling Zhong, 2020 (China) [26]	✓	✓	✓	✓	✓	✓
Hao Liang, 2020 (China) [27]	✓	✓	✓	✓	×	×
Jie Wang, 2013 (China) [28]	✓	✓	✓	×	×	×
Xiandu Pan, 2021 (China) [29]	✓	✓	×	✓	✓	×
Yang Song, 2021 (China) [30]	✓	✓	✓	✓	×	×
Yuanyuan Guan, 2020 (China) [31]	✓	✓	×	×	×	×
Ziyu Lian, 2017 (China) [32]	✓	✓	✓	✓	×	×
Lu Cai, 2016 (China) [33]	✓	✓	✓	×	×	×
Chengji Jin, 2018 (China) [34]	✓	✓	×	✓	✓	×
Hongguo Li, 2011 (China) [35]	✓	✓	×	×	×	×
Yeting Zhang, 2017 (China) [36]	✓	✓	×	×	×	×
Yongpeng Zhang, 2019 (China) [37]	✓	✓	×	✓	×	×

Note:✓, low risk; ×, high risk.

researches can more strongly demonstrate the rigor of the literature screening process. The authors of the 8 SRs/MAs did not consider the risk of bias of the included RCTs when interpreting or discussing the study results, which may reduce the reliability of the final results. The ROBIS scale was used to assess the risk of bias of the included SRs/MAs. Among the included SRs/MAs, only one SR/MA was rated as low risk, and the remaining lacked a reasonable explanation for the risk of bias, which affected the quality of SRs/MAs and reduced the utility of evidence. Similar to the results of the AMSTAR-2 assessments, the PRISMA assessment results indicated a lack of registration of the programs. In addition, only search keywords were provided but no specific search strategies were provided, which reduced the reproducibility and credibility of the research.

Based on the GRADE assessment for the 69 outcome indicators, 3 were rated as high, 15 moderate, 29 low, and 22 very low in terms of the evidence quality. The main downgrading factors were inconsistency, publication bias, and risk of bias. Further analysis revealed high inconsistency in many outcomes, possibly due to the large clinical and methodological differences in the included RCTs, such as the duration, frequency, and pattern of TC exercise that varied widely across these studies. Besides, the absence of an

Table 6: Results of the PRISMA checklist.

Section/ topic	Items	Dongling Zhong, 2020 (China) [26]	Hao Liang, 2020 (China) [27]	Jie Wang, 2013 (China) [28]	Xiandu Pan, 2021 (China) [29]	Yang Song, 2021 (China) [30]	Yuanyuan Guan, 2020 (China) [31]	Ziyu Lian, 2017 (China) [32]	Lu Cai, 2016 (China) [33]	Chengji Jin, 2018 (China) [34]	Hongguo Li, 2011 (China) [35]	Yeting Zhang, 2017 (China) [36]	Yongpeng Zhang, 2019 (China) [37]	Number of yes (%)
Title	Q1. Title	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
Abstract	Q2. Structured summary	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
Introduction	Q3. Rationale	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q4. Objectives	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
Methods	Q5. Protocol and registration	Y	Y	N	N	Y	N	N	N	N	N	N	N	25
	Q6. Eligibility criteria	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q7. Information sources	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q8. Search	Y	Y	N	N	N	N	N	N	N	N	N	N	16.67
	Q9. Study selection	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q10. Data collection process	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q11. Data items	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q12. Risk of bias in individual studies	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q13. Summary measures	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q14. Synthesis of results	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	91.67
	Q15. Risk of bias across studies	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	Y	83.33
	Q16. Additional analyses	Y	Y	N	Y	N	N	Y	N	Y	N	N	Y	50
Results	Q17. Study selection	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q18. Study characteristics	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100

Table 6: Continued.

Section/ topic	Items	Dongling Zhong, 2020 (China) [26]	Hao Liang, 2020 (China) [27]	Jie Wang, 2013 (China) [28]	Xiandu Pan, 2021 (China) [29]	Yang Song, 2021 (China) [30]	Yuanyuan Guan, 2020 (China) [31]	Ziyu Lian, 2017 (China) [32]	Lu Cai, 2016 (China) [33]	Chengji Jin, 2018 (China) [34]	Hongguo Li, 2011 (China) [35]	Yeting Zhang, 2017 (China) [36]	Yongpeng Zhang, 2019 (China) [37]	Number of yes (%)
	Q19. Risk of bias within studies	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q20. Results of individual studies	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q21. Synthesis of results	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	91.67
	Q22. Risk of bias across studies	Y	Y	N	Y	N	Y	Y	N	Y	N	N	Y	58.33
	Q23. Additional analysis	Y	Y	Y	Y	Y	Y	N	N	Y	N	N	Y	66.67
Discussion	Q24. Summary of evidence	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q25. Limitations	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
	Q26. Conclusions	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	100
Funding	Q27. Funding	Y	Y	Y	Y	Y	Y	Y	N	N	N	Y	N	66.67

Note: Y, yes; N, no.

Table 7: Results of evidence quality.

Author, year (country)	Outcomes	Studies (participants)	Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Relative effect (95% CI)	Quality
Dongling Zhong, 2020 (China)	SBP (TC vs. HE/NT)	9 (974)	−1①	−1②	0	0	−1④	MD = −14.784 (−19.587, −9.981)*	Very low
	DBP (TC vs. HE/NT)	9 (974)	−1①	0	0	0	−1④	MD = −7.035 (−9.083, −4.988)*	Low
	SBP (TC vs. OE)	5 (352)	−1①	−1②	0	−1③	−1④	MD = −7.934 (−14.221, −1.674)*	Very low
	DBP (TC vs. OE)	5 (352)	−1①	0	0	−1③	−1④	MD = −3.856 (−6.544, −1.168)*	Very low
	SBP (TC vs. AHD)	15 (1,508)	−1①	−1②	0	0	0	MD = −9.070 (−14.033, −4.108)*	Low
	DBP (TC vs. AHD)	15 (1,508)	−1①	−1②	0	0	−1④	MD = −5.625 (−8.836, −2.414)*	Very low
	TCL (TC vs. HE/NT)	3 (362)	−1①	0	0	0	−1④	MD = −0.753 (−1.161, −0.345)*	Low
	TG (TC vs. HE/NT)	3 (362)	−1①	−1②	0	−1③	−1④	MD = −0.373 (−0.795, 0.049)	Very low
	HDL (TC vs. HE/NT)	3 (362)	−1①	−1②	0	−1③	−1④	MD = 0.269 (−0.184, 0.722)	Very low
	LDL (TC vs. HE/NT)	3 (362)	−1①	−1②	0	−1③	−1④	MD = −1.048 (−1.650, −0.447)*	Very low
	TG (TC vs. AHD)	4 (365)	−1①	−1②	0	−1③	−1④	MD = −2.238 (−3.889, −0.587)*	Very low
Hao Liang, 2020 (China)	SBP	15 (1,543)	−1①	0	0	0	0	MD = −12.47 (−16.00, −8.94)*	Moderate
	DBP	15 (1,543)	−1①	−1②	0	0	0	MD = −6.46 (−8.28, −4.64)*	Low
	QOL	7 (955)	−1①	−1②	0	0	0	SMD = 0.62 (0.35, 0.90)*	Low
	TCL	5 (846)	−1①	0	0	0	0	MD = −0.49 (−0.62, −0.37)*	Moderate
	TG	5 (846)	−1①	−1②	0	0	0	MD = −0.49 (−0.92, −0.07)*	Low
	LDL	5 (846)	−1①	−1②	0	0	0	MD = −0.86 (−1.30, −0.43)*	Low
	HDL	5 (846)	−1①	−1②	0	−1③	0	MD = −0.92 (−2.21, 0.37)	Very low
	Blood glucose	4 (612)	−1①	−1②	0	0	−1⑤	MD = −0.91 (−1.59, −0.23)*	Very low
Jie Wang, 2013 (China)	Efficient (TC vs. RC)	4 (220)	−1①	−1②	0	−1③	−1④	RR = 3.39 (1.81, 6.34)*	Very low
	SBP (TC vs.RC)	10 (896)	−1①	−1②	0	0	−1④	MD = −12.43 (−12.62,−12.24)*	Very low
	SBP (TC +AHDvs. AHD)	2 (72)	−1①	−1②	0	−1③	−1④	MD = −9.34 (−10.89, −7.79)*	Very low
	DBP (TC vs.RC)	10 (896)	−1①	−1②	0	0	−1④	MD = −6.03 (−6.16 , −5.90)*	Very low
	DBP (TC +AHDvs. AHD)	2 (72)	−1①	−1②	0	−1③	−1④	MD = −7.16 (−7.71, −6.60)*	Very low
Xiandu Pan, 2021 (China)	SBP	24 (2,107)	0	−1②	0	0	0	SMD = −1.05 (−1.44, −0.67)*	Moderate
	DBP	24 (2,107)	0	−1②	0	0	0	SMD = −0.91 (−1.24, −0.58)*	Moderate
	BMI	6 (790)	0	0	0	0	−1④	SMD = −0.08 (−0.35, −0.19)*	Moderate
	Physical function	7 (853)	0	−1②	0	0	−1④	SMD = 0.86 (0.36, 1.37)*	Low
	Role physical	7 (853)	0	0	0	0	−1④	SMD = 0.86 (0.61, 1.11)*	Moderate
	General health	7 (853)	0	−1②	0	0	−1④	SMD = 0.75 (0.32, 1.17)*	Low

Table 7: Continued.

Author, year (country)	Outcomes	Studies (participants)	Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Relative effect (95% CI)	Quality
	Bodily pain	7 (853)	0	−1②	0	0	−1④	SMD = 0.65 (0.29, 1.00)*	Low
	Vitality	7 (853)	0	−1②	0	0	−1④	SMD = 0.71 (0.34, 1.07)*	Low
	Social function	7 (853)	0	−1②	0	0	−1④	SMD = 0.63 (0.07, 1.19)*	Low
	Role emotional	7 (853)	0	−1②	0	0	−1④	SMD = 0.64 (0.22, 1.06)*	Low
	Mental health	7 (853)	0	−1②	0	0	−1④	SMD = 0.73 (0.31, 1.16)*	Low
Yang Song, 2021 (China)	Physical function	8 (981)	0	0	0	0	−1④	MD = 7.54 (5.65, 9.43)*	Moderate
	Role physical	8 (981)	0	−1②	0	0	−1④	MD = 10.07 (6.64, 13.49)*	Low
	Bodily pain	7 (859)	0	−1②	0	0	−1④	MD = 9.40 (4.67, 14.13)*	Low
	General health	8 (981)	0	−1②	0	0	−1④	MD = 6.95 (2.51, 11.39)*	Low
	Vitality	7 (859)	0	0	0	0	−1④	MD = 9.40 (7.87, 10.93)*	Moderate
	Social function	7 (859)	0	−1②	0	0	−1④	MD = 9.56 (2.84, 16.28)*	Low
	Role emotional	7 (859)	0	−1②	0	0	−1④	MD = 9.09 (3.62, 14.55)*	Low
	Mental health	8 (981)	0	0	0	0	−1④	MD = 9.85 (7.08, 12.61)*	Moderate
Yuanyuan Guan, 2020 (China)	SBP	13 (1,461)	−1①	0	0	0	0	MD = −6.58 (−8.14, −5.02)*	Moderate
	DBP	13 (1,461)	−1①	0	0	0	0	SMD = −0.57 (−0.77, −0.37)*	Moderate
	TCL	4 (476)	−1①	−1②	0	0	0	SMD = −0.29, (−0.73, 0.15)	Low
	TG	3 (448)	0	0	0	0	0	SMD = −0.19, (−0.22, −0.16)*	High
	HDL	4 (612)	0	−1②	0	−1③	0	SMD = 0.59, (−0.12, 1.29)	Low
	LDL	3 (448)	0	0	0	0	0	SMD = −12.55, (−15.96, −9.14)*	High
	BMI	7 (1,039)	−1①	−1②	0	−1③	0	SMD = −0.11, (−0.75, 0.52)	Very low
	WC	4 (638)	0	0	0	0	0	SMD = −0.37, (−0.63, −0.10)*	High
Ziyu Lian, 2017 (China)	DBP(TC vs. NT)	10 (875)	−1①	0	0	0	0	SMD = −0.84, (−1.18, −0.50)*	Moderate
	BMI(TC vs. NT)	4 (451)	−1①	0	0	0	−1④	SMD = −0.39, (−0.73, −0.06)*	Low
	WC(TC vs. NT)	3 (375)	−1①	0	0	0	−1④	SMD = −0.53, (−0.74, −0.32)*	Low
	SBP(TC vs. AHD)	3 (210)	0	−1②	0	−1③	0	SMD = −0.81, (−1.40, −0.22)*	Low
	DBP(TC vs. AHD)	3 (210)	0	−1②	0	−1③	0	SMD = −0.75, (−1.60, −0.10)*	Low
Lu Cai, 2016 (China)	SBP(TC vs. HE/NT)	6 (881)	0	−1②	0	0	−1④	MD = −9.56 (−15.29, −3.82)*	Low
	DBP(TC vs. HE/NT)	6 (881)	0	−1②	0	−1③	−1④	MD = −4.79 (−9.83 , 0.26)	Very Low
	SBP(TC +AHDvs. AHD)	2 (182)	−1①	0	0	−1③	−1④	MD = −13.97 (−16.73 , −11.22)*	Very low
	DBP(TC +AHDvs. AHD)	2 (182)	−1①	0	0	−1③	−1④	MD = −10.31 (−12.15 , −8.46)*	Very low
Chengji Jin, 2018 (China)	SBP	19 (1,545)	0	−1②	0	0	0	MD = 11.14 (7.82,14.47)*	Moderate
	DBP	19 (1,545)	0	−1②	0	0	0	MD = 5.64 (3.34,7.94)*	Moderate
Hongguo Li, 2011 (China)	SBP	2 (104)	0	−1②	0	−1③	−1④	MD = 18.93 (8.16,29.71)*	Very low
	DBP	2 (104)	0	0	0	−1③	−1④	MD = 8.95 (5.61 , 12.3)*	Low
	Efficient	4 (298)	−1①	0	0	−1③	−1④	OR = 4.59 (2.55, 8.24)*	Very low
Yeting Zhang, 2017 (China)	SBP	6 (434)	0	0	0	0	−1④	MD = 14.30 (11.74, 16.86)*	Moderate

Table 7: Continued.

Author, year (country)	Outcomes	Studies (participants)	Limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Relative effect (95% CI)	Quality
Yongpeng Zhang, 2019 (China)	DBP	5 (354)	0	0	0	−1③	−1④	MD = 5.48 (4.07, 6.90)*	Low
	SBP	15 (732)	−1①	−1②	0	0	0	SMD = 1.22(1.07, 1.37)*	Low
	DBP	15 (732)	−1①	−1②	0	0	−1④	SMD = 0.63(0.49, 0.77)*	Very low

Note: ①The included studies had a large bias in methodology such as randomization, allocation concealment, and blinding. ②The confidence interval overlapped less or the I^2 value of the combined results was larger. ③The sample size from the included studies did not meet the optimal sample size or the 95% confidence interval crossed the invalid line. ④The funnel chart was asymmetry. ⑤Few studies were included, and their results were all positive, which may result in a large publication bias. *The 95% confidence interval did not cross the invalid line.

assessment of publication bias also led to the downgrading of the quality of the evidence, which affected the confidence of the results. In addition to this, the risk of bias was also an important factor that lowered the quality of evidence, implying that the quality of the RCTs included in the SRs/MAs was low. According to the assessment of the methodological quality of included RCTs, most only referred to randomization without providing a specific method of random sequence generation. Most RCTs did not explicitly state how the treatment assignment, as well as the patients and researchers were blinded.

Descriptive analysis showed that TC is an effective and safe method for the treatment of EH, especially in the control of blood pressure in patients. Due to the low quality of methodology and evidence from the included studies, the conclusions of SRs/MAs may deviate from the real results, so we cannot draw firm conclusions about TC for EH.

4.2. Implications for Practice and Research. Previous studies have shown that TC exercise can increase the central excitability of respiration, spread the excitatory focus to the parasympathetic nerves, relax small peripheral pulses, and reduce spasticity, blood flow resistance, and blood pressure [40]. Besides, during TC exercise, patients may benefit in the following two ways: firstly, the loss amount of sodium may exceed the normal intake level [41]; secondly, the plasma nitric oxide (NO) metabolite levels are higher than normal [42], and both factors can lead to improved blood pressure.

Through a comprehensive assessment of all aspects of the included MAs using AMSTAR-2, PRISMA, ROBIS, and GRADE, it was found that the methodological and evidentiary quality was not satisfactory, which implied that there was considerable scope for addressing the quality issues in the process of conducting SRs/MAs. Researchers should register or publish research protocols in advance when conducting SRs/MAs to minimize the risk of bias and ensure the accuracy of SRs/MAs results, and they should also provide a list of excluded literature as well as explanations to ensure transparency and avoid publication bias. For literature at high risk of bias, researchers should conduct separate analyses and provide reasonable explanations to ensure the quality of the evidence. In addition, a complete assessment of publication bias would also improve the accuracy of the SRs/MAs results. Although the specificity of TC treatment may make blinding difficult, a well-designed and rigorously executed RCT is believed to be the gold standard for evaluating interventions to minimize or avoid bias [43], and future RCTs should employ a more rigorous and scientific method to solve the above problems.

TC originated from traditional Chinese medicine theory, and the duration, frequency, and mode of TC movement vary greatly in different studies. Therefore, we propose to use a standardized TC training program, including fixed duration, frequency, and pattern to better study the impact of TC on EH, which can also effectively reduce the inconsistency of SRs/MAs and enhance the credibility of the evidence. In addition, currently published SRs/MAs ignore the evaluation of blood NO and endothelin-1 levels, and the evaluation of these vascular endothelial function-related indicators can also help us to better understand the underlying mechanism of TC intervention. Future studies should complement the assessment of endothelial function by adding the assessment of circulating biochemical markers. Therefore, in future RCTs of TC interventions for EH, researchers are expected to address the issue of blinding; standardize the duration, frequency, and pattern of TC exercise; and pay attention to circulating biochemical markers so as to better explore the intrinsic mechanisms by which TC exercise exerts its efficacy.

4.3. Strength and Limitations. Our overview is the first to use AMSTAR2, ROBIS, PRISMA, and GRADE to evaluate SRs/MAs regarding the effect of TC on EH. The evaluation process revealed clear limitations of the current relevant SRs/MAs and RCTs, which may help guide future high-quality clinical studies. However, this overview has certain limitations because of the subjectivity of the assessment. While our assessments were assessed and reviewed by two independent assessors, different assessors may have their own judgment on each factor, so the results may vary. Although the choice of AMSTAR-2 for quality assessment is an advantage of this study, it also comes with a shortcoming, e.g., 25% of the included SRs/MAs were published before the release of AMSTAR-2, so some authors may not follow the rules, which may partly contribute to the low quality of the assessment. Besides, only one SR/MA methodology was considered to be of high quality, and therefore the evidence for the impact of TC on EH should be approached with caution.

5. Conclusion

In conclusion, TC is beneficial and safe for EH. However, due to the generally low quality of methodology and evidence in the included MAs, clinicians should approach this finding with caution in their practice.

Data Availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Disclosure

Hongshuo Shi and Zixuan Wu are the co-first authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

LT and SGM participated in the research design. SHS, DCD, and WD conducted a literature search and screened data extraction. WZX, WD, DCD, and SHS analyzed the data, did a statistical analysis, and wrote the manuscript. DCD, LPL, and SHS participated in the correction of the manuscript. All authors reviewed the manuscript. All authors read and approved the final version of the manuscript.

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Research Article

Impact of MMP-9 Genetic Polymorphism and Concentration on the Development of Coronary Artery Disease in Ukrainian Population

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Coronary artery disease (CAD) is one of the leading causes of death in Europe. It is known that atherosclerosis is the primary risk factor of CAD development. MMP-9 is involved in all stages of atherosclerosis and thus may contribute to CAD emergence. To investigate the influence of MMP-9 on the (CAD) development 25 patients with intact coronary arteries (CA), 40 patients with acute coronary syndrome (ACS), and 63 patients with chronic coronary syndrome (CCS) were enrolled in the study. Real-time PCR was carried out for genotyping on the rs17567-polymorphic locus, and ELISA study was performed to measure the MMP-9 plasma concentration. It was found the lower risk of MI occurrence for AG-carriers ($P_a = 0.023$; $OR_a = 0.299$, 95% CI = 0.106–0.848) in Ukrainian population.

1. Introduction

Coronary artery disease (CAD) is one of the main causes of death in European countries. To illustrate the sharp contrast between Eastern and Western Europe, the authors of the third edition of statistics on cardiovascular morbidity and mortality in European countries cite the example of Ukraine, where mortality from CAD among men under age 65 is 14 times higher and among women of the same age, it is 25 times higher than in men and women in France [1].

Atherosclerosis is characterized by a complex multifactorial pathophysiology that is the major cause of CAD [2]. This process starts with the accumulation of lipids, smooth muscle cell (SMC) proliferation, cell apoptosis, necrosis, and fibrosis [3]. Matrix metalloproteinases (MMPs) form a family of zinc-dependent enzymes with proteolytic activity against connective tissue proteins such as collagen, proteoglycans, and elastin. MMPs plays a key role in all stages of atherosclerosis through vascular inflammation, endothelial dysfunction, SMC migration, proliferation, and migration of vascular smooth muscle cells (VSMCs), increase of intima-media thickness, vascular calcification, extracellular matrix degradation, and promotion of endothelial cell apoptosis. MMPs participate in oxidative modification, low density lipoprotein effect, plaque activation, and destabilization [4–6]. Hypoxia and inflammation in the lesion can induce plaque neovascularization [7]. These pathological microvessels are more prone to rupture [8]. Plaque rupture or erosion may induce thrombus formation, leading to myocardial infarction or ischemic stroke [9]. Ruptured plaques are characterized by a large lipid-rich core, a thin fibrous cap that contains few SMCs and many macrophages,

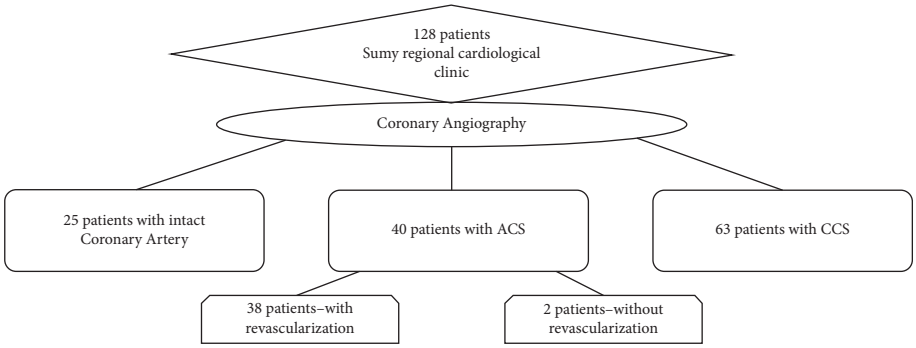

Figure 1: Scheme of patient recruiting and including to the groups.

angiogenesis, and inflammation. Macrophages, the primary cells of the atherosclerotic lesions, express MMPs as proteolytic enzymes that may influence rates of atherogenesis and the stability of atherosclerotic plaques [10, 11]. Among MMPs, MMP-9 plays a crucial role in the development of atherosclerosis.

MMP-9, also known as gelatinase B or 92 kDa type IV collagenase, is one of the important members of MMPs that may contribute to the breakdown of the extracellular matrix (ECM). MMP-9 is characterized by wide substrates, but it possesses specialized proteolytic activity against type IV collagen, a major component of the coronary artery basement membrane underlying the endothelium and surrounding each VSMC [12]. MMP-9 is secreted from macrophages in the fibrous cap and has been suggested to be involved in the remodeling processes associated with atherosclerosis and plaque rupture [13, 14]. MMP-9 not only degrades ECM but also conducts a connection between the cell surface and the matrix [12, 15]. MMP-9 expression increases after vascular injury [16, 17] and is particularly evident in inflammatory atherosclerotic lesions [18]. MMP-9 contributes to plaque vulnerability, and its high expression of MMP-9 has been associated with coronary plaque destabilization [19].

It is very important to find specific mechanisms of atherosclerosis progression, the impact of genetic and external factors which influence the development of CAD in different populations. Therefore, the aim of this study was to investigate the influence of MMP-9 concentration and functional rs17576 single nucleotide polymorphism (SNP) on the risk of CAD development in the Ukrainian population.

2. Materials and Methods

2.1. Subjects. In our study, we enrolled 128 patients after coronary angiography from February to October 2019, regardless of the clinical presentation of acute or chronic chest pain, in the "Sumy Regional Cardiological Clinic" (Sumy, Ukraine). All medical procedures were provided in accordance with the Declaration of Helsinki (1964) and each participant was required to provide a written informed consent. The study protocol was approved by the Ethics Committee of the Medical Institute of Sumy State University.

After the coronary angiography, all patients were divided into three groups (Figure 1): group 1, 25 patients with angiographically normal (intact) coronary arteries; group 2, 40 patients with acute coronary syndrome (ACS); and group3, 63 patients with chronic coronary syndrome (CCS). The diagnosis of ACS and CCS and inclusion criteria of patients are provided according to the criteria described in the relevant ESC Guidelines [20–23]. The key exclusion criteria were as follows: any systemic connective tissue diseases, chronic obstructive pulmonary disease, bronchial asthma, and oncological pathology.

As a clinical routine, we recorded clinical data, including age, gender, weight, height, smoking habit, and the presence or absence of arterial hypertension (defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg and/or if subjects were receiving antihypertensive medication). All patients were examined using electrocardiogram (ECG) and echocardiography, and body mass index (BMI) was also calculated.

Troponin test was conducted for patients with ACS immediately after admission in hospital. Overnight fasting venous blood samples were collected from each subject for total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL—cholesterol), high-density lipoprotein (HDL—cholesterol), AST, ALT, glucose, fibrinogen, erythrocytes, hemoglobin, leucocytes. The glomerular filtration rate was calculated with the QxMD Calculator (https://qxmd.com/calculate). Blood for detection of MMP-9 and rs 17567 MMp-9 polymorphism was collected under standardized conditions before coronary angiography.

38 patients with ACS received revascularization. Their medications included dual antiplatelet therapy (aspirin and P2Y12 receptor antagonist clopidogrel or ticagrelor), unfractionated heparin (only before revascularization), beta blockers (if they had no contraindications), rosuvastatin, and morphine. Nitroglycerine (iv) was added for some of those who had reccurent ischemia. ACE-inhibitors were prescribed to patients suffering from arterial hypertension or heart failure. 2 patients who did not receive revascularization have continued taking those medications in maintenance doses: aspirin and clopidogrel or ticagrelor, enoxaparin (5–7 days), beta-blockers, rosuvastatin, and ACE-inhibitors.

2.2. ELISA Study. Collected whole blood was kept in a tube without additives at room temperature for 30 min, followed by centrifugation at 3000 rpm at 4°C for 15 min. Serum was immediately transferred into a clean polypropylene tube and frozen at −80°C till further analysis, and recentrifuged after thawing before the assay. Levels of MMP-9 were assessed using a commercial ELISA immunoassay following the manufacturer's instructions (Platinum ELISA, Affymetrix eBioscience, BMS2016/2 MMP-9, Bender MedSystems GmbH). After reading each microwell's absorbance on a spectro-photometer, Thermo Scientific Multiskan FC (Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm, the average absorbance values for each set of standards and samples in duplicate were calculated. To determine the concentration of circulating human MMP-9 (ng/ml) for each sample, a standard curve using computer software (Skanlt Software 4.1 for Microplate Readers) capable of generating a five parameter logistic (5-PL) curve-fit was prepared, taking into account the final dilution factor for prediluted and undiluted samples.

2.3. Genotyping. DNA extraction was performed from whole venous blood using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania). The genotyping for *MMP-9* rs17567-single nucleotide polymorphism was done by Real-time PCR using the 7500 Fast Real-time PCR System (Applied Biosystems, Foster City, USA) and Taq-Man Assays (TaqMan®SNP Assay C_11655953_10). The PCR conditions were as follows: denaturation step at 95°C for 45 s, treatment at 95°C for 15 s, and at 60°C for 30 s (45 cycles). The data obtained were processed with the 7500 Fast Real-time PCR Software.

2.4. Statistical Analysis. All statistical calculations were carried out using the Statistical Package for the Social Sciences software (SPSS, version 22.0, Chicago, IL, USA). The distribution normality of continuous variables was checked using the Kolmogorov–Smirnov test. Continuous parameters were presented as the mean ± standard deviation (SD) or median with interquartile range. Categorical variables were presented as absolute and percentage values. Normally distributed continuous parameters were compared using a two-tailed Student's *t*-test. The Mann–Whitney test was used to compare the continuous data with other types of distributions. All categorical variables, allele and genotype frequencies were compared using a chi-squared ($\chi 2$) test. The allele distribution in accordance with the Hardy–Weinberg equilibrium was detected by the calculator of Hardy–Weinberg equilibrium (https://wpcalc.com/en/equilibrium-hardy-weinberg/) for each group. The association analysis between MMP-9 rs17567-polymorphism as well as MMP-9 serum concentration and CAD development was performed using logistic regression. Genetic studies were carried out under dominant, recessive, overdominant, and additive models of inheritance. Further adjustments for age, sex, body mass index (BMI), the presence of arterial hypertension (AH), and smoking habit were incorporated to increase the reliability of the calculations. The value $P < 0.05$ was accepted as significant.

3. Results

Clinical and laboratory characteristics of various groups in the study are shown in Table 1. There were no statistically significant differences in gender distribution, as well as the number of patients with AH and smokers in the groups under comparison. Furthermore, the study also showed that various indices, namely, BMI, the levels of cholesterol, TG, LDL—cholesterol, HDL—cholesterol, glomerular filtration rate (GFR-EPI), erythrocytes, and hemoglobin are statistically insignificant both for ACS and CCS compared with the control group. In contrast, patients with ACS showed significant differences compared to the control group with respect to the following test parameters: glucose concentration ($P = 0.001$), fibrinogen, ALT, AST, and MMP-9. Patients with CCS were significantly different from patients with intact coronary arteries with respect to age, concentration of glucose, concentration ща fibrinogen, and MMP-9.

The highest MMP-9 concentration was found in patients with ACS, the lowest in patients with intact coronary arteries, and patients with CCS had a middle range of MMP-9. Therefore, it indicated that a high concentration of MMP-9 is a risk factor for the progression of CAD and the development of its complication, ACS.

The results of association analysis between MMP-9 rs17576 and CAD development are shown in Table 2. There was a weak link with borderline significance between *MMP-9* concentration and ACS presence both before ($P_c = 0.026$; $OR_c = 1.003$, 95% CI = 1–1.006) and after the adjustment for covariates ($P_a = 0.04$; $OR_a = 1.003$, 95% CI = 1–1.006). In contrast, no significant association was detected for the CCS group ($P_a > 0.05$).

The results of the comparison of genotypes and alleles are shown in Table 3. There were no statistically significant differences in genotype and allele frequencies in the compared groups ($P > 0.05$). Genotypes are distributed in accordance with the Hardy–Weinberg equilibrium in each group ($P_{HWE} > 0.05$).

The association between *MMP-9* rs17576-polymorphic variant and the development of CAD was investigated using logistic regression (Table 4). There was no statistically significant link for both the ACS and CCS groups ($P_a < 0.05$).

The next step was to investigate the risk of myocardial infarction (MI) in patients with current CAD (47 among the 63 patients with CAD had previous MI in anamnesis). There was no statistically significant association between MMP-9 serum concentration and MI occurrence (Table 5).

A comparison of genotypes and alleles is indicated in Table 6. Statistically significant differences in genotype distribution ($P = 0.025$) were found, while the allele distribution was similar ($P > 0.05$) among the compared groups.

Table 1: Baseline characteristic of subjects.

Index	ACS, $n = 40$	CCS, $n = 63$	Intact, $n = 25$	P_1	P_2
Age, years[2]	58.35 ± 9.17	60.35 ± 7.95	55.4 ± 9.03	0.209[b]	0.013[b]
Gender, men/women	35/5	55/8	20/5	0.415	0.384
BMI, kg/m2[2]	28.69 ± 4.08	30.24 ± 4.97	31.18 ± 5.99	0.051[b]	0.452[b]
AH, n (%)	29 (72.5)	51 (81)	19 (76)	0.755	0.603
Smoker, n (%)	17 (42.5)	11 (17.5)	5 (20)	0.062	0.781
Cholesterol, mmol/L[2]	4.43 ± 1.15	4.44 ± 1.18	4.76 ± 1.67	0.365[b]	0.324[b]
Triglyceride, mmol/L	1.44 ± 0.75[1]	1 (0.84–1.29)[2]	1.24 ± 0.58[1]	0.295[b]	0.369[a]
HDL, mmol/L[2]	1.02 (0.89–1.14)[2]	1.09 ± 0.31[1]	1.07 (0.91–1.44)[2]	0.279[a]	0.439[b]
LDL, mmol/L	2.61 (2.2–3.38)	2.73 (2.07–3.44)	2.8 (1.99–3.36)	0.805[a]	0.628[a]
Glucose, mmol/L[1]	5.35 (4.6–6.9)	4.7 (4.2–5.78)	3.89 (3.53–4.58)	0.001[a]	0.01[a]
Fibrinogen, g/L[1]	2.2 (2–2.8)	3.7 (2.4–27.1)	3 (2.55–3.2)	0.003[a]	0.038[a]
GFR (EPI), ml/min[2]	70.89 ± 21.87	74.41 ± 18.83	79.91 ± 27.8	0.242[b]	0.395[b]
ALT, U/L[1]	99.05 (52.98–212.88)	26.35 (19.18–36.08)	25.35 (19.3–33.28)	<0.001[a]	0.811[a]
AST, U/L[1]	45.2 (26.78–66.58)	28 (22.6–35.9)	22.25 (19.88–27.73)	0.003[a]	0.168[a]
MMP-9,[1]	449.4 (151.15–624.5)	354.35 (149.98–575.58)	67.97 (34.88–303.6)	0.002[a]	0.004[a]
Erythrocytes, $\times 10^{12}$/L,[2]	4.52 ± 0.54	4.41 ± 0.51	4.46 ± 0.7	0.749[b]	0.733[b]
Hemoglobin, g/L[2]	148.34 ± 16.79	145.4 ± 14.84	147.11 ± 16.85	0.799[b]	0.679[b]

ACS: acute coronary syndrome; CCS: chronic coronary syndrome; intact: patients with intact coronary arteries; n: number of cases; BMI: body mass index; AH: arterial hypertension; HDL: high-density lipoprotein; LDL: low-density lipoprotein; GFR (EPI): glomerular filtration rate (according to the chronic kidney disease epidemiology collaboration); ALT: alanine aminotransferase; AST: aspartate aminotransferase. [1]Data are given in the form of median and interquartile range. [2]Data are given as mean and standard deviation. [a]Comparison was performed using the Mann–Whitney criterion. [b]Comparison was performed using Student's t-criterion. P_1: P value for ACS and intact group comparison; P_2: P value for CCS and intact group comparison.

Table 2: Analysis of the association between MMP-9 serum concentration and the development of CAD.

Predictor	P_c	OR_c (95% CI)	P_a	OR_a (95% CI)
MMP-9[a]	0.026	1.003 (1–1.006)	0.04	1.003 (1–1.006)
	0.058	1.003 (1–1.005)	0.06	1.003 (1–1.006)

CAD: coronary artery disease; P_c: crude P value; P_a: P value adjusted for age, sex, body mass index, the presence of hypertension, and smoking habits. [a]Upper row represents the results for group with acute coronary syndrome and lower—for group with chronic coronary syndrome.

Table 3: Distribution of genotypes and alleles in comparison groups.

	ACS		CCS		Intact CA		P_1 (χ^2)	P_2 (χ^2)
	n	%	N	%	N	%		
				Genotypes				
AA	18	51.4	21	38.9	8	36.4		
AG	12	34.3	27	50	11	50	0.472 (1.503)	0.946 (0.11)
GG	5	14.3	6	11.1	3	13.6		
				Alleles				
A	48	68.6	69	63.9	27	61.4	0.43 (0.624)	0.77 (0.086)
G	22	31.4	39	36.1	17	38.6		
				Hardy–Weinberg equilibrium				
P_{HWE} (χ^2)	0.226 (1.464)		0.539 (0.378)		0.798 (0.065)		—	—

ACS: acute coronary syndrome; CCS: chronic coronary syndrome; intact: patients with intact coronary arteries; n: number of cases.

It was found that AG-carriers had the lower risk of MI development in crude ($P_c = 0.033$; $OR_c = 0.359$, 95% CI = 0.14–0.922) and adjusted ($P_a = 0.023$; $OR_a = 0.299$, 95% CI = 0.106–0.848) overdominant model of inheritance (Table 7).

4. Discussion

The matrix-degrading activity of MMPs contributes to complications of atherosclerotic lesions. Vulnerable atherosclerotic plaques are responsible for life-threatening clinical endpoints: myocardial infarction and ischemic stroke [9, 24]. Plaque rupture is the most common cause of coronary thrombosis [25]. Thus, MMP-9 not only degrades ECM but may also contribute to plaque vulnerability [26].

Our study demonstrated that the concentration of MMP-9 is the highest in patients with ACS (449.4 ng/ml (95% CI = 151.15–624.5)) compared with patients with CCS (354.35 ng/ml (95% CI = 149.98–575.58)) and patients with intact coronary arteries (67.97 ng/ml (95% CI = 34.88–303.6)). Many other studies have shown that MMP-9 can serve as a biomarker of vulnerable plaques [18, 27–30]. It was revealed that patients with plaque rupture and ACS (myocardial infarction versus unstable angina pectoris) had significantly higher levels of MMP-9 than patients who did not have plaque rupture. Kobayashi et al. demonstrated that MMP-9 levels are elevated earlier than high-sensitive troponin T and have a higher diagnostic value for early stage of ACS [27].

Hamed and Abdel Fattah reported that patients with ACS who had adverse cardiovascular events had a higher level of MMP-9 [16]. MMP-9 levels can also be used to predict ischemic stroke and cardiovascular death in patients with 50% and more carotid stenosis [31]. Blankenberg et al. demonstrated a strong association between baseline MMP-9 levels and future risk of cardiovascular death [32]. We found a weak borderline association between MMP-9 serum concentration and CAD development that needs to be investigated in further studies with extended groups of comparison.

Table 4: Association analysis between *MMP-9* rs17576-single nucleotide polymorphism and CAD development.

Model[a]		P_c	OR_c (95% CI)	P_a	OR_a (95% CI)
Dominant		0.269	0.54 (0.181–1.609)	0.307	0.533 (0.159–1.781)
		0.837	0.898 (0.322–2.507)	0.611	0.751 (0.249–2.265)
Recessive		0.945	1.056 (0.226–4.936)	0.52	0.533 (0.078–3.628)
		0.758	0.792 (0.179–3.492)	0.787	0.793 (0.147–4.27)
Overdominant		0.242	0.522 (0.176–1.55)	0.498	0.647 (0.184–2.275)
		—	—	—	—
Additive	AG vs. AA	0.224	0.485 (0.151–1.558)	0.337	0.53 (0.145–1.938)
		0.903	0.935 (0.319–2.738)	0.67	0.781 (0.251–2.431)
	GG vs. AA	0.722	0.741 (0.141–3.88)	0.338	0.317 (0.03–3.319)
		0.74	0.762 (0.153–3.802)	0.949	0.936 (0.125–6.997)

CAD: coronary artery disease; P_c: crude P value; P_a: P value adjusted for age, sex, body mass index, the presence of hypertension, and smoking habits. [a]Upper row represents the results for the group with acute coronary syndrome, and the lower one for group with chronic coronary syndrome.

Table 5: Association analysis between MMP-9 serum concentration and MI development among patients with CAD.

Predictor	P_c	OR_c (95% CI)	P_a	OR_a (95% CI)
MMP-9	0.169	1.001 (0.999–1.004)	0.237	1.001 (0.999–1.004)

MI: myocardial infarction; CAD: coronary artery disease; P_c: crude P value; P_a: P value adjusted for age, sex, body mass index, the presence of hypertension, and smoking habits.

Table 6: Distribution of genotypes and alleles in comparison groups.

	With MI		Without MI		P (χ^2)
	N	%	N	%	
			Genotypes		
AA	29	46	10	38.5	
AG	23	36.5	16	61.5	0.025 (7.412)
GG	11	17.5	0	0	
			Alleles		
A	81	64.3	36	69.2	0.527 (0.4)
G	45	35.7	16	30.8	

MI: myocardial infarction; n: number of cases.

Table 7: Association analysis between *MMP-9* rs17576-single nucleotide polymorphism and MI development among patients with CAD.

Model	P_c	OR_c (95% CI)	P_a	OR_a (95% CI)
Dominant	0.513	0.733 (0.288–1.862)	0.523	0.725 (0.271–1.942)
Overdominant	0.033	0.359 (0.14–0.922)	0.023	0.299 (0.106–0.848)
Additive	0.152	0.496 (0.19–1.296)	0.114	0.425 (0.147–1.23)

MI: myocardial infarction; CAD: coronary artery disease; P_c: crude P value; P_a: P value adjusted for age, sex, body mass index, the presence of hypertension, and smoking habits.

In our studies there was no statistically significant association between MMP-9 serum concentration and MI occurrence among comparison groups of patients who had MI in the past or at present and without MI ($P_a > 0.05$). Thus, the concentration of MMP-9 is not a prognostic factor for the risk of acute MI in a patient with current CAD among the Ukrainian population. The role of MMP-9 concentration in the risk of development of MI in patients who have current CAD has not been confirmed. The same results were obtained by Eldrup et al. [31]. However, Blankenberg et al. showed that high levels of MMP-9 in patients with stable and unstable angina were directly correlated with a high risk of cardiovascular death [32].

The human *MMP-9* gene, which is located on chromosome 20q12.2–13.1, contains 13 exons and 12 introns. Common polymorphic variants in the promoter and exon sequences have been reported to be associated with CAD in recent years. Zhang et al. showed that there was a functional single-nucleotide polymorphism (SNP) rs3918242: this nucleotide variation from C to T gave rise to a two-fold increase in the promoter activity and the T allele carriers tended to have a higher risk of severe CAD [33]. Another common SNP—rs17576 (or R279Q) located at exon 6, is an A to G substitution that results in change of positively charged arginine (R) to uncharged glutamine (Q) in the catalytic domain of the MMP-9 enzyme. Such alteration may have an effect on the activity of the enzyme, although few functional data are available [33–35]. It was assumed that the SNP may presumably be involved in the binding of the enzyme to its substrate elastin. Further bioinformatics studies identified the spectrum of microRNAs (miRNAs) that bind to SNPs within Chromosome 20 in an allele-dependent manner. Researchers have indicated 41 SNP-specific miRNAs that target MMP-9 polymorphic loci most of which (95%) are concentrated in the coding exon. It was shown that the stringent pairing between G-allele (rs 17576 SNP) containing MMP-9 transcript and hsa-miR-3934-5p (δscore = 167 and δMFE = −20.99 kcal/mol) means that miRNA preferentially binds to mRNA with SNP-allele. It can be assumed that further hsa-miR-3934-5p-dependent deadenylation and decapping of MMP-9 transcript with G-allele will decrease the stability of mRNA and inhibit the protein translation. Thus, the potential mechanism of the posttranscriptional regulation of MMP-9 expression is carried out by miRNAs in an SNP-specific manner [36].

There were 7 case-control studies with 5525 cases of CAD and 2497 controls (Asian and European) related to the MMP-9 (R279Q) SNP concerning the risk of CAD [37]. The pooled results indicated a negative, but not significant,

association between *MMP-9* (R279Q) gene polymorphism and CAD risk under all genotype models for the overall population and subgroup analysis. According to these data, heterozygotes (47%) predominated among the representatives of the European population with ACS, while homozygotes by AA genotype amounted to 42.5% in this group [14]. Homozygotes by AA genotype accounted for the majority (46.9–48.1%) among Asians with ACS, and heterozygotes were 39–43.9% [38]. The data are contradictory for the European population of patients with stable CAD: carriers of the AG genotype predominated (47.4%) among Norwegians, but in the Italian population the AA genotype is the most frequent (44.3%) [39, 40]. There are conflicting data on the distribution of genotypes by rs17567 *MMP-9* polymorphism among individuals with stable forms of CAD in the Asian population and among control groups of both populations [14, 35, 38–42]. In our study, we found that the minor allele frequency (MAF) in the ACS group was 31.4%, while in the CCS group it was 36.1%. That did not significantly differ from patients with intact coronary arteries (38.6%; $P > 0.05$).

The rs17567 MMP-9 (R279Q) polymorphism was evaluated in association with carotid artery stenosis [43, 44] and hypertension [34, 45]. The authors reported a strong association between the MMP9 279Q allele and the presence of atherosclerotic plaques in men. Although Blankenberg et al., revealed the association of the R279Q polymorphism with cardiovascular death and nonfatal MI ($P = 0.02$) in the subgroup of patients with stable angina [32]. They showed that patients carrying the 279Q allele have a higher risk than patients homozygous for the 279R allele.

In current research, we did not find any significant link between rs17567 *MMP-9* and ACS or CCS development ($P_a > 0.05$).

Several studies have shown that R279Q SNP significantly increased the risk of MI in the premature CAD group [46, 47] as well as C-1562T polymorphism may increase susceptibility to myocardial ischemia [48]. When we compared the distribution of genotypes in groups of patients with and without MI, the statistically significant differences were found in genotypes ($P = 0.025$) but not alleles ($P > 0.05$) distribution. Logistic regression analysis showed that AG-carriers had a lower risk of MI development in crude ($P_c = 0.033$; $OR_c = 0.359$; 95% CI = 0.14–0.922) and adjusted ($P_a = 0.023$; $OR_a = 0.299$; 95% CI = 0.106–0.848) overdominant models of inheritance. These results could be explained by the existence of ethnical features for different populations.

It should be noted that current research has several limitations. Further studies with extended groups are necessary to confirm the results of this study. Moreover, it would be interesting to measure the expression rates of *MMP-9* for each polymorphic variant.

5. Conclusions

In the present research, we have analyzed the link of MMP-9 serum concentration and *MMP-9* rs17567-polymorphic variant with CAD development among Ukrainians. We found a lower risk of MI occurrence for AG-carriers. However, further studies are required to confirm this observation.

Data Availability

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

Disclosure

The materials of this manuscript were reported at the European Cardiology Congress-2021 and published as an abstract in the European Heart Journal (https://academic.oup.com/eurheartj/article/42/Supplement_1/ehab724.1338/6393113).

Conflicts of Interest

The authors declare no conflicts of interest.

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Research Article

Characteristics of Patent Foramen Ovale: Analysis from a Single Center

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Objective. To collect and analyze data of patent foramen ovale (PFO). *Methods*. This study included a total of 260 patients diagnosed with PFO. We analyzed basic clinical data such as sex, age, transesophageal echocardiography as well as other symptoms. *Results*. Our data showed that females accounted for the highest proportion of PFO (166 females, 64%), with the highest number of patients (65 patients) having between 45 and 55 years. Transesophageal echocardiography examination demonstrated frequent occurrence of tunnel-like anatomical structures. In addition, PFO was associated with symptoms such as migraine, stroke or TIA, syncope, chest tightness, and palpitations, with dizziness being the most common symptom in the patients with PFO. *Conclusion*. Our data demonstrated that females accounted for the highest proportion of PFO patients, with those aged between 45 and 55 years being most affected. The most frequently encountered clinical symptom was dizziness. Taken together, these findings may help doctors to better understand and screen for PFO patients.

1. Introduction

Foramen ovale is the passage that connects the left and right atria in the fetus. Before birth, the fetus does not breathe through the lungs. Oxygen-carrying maternal blood enters the left atrium from the right atrium through the foramen ovale, which is then supplied to all parts of the body. After birth, there is oxygen filling of the alveoli which expands the pulmonary arterioles, leading to suppression of pulmonary circulation resistance and right atrial pressure. At the same time, increase in return blood volume after the pulmonary circulation leads to an increase in the left atrial pressure, which together promote the closure of the foramen ovale [1–3]. Under normal circumstances, the foramen ovale is closed within the first year of life. Lack of closure of the foramen ovale at the age of 3 years leads to patent foramen ovale (PFO). About 20–25% of adults have incomplete foramen ovale closure [4–8].

Previous data showed that PFO had little effect on cardiac hemodynamics and could be left untreated. However, recent studies have demonstrated that the presence of PFO is closely associated with occurrence of paradoxical embolism, stroke, migraine, and other diseases. Besides, presence of atrial septal herniation with PFO is a risk factor for cerebral infarction [2–4, 8–13]. Therefore, many studies have analyzed the role of PFO on stroke and migraine [10, 14–19]. However, PFO can also lead to other symptoms which include dizziness, syncope, chest tightness, and palpitations [20–28]. Therefore, it is important to characterize and understand the clinical characteristics of PFO. In this study, we collected and analyzed clinical data of PFO patients in our center. Findings of this study might help doctors to better understand and screen for PFO patients.

2. Materials and Methods

A total of 260 patients diagnosed with PFO patients were included in this study. The patients underwent transesophageal echocardiography, the mainstay diagnostic tool for PFO [29]. We collected and analyzed the basic clinic information including sex, age, transesophageal echocardiography, and associated symptoms to characterize the PFO. This study was approved by the Administrative Committee of Experimental Animal Care and Use of our hospital.

2.1. Gender Composition Analysis. We analyzed the number and proportion of males and females in the 260 enrolled PFO patients.

2.2. Age Distribution. The patients were divided into different groups according to their age, at an interval of 10 years (< 15 years old, 15–25 years old, 25–35 years old, 35–45 years old, 45–55 years old, 55–65 years old, 65–75 years old). Age distribution was used to demonstrate the predisposing or common population in PFO.

2.3. Anatomical Classification Based on Transesophageal Echocardiography. Patients undergoing transesophageal echocardiography with sedation are required to abstain from food and beverages (other than clear liquids) for a minimum of 6 hours before the planned procedure and restrain from all intake for 3 hours before the procedure [29, 30]. Patients with delayed gastric emptying and other aspiration risks may need a longer period of fasting or preprocedural administration of agents, such as metoclopramide, to minimize the risk for residual gastric contents and aspiration [29]. The patients first used dyclonine for local throat anesthesia and then inserted it with an ultrasound probe for examination. Based on the transesophageal echocardiography results, anatomical classification was divided into long-tunnel, crevice-like, and other types. Furthermore, they were divided into those with or without aneurysmal septum. The number and proportion of the different types were analyzed.

2.4. Symptom Analysis. The 260 PFO patients were interviewed and recorded, and 5 kinds of symptoms: dizziness, migraine, syncope, stroke or TIA, chest tightness, and palpitations were identified. The number and proportion of these five types of symptoms were analyzed.

3. Results

3.1. Gender Composition. Out of the 260 PFO patients, 166 were females, which accounted for 64% while 94 (36%) were males (Figure 1).

3.2. Age Distribution. The analysis showed that there were 5 PFO patients for age < 15 years old, 29 for 15–25 years old, 43 for 25–35 years old, 46 for 35–45 years old, 65 for 45–55 years old, 60 for 55–65 years old, and 14 for 65–75 years old (Figure 2).

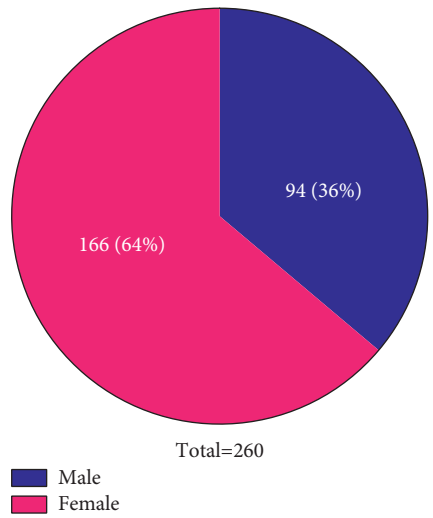

Figure 1: Gender composition. In the pie chart, blue represents men and red represents women. White font represents the number and percentage of each part.

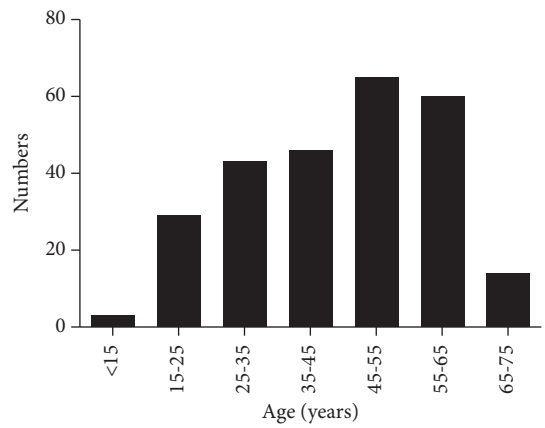

Figure 2: Age distribution. The histogram represents the number and distribution of patients in different age groups.

3.3. Anatomical Classification Based on Transesophageal Echocardiography. Out of the total 260 PFO patients, 195 (75%) were long-tunnel type, while 31 were crevice-like type, and 34 included other types, accounting for 36%. 8 PFO patients were accompanied with aneurysmal septum, while 252 were normal without aneurysmal septum (Figure 3).

3.4. Symptoms Recorded and Analysis

3.4.1. Dizziness. Out of the 260 PFO patients, 163 (63%) were accompanied with dizziness, while 97 (37%) did not experience dizziness (Figure 4(a)).

3.4.2. Migraine. Out of the 260 PFO patients, 89 (34%) had migraine, while 171 (66%) were without migraine (Figure 4(b)).

3.4.3. Stroke or TIA. In the 260 PFO patients, 66 (25%) were accompanied with stroke or TIA, while 194 (75%) did not experience dizziness (Figure 4(c)).

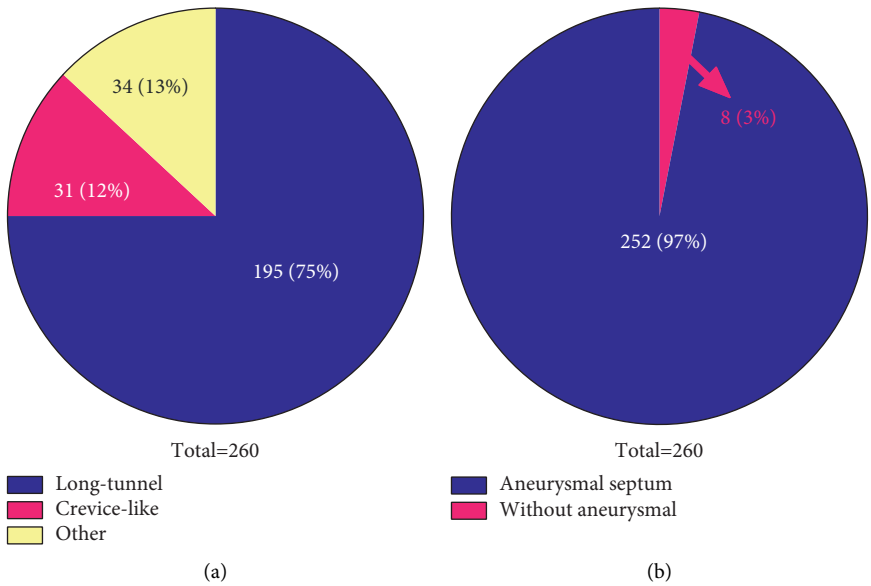

Figure 3: Anatomical classification based on transesophageal echocardiography. (a) Blue represents long-tunnel type, red represents crevice-like type, and yellow represents other type. (b) Red represents accompanied with aneurysmal septum and blue represents without aneurysmal septum.

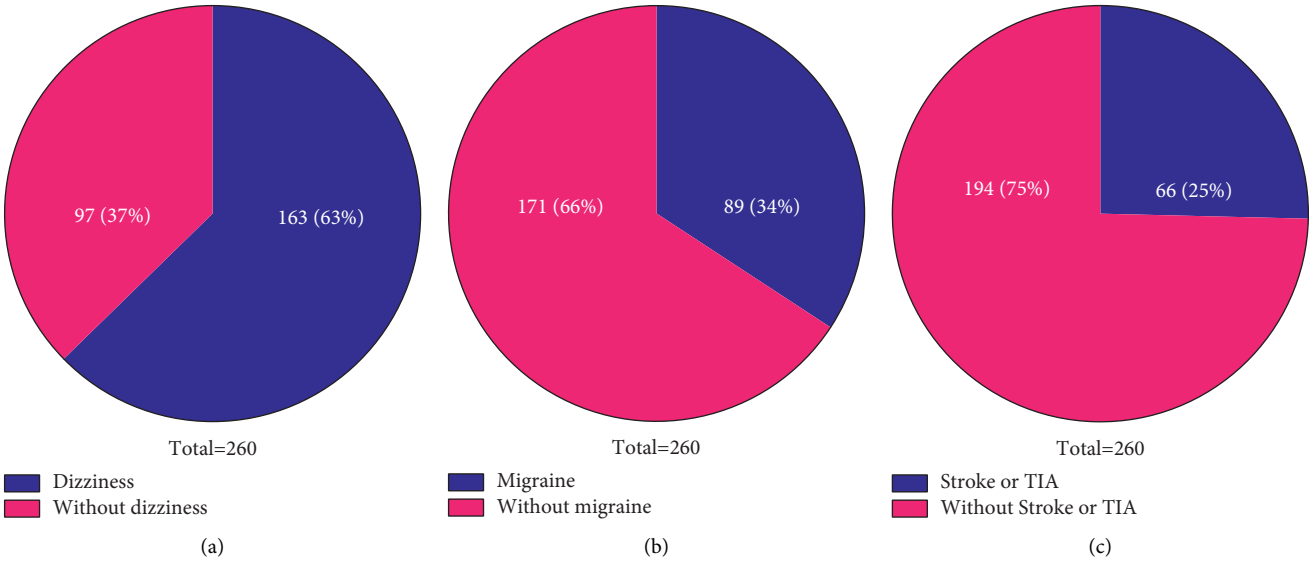

Figure 4: Continued.

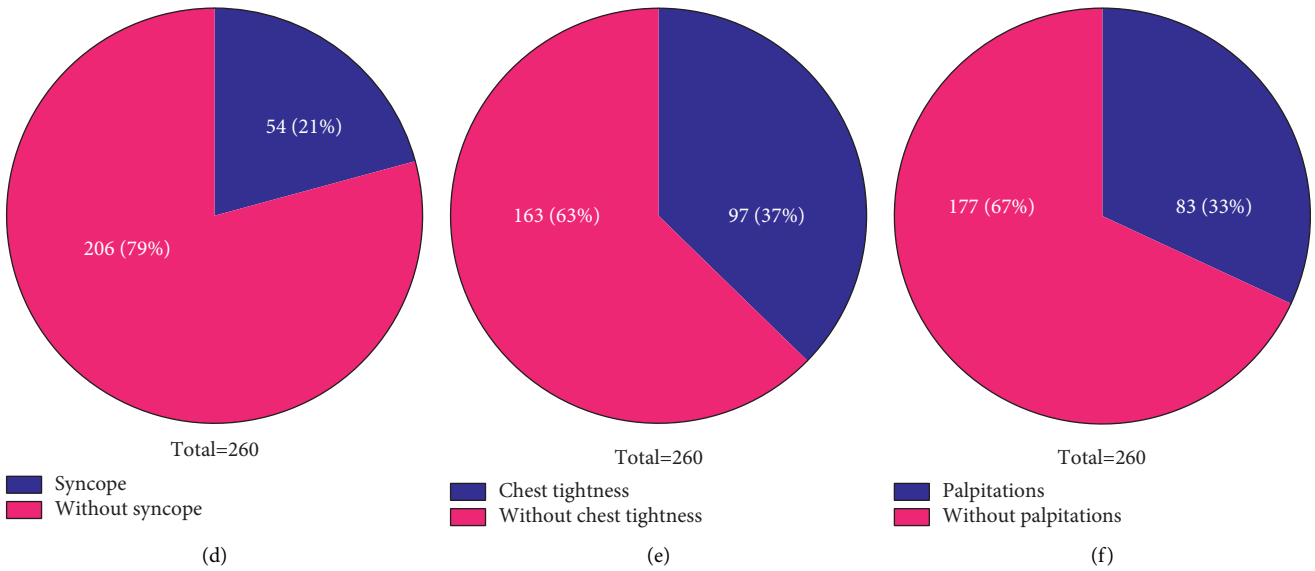

Figure 4: Symptoms recorded and analysis. (a) Blue represents patients accompanied with dizziness while red represents patients without dizziness. (b) Blue represents patients accompanied with migraine while red represents patients without migraine. (c) Blue represents patients accompanied with stroke or TIA while red represents patients without stroke or TIA. (d) Blue represents patients accompanied with syncope while red represents patients without syncope. (e) Blue represents patients accompanied with chest tightness while red represents patients without chest tightness. (f) Blue represents patients accompanied with palpitations while red represents patients without palpitations.

3.4.4. Syncope. Out of the 260 PFO patients, 54 (21%) were accompanied with syncope, while 206 (79%) were without syncope (Figure 4(d)).

3.4.5. Chest Tightness. Out of the 260 PFO patients, 97 (37%) were accompanied with chest tightness, while 163 (63%) did not have chest tightness (Figure 4(e)).

3.4.6. Palpitations. Out of the 260 PFO patients, 83 (33%) were accompanied with palpitations, while the remaining 67% did not have palpitations (Figure 4(f)).

3.5. Composition of the Symptoms. As shown in Figure 5(a), out of the 260 PFO patients, 5 did not have any symptom. 67 patients were accompanied with only one kind of symptom, 110 had 2 kinds of symptoms, 52 were accompanied with 3 kinds of symptoms, and 21 had 4 kinds of symptoms while the remaining 5 were accompanied with 5 kinds of symptoms. Out of the 67 patients with only one symptom, the most common symptoms were dizziness (21 patients), migraine (13 patients), syncope (7 patients), stroke or TIA (14 patients), chest tightness (7 patients), and palpitations (5 patients). Figure 5(c) shows in all patients, 163 patients had the dizziness, 89 had migraine, 54 had syncope, 66 had stroke or TIA, 97 had chest tightness, and 83 had palpitations.

4. Discussion

This study showed that there were more females with PFO in the clinic. Besides, patients aged between 45 and 55 years old had the highest burden of PFO. Analysis by transesophageal echocardiography demonstrated that the most common type of anatomy was the tunnel type, with few having aneurysmal septum. Out of all the symptoms, dizziness was the most common symptom in the patients with PFO.

Previous data indicated that PFO had little effect on cardiac hemodynamics and thus could be left untreated. However, recent studies have shown that PFO is of clinical significance as it is a source of thrombus formation or could be a conduit for paradoxical embolism [31]. Previous reports showed an association between ischemic stroke and PFO because of paradoxical venous thromboembolism [32–35]. In addition, recent analyses have focused on stroke, TIA, or migraine [9, 10, 14–19, 31, 32, 36–41]. The data showed that besides cryptogenic stroke, paradoxical embolism, and migraine, PFO can result in dizziness, syncope, chest tightness, and palpitations. This study showed that the most common symptom was dizziness, stroke, or TIA, as well as migraine. Therefore, there is need to not only pay attention to serious complications such as stroke or TIA but also related symptoms such as dizziness, syncope, chest tightness, and palpitations. These symptoms can seriously affect the quality of life of the PFO patients. On the other hand, it reminds doctors that when they observe the above-mentioned symptoms in the clinic, without other positive from related tests or systems, they should screen for PFO, especially in females between the ages of 15 and 65 years. Since early diagnosis and treatment can improve the patients' symptoms and quality of life and prevent the occurrence of stroke, in sync, this study analyzed the gender, age, and symptoms of the PFO patients to guide the screening and treatment of the PFO patients.

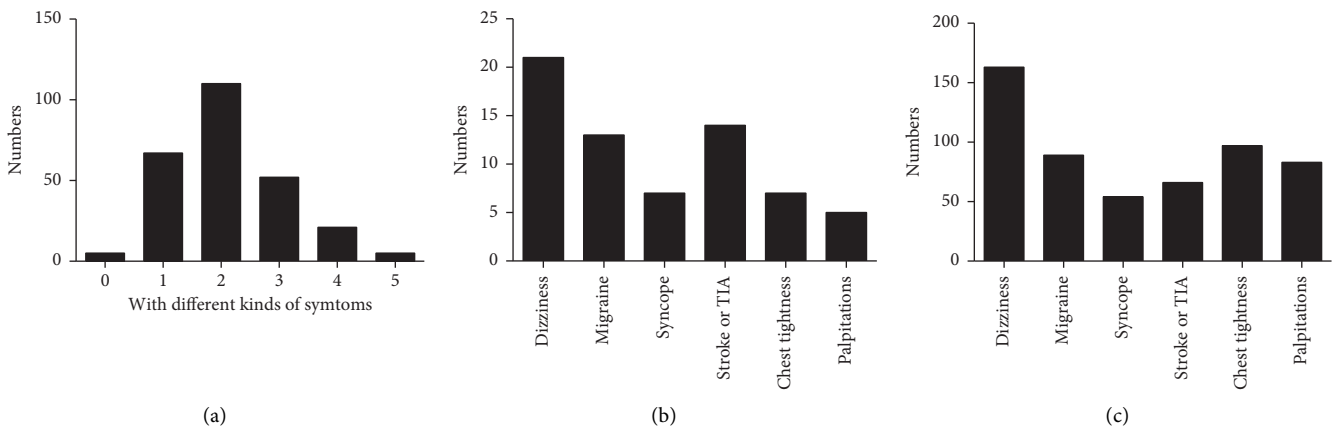

Figure 5: Composition of different kinds of symptoms. (a) The number of patients with different kinds of symptoms. (b) The composition of the 67 patients with only one symptom. (c) The number of patients with different symptoms.

Although this study highlights important findings, it analyzed data from a single center, and thus there is a need for a larger sample size. Besides, we did not evaluate related effects of interventional closure therapy, especially improvement of symptoms other than stroke or TIA.

5. Conclusion

Our data showed that females accounted for the highest proportion of PFO patients, and most patients were in the 45–55 age group. PFO was associated with symptoms such as dizziness, migraine, stroke or TIA, syncope, chest tightness, and palpitations. The most frequently encountered clinical symptom was dizziness. Together, characterizing the PFO patients in terms of gender, age, and associated symptoms may help doctors to better understand and screen for PFO.

Data Availability

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

Ethical Approval

The study was approved by the Ethics Committee of Jiangmen central hospital.

Consent

The patients provided their written informed consent to participate in this study.

Conflicts of Interest

The authors declare that they do not have any conflicts of interest in connection with the work submitted.

Authors' Contributions

Bin Zhang and Dong Li did the collection, analysis of data, and manuscript preparation; Anjian Song, Qiang Ren, Shangan Cai, Peng Wang, and Wenfeng Tan collected the data and helped analyzing the data; Gaoxing Zhang and Jun Guo did the study design, data analysis, interpretation, and manuscript preparation. Bin Zhang and Dong Li are equal contributors.

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Research Article

TMAO-Activated Hepatocyte-Derived Exosomes Are Widely Distributed in Mice with Different Patterns and Promote Vascular Inflammation

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Background. Trimethylamine-N-oxide (TMAO) has been shown to be an important player in cardiovascular disease (CVD) by promoting vascular inflammation and endothelial dysfunction. We recently found that exosomes (Exos) released from TMAO-activated hepatocytes (TMAO-Exos) could significantly induce inflammation and endothelial dysfunction. However, understandings of how are the Exos secreted by hepatocytes, where are they distributed *in vivo*, and what effects will they have on vascular inflammation remain limited. The present study aimed to explore the hub genes involved in the production of TMAO-Exos and their distributions *in vivo* and effects on inflammation. *Methods*. The transcriptome profiles of primary rat hepatocytes stimulated with TMAO were obtained from the GSE135856 dataset in the Gene Expression Omnibus repository, and the hub genes associated with Exos were screened and verified by qPCR. Next, Exos derived from TMAO-treated hepatocytes were isolated using differential centrifugation and given intravenously to mice. After 24 h, the distributions of DiI-labelled Exos were visualized with a fluorescence microscope, and the levels of proinflammatory genes in the aorta were detected by qPCR. *Results*. *Phgdh*, *Mdh*2, *Echs*1, *Rap*2*a*, *Gpd*1*l*, and *Slc3a*2 were identified as hub genes that may be involved in the production of TMAO-Exos. And TMAO-Exos were found to be efficiently taken up by cardiomyocytes, hepatocytes, and endothelial cells in the aorta and gastrocnemius muscle. Furthermore, TMAO-Exos, but not control-Exos, could significantly promote the mRNA expressions of *Tnf*, *Icam*1, *Sele*, and *Cox*-2 in the aorta. *Conclusions*. We provided clues about how TMAO may stimulate hepatocytes to produce Exos and further offered evidence that Exos secreted by TMAO-treated hepatocytes could be widely distributed *in vivo* and promote vascular inflammation.

1. Introduction

There is increasing evidence that gut microbiota and its metabolites play a key role in the pathogenesis and development of cardiovascular disease (CVD) [1]. Thereinto, trimethylamine-N-oxide (TMAO) has been found to be an independent risk factor for adverse cardiovascular events [2–6], which may be related to excessive vascular inflammation and endothelial dysfunction provoked [7–10]. It has been shown that dietary precursors such as choline, betaine, and L-carnitine can be metabolized into trimethylamine in the gut flora and further catalyzed into trimethylamine-N-oxide (TMAO) in the liver [2, 5, 11]. However, the mechanisms underlying TMAO are still not completely understood.

Exosomes (Exos) have gained a growing concern for the pivotal roles in cardiovascular physiology [12]. Exos are nanosized membrane vesicles produced by nearly all types of cells, ranging from approximately 30 to 100 nm in diameter and containing a variety of bioactive molecules [12–14]. Recent research has found that Exos derived from hepatocytes play an important role in inflammation, endothelial

function, and metabolic disorders [15–19]. In the latest work, we found that Exos secreted by TMAO-treated hepatocytes (TMAO-Exos) contained a distinctive profile of miRNAs compared to those from the TMAO-free group (control-Exos), and furthermore, TMAO-Exos could notably promote inflammation, damage vascular endothelial cells (VECs), and impair endothelium-dependent vasodilation [20]. However, understandings of how these Exos are produced by hepatocytes, where are they distributed *in vivo*, and what effects will they have on vascular inflammation remain limited.

In the present study, we first obtained the transcriptome profiles of primary rat hepatocytes stimulated with or without TMAO [21] and then identified the hub genes related to Exos. Next, Exos were isolated from hepatocytes treated with or without TMAO and given intravenously to mice, and then their distributions in the heart, liver, aorta, and gastrocnemius muscle and effects on vascular inflammation were examined.

2. Materials and Methods

2.1. Hepatic Gene Expressions Induced by TMAO. The transcriptome profiles of primary rat hepatocytes stimulated with or without TMAO (50 μmol/L) for 30 h were obtained from the GSE135856 dataset in the Gene Expression Omnibus repository [21, 22]. The microarray experiments were performed using a single Affymetrix Clariom S Rat array (Affymetrix, Santa Clara, CA, USA) with three biological repetitions. The open-source Bioconductor packages, affy and limma, were used to process the data [21].

2.2. Enrichment Analysis. The differentially expressed genes ($P < 0.05$) related to Exos were screened and visualized on a heatmap constructed in R. DAVID (Database for Annotation, Visualization, and Integrated Discovery) was used for investigating the functional annotation of genes. GO (gene ontology) analysis was performed to elaborate the biological process, STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database (v11.0) [23] was used for analyzing the protein-protein interaction (PPI), and networks were constructed on Cytoscape platform (v3.8.2) [24]. CytoHubba plugin was used to identify hub genes with a threshold value >0.4, and the color of the nodes represented the degree of gene interaction.

2.3. Cell Culture and Exos Isolation. The procedures were performed as described in our recent study [20]. In brief, AML12 cells (iCell Bioscience Inc., Shanghai) were cultured in DMEM/F12 (iCell Bioscience Inc., Shanghai) containing Exos-depleted serum (ViVaCell, Shanghai) and treated with TMAO (Tokyo Chemical Industry Co., Ltd.) at a physiological concentration of 50 μmol/L for 48 h (TMAO-Exos). The untreated group served as the control (control-Exos). Exos were isolated and purified from the culture supernatant using differential centrifugation and then resuspended in PBS.

2.4. Exos Identification and Labelling. The protein levels of the Exos were measured using the BCA protein assay kit (Thermo Fisher Scientific, MA, USA). The ultrastructure of the Exos was inspected using a transmission electron microscope (JEM 1200-EX, Japan). In brief, Exos suspensions were loaded on 200-mesh formvar-coated grids and then negatively stained with phosphotungstic acid. The samples were observed under a transmission electron microscope at a voltage of 100 kV. The concentration and size distribution of the Exos were detected by nanoparticle tracking analysis (Nanosight NS300, Malvern, UK). Exosomal markers of CD9 and TSG101 and the negative marker of calnexin were detected by western blotting. In brief, the samples were separated by SDS-PAGE and transferred onto Millipore polyvinylidene difluoride membranes. The membranes were incubated overnight at 4°C with the primary antibodies of CD9 (Zenbio, Chengdu, China), TSG101 (Zenbio, Chengdu, China), and calnexin (Affinity Biosciences, Jiangsu, China) and visualized with enhanced chemiluminescence reagent (Millipore, MA, USA). Exos were labelled with DiI (Beyotime Biotechnology, Shanghai, China) for *in vivo* tracer experiments.

2.5. Animal Experiments. All experiments conform to the protocols approved by the Institutional Animal Care and Use Committee of Guangdong Provincial People's Hospital. Male wild-type C57BL/6 mice (six weeks old) were purchased from the Experimental Animal Center of Sun Yat-sen University. The mice were intravenously injected with 30 μg of Exos resuspended in 100 μL of PBS. After 24 h, the mice were anesthetized by pentobarbital sodium (50 mg/kg), and then the hearts, thoracic aortas, livers, and gastrocnemius muscles were collected for subsequent study.

2.6. Fluorescence Detection. The hearts, thoracic aortas, livers, and gastrocnemius muscles were fixed in 4% paraformaldehyde and then dehydrated and embedded in paraffin. Samples underwent dewaxing and antigen retrieval. The slides were blocked in 10% goat serum for 30 min at room temperature and then incubated with the primary antibody of CD31 (Abcam, MA, USA) overnight. Slides were incubated with Alexa Fluor® 488 donkey anti-rabbit IgG (H + L). Wheat germ agglutinin (WGA, Sigma-Aldrich, USA) staining was used to outline the cardiomyocytes. Slides were then washed and stained with DAPI (Solarbio, Beijing, China). The positive signals were detected with a fluorescence microscope (Olympus, Tokyo, Japan).

2.7. Quantitative Polymerase Chain Reaction. Quantitative polymerase chain reaction (qPCR) was performed as described in our previous study [25]. In brief, total RNA was extracted using TRIzol reagent (Invitrogen, USA), and concentration was measured using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA). Then, RNA was reversely transcribed into cDNA using Color Reverse Transcription Kit (EZBioscience, CA, USA),

Table 1: qPCR primers for mRNA (*Mus musculus*) used in the study.

Name	Forward sequence	Reverse sequence
Phgdh	CCAGGTGGTTACACAAGGAACA	TTCACGTCTGCCTGCTTAGATG
Mdh2	TTTGTGGCAGAGCTAAAGGGTT	GTACACTGAGAGATCAGGGGGA
Echs1	AATGGAGATGGTCCTCACTGGT	TCTGCACATTGGATGGCTTCTT
Rap2a	CAGCAGAGCTTCCAAGACATCA	CTCTCCAGGTCCACTTTGTTCC
Gpd1l	TCCGACATCATCCGAGAGAAGA	AAGGCCGTTCTGCATCACTTT
Slc3a2	GGCCCAATTCACAAGAACCAGA	TGGGAGTGAGGTCCAAAATGATG
Hyou1	GATCTTCGGGTATTTGGCTCCC	TAAAGTGGGCCTTGATGCCTTT
Hspa9	AACTCCTGTGTGGCTGTTATGG	CAAGTCGTTCTCCATCTGCTGT
Psat1	GGGTGGAGTTTGACTTCGTACC	TCTTCTGAGCACCAGCGAAAAT
Pck2	GAGGCTGAGAACACTGCCATAC	TGCGAAGGAGTTACAATCACCG
Tnf	CTGTAGCCCACGTCGTAGC	TTGAGATCCATGCCGTTG
Icam1	CCCACGCTACCTCTGCTC	GATGGATACCTGAGCATCACC
Sele	ATGCCTCGCGCTTTCTCTC	GTAGTCCCGCTGACAGTATGC
Cox-2	TTCAACACACTCTATCACTGGC	AGAAGCGTTTGCGGTACTCAT
Gapdh	ACTCTTCCACCTTCGATGCC	TGGGATAGGGCCTCTCTTGC

Phgdh: 3-phosphoglycerate dehydrogenase; Mdh2: malate dehydrogenase 2, NAD (mitochondrial); Echs1: enoyl coenzyme A hydratase, short chain, 1, mitochondrial; Rap2a: Ras-related protein 2a; Gpd1l: glycerol-3-phosphate dehydrogenase 1-like; Slc3a2: solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2; Hyou1: hypoxia upregulated 1; Hspa9: heat shock protein 9; Psat1: phosphoserine aminotransferase 1; Pck2: phosphoenolpyruvate carboxykinase 2 (mitochondrial); Tnf: tumor necrosis factor; Icam1: intercellular adhesion molecule-1; Sele: selectin, endothelial cell; Cox-2: cyclooxygenase-2; Gapdh glyceraldehyde-3-phosphate dehydrogenase.

and qPCR was performed on Bio-Rad CFX-96 (Bio-Rad, CA, USA) with Color SYBR Green qPCR Master Mix (EZBioscience, CA, USA). The expressions were normalized to Gapdh. The qPCR primers used in the study are listed in Table 1.

2.8. Statistical Analysis. Statistical analysis was conducted using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA), and the graphs were plotted by GraphPad Prism (version 9.3.0, San Diego, USA). Data were presented as mean ± standard error of the mean (SEM). For continuous variables with normal distribution, the comparisons between two groups were performed with independent *t*-test. For continuous variables with nonnormal distribution, the comparisons between two groups were performed with Wilcoxon rank-sum test. A P value <0.05 was considered statistically significant.

3. Results

3.1. Exos-Related Genes in Hepatocytes Were Dysregulated by TMAO. Microarray assay was performed to determine the expression profiles of genes in primary rat hepatocytes stimulated with TMAO. Compared to the untreated group, a total of 101 genes related to Exos changed significantly ($P < 0.05$), and among these genes, 43 were upregulated and 58 were downregulated (Figure 1(a)). GO analysis was used to explore the predominant biological processes, and the results showed that the differentially expressed genes (DEGs) were significantly enriched in secretion, peptide metabolic process, export from the cell, membrane fusion, and small-molecule biosynthetic process (Figure 1(b)). Besides, the PPI networks were constructed to further elaborate the interactions among the DEGs and identified *Phgdh*, *Mdh*2, *Echs*1, *Rap*2*a*, *Gpd*1*l*, *Slc*3*a*2, *Hyou*1, *Hspa*9, *Psat*1, and *Pck*2 as the top 10 hub genes (Figure 1(c)).

3.2. Verification of the Hub Genes. To verify the changes of the hub genes, AML12 cells were stimulated with or without TMAO for 48 h, and the mRNA levels of the top 10 hub genes were detected by qPCR. It was shown that TMAO remarkably enhanced the mRNA expressions of *Phgdh*, *Mdh*2, *Echs*1, *Rap*2*a*, and *Gpd*1*l* (Figures 2(a)–2(e)) and reduced *Slc*3*a*2 levels (Figure 2(f)). However, *Hyou*1, *Hspa*9, *Psat*1, and *Pck*2 remained unchanged (Figures 2(g)–2(j)).

3.3. Isolation and Identification of Exos. Nanovesicles with a cup-shaped morphology and a typical size around 100 nm were isolated and purified from the cell culture supernatant (Figure 3(a)). The size distribution profiles and concentrations of the Exos showed no significant differences between the two groups (Figure 3(b)). Exosomal markers of CD9 and TSG101 were mainly enriched in control-Exos and TMAO-Exos, and the negative marker of calnexin was detected only in the whole cell lysate (Figure 3(c)).

3.4. Exos Were Widely Distributed In Vivo with Different Patterns. TMAO-Exos were labelled with DiI and administered intravenously. The results clearly showed that Exos could be efficiently taken up by cardiomyocytes (Figure 4(a)), hepatocytes (Figure 4(b)), and endothelial cells in the aorta (Figure 4(c)). It was worth noting that these Exos were predominantly localized in the nuclei of hepatocytes. And unlike in cardiomyocytes, these Exos appeared to keep themselves out of the skeletal muscle cells and preferentially located in the endothelial cells, as indicated by the same subcellular localizations of Exos and CD31 proteins in the gastrocnemius muscle (Figure 4(d)).

3.5. TMAO-Exos Promoted Vascular Inflammation. The *in vivo* tracer experiments clearly showed that TMAO-Exos could target endothelial cells in the aorta and gastrocnemius

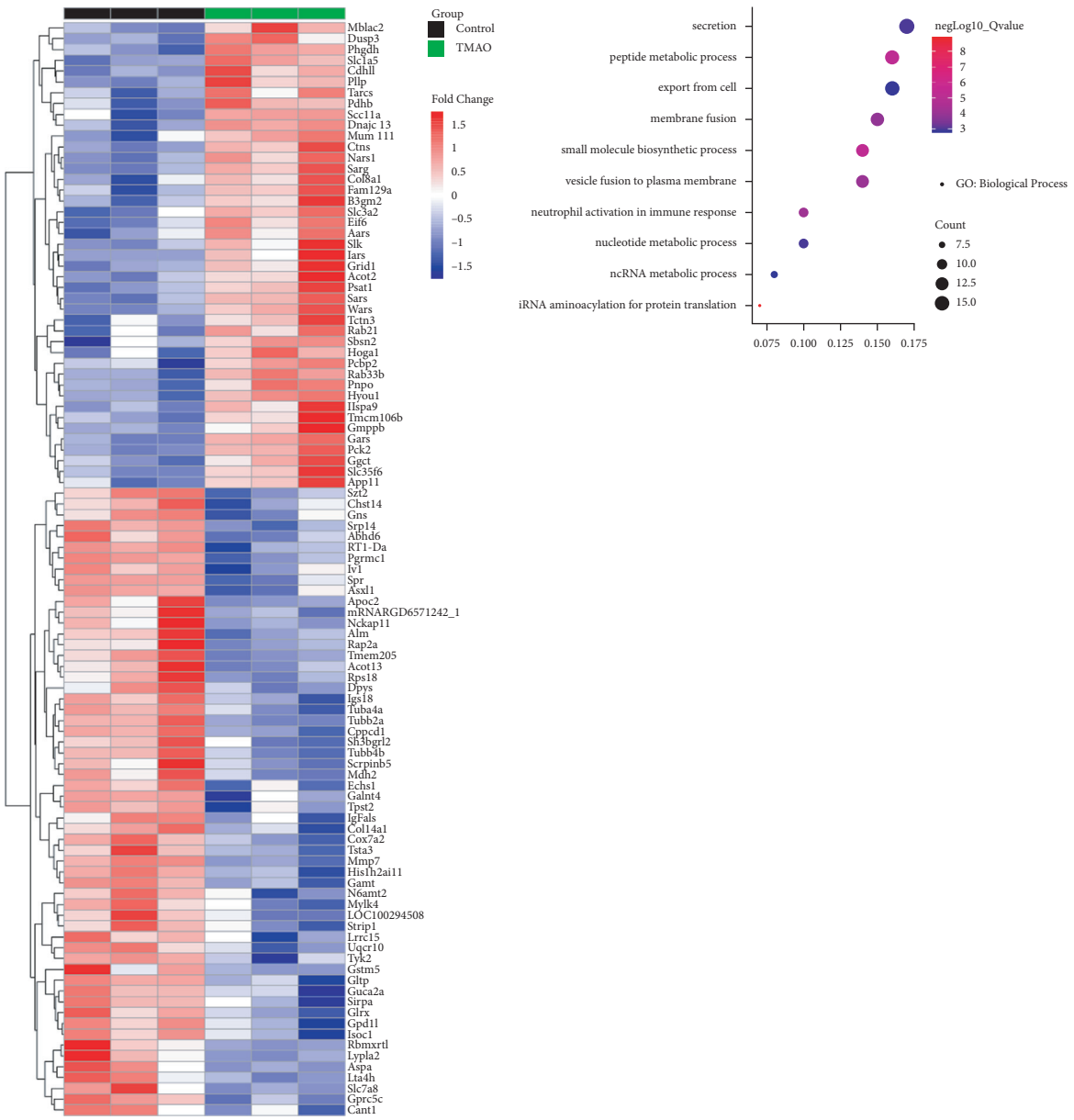
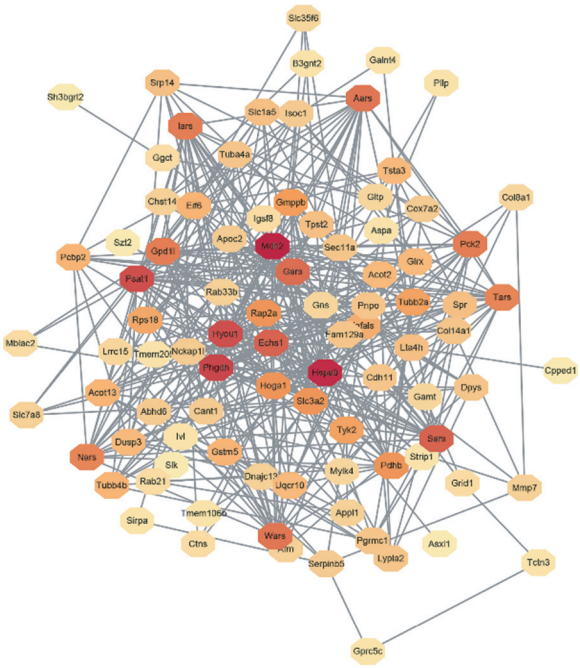


Figure 1: Continued.



(c)

Figure 1: Exos-related genes in hepatocytes were dysregulated by TMAO. (a) A total of 101 genes related to Exos changed significantly ($P < 0.05$) after TMAO treatment. (b) The differentially expressed genes were significantly enriched in the biological processes of secretion, peptide metabolic process, export from the cell, membrane fusion, and small-molecule biosynthetic process. (c) *Phgdh*, *Mdh*2, *Echs*1, *Rap2a*, *Gpd*1*l*, *Slc3a*2, *Hyou*1, *Hspa*9, *Psat*1, and *Pck*2 were identified as the top 10 hub genes.

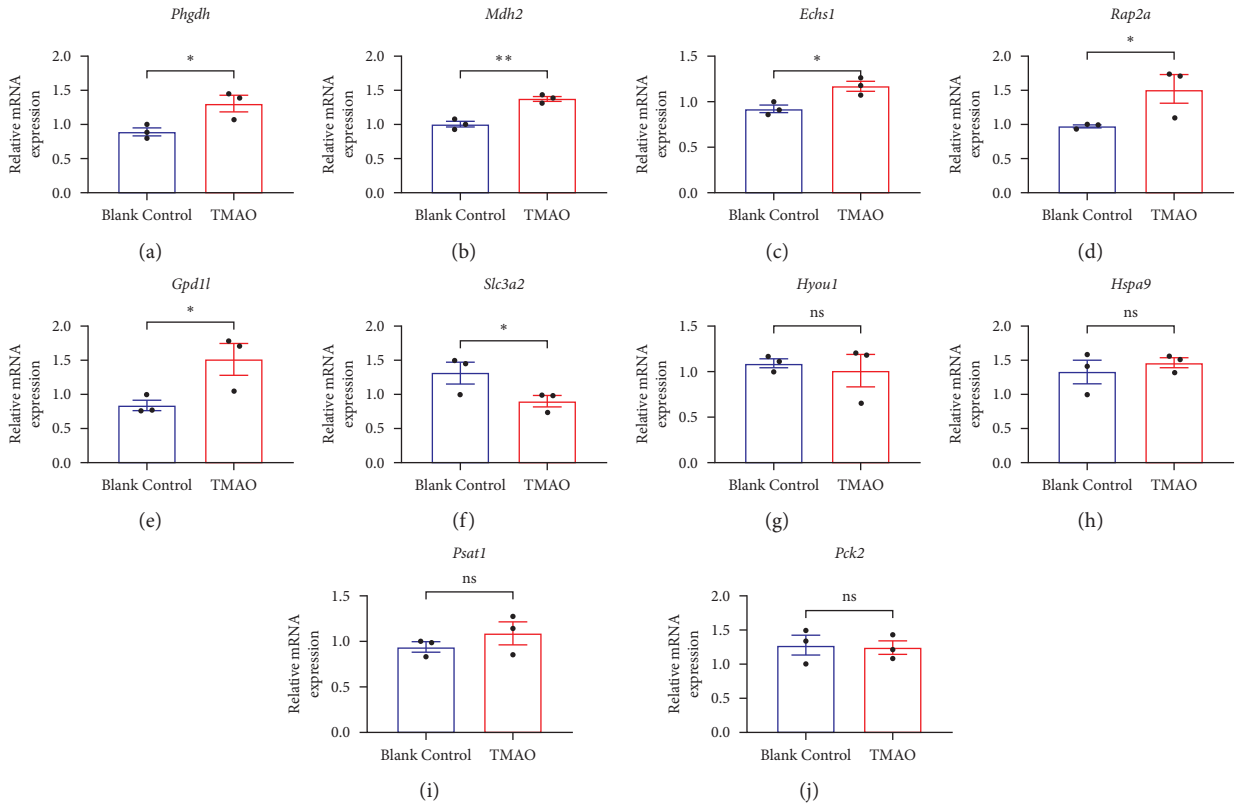


Figure 2: Verification of the top 10 hub genes. (a–e) TMAO remarkably enhanced the mRNA expressions of *Phgdh*, *Mdh*2, *Echs*1, *Rap2a*, and *Gpd*1*l* and (f) reduced *Slc*3*a*2 levels. (g–j) *Hyou*1, *Hspa*9, *Psat*1, and *Pck*2 remained unchanged. qPCR was used to determine the mRNA expressions ($n = 3$). $^{*}P < 0.05$ vs. the blank control group. ns indicates not significant.

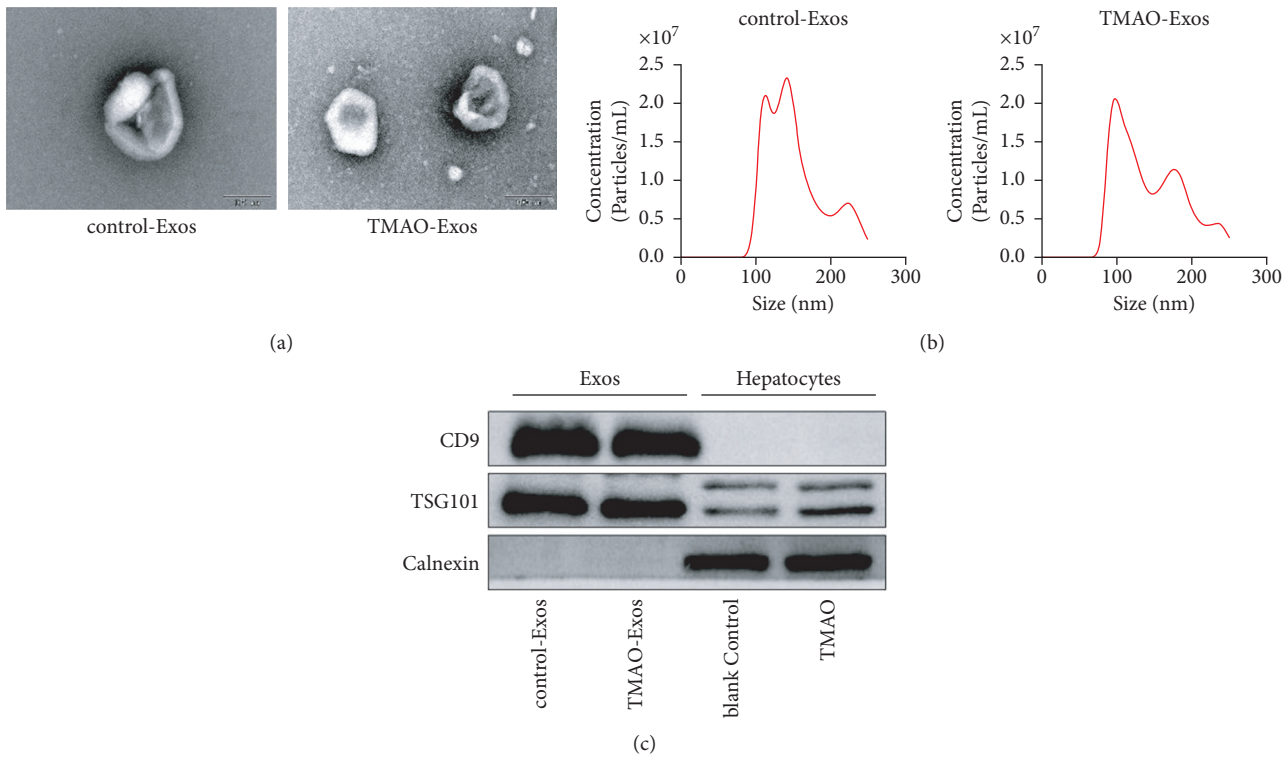


Figure 3: Isolation and identification of Exos. (a) Nanovesicles with a cup-shaped morphology and a typical size around 100 nm were isolated and purified from the cell culture supernatant. Bar: 100 nm. (b) The size distribution of the Exos showed no significant difference between control-Exos and TMAO-Exos. (c) Exosomal markers of CD9 and TSG101 were enriched in Exos groups, and the negative marker of calnexin was detected only in the whole cell lysate.

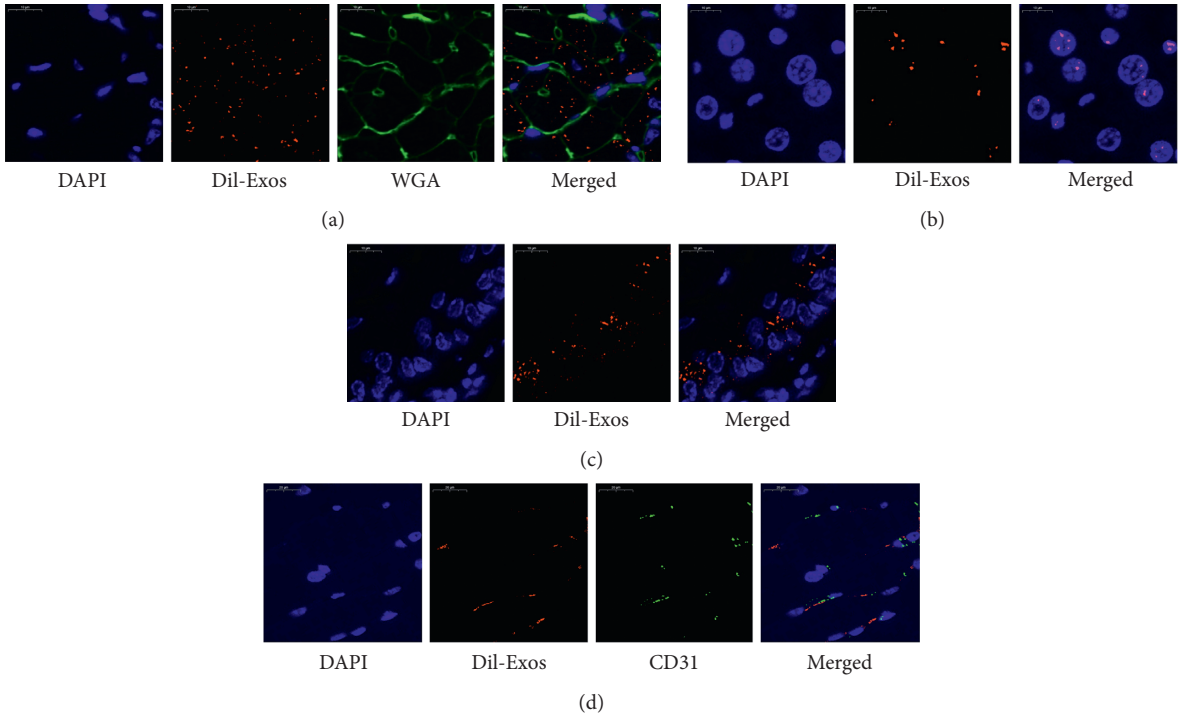


Figure 4: Exos were widely distributed *in vivo* with different patterns. Exos secreted by TMAO-treated hepatocytes could be efficiently captured by (a) cardiomyocytes, (b) hepatocytes, and (c) endothelial cells in the aorta. (d) The Exos appeared to keep themselves out of the skeletal muscle cells and preferentially located in the endothelial cells.

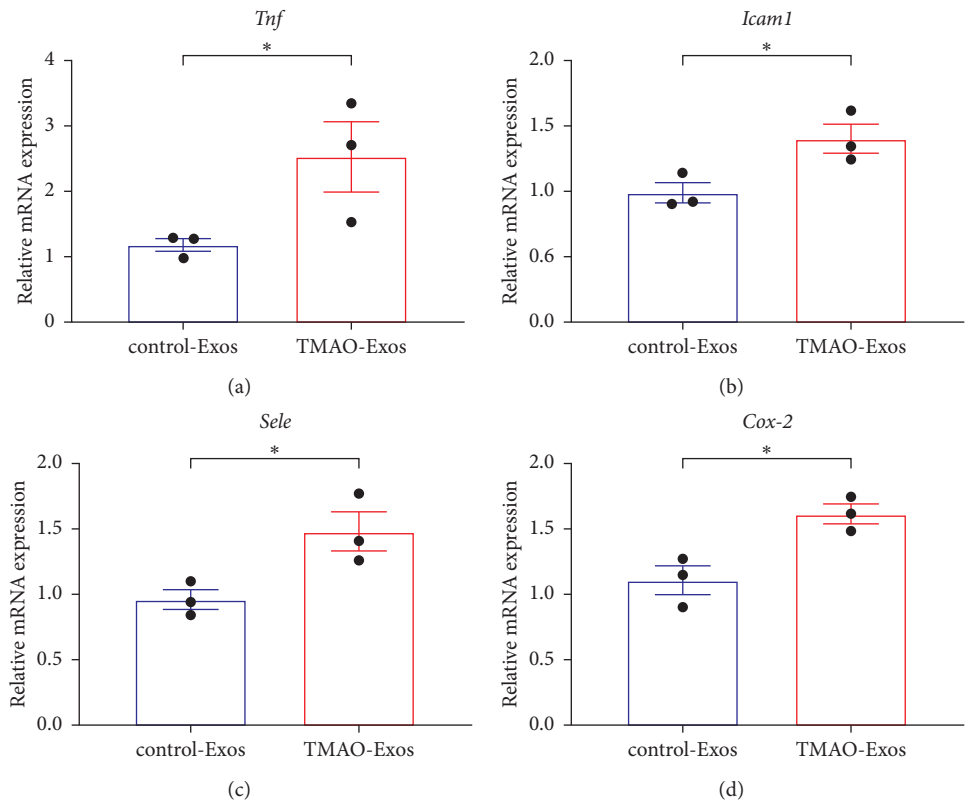


Figure 5: TMAO-Exos promoted the mRNA expressions of inflammatory genes in aortas. TMAO-Exos notably enhanced the mRNA levels of (a) *Tnf*, (b) *Icam*1, (c) *Sele*, and (d) *Cox*-2. qPCR was used to determine the mRNA expressions ($n = 3$). $^*P < 0.05$ vs. the control-Exos group.

muscle, so we assessed the effects of TMAO-Exos on the mRNA expressions of inflammatory genes in aortas. Compared to control-Exos, TMAO-Exos significantly promoted the mRNA expressions of *Tnf*, *Icam*1, *Sele*, and *Cox*-2 (Figures 5(a)–5(d)).

4. Discussion

In the current work, we first identified the hub genes in hepatocytes after TMAO treatment, which may be related to the production of TMAO-Exos. Next, we provided evidence that TMAO-Exos could be taken up by several tissues including the heart, liver, aorta, and gastrocnemius muscle with different distribution patterns. And finally, we found that TMAO-Exos, but not control-Exos, could significantly promote the mRNA expressions of *Tnf*, *Icam*1, *Sele*, and *Cox*-2 in the aorta.

There is a close relationship between TMAO and the liver. TMAO, at a physiological concentration of 50 μmol/L, has been shown to be able to target hepatocytes and result in the abnormal expressions of hepatic genes, thus exerting an influence on metabolic disorder [21]. Moreover, we recently found that TMAO (50 μmol/L) could stimulate hepatocytes to release functional Exos [20]. However, the molecular pathways and hub genes behind it remain unknown. It was found that the fusion of multivesicular bodies (MVBs) with the plasma membrane is critical for the extracellular secretion of Exos, and intracellular molecules and pathways such as Rab27a/b and V-ATPase-mediated acidification of MVBs are implicated [26]. An interference study showed that HGS, Alix, TSG101, and nSmase2 were necessary for the Exos production by normal or ethanol-treated hepatocytes [27]. And other studies revealed that Tsg101 and Vps4a played a pivotal role in exosomal secretion [28, 29]. Based on the aforementioned study [21], a Exos-related gene set was selected to identify the predominant biological processes and hub genes which responded to TMAO treatment. The results showed that the DEGs were mainly enriched in secretion, peptide metabolic process, export from the cell, membrane fusion, and small-molecule biosynthetic process, which suggested that these DEGs may be related to the synthesis and secretion of TMAO-Exos. Furthermore, we verified that *Phgdh*, *Mdh*2, *Echs*1, *Rap*2*a*, and *Gpd*1*l* were upregulated, and *Slc*3*a*2 was downregulated by TMAO. *Phgdh* and *Mdh*2 are classified into the biological process of "small-molecule biosynthetic process." The protein encoded by *Phgdh* is essential for serine synthesis and contributes to glucose and lipid homeostasis [30–32]. And gene *Mdh*2 encodes a mitochondrial enzyme that plays a pivotal role in the malate-aspartate shuttle and glucose homeostasis [33, 34]. In addition, *Slc*3*a*2 encodes a membrane protein and is involved in amino acid transport and endoplasmic reticulum stress [35–37]. However, the results simply provided some early clues about how TMAO may stimulate hepatocytes to

produce Exos, and the exact mechanisms remain to be further elucidated by using the gain- and loss-of-function strategies.

Similarly, the distribution patterns of Exos remain poorly defined. It was suggested that the processes may depend on the distinctive factors or receptors located on the recipient cell surfaces [38]. However, Horibe et al. found that Exos could be nonselectively incorporated into recipient cells via a different mechanism, which may depend on the recipient cells rather than the donor cells [39]. In the present work, we showed that TMAO-Exos could be distributed in tissues including the heart, liver, aorta, and gastrocnemius muscle after systemic administration, suggesting that these Exos spread widely *in vivo* through the bloodstream. However, it seemed that the distribution of these Exos varied depending on the recipient cell type, as indicated by the observations that they could be incorporated by cardiomyocytes, but appeared to keep themselves out of the skeletal muscle cells and preferentially located in the endothelial cells. In addition, it was interesting to see that these Exos were able to be captured by hepatocytes and localized in the nuclei. These findings were consistent with previous research, in which Exos were able to be captured by the donor cells themselves [27, 39]. Furthermore, we found that TMAO-Exos could significantly promote the mRNA expressions of *Tnf*, *Icam*1, *Sele*, and *Cox*-2 in the aortas. These proinflammatory genes have been shown to play pivotal roles in the regulation of inflammation, endothelial dysfunction, and atherosclerosis [40–43].

5. Conclusions

In the current study, we provided clues about how TMAO may stimulate hepatocytes to produce Exos and further offered evidence that Exos secreted by TMAO-treated hepatocytes could be widely distributed *in vivo* and promote vascular inflammation. These findings may help to advance our understanding of the mechanisms by which TMAO promotes CVD.

Data Availability

The data relevant to this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Jimei Chen contributed to the concept, designed the study, directed the research, and revised the manuscript. Xiang Liu, Jiazichao Tu, Ziqin Zhou, Bingxin Huang, and Jianrong Zhou performed the experiments and analyzed the data. Xiang Liu drafted the manuscript. All authors read and approved the final manuscript.

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