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Original Article

Changes of miR-130a and ET-1 and Their Predictive Value for In-Stent Restenosis after Percutaneous Coronary Intervention

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Abstract

Background: To explore the changes of miR-130a and endothelin -1 (ET-1) and their predictive value for in-stent restenosis (ISR) after percutaneous coronary intervention (PCI).

Methods: Overall, 253 patients with coronary heart disease (CHD) treated with PCI in Lianshui County People's Hospital, Huaian, China from April 2013 to May 2016 were selected. The changes of miR-130a and ET-1 levels before and after PCI were compared. The predictive value of miR-130a and ET-1 for ISR was analyzed by receiver operating characteristic (ROC) curves, and the correlation between ISR and miR-130a, ET-1 was analyzed by Kaplan-Meier (K-M) curve. The risk factors of ISR in CHD patients were evaluated by logistics regression analysis.

Results: The postoperative levels of miR-130a and ET-1 were significantly increased ($P<0.05$). The levels of miR-130a and ET-1 in peripheral blood of patients with ISR were higher than those in patients without ISR ($P<0.05$). The ROC curves showed that the area under curve (AUC), sensitivity, specificity and critical value of miR-130a in predicting ISR were respectively 0.912, 92.02%, 73.47%, 1.457 pmol/L, and those of ET-1 were 0.814, 87.63%, 63.27%, 2.245 pmol/L, respectively. The K-M curve showed that the incidence of ISR in patients with high expression of miR-130a or ET-1 was significantly higher than that in patients with low expression ($P<0.05$). miR-130a and ET-1 were independent risk factors for ISR ($P<0.05$).

Conclusion: MiR-130a and ET-1 have high predictive value for ISR after PCI and are independent risk factors for CHD patients, which are worthy of clinical application.

Keywords: Coronary heart disease; Interventional therapy; miR-130a; Human; In-stent restenosis; Prediction

Introduction

Cardiovascular disease (CVD) is a group of diseases involving heart and blood vessels. In developed countries, coronary heart disease (CHD) is the main cause of death and disability (1, 2). Although the death rate has gradually decreased in western countries in the last few decades, one third of the population over 35 still died of this disease (3, 4). Of the 58 million deaths worldwide in 2005, 17 million died of CVD, of which 7.6 million died of CHD (5, 6). Interventional therapy is an important method to reduce the area of myocardial infarction clinically. In non-acute cases, the potential benefits of percutaneous coronary intervention (PCI) for stable CHD patients have been debated for more than ten years. One of the safety issues is in-stent restenosis (ISR), which has an incidence of 26.4%, seriously affecting the treatment effect and quality of life of pa-

tients (7, 8). Therefore, ISR prevention after interventional therapy is a critical clinical problem, but there is currently no method with high specificity except angiography.

It is of great significance to find simple and minimally invasive prevention indicators for clinical prevention of ISR. In recent years, microRNAs (miRs), such as miR-143 and miR-145, could be used as noninvasive biomarkers for ISR (9, 10). In addition, miR-130a had the function of regulating angiogenesis and an up-regulated expression in carotid restenosis, which may promote the proliferation of VSMCs by down-regulating the expression levels of Gax and PTEN (11-13). Endothelin -1 (ET-1) is significantly increased in peripheral blood of patients after interventional therapy, and is associated with arterial injury and endothelial dysfunction, which promotes the occurrence of ISR (14, 15).

Therefore, this study aimed to confirm our hypotheses that miR-130a and ET-1 may have predictive value in the occurrence of ISR in CHD patients after interventional therapy.

Methods

Research object

A total of 253 CHD patients undergoing PCI treatment in Lianshui County People's Hospital, Huaian, China from April 2013 to May 2016 were selected. Inclusion criteria were referring to the 2012 WHO diagnostic criteria for CHD; patients aged 50-70 years old; patients voluntarily participating in this study (16). Exclusion criteria were patients with unstable CHD, intermittent or acute myocardial infarction and infectious diseases; patients with thyroid dysfunction, severe cardiac insufficiency, tumors, grade III hypertension, and liver and kidney failure; patients with incomplete case data; patients with mental or learning dysfunction. This study was approved by the Ethics Committee of Lianshui County People's Hospital, and patients or their families signed an informed consent form.

qRT-PCR

Peripheral blood of all patients before and after treatment (24 h) was collected and centrifuged to obtain the serum. Total RNA was extracted using TRIzol (Guangzhou Labgene Biotechnology Co., Ltd.) referring the kit instruction. A micrometer ultraviolet spectrophotometer DanoProp1000 (Tuomorgan Biotechnology Co., Ltd.) was used to analyze the concentration and purity of the extracted RNA. 3% agarose gel electrophoresis (Shanghai Jingke Chemical Technology Co., Ltd.) was used to analyze the integrity of RNA, A260/A280 value between 1.8 and 2.1 being considered to meet the experimental requirements. qRT-PCR reaction was carried out after RNA extraction. The reverse transcription reaction system contained 2 μL of 5 * Primerscript Buffer, 0.5 μL of Primerscript RT Enzyme Mix, 0.5 μL of Random 6mers (100 μM), 0.5 μL of Oligo dT Primer (50 μM), 2 μg of total RNA, and ribonuclease-free distilled water added to 10 μL. Reverse transcription reaction was carried out at 37℃ for 15 min, deactivation of reverse transcriptase at 85℃ for 5 s, reaction completed at 4 ℃. PCR amplification was performed after reverse transcription reaction. The PCR amplification system: 4 μL of cDNA template, 25 μL of SYBR Green Mix (2x), 1 μL of each upstream primer and downstream primer, 1 μL of Reference Dye (optional), and double distilled water added to 50 μL. PCR conditions: predenaturation at 95 ℃ for 3 min, denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, extension at 72℃ for 60 s, for 30 cycles, and extension at 72 ℃ for 5 min after cycle completion. U6 was used as an internal reference and the $2^{-\triangle\triangle CT}$ method was used to analyze the data. The primer sequences were designed and synthesized by Hepeng (Shanghai) Biotechnology Co., Ltd (Table 1).

Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to detect ET-1 level in peripheral blood of patients before and after PCI treatment (24 h). The detection kit was purchased from Shanghai Hengfei Biotechnology Co., Ltd. (cat no E7920). Specific steps referred to the kit specification.

Table 1: Primer sequence

	Forward primer	*Reverse primer*
miR-130a	GGGGCAGTGCAATGTTAAAA	GTGCGTGTCGTGGAGTCG
U6	GCGCGTCGTGAAGCGTTC	GTGCAGGGTCCGAGGT

Outcome measures

The changes of miR-130a and ET-1 levels before and after interventional therapy were compared. The incidence of ISR within 6 months after operation in the two groups was recorded. MiR-130a and ET-1 levels in the peripheral blood between patients with and without ISR were compared. Receiver operating characteristic (ROC) curve was used to analyze the predictive value of miR-130a and ET-1 for ISR 24 hours after operation. Taking cut-off value as critical value, ≤ cut-off was considered as low expression group, > cut-off as high expression group. The Kaplan-Meier (K-M) curve was used to compare the difference in the ISR incidence between the two groups, and logistics regression was applied to analyze the risk factors of ISR in CHD patients after PCI.

Criteria for in-stent restenosis

The patient underwent coronary angiography within 6 months after PCI, and the results showed that the diameter of ISR exceeded 50% (17).

Statistical analysis

SPSS 19.0 (Asia Analytics Formerly SPSS China) was used to analyze the collected data. Measurement data were expressed in rate (%) and the comparison of rates adopted χ^2 test. The counting data were expressed in mean ± standard deviation (mean±sd). Independent sample *t* test was used for comparison between the two groups, and paired sample *t* test was used for analysis before and after treatment. ROC was used to analyze the predictive value of miR-130a and ET-1 for ISR. Taking cut-off value as critical value, ≤ cut-off was considered as low expression group, > cut-off as high expression group. K-M curve was used to compare the difference in the ISR incidence between the two groups, and logistics regression to analyze the risk factors of of ISR in CHD patients after PCI. A value of $P < 0.05$ indicated a statistical significance.

Results

General data

A total of 253 CHD patients received PCI treatment, including 173 males (68.38%) and 80 females (31.62%). There were 89 patients under 60 years old (35.18%) and 164 over 60 (64.82%), 180 with BMI ≥ 24 kg/m^2 (71.15%) and 73 < 24 kg/m^2 (28.85%) (Table 2).

Changes of miR-130a and ET-1 levels after PCI

The postoperative levels of miR-130a and ET-1 in patients were significantly higher than the preoperative ones ($P < 0.05$) (Fig. 1).

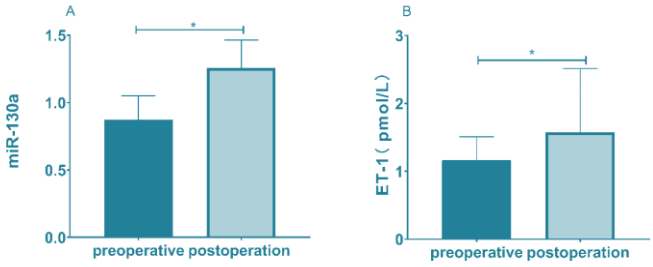

Fig. 1: Changes of miR-130a and ET-1 levels after PCI **A:** Changes of miR-130a level after PCI. **B:** Changes of ET-1 level after PCI. * indicates that $P<0.05$

Table 2: General data [n(%)]

Variable	*Research object (n=253)*
Sex	
Male	173(68.38)
Female	80(31.62)
Age (yr)	
≤60	89(35.18)
>60	164(64.82)
BMI (kg/m²)	
≥24	180(71.15)
<24	73(28.85)
Coronary artery lesions	
Single branch	70(27.67)
Multiple branches	183(72.33)
Smoking history	
Yes	182(71.94)
No	71(28.06)
Length of lesion vessel (mm)	9.36±1.13
Preoperative arterial stenosis (%)	77.42±4.25
Stent type	
Drug-eluting stent	182(71.94)
Bare stent	71(28.06)
Number of stents implanted	
1	46(18.18)
≥2	207(81.82)

Incidence of ISR within 6 months after operation

Of 253 patients, 49 developed ISR within 6 months after operation (19.38%).

MiR-130a and ET-1 levels in patients with ISR after operation

Compared with patients without ISR, the expression levels of miR-130a and ET-1 in peripheral blood of patients with ISR were significantly increased ($P < 0.05$) (Fig. 2).

Predictive value of miR-130a and ET-1 for ISR

ROC analysis showed that the area under curve (AUC), sensitivity, specificity and critical value of miR-130a in predicting ISR were respectively 0.912, 92.02%, 73.47%, 1.457 pmol/L, and those of ET-1 were 0.814, 87.63%, 63.27%, 2.245 pmol/L, respectively (Fig. 3).

Difference analysis of ISR incidence between the two groups

Taking cut-off value as critical value, ≤ cut-off was considered as low expression group, > cut-off as high expression group. The K-M curve showed that the ISR incidence in patients with high expression of miR-130a or ET-1 was significantly higher than that in patients with low expression ($P < 0.05$) (Fig. 4).

Analysis on risk factors of ISR

Body mass index (BMI), miR-130a and ET-1 were risk factors of ISR after PCI in CHD patients ($P < 0.05$), of which miR-130a and ET-1 were independent risk factors ($P < 0.05$) (Tables 3-5).

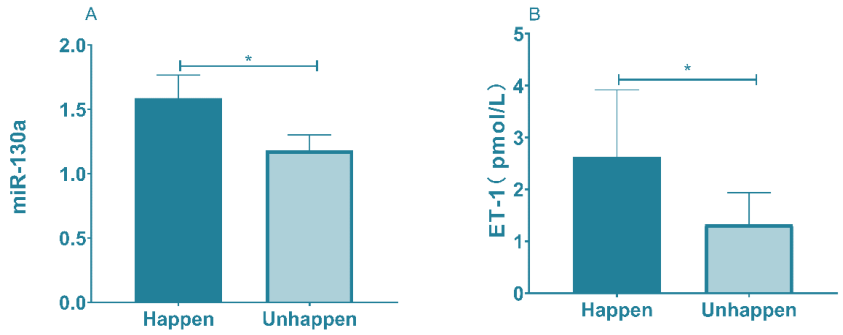

Fig. 2: miR-130a and ET-1 levels in patients with ISR after operation **A:** miR-130a level in patients with ISR after surgery. **B:** ET-1 level in patients with ISR after surgery. * indicates that $P < 0.05$

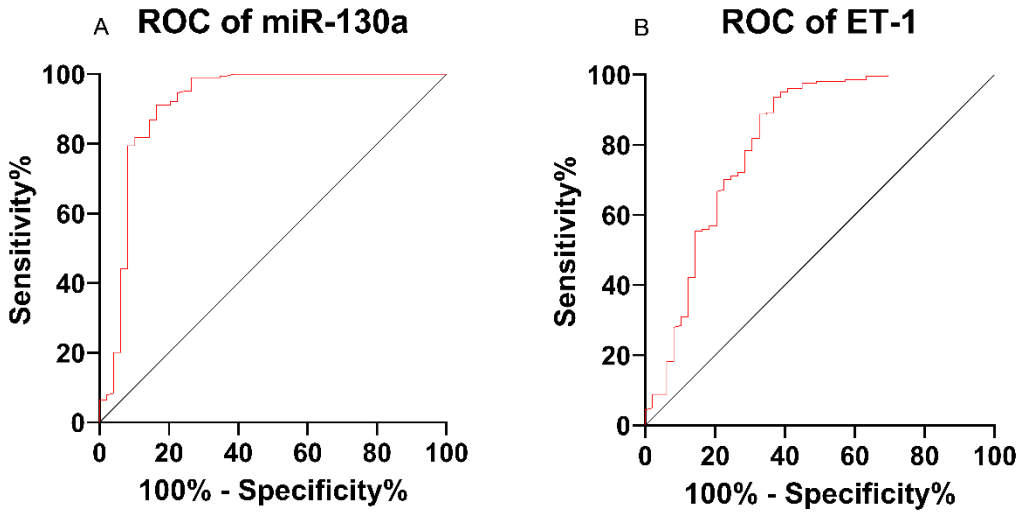

Fig. 3: Predictive value of miR-130a and ET-1 for ISR **A:** The predictive value of miR-130a for ISR. AUC (miR-130a) = 0.912. **B:** The predictive value of ET-1 for ISR. AUC (ET-1) = 0.814

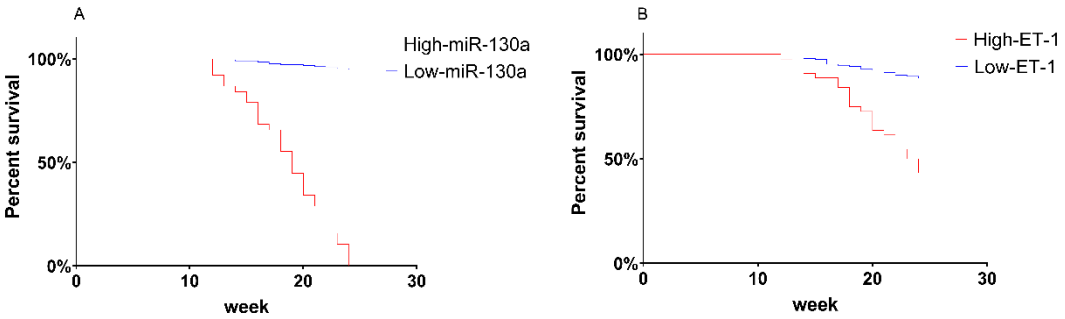

Fig. 4: Difference analysis of ISR incidence between the two groups **A:** Relationship between miR-130a and ISR incidence. The ISR incidence in patients with high expression of miR-130a was significantly higher than that in patients with low expression ($P < 0.05$). **B:** Relationship between ET-1 and ISR incidence. The ISR incidence in patients with high expression of ET-1 was significantly higher than that in patients with low expression ($P < 0.05$)

Table 3: Assignment Table

Sex	***male=1, female=0***
Age (yr)	≤60=1, >60=0
BMI (kg/m²)	≥24=1, <24=0
Coronary artery lesions	single branch =1, multiple branches =0
Smoking history	yes =1, no =0
Length of lesion vessel (mm)	continuous variable
Preoperative arterial stenosis (%)	continuous variable
Type of bracket	drug-eluting stent =1, bare stent =0
Number of stents implanted	1=1, ≥2=0
miR-130a	>1.457=1, ≤1.457=0
ET-1 (pmol/L)	>2.245=1, ≤2.245=0

Table 4: Univariate analysis

Variable	***Happen (n=49)***	***Unhappen (n=204)***	***χ²/t***	**P**
Sex			0.728	0.394
Male	36 (73.47)	137 (67.16)		
Female	13 (26.53)	67 (32.84)		
Age (yr)			0.556	0.456
≤60	15 (30.61)	74 (36.27)		
>60	34 (69.39)	130 (63.73)		
BMI (kg/m²)			4.656	0.031
≥24	41 (83.67)	139 (68.14)		
<24	8 (16.33)	65 (31.86)		
Coronary artery lesions			0.755	0.385
Single branch	16 (32.65)	54 (26.47)		
Multiple branches	33 (67.35)	150 (73.53)		
Smoking history			2.830	0.093
Yes	40 (81.63)	142 (69.41)		
No	9 (18.37)	62 (30.39)		
Length of lesion vessel (mm)	9.46±1.16	9.37±0.19		
Preoperative arterial stenosis (%)	77.31±4.22	77.43±0.39		
Stent type			0.384	0.585
Drug-eluting stent	37 (75.51)	145 (71.08)		
Bare support	12 (24.49)	59 (28.92)		
Number of stents implanted			0.620	0.431
1	7 (14.29)	39 (19.12)		
≥2	42 (85.71)	165 (80.88)		
miR-130a			Fisher	<0.001
>1.457	38 (77.55)	0 (0.00)		
≤1.457	11 (22.45)	204 (100.00)		
ET-1 (pmol/L)			47.836	<0.001
>2.245	25 (51.02)	19 (9.31)		
≤2.245	24 (48.98)	185 (90.69)		

Table 5: Multivariate analysis

Variable	*B*	*SE*	*Wals*	*df*	*Sig.*	*Exp (B)*	*95%CI*	
							Lower limit	*Upper limit*
BMI	-0.335	0.862	0.151	1	0.697	0.715	0.132	3.874
miR-130a	21.908	3.989	30.164	1	0.001	12.465	4.387	20.762
ET-1	1.808	0.543	11.081	1	0.001	6.099	2.103	17.687

Discussion

ISR usually occurs 3-6 months after PCI, which seriously affects the prognosis of patients (18). Coronary angiography is an important method for clinical judgment of ISR after PCI. However, due to its invasive and complicated operation, the prediction value for ISR is not high, and there is a lack of specific prediction indicators (19). Therefore, this study aimed to explore the prediction value of miR-130a and ET-1 for ISR after PCI for clinical reference.

A total of 253 patients with CHD were included in this study through strict inclusion and exclusion criteria, 49 of whom suffered from ISR after PCI (19.38%). The levels of miR-130a and ET-1 in peripheral blood of patients increased significantly after PCI. It may be related to vascular injury, because stent implantation often causes endothelial injury due to inflammatory stress (20). miR-130a and ET-1 are increased after vascular injury (21, 22).

VSMCs have the characteristic of phenotypic transformation. In peripheral vascular obstructive diseases, there are vascular smooth muscle contraction and decreased expression of marker genes, as well as increased expression of proliferation, migration-related genes, which are also closely related to ISR in patients after operation (23). MiR is a new endogenous non-protein coding small molecule RNA that can regulate hundreds of genes (24). MiR-130a has been reported to promote proliferation of VSMCs (25). In addition, ET-1 is increased in giant cell arteritis, which promotes VSMCs migration to the intima, thus leading to intimal hyperplasia and vascular occlusion (26).

miR-130a and ET-1 have good predictive value for ISR, which is also confirmed in our results. ROC analysis results showed that the AUC, sensitivity and specificity of miR-130a in predicting ISR were respectively 0.912, 92.02%, 73.47%, and those of ET-1 were 0.814, 87.63%, 63.27%, respectively. At present, there are few reports on the prediction of ISR by miR-130a. The AUC of miRNA-143 in predicting ISR was 0.866, and sensitivity and specificity were 83.7% and 82.6% respectively (10), both lower than our results. In 20 cases of peripheral arterial occlusive diseases, the serum ET-1 level 1 hour after operation had the best predictive value for ISR (diagnostic threshold: 0.1089 ng/ml; sensitivity and specificity: both 85%) (27). Compared with our results, the reported specificity was higher, which may be related to the detection time.

In this study, the ET-1 level at 24 hours after operation was used to predict ISR. Therefore, the difference between these results needs to be further analyzed at more time points, but our study included more research subjects. Moreover, this study also further analyzed the relationship between miR-130a, ET-1 and ISR. Taking the cut-off value as the critical value, the K-M curve showed that the ISR incidence in patients with higher miR-130a, ET-1 cut-off values was significantly higher than that in patients with lower cut-off values, which again verifies the predictive value of miR-130a, ET-1 for ISR. Our analysis results also showed that miR-130a and ET-1 were independent risk factors for ISR after PCI in CHD patients, which is rarely reported in other studies.

There are some deficiencies in our study. This study was a single-center prospective analysis, so there might be some bias in the inclusion of pa-

tients. Besides, compared with the big data study, this study includes few subjects and some of the results are lack of supporting documents. Therefore, the conclusion still needs more data analysis for verification. Moreover, the collection of peripheral blood at different time points after surgery may also affect the analysis results. Further improved research process and more time points are needed for analysis. In addition, in a study (9), the predictive value of miR-130a for ISR was not found, which needs to be further analyzed. We hope to expand sample size and carry out related animal experiments to solve our puzzles.

Conclusion

miR-130a and ET-1 have high predictive value for ISR after PCI and are independent risk factors for CHD patients, which are worthy of clinical application.

Ethical Considerations

Ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely considered by the authors.

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There was no financial source.

Conflicts of interests

The authors declare that there is no conflict of interests.

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