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Serum Exosomal MicroRNA-186-5p Positively Correlates with Lipid Indexes, Coronary Stenosis Degree, and Major Adverse Cardiovascular Events in Coronary Heart Disease

Lingyun Ren,[1,*] Wei Liu,[1,*] Shanshan Chen[2] and Haibo Zeng[1]

[1]Department of Anesthesiology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China
[2]Key Laboratory for Molecular Diagnosis of Hubei Province, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

Our previous study finds that exosomal microRNA (miR)-186-5p promotes viability and invasion of vascular smooth muscle cells to accelerate atherosclerosis via inactivating phosphoinositide 3 kinase/protein kinase B/mammalian target of rapamycin pathway. Subsequently, this study aimed to identify the linkage of serum exosomal miR-186-5p with clinical features and major adverse cardiovascular events (MACE) in coronary heart disease (CHD) patients. Serum exosomal miR-186-5p was quantified in 175 CHD patients and 50 healthy controls (HCs) via reverse transcription quantitative polymerase chain reaction. Our study revealed that serum exosomal miR-186-5p was enhanced in CHD patients vs. HCs ($P < 0.001$). In CHD patients, serum exosomal miR-186-5p was positively correlated with total cholesterol ($P = 0.002$) and low-density lipoprotein cholesterol ($P = 0.003$). Elevated serum exosomal miR-186-5p was linked with increased Gensini score ($P = 0.028$) and stenosis degree categorized by the Gensini score ($P = 0.018$). Regarding MACE, the 1-year and 2-year accumulating MACE rate was 6.6% and 15.6%, respectively. Serum exosomal miR-186-5p was elevated in CHD patients with MACE vs. those without ($P = 0.042$). By Kaplan-Meier curves and log-rank analyses, serum exosomal miR-186-5p > 1.000 ($P = 0.404$) and > 1.610 ($P = 0.328$) was not related to accumulating MACE. While serum exosomal miR-186-5p > 3.390 exhibited a correlative trend with increased accumulating MACE, but not achieving statistical significance ($P = 0.071$). The 1-year and 2-year accumulating MACE rate of patients with serum exosomal miR-186-5p > 3.390 was 11.5% and 21.5%, respectively; while the rate was 3.3% and 11.5% in patients with serum exosomal miR-186-5p ≤ 3.390, accordingly. Conclusively, serum exosomal miR-186-5p positively associates with lipid level, coronary stenosis degree, and the risk of MACE in CHD patients.

Keyword: clinical features; coronary heart disease; coronary stenosis degree; exosomal microRNA-186-5p; major adverse cardiovascular events
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Introduction

Coronary heart disease (CHD), with an increasing incidence in many countries, is caused by artery stenosis or occlusion due to coronary atherosclerosis (Ralapanawa and Sivakanesan 2021). Factors such as elevated low-density lipoprotein cholesterol (LDL-C), inflammation, and blood pressure are recognized as causal contributors to CHD (Fuchs and Whelton 2020; Ebadi et al. 2022; Shaya et al. 2022). The therapeutic goal for CHD is to enhance coronary flow capacity and prevent infarction, and the basic treatments encompass conservative measures, percutaneous

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*These two authors contributed equally to this work.
Correspondence: Haibo Zeng, Department of Anesthesiology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, No. 26, Shengli Street, Wuhan, Hubei 430013, China.
e-mail: zenghaibowuhan@126.com
Shanshan Chen, Key Laboratory for Molecular Diagnosis of Hubei Province, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430013, China.
e-mail: chenshanshan_wuhan@126.com

coronary intervention, and coronary artery bypass grafting (Doenst et al. 2022; Sulava and Johnson 2022). However, even with appropriate treatment, some CHD patients still face the risk of experiencing major adverse cardiovascular events (MACE), of which incidence ranges from 6.0% to 63.8% worldwide, especially those with aggravated coronary obstructions (Bauersachs et al. 2019; Akyea et al. 2022; Zhang et al. 2022). Thus, finding some biomarkers associated with the occurrence of MACE is helpful for CHD management.

MicroRNAs (miRNAs), with approximately 18-24 nucleotides, are a series of short endogenous noncoding single-stranded RNA molecules (Islas and Moreno-Cuevas 2018). They regulate several biological processes in the development and progression of cardiovascular diseases, such as cardiomyocyte contraction, lipid metabolism, and inflammatory response (Islas and Moreno-Cuevas 2018; Kalayinia et al. 2021). MiR-186-5p is reported to participate in atherosclerosis (Sun et al. 2020; Zhang et al. 2020). For instance, one study reveals that serum miR-186-5p regulates the proliferation and migration of proatherogenic vascular endothelial cells (Zhang et al. 2020). Another study elucidates that serum miR-186-5p modulates vascular smooth muscle cell proliferation and migration to facilitate atherosclerosis (Sun et al. 2020). Clinically, some studies disclose that the level of serum miR-186-5p is aberrant in patients with carotid artery stenosis or acute coronary syndrome (ACS) (Wang et al. 2016; Li et al. 2019; Lv et al. 2020).

Exosomes are a class of nanoscale vesicles containing cell molecules, such as proteins and RNA (Zheng et al. 2021). The predominant secretions of exosomes are miRNAs, which are released as intercellular communication mediators and subsequently facilitate the exchange of regulatory signals between neighboring or distant cells to affect biological activities (Zheng et al. 2021). Our previous study has explored the effect of exosomal miR-186-5p from oxidized low-density-lipoprotein-induced macrophage in atherosclerosis (Ren et al. 2022). The findings show that exosomal miR-186-5p promotes vascular smooth muscle cell viability and invasion to accelerate atherosclerosis by inactivating Src homology 2 domain-containing inositol polyphosphate 5-phosphatase 2 (SHIP2) mediated phosphoinositide 3 kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway (Ren et al. 2022). More importantly, serum free miRNAs are unstable and likely to be degraded, while serum exosomal miRNAs are more stable since they are packaged into lipid bilayer membranes of exosomes (Aiso et al. 2018; Susilawati 2019). Consequently, exosomal miRNAs may serve as promising clinical biomarkers. Nevertheless, the clinical role of serum exosomal miR-186-5p in CHD patients lacks evidence.

Hence, this study detected serum exosomal miR-186-5p, aiming to investigate its linkage with clinical features, coronary stenosis, and the risk of MACE in CHD patients.

Table 1. Clinical characteristics of coronary heart disease (CHD) patients.

Items	CHD patients (N = 175)
Age (years), median (range)	65.0 (45.0-83.0)
Male, n (%)	120 (68.6)
BMI (kg/m2), mean ± SD	25.6 ± 3.0
Smoke, n (%)	
Never	94 (53.7)
Former	53 (30.3)
Current	28 (16.0)
Hypertension, n (%)	127 (72.6)
Hyperlipidemia, n (%)	81 (46.3)
Diabetes mellitus, n (%)	43 (24.6)
Chronic kidney disease, n (%)	30 (17.1)
HR (bpm), mean ± SD	75.8 ± 9.5
DBP (mmHg), mean ± SD	134.3 ± 14.1
SBP (mmHg), mean ± SD	77.7 ± 9.9
FBG (mmol/L)	
Median (IQR)	6.0 (5.3-7.1)
Range	4.1-9.9
Scr (μmol/L)	
Median (IQR)	85.4 (75.8-93.7)
Range	54.0-174.2
SUA (μmol/L)	
Median (IQR)	354.0 (312.0-399.0)
Range	248.0-596.0
TG (mmol/L)	
Median (IQR)	1.9 (1.1-2.7)
Range	0.5-3.7
TC (mmol/L)	
Median (IQR)	4.8 (4.2-5.7)
Range	3.2-8.1
LDL-C (mmol/L)	
Median (IQR)	3.4 (2.8-4.3)
Range	1.7-6.7
HDL-C (mmol/L)	
Median (IQR)	0.9 (0.8-1.1)
Range	0.4-1.7
CRP (mg/L)	
Median (IQR)	5.4 (4.0-7.8)
Range	0.4-25.0
Gensini score, median (IQR)	30.0 (16.0-48.0)
Gensini score, n (%)	
0-10	4 (2.3)
11-40	114 (65.1)
> 40	57 (32.6)
Stenosis degree, n (%)	
Mild	88 (50.3)
Moderate	52 (29.7)
Severe	35 (20.0)

BMI, body mass index; SD, standard deviation; HR, heart rate; DBP, diastolic blood pressure; SBP, systolic blood pressure; FBG, fasting blood glucose; IQR, interquartile range; Scr, serum creatinine; SUA, serum uric acid; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein.

Methods

Participants

Between February 2021 and July 2023, 175 CHD patients were consecutively enrolled. The inclusion criteria were as follows: i) diagnosed as CHD by angiography; ii) aged ≥18 years; iii) willing to provide peripheral blood (PB) for serum exosomal miR-186-5p detection. The exclusion criteria were as follows: i) had a solid tumor or malignant hematological diseases; ii) participated in other clinical studies at the time of enrollment; iii) had acute and critical diseases, such as acute myocardial infarction or serious infection; iv) had liver or kidney failure. This study enrolled 50 healthy subjects as healthy control (HCs) during the same period, whose age and sex information was consistent with the CHD patients. It had fewer number of HCs than number of patients in order to save cost during the study. The inclusion criteria were: i) normal results of the physical examination; ii) aged ≥ 18 years; iii) was willing to provide PB. The exclusion criteria were the same as the CHD patients. The study received approval from the Ethics Committee of The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology. All participants submitted informed consent.

PB collection and detection

The PB was collected from the CHD patients and HCs. Within 4 hours, the PB was centrifuged to obtain serum, and exosomes were isolated from serum using Total Exosome Isolation (from serum) (No. 4478360, Invitrogen™, Cincinnati, OH, USA). The expression of serum exosomal miR-186-5p in exosomes was quantified by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). U6 was used as an internal control. Results were calculated based on the $2^{-\Delta\Delta Ct}$ method. The primer sequences of serum exosomal miR-186-5p were: forward primer (5′ to 3′), ACACTCCAGCT GGGGCAAAGAATTCTCCTT; reverse primer (5′ to 3′), TGTCGTGGAGGGGCAAAGAATTCTCCTT. The primer sequences of U6 were: forward primer (5′ to 3′), GCTTCG GCAGCACATATACTAA; reverse primer (5′ to 3′), CG AATTTGCGTGTCATCCTT (Ren et al. 2022). The experimental procedures were strictly performed according to the instructions.

Data collection and follow-up

Clinical characteristics, physical examination findings, and comorbidities of CHD patients were collected. The Gensini score ≤ 10 was defined as mild, > 10 and ≤ 40 was defined as moderate, and > 40 was defined as severe (Karacaglar et al. 2020). In particular, stenosis degree was assessed according to the Gensini score, with a Gensini score < 32 defined as mild, a Gensini score of 32 to 56 defined as moderate, and a Gensini score > 56 defined as severe. Patients had a normal follow-up. The follow-up was made every month for the first three months, and after three months, the follow-up was made every three months. The median follow-up duration was 14.8 months. The final follow-up date was August 2023. The MACE of the CHD patients was recorded.

Data analysis

The program used for data analysis was SPSS 23.0 (IBM Corp., Armonk, NY, USA). Comparative analyses were performed using the Mann-Whitney U test. The correlation analysis of continuous variables was performed using the Spearman test. The ability of serum exosomal miR-186-5p to distinguish between CHD patients and HCs was demonstrated using Receiver Operating Characteristic (ROC) curves. The association of serum exosomal miR-186-5p with patient prognosis was demonstrated using Kaplan-Meier curves. The cut-off values of serum exosomal miR-186-5p in CHD patients were selected as 1.000 (median serum exosomal miR-186-5p of HCs), 1.610 (3/4

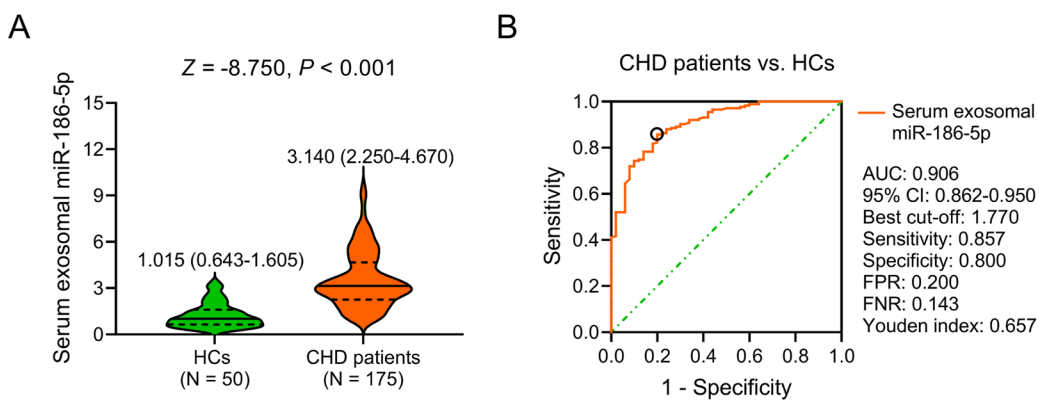

Fig. 1. Serum exosomal miR-186-5p was elevated in coronary heart disease (CHD) patients compared to healthy controls (HCs).
(A) Comparison of serum exosomal miR-186-5p between CHD patients and HCs. Data are shown as median (IQR). (B) Receiver Operatorating Characteristic (ROC) curve of serum exosomal miR-186-5p to differentiate CHD patients from HCs. AUC, area under the curve; CI, confidence interval; FPR, false positive rate; FNR, false negative rate. Youden index was calculated as the sum of sensitivity and specificity minus 1.

Table 2. The correlation of serum exosomal miR-186-5p with comorbidities in coronary heart disease (CHD) patients.

Items	Serum exosomal miR-186-5p	*Z* value	*P* value
Hypertension		−1.132	0.258
No	2.870 (2.038-4.568)		
Yes	3.190 (2.390-4.850)		
Hyperlipidemia		−1.784	0.074
No	2.880 (2.005-4.263)		
Yes	3.280 (2.595-5.115)		
Diabetes mellitus		−1.735	0.083
No	3.125 (2.155-4.348)		
Yes	3.400 (2.590-5.620)		
Chronic kidney disease		−1.356	0.175
No	3.040 (2.160-4.555)		
Yes	3.435 (2.543-5.678)		

Serum exosomal miR-186-5p levels are shown as median (IQR).
miR-186-5p, microRNA-186-5p.

Table 3. The correlation of serum exosomal miR-186-5p with physical examination findings and biochemical indexes in coronary heart disease (CHD) patients.

Items	*r* value	*P* value
HR (bpm)	0.105	0.166
DBP (mmHg)	0.121	0.111
SBP (mmHg)	0.134	0.076
FBG (mmol/L)	0.057	0.456
Scr (μmol/L)	0.087	0.254
SUA (μmol/L)	0.135	0.076
TG (mmol/L)	0.126	0.096
TC (mmol/L)	0.230	0.002
LDL-C (mmol/L)	0.220	0.003
HDL-C (mmol/L)	−0.077	0.310
CRP (mg/L)	0.109	0.149

miR-186-5p, microRNA-186-5p; HR, heart rate; DBP, diastolic blood pressure; SBP, systolic blood pressure; FBG, fasting blood glucose; Scr, serum creatinine; SUA, serum uric acid; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein.

quartile serum exosomal miR-186-5p of HCs), and 3.390 (maximum serum exosomal miR-186-5p of HCs). The analyses were performed using the log-rank test. *P*-values < 0.050 were considered to be significantly different.

Results

Baseline features of CHD patients

The median age of CHD patients was 65.0 years, ranging from 45.0 to 83.0 years. There were 120 (68.6%) male patients. The median (range) triglyceride (TG), total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), and C-reactive protein (CRP) was 1.9 (0.5-3.7) mmol/L, 4.8 (3.2-8.1) mmol/L, 3.4 (1.7-6.7) mmol/L, 0.9 (0.4-1.7) mmol/L, and 5.4 (0.4-25.0) mg/L, respectively. The median (interquartile range, IQR) Gensini score was 30.0 (16.0-48.0). In detail, Gensini score was 0-10 in 4 (2.3%) patients, 11-40 in 114 (65.1%) patients, and > 40 in 57 (32.6%) patients. A total of 88 (50.3%), 52 (29.7%), and 35 (20.0%) patients had mild, moderate, and severe stenosis, accordingly. The detailed clinical features of CHD patients were listed in Table 1.

Serum exosomal miR-186-5p between CHD patients and HCs

Serum exosomal miR-186-5p was enhanced in CHD patients vs. HCs ($P < 0.001$). The median (IQR) serum exosomal miR-186-5p was 3.140 (2.250-4.670) and 1.015 (0.643-1.605) in CHD patients and HCs, accordingly (Fig. 1A). Serum exosomal miR-186-5p exhibited an excellent value for estimating the risk of CHD [area under curve (AUC): 0.906, 95% confidence interval (CI): 0.862-0.950], with the best cut-off value of 1.770 (sensitivity: 0.857, specificity: 0.800) (Fig. 1B).

Correlation of serum exosomal miR-186-5p with clinical features in CHD patients

In CHD patients, serum exosomal miR-186-5p was not associated with hypertension ($P = 0.258$), hyperlipidemia ($P = 0.074$), diabetes mellitus ($P = 0.083$), or chronic kidney disease ($P = 0.175$) (Table 2). Increased serum exosomal miR-186-5p was related to elevated TC ($P = 0.002$) and LDL-C ($P = 0.003$), but it was not related to heart rate, diastolic blood pressure, systolic blood pressure, fasting blood glucose, serum creatinine, serum uric acid, TG, HDL-C, or CRP (all $P > 0.050$) (Table 3).

Correlation of serum exosomal miR-186-5p with coronary stenosis in CHD patients

In CHD patients, serum exosomal miR-186-5p was positively linked with Gensini score ($P = 0.028$) (Fig. 2A). Elevated serum exosomal miR-186-5p was related to increased stenosis degree ($P = 0.018$). In detail, serum exo-

somal miR-186-5p was lowest in patients with mild stenosis, followed by those with moderate stenosis, and the highest in those with severe stenosis (Fig. 2B).

Correlation of serum exosomal miR-186-5p with MACE in CHD patients

In CHD patients, the accumulating MACE rate at 1 year and 2 years was 6.6% and 15.6%, correspondingly (Fig. 3A). Serum exosomal miR-186-5p was enhanced in patients who experienced MACE vs. those who did not (P = 0.042) (Fig. 3B). However, serum exosomal miR-186-5p > 1.000 (P = 0.404) (Fig. 3C) and > 1.610 (P = 0.328) (Fig. 3D) was not related to accumulating MACE. Serum exosomal miR-186-5p > 3.390 exhibited a correlative trend with increased accumulating MACE, but did not achieve statistical significance (P = 0.071) (Fig. 3E).

Discussion

Serum miRNA, likely to be degraded, is released during cell activities and transmitted by circulatory system to regulate biological processes (Aiso et al. 2018). Additionally, exosomes, containing many proteins, lipids,

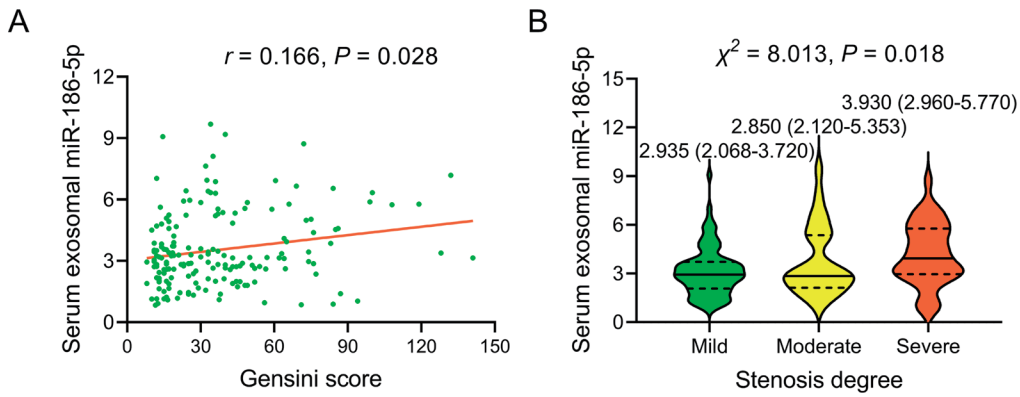

Fig. 2. Serum exosomal miR-186-5p was positively correlated with Gensini score and stenosis degree in coronary heart disease (CHD) patients.
(A) Correlation of serum exosomal miR-186-5p with Gensini score in CHD patients. (B) Stenosis degree in CHD patients. Data are shown as median (IQR).

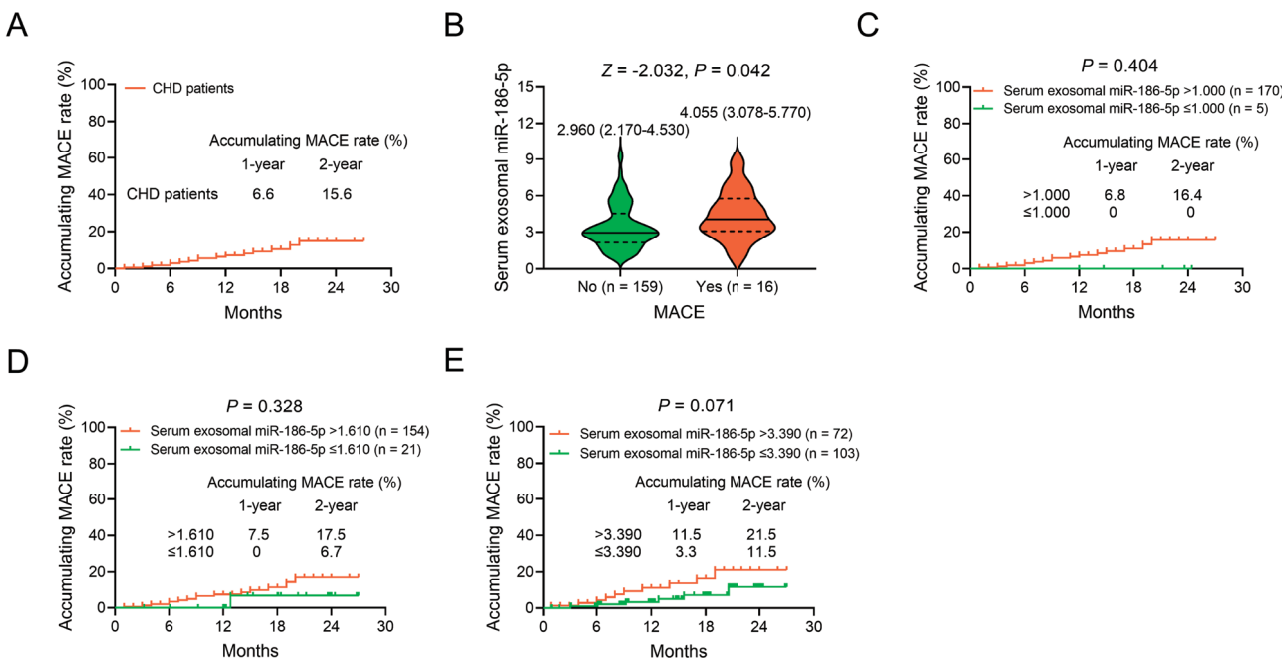

Fig. 3. Serum exosomal miR-186-5p was elevated in coronary heart disease (CHD) patients who experienced major adverse cardiovascular events (MACE) compared to those who did not.
(A) The 1-year and 2-year accumulating MACE rate of CHD patients. (B) Comparison of serum exosomal miR-186-5p between CHD patients who experienced MACE and those who did not. Data are shown as median (IQR). (C-E) Kaplan-Meier curves reflecting the value of serum exosomal miR-186-5p; the value > 1.000 (C), the value > 1.610 (D), and the value > 3.390 (E) for predicting MACE in CHD patients.

and nucleic acids, mediate intercellular communication and various physiological and pathobiological mechanisms (Ludwig and Giebel 2012). Different from serum free miRNA, exosomal miRNA is protected by lipid bilayer membranes of exosomes and influences cells via intercellular communication processes (Susilawati 2019). Previously, abnormal level of miR-186-5p is reported in patients with cerebro-cadiovascular diseases from many studies (Wang et al. 2018; Li et al. 2019; Sun et al. 2020). For example, one study reveals that serum miR-186-5p is enhanced in atherosclerosis patients compared to healthy people (Sun et al. 2020). Another study indicates that serum miR-186-5p is increased in ACS patients upon admission compared to the controls (Li et al. 2019). Besides, it is stated that serum miR-186-5p is enhanced in patients with ischemia stroke vs. healthy donors (Wang et al. 2018). Similarly, this study disclosed that serum exosomal miR-186-5p was elevated in CHD patients vs. HCs. The possible reasons could be: Based on our previous study, miR-186-5p promoted atherosclerosis by suppressing SHIP2-mediated PI3K/AKT/mTOR pathway (Ren et al. 2022). In addition, the current study suggested that serum exosomal miR-186-5p exhibited an excellent ability to differentiate CHD patients from HCs, and its value of 1.770 had relatively good sensitivity and specificity, indicating its potency as a predictor for the risk of CHD. But further validations were warranted.

Lipids exacerbate atherosclerosis as well as stenosis degree by promoting plaque accumulation and instability, accelerating the progression of CHD; thereby, CHD patients have aberrant lipid levels (Liu et al. 2019; Shaya et al. 2022). The findings in this study showed that serum exosomal miR-186-5p was positively related to TC and LDL-C in CHD patients. The possible explanations could be: (1) MiR-186-5p might promote lipid peroxidation through inhibiting PI3K/AKT signaling, which could increase the level of blood cholesterol (Guo et al. 2019; Zhao et al. 2022). (2) MiR-186-5p decreased insulin-like growth factor 1, and the latter facilitated lipid metabolism (Aguirre et al. 2016; Wang et al. 2018). Therefore, elevated miR-186-5p was correlated with increased TC and LDL-C in CHD patients. Additionally, the current study also revealed a positive linkage of serum exosomal miR-186-5p with Gensini score and stenosis degree in CHD patients, which could be explained by that: miR-186-5p exacerbated lipid accumulation and atherosclerosis as mentioned before, which further aggravated coronary stenosis.

The incidence of MACE at 1 year and 2 years ranges from 2.7% to 12% and 6% to 30%, accordingly, in CHD patients by other studies (Ferencik et al. 2018; Bauersachs et al. 2019; Berger et al. 2020; Puymirat et al. 2021). In this study, the accumulating MACE rate at 1 year was 6.6% and the rate at 2 years was 15.6% in CHD patients. Moreover, this study observed that serum exosomal miR-186-5p was enhanced in CHD patients who experienced MACE compared to those who did not. The possible explanations could be: (1) MiR-186-5p was positively associated with vascular stenosis as discussed above, and the latter could exacerbate myocardial ischemia, which could increase the risk of MACE (Dettori et al. 2021; Kato et al. 2022). (2) MiR-186-5p was correlated with aberrant lipid level as revealed in the current study, which was considered a contributor to MACE occurrence (Zhao et al. 2021). In addition, serum exosomal miR-186-5p > 3.390 had a correlative trend (without statistical significance) with elevated accumulating MACE in CHD patients. The probable explanation could be: The follow-up duration was relatively short in the current study (median: 14.8 months), and only 16 end-point events had occurred by the last follow-up date, partially weakening the statistical power.

The current study existed some limitations. To begin with, the sample size of the current study was relatively small, which weakened the statistical power. Additionally, this study only detected serum exosomal miR-186-5p after enrollment; thus, its longitudinal changes and corresponding prognostic value needed further detection. Finally, this study did not set disease controls (such as angina pectoris) to eliminate some potential confounders.

To sum up, serum exosomal miR-186-5p positively correlates with TC, LDL-C, coronary stenosis degree, and the risk of MACE in CHD patients, indicating its potency as a prognostic biomarker to assist in the promotion of CHD individualized management. Since our previous study indicates that exosomal miR-186-5p regulates macrophage, further study is required for verifying its clinical role on type 1 macrophage polarization in these patients.

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Conflict of Interest

The authors declare no conflict of interest.

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