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Original Article

Application values of MMP-9 and miR-126 detection for diagnosis of coronary heart disease in hypertensive patients

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Abstract: Objective: This paper aims to explore the application value of matrix metalloproteinase-9 (MMP-9) and miR-126 detection for the diagnostic and severity predictions of coronary heart disease (CHD). Methods: Altogether 100 hypertensive patients were selected, of whom 25 patients without CHD were placed into an observation group 1, and the remaining 75 patients complicated with CHD were placed into an observation group 2. A further 60 people undergoing physical examination at the same time were placed into a control group. The correlation of the relative expression levels of MMP-9 and miRNA-126 with the severity of CHD and the severity of coronary artery stenosis was analyzed. Results: The relative expression level of MMP-9 in the observation group 2 was significantly higher than that in the control group and the observation group 1 ($P<0.001$), while the relative expression level of miRNA-126 was significantly lower than that in the control group and the observation group 1 ($P<0.001$). The area under the receiver operating characteristic (ROC) curve (AUC) of MMP-9 for the diagnosis of CHD in hypertensive patients was 0.858, 95% CI (80.2%-91.5%) and the Youden index was 0.560, with the sensitivity of 0.950 and the specificity of 0.610. The AUC of miRNA-126 for the diagnosis was 0.879, 95% CI (82.6%-93.2%) and the Youden index was 0.650, with the sensitivity of 0.900 and the specificity of 0.750. The AUC of MMP-9 combined with miRNA-126 for the diagnosis was 0.949, 95% CI (91.8%-98.1%) and the Youden index was 0.870, with the sensitivity of 0.890 and the specificity of 0.917. The more severe the coronary artery stenosis was, the higher the relative expression level of MMP-9 was and the lower the relative expression level of miRNA-126 was ($P<0.001$). Hypertensive patients with myocardial infarction (MI) had high relative expression levels of MMP-9 but low relative expression levels of miRNA-126 ($P<0.001$), especially for the patients with ST-segment elevation myocardial infarction (STEMI). The AUC of MMP-9 for the diagnosis of MI was 0.898, 95% CI (82.4%-97.2%) and the Youden index was 0.644, with the sensitivity of 0.800 and the specificity of 0.844. The AUC of miRNA-126 for the diagnosis was 0.958, 95% CI (91.7%-99.9%) and the Youden index was 0.856, with the sensitivity of 0.889 and the specificity of 0.967. The AUC of MMP-9 combined with miRNA-126 for the diagnosis was 0.997, 95% CI (99.7%-100.1%) and the Youden index was 0.967, with the sensitivity of 0.967 and the specificity of 1.000. Conclusion: Detection of the relative expression levels of serum MMP-9 and miRNA-126 has a high diagnostic value for hypertensive patients complicated with CHD.

Keywords: MMP-9, miR-126, coronary heart disease, diagnostic value

Introduction

The incidence of coronary heart disease (CHD) is increasing year by year. A recent study showed that the most common cause of death is due to cardiovascular events, and myocardial infarction (MI) is a leading one [1]. The early prediction and intervention in cardiovascular disease has a positive effect on the prognosis of patients. Matrix metalloproteinases (MMPs), are highly conserved proteases from natural evolution, and are of great significance to the declined stability of carotid plaques [2]. A previous study found that matrix metalloproteinase-9 (MMP-9) is closely related to ischemic cerebral infarction [3]. According to other studies, MMP-9 is also correlated with heart failure and MI [4-6]. After myocardial cells are injured, certain proteins and molecular substances from cells enter the blood, which are commonly

known and used as clinical indicators, such as cardiac troponin I, creatine kinase, creatine kinase isoenzyme and myoglobin [7]. The first one, cardiac troponin, is the gold standard for diagnosing MI [8]. The latter three can also be detected in the blood of patients without ischemia, and they have poor specificity and sensitivity [9, 10]. With the development of gene detection technologies, the abnormal expression of some non-coding RNA (MicroRNA, miRNAs) have been found in the serum of patients with MI [11]. According to previous studies, miR-126 inhibits apoptosis and has abnormal expression in patients with tumors [12-14]. A cardiovascular system-related study revealed the relationship between miR-126 and CHD for the first time. It also found that in patients with early MI, the changes in miR-126 expression level are in the same time window as that of myocardial troponin I, but the former reaches the its lowest point earlier [15]. Additionally, the expression of miR-126 is stable in human body fluids [16]. In this study, the expression of MMP-9 and miR-126 in the circulated blood of patients with CHD was monitored, to explore the correlation of the expression of these markers with the severity of CHD. The report is as follows.

Materials and methods

General information

A total of 100 patients with grade 1-2 hypertension admitted to the Department of Cardiology of Zhangye People's Hospital Affiliated with Hexi University from June 2017 to December 2018 were collected. Among them, 25 patients without CHD were placed into the observation group 1, and 75 patients complicated with CHD were placed into the observation group 2; with an age range of 40-80 years old and an average age of 62.5±8.2 years old. A further 60 people undergoing physical examination at the same time were selected as the control group, with an average age of 64.3±7.6 years old. All patients signed an informed consent form. This study was approved by the Ethics Committee of Zhangye People's Hospital Affiliated with Hexi University.

Inclusion and exclusion criteria

Inclusion criteria: (1) patients who met the diagnostic criteria and classification of hypertension and CHD, referring to the criteria formulated by the Chinese Society of Cardiology, Chinese Medical Association in 2007 [17]; (2) patients older than 18 years old. Exclusion criteria: (1) patients with incomplete clinical data; (2) patients with severe malnutrition and tumors; (3) patients with mental disorders or cerebrovascular diseases who could not cooperate; (4) patients who had taken glucocorticoids or immunosuppressants recently; (5) patients complicated with impairment of major organs.

Grouping

According to the severity of coronary artery stenosis, 100 hypertensive patients were divided into four groups with stenosis ≤ 30%, 31%-49%, 50%-69% and over 70%. According to the diagnostic criteria, 75 patients with CHD were divided into groups: 25 patients with stable angina pectoris (group A), 20 patients with unstable angina pectoris (group B), 17 patients with non-ST-segment elevation myocardial infarction (NSTEMI) (group C), and 13 patients with ST-segment elevation myocardial infarction (STEMI) (group D).

Extraction of miRNA

In this study, a Trizol kit (Molecular Research Center, USA) was used to extract the total RNA from plasma and to detect the concentration and integrity of the RNA. The upstream and downstream primers were provided by Guangzhou Rainbow Biotech Co., Ltd. [18]. Then, a reverse transcription kit (Fernentas, Canada) was used to reverse transcribe miRNA into cDNA, which was used as a template to amplify the DNA. Finally, fluorescence probe quantitative PCR was used to measure the expression of MMP-9 and miRNA-126 in the serum samples. The specific steps were as follows: (1) the included patients fasted from food and liquid after 10 p.m. In the next morning, 2 mL of peripheral venous blood was collected in two test tubes (4 mL in total) from the fasting patients. Before the interventional operation, 2 mL of peripheral venous blood was collected in two test tubes (4 mL in total) from patients with acute myocardial infarction (AMI). The tubes were mixed with EDTA anticoagulants to keep the cell integrity. After that, the venous blood in the tubes was shaken evenly to make sure the blood cells were fully in contact with the antico-

Table 1. Comparison of general information and baseline data

	Control group	Observation group 1	Observation group 2	χ^2/F	P
Gender (male/female)	32/28	15/10	40/35	0.682	0.711
Age (year)	64.30±7.60	61.80±9.50	63.40±7.60	0.927	0.398
Systolic pressure (mmHg)	108.93±7.65	151.94±8.10***	154.03±7.23***,#	658.681	<0.001
Diastolic pressure (mmHg)	68.60±5.09	86.94±8.10	89.11±6.98***,#	176.117	<0.001
Triglyceride (mmol/L)	1.18±0.69	1.83±0.66***	1.83±0.66***,#	17.542	<0.001
Total cholesterol (mmol/L)	4.58±0.44	5.88±0.44***	5.89±0.44***,#	168.493	<0.001
High-density lipoprotein (mmol/L)	1.52±0.44	1.05±0.34***	1.12±0.34***,#	23.237	<0.001
Low density lipoprotein (mmol/L)	2.42±0.42	3.82±0.87***	3.82±0.86***,#	69.644	<0.001
Hemoglobin (gl/L)	131.68±10.27	102.08±9.57***	102.79±10.28***,#	153.097	<0.001
BMI	26.72±1.96	26.99±2.03	27.27±2.13	0.769	0.465
Blood glucose (mmol/L)	5.70±0.78	5.58±0.77	5.56±0.77	0.561	0.572

Note: Compared with the control group, ***P<0.001; compared with the observation group 1, #P>0.05. BMI, body mass index.

agulants. (2) The treated tubes were placed into a refrigerator at 4°C, and the plasma was separated within 2 hours. (3) The plasma was centrifuged for 10 min using a high-speed centrifuge (Shanghai Generay Biotech Co., Ltd.), and then the supernatant was sucked out and placed into an Eppendorf tube. (4) The supernatant was centrifuged again for 10 min. (5) The plasma after being separated twice was placed into a freezer at -80°C and sub-packaged (500 μL). (6) A Trizol kit was used to extract the total RNA, which was then reverse transcribed using stem loop reverse transcription and M-MLV reverse transcriptase. After that, stem-loop primers for Has miR-126 were used. The upstream primer sequences of miR-126 and MMP-9 were 5'-TATCCAGTGATTCCGACCGCCGCATGGAGTCTG-3' and 5'-CGTGAACATCTTCGACGCCAT-3', and the downstream primer sequences of them were 5'-TCCGCTCTTGGTGTGGTGTGAGTCGC-3' and 5'-TCCTCAAAGACCGACTCCAGC-3'. The primer for the reference gene U6 was 5'-TCCGCTCTTGGTGT-GGTGTGAGTCGC-3'. The 25 μL of the recycle system was as follows: 12.5 μL of SYBR premix (2X), each 0.5 μL of upstream and downstream primers of the target gene, 2.0 μL of cDNA template and 9.5 μL of ddH_2O. Reaction conditions were as follows: pre-denaturation at 94°C for 4 min, 95°C for 40 s, 60°C for 30 s and 72°C for 30 s, for 35 cycles, then extension at 72°C for 1 min. Agarose electrophoresis was used to detect the PCR amplification products. The relative expression of U6 snRNA was used as the standard and $2^{-\Delta\Delta C(T)}$ was used for analysis. Finally, the relative expression of miRNA-126 was determined.

Statistical methods

SPSS 22.0 was used for statistical analysis. Continuous variables were expressed by mean ± standard deviation ($\bar{x}$ ± sd). Paired t test was used for the comparison of data confirming to normal distribution and homogeneity of variance within groups, rank sum test was used for data which did not confirm to normal distribution and homogeneity of variance, one-way ANOVA was used for comparison between multiple groups, Bonferroni method was used for post hoc pairwise comparison between groups if there was a difference. Pearson's product moment correlation was used for analyzing the linear correlation between two variables. P< 0.05 indicates a statistically significant difference.

Results

Comparison of general information and baseline data

There were significant differences among the observation group 1, the observation group 2 and the control group in terms of systolic blood pressure, diastolic blood pressure, triglycerides, total cholesterol, low-density lipoprotein, high-density lipoprotein and hemoglobin (P< 0.05), but there was no statistically significant difference in age, gender, body mass index (BMI) and blood glucose (P>0.05). There was no difference in the above indexes between observation groups 1 and 2 (P>0.05), which were comparable. More details are shown in **Table 1**.

Table 2. Comparison of relative expression levels of MMP-9 and miRNA-126 between observation groups 1 and 2

	Case	Relative expression level of MMP-9	Relative expression level of miRNA-126
Control group	60	1.67±0.79	0.38±0.05
Observation group 1	25	2.51±0.76***	0.37±0.05***
Observation group 2	75	3.18±0.86***,###	0.24±0.04***,###
F		52.181	176.183
P		<0.001	<0.001

Note: Compared with the control group, ***P<0.001; compared with the observation group 1, ###P>0.05.

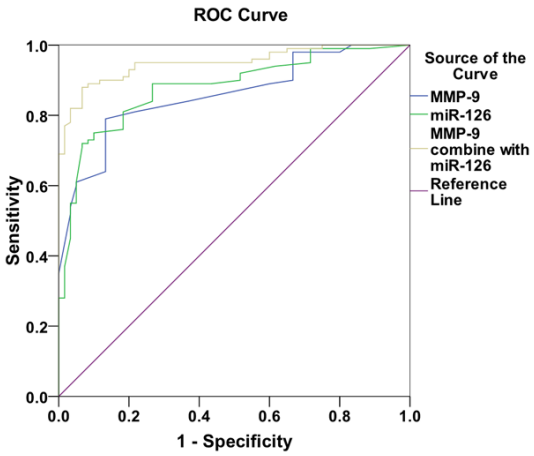

Figure 1. ROC curve of MMP-9 and miRNA-126 in the diagnosis of coronary heart disease in patients with hypertension.

Comparison of relative expression levels of MMP-9 and miRNA-126 between observation groups 1 and 2

The relative expression level of MMP-9 in observation group 2 was significantly higher than that in the control group and observation group 1; while the relative expression level of miRNA-126 was significantly lower than that in the control group and observation group 1 (P<0.001). More details are shown in **Table 2**. The AUC of the ROC curve of MMP-9 for the diagnosis of CHD in hypertensive patients was 0.858, 95% CI (80.2%-91.5%) and the Youden index was 0.560, with the sensitivity of 0.950 and the specificity of 0.610. The AUC of the ROC curve of miRNA-126 for the diagnosis was 0.879, 95% CI (82.6%-93.2%) and the Youden index was 0.650, with the sensitivity of 0.900 and the specificity of 0.750. The AUC of MMP-9 combined with miRNA-126 for the diagnosis was 0.949, 95% CI (91.8%-98.1%) and the Youden index was 0.870, with the sensitivity of 0.890 and the specificity of 0.917. More details are shown in **Figure 1**.

Correlation of relative expression levels of MMP-9 and miRNA-126 with coronary artery stenosis in hypertension patients

The more severe the coronary artery stenosis was, the higher the relative expression level of MMP-9 was and the lower the relative expression level of miRNA-126 was (P<0.001). More details are shown in **Table 3**.

Comparison of relative expression levels of MMP-9 and miRNA-126 between CHD patients with different lesions

Compared with the groups A and B, patients in the groups C and D had higher relative expression level of MMP-9 but lower relative expression level of miRNA-126 (P<0.001). More details are shown in **Table 4**. The AUC of MMP-9 for the diagnosis of MI was 0.898, 95% CI (82.4%-97.2%) and the Youden index was 0.644, with the sensitivity of 0.800 and the specificity of 0.844. The AUC of miRNA-126 for the diagnosis of MI was 0.958, 95% CI (91.7%-99.9%) and the Youden index was 0.856, with the sensitivity of 0.889 and the specificity of 0.967. The AUC of MMP-9 combined with miRNA-126 for the diagnosis MI was 0.997, 95% CI (99.7%-100.1%) and the Youden index was 0.967, with the sensitivity of 0.967 and the specificity of 1.000. More details are shown in **Figure 2**.

Discussion

MMP-9 as an important member of MMP, it degrades the dependence of the extracellular matrix on zinc. A study showed that MMP-9 plays an important role in the formation and rupture of atherosclerotic plaques [19]. MMP-9 activates type V gelatinase and then induces it to enter smooth muscle cells, so as to promote a rupture of atherosclerotic plaques and makes them easy to detach due to their declined stability [20, 21]. The detachment of carotid plaques is closely related to AMI [22]. According to a recent study, MMP-9, which is correlated with cardiovascular events, significantly increa-

Table 3. Correlation of relative expression levels of MMP-9 and miRNA-126 with coronary artery stenosis in hypertension patients

Severity of coronary artery stenosis	Case	Relative expression level of MMP-9	Relative expression level of miRNA-126
≤ 30%	13	2.01±0.72	0.36±0.03
31-49%	12	2.46±0.835***	0.32±0.06***
50-69%	45	2.83±0.53***,###	0.23±0.05***,###
Over 70%	30	3.36±0.21***,###,&&&	0.20±0.03***,###,&&&
F		44.525	44.525
P		<0.001	<0.001

Note: Compared with ≤ 30%, ***P<0.001; compared with 31-49%, ###P>0.01; compared with 50-69%, &&&P>0.01.

Table 4. Comparison of relative expression levels of MMP-9 and miRNA-126 between CHD patients with different lesions

	Case	Relative expression level of MMP-9	Relative expression level of miRNA-126
Group A	25	2.42±0.74	0.25±0.06
Group B	20	3.00±0.54***	0.23±0.01
Group C	17	3.61±0.58***,###	0.20±0.01***
Group D	13	4.02±0.56***,###,&&&	0.17±0.01***,###
F		23.138	16.396
P		<0.001	<0.001

Note: Compared with group A, ***P<0.001; compared with group B, ###P>0.01; compared with group C, &&&P>0.01. Group A, patients with stable angina pectoris; group B, patients with unstable angina pectoris; group C, patients with non-ST-segment elevation myocardial infarction; group D, patients with ST-segment elevation myocardial infarction.

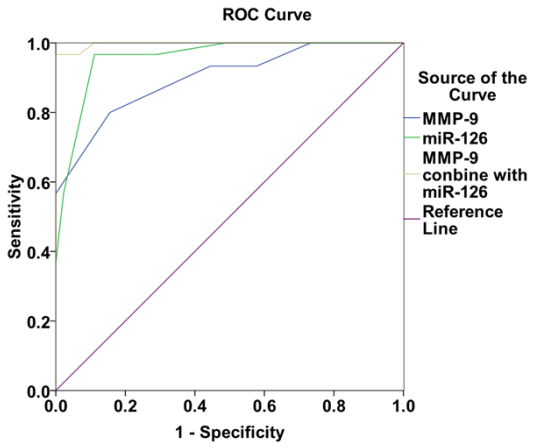

Figure 2. ROC curve of MMP-9 and miRNA-126 in the diagnosis of myocardial infarction in patients with hypertension.

ses in patients with MI but gradually decreases after the patients' blood vessels are opened [23]. In a study on miRNA in the cardiovascular system, the detection of miRNA expression has a diagnostic value for the early judgment and prognosis of CHD, MI, heart failure and atrial fibrillation [24]. Some studies show that miRNA has stable expression in human body fluids, and has great potential and values in diagnosing cardiovascular diseases [16, 25, 26]. In a study on arteriosclerosis, miRNA-126 inhibits the activation of endothelial cells and thereby causes damage to the cardiovascular system [27]. The damaged endothelial cells increase their vascular permeability, which results in the deposition of low-density lipoproteins on the vascular wall and promotes arteriosclerosis [28, 29]. In this study, after the measurement of the severity of coronary artery stenosis in patients with hypertension, it was found that the more severe the coronary artery stenosis was, the higher the relative expression level of MMP-9 was and, the lower the relative expression level of miRNA-126 was. This is consistent with the findings of the above studies.

According to recent studies, the severity of coronary artery lesions is closely related to the stability of plaques rather than their size. MMP-9 causes plaque rupture and reduces the stability, and thus involves in the formation of coronary atherosclerosis [30-32]. MiRNA-126 expression is found to significantly decrease in patients having CHD for the first time [33]. MiRNA-126 inhibits the activation of endothelial cells and the release of apoptotic bodies, which significantly reduces miRNA-126 released by endothelial cells [34, 35]. In this study, the relative expression level of MMP-9 increased while the relative expression level of miRNA-126 decreased in hypertensive patients

complicated with CHD, which is consistent with the results of above studies. Moreover, according to the diagnosis of CHD, the sensitivity of MMP-9 was 0.950 and the specificity was 0.610; the sensitivity of miRNA-126 was 0.900 and the specificity was 0.750; the sensitivity of MMP-9 combined with miRNA-126 was 0.890 and the specificity was 0.917. Therefore, MMP-9 and miRNA-126 have high diagnostic values for hypertensive patients complicated with CHD.

A study shows that MMP-9 in patients with MI significantly increases before the patients' blood vessels are opened, but it significantly decreases 3 days to 3 months after the opening [5]. In patients with early MI, the changes in miR-126 expression level are the same as the time window of myocardial troponin I changes, but the former reaches the lowest point earlier [15]. In this study, patients with MI had high relative expression level of MMP-9 but low relative expression level of miRNA-126, especially for the patients with STEMI, which is consistent with the results of the above studies. In the diagnosis of MI, the sensitivity and specificity of MMP-9 were 0.800 and 0.844 respectively; those of miRNA-126 were 0.889 and 0.967 respectively; those of MMP-9 combined with miRNA-126 were 0.967 and 1.000 respectively. Therefore, MMP-9 and miRNA-126 have high diagnostic values for hypertensive patients complicated with MI.

The sample size in this study is small, so it can be enlarged for later multi-center studies.

In summary, detection of the relative expression levels of serum MMP-9 and miRNA-126 has a high diagnostic value for coronary artery lesions in hypertensive patients.

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Disclosure of conflict of interest

None.

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