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Original Article

Increased plasma miR-16 is associated with poor prognosis for acute myocardial infarction

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Abstract: Objective: MicroRNAs (miRNAs) play a crucial role in regulating many physiological and pathological processes. Although increasing number of biomarkers have been identified for early detection and predicating the prognosis of acute myocardial infarction (AMI), they are still not ideal enough. Method: The aim of current study was to evaluate the expression pattern of plasma miR-16 in patients with AMI using real-time PCR and to explore its potential clinical value in AMI progression. Results: Our results showed that plasma miR-16 was significantly elevated in AMI patients compared to the healthy volunteers. In addition, plasma miR-16 level distinguished AMI patients from controls with an area under a receiver operating characteristic (ROC) curve (AUC) value of 0.813. Plasma miR-16 level was also found to associated with many clinical variables including left ventricular ejection fraction, GRACE score and Killip class. Moreover, patients in the high plasma miR-16 group had more unfavorable three-year overall survival and major adverse cardiac events (MACE) than those in the low plasma miR-16 group. High plasma miR-16 was demonstrated to be an independent risk factor for AMI. Conclusion: Collectively, the peripheral plasma miR-16 level remarkably increases following cardiac infarction and might serve as a novel biomarker for the diagnosis and prognosis of AMI.

Keywords: Acute myocardial infarction, biomarker, diagnosis, plasma miR-16, prognosis

Introduction

Acute myocardial infarction (AMI) is still one of the most frequent cardiovascular diseases that leads to high mortality and morbidity worldwide. Currently characteristic clinical symptoms, electrocardiogram (ECG) changes, and cardiac markers elevation are the basis for the diagnosis and prognosis prediction. However, they have significant limitation [1]. Accurate diagnosis as well as stratification of patients with AMI is crucial for the effective evidence-based therapeutic intervention. Therefore, it is urgent to explore novel molecular biomarker meet the clinical requirement.

MiRNAs are a group of highly conserved, small non-coding, single-stranded RNAs which negatively regulate gene expression at the post-transcriptional level [2]. These molecules involve in a variety of biological processes such as proliferation, differentiation, angiogenesis and metabolism [3]. Numerous studies have demonstrated that miRNAs are key regulators of many human diseases including cardiovascular diseases [4-6]. In addition, miRNAs are extremely stable in the circulatory system [7]. Thus, miRNAs have become attractive biomarkers for prediction and prognosis for AMI. For instance, the expression levels of circulating miR-146a and miR-21 were remarkably increased in AMI patients compared with the healthy volunteers. In addition, miR-146a and miR-21 levels were both independent predictors of left ventricular remodeling development in patients suffering from AMI [8]. Similarly, miR499a-5p level was significantly elevated in the patients with ST elevation myocardial infarction (STEMI) in comparison with the healthy controls. Moreover, it remained to be an independent prognostic factor of STEMI even after adjusting various kinds of risk variables [9].

Previous studies have reported that miR-16 was aberrantly expressed during the development of cardiovascular diseases [10]. However, whether plasma miR-16 had clinical significance remains unclear. Therefore, the goal of

Table 1. The clinical characteristics of AMI patients and controls

Variables	AMI patients (n = 116)	Healthy controls (n = 30)	*P*
Gender (male)	74 (63.8%)	14 (46.7%)	0.088 (z = 1.709)
Age	57.36±8.21	56.72±12.39	0.265 (t = 1.120)
Obesity	28 (24.1%)	5 (16.7%)	0.383 (z = 0.872)
Hypertension	69 (59.5%)	16 (53.3%)	0.543 (z = 0.609)
Hyperlipidemia	36 (31.0%)	8 (26.7%)	0.642 (z = 0.465)
DM	32 (27.6%)	9 (30.0%)	0.793 (z = 0.262)
Smoking	53 (45.7%)	5 (16.7%)	0.004 (z = 2.896)
Stroke	5 (4.3%)	0 (0%)	0.247 (z = 1.157)
Prior MI	13 (11.2%)	0 (0%)	0.055 (z = 1.921)
TC, mmol/L	4.42 (1.74-6.81)	4.16 (1.82-7.03)	0.482 (t = 0.705)
LDL, mmol/L	3.51(0.39-5.66)	1.98 (0.57-3.54)	<0.001 (t = 3.863)
HDL, mmol/L	1.06 (0.34-2.13)	1.12 (0.49-2.28)	0.327 (t = 0.984)
TG, mmol/L	1.48 (0.41-7.53)	1.45 (0.36-5.38)	0.813 (t = 0.237)
Creatinine, μmol/L	67 (37-205)	65 (41-108)	0.139 (t = 1.489)
LVEF %	45 (23-58)	60 (57-64)	<0.001 (t = 3.452)
eGFR	105 (34-206)	106 (49-202)	0.621 (t = 0.496)
cTnI, ng/mL	4.42 (0.005-116.800)	0.02 (0-0.152)	<0.001(t = 8.247)

DM: diabetes mellitus; MI: myocardial infarction; TC: total cholesterol; TG: trigliserid; HDL: high density lipoprotein; LDL: low density lipoprotein; LVEF: left ventricular ejection fraction; eGFR: estimated glomerular filtration rate; cTnI: cardiac troponin I.

current study was to investigate the prognostic value of plasma miR-16 in patients after AMI.

Materials and methods

Study population

This study was conducted in accordance with the Helsinki declaration and approved by the Ethics Committee of Xi'an No. 1 Hospital. All the participants signed the informed consent. A total of 116 patients with STEMI or non-STEMI were enrolled in this study. The diagnosis of AMI was based on characteristic clinical symptoms, electrocardiogram (ECG) changes, and cardiac troponin I elevation. Patients with malignant diseases or other critical diseases such as liver failure and end-stage renal disease were excluded from this study. The control group was made up of 30 healthy people who received routine physical examination in our hospital. All the clinical data were retrieved from computerized medical records. The clinical characteristics of AMI patients and healthy controls were summarized in **Table 1**.

Sample collection

Peripheral blood (5 mL) was obtained from all the subjects and processed within 2 h after sample collection. The plasma samples from AMI patients were withdrawn within 12 h following infarction. The procedures to obtain plasma were as follows: briefly, the blood samples were centrifuged at 400× g for 20 min at 4°C, followed by centrifugation of the supernatants at 800× g for 20 min at 4°C. Resulting supernatants were transferred to new RNase-free tubes and stored at -80°C.

Quantitative real-time PCR

Total RNA was extracted from 300 μL plasma using an miRNeasy serum/plasma kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA concentration was determined by NanoDrop 2000 Spectrophotometer (NanoDrop Technologies, Waltham, MA). The amounts of miR-16 in the plasma samples were quantified by qRT-PCR using human TaqMan MicroRNA Assay Kits (Applied Biosystems, Foster City, CA, USA). RNA was first reverse transcribed to cDNA and then undergone PCR amplification. The reaction was run on a 7300 Real-time PCR system (Applied Biosystems) and it conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min. All samples were run in triplicate. Relative expression of miR-16 was nor-

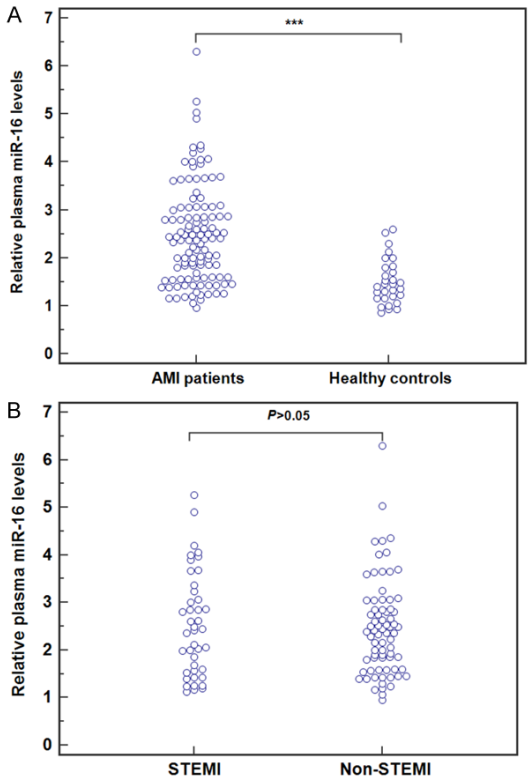

Figure 1. Plasma miR-16 was upregulated in patients with AMI.

malized to the U6 snRNA endogenous control and was calculated by the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA) for Windows and Medcalc 8.3.1.1 (MedCalc Software, Mariakerke, Belgium). The differences of continuous data between groups were analyzed by the Mann-Whitney U-test or Student's t-test. Categorical variables were compared using the Chi-squared test. The area under the receiver operating characteristic (ROC) curve (AUC) was used to assess the performance of plasma miR-16 to distinguish AMI patients from healthy controls. Kaplan-Meier survival analysis was conducted to compare the difference in three-year overall survival and major adverse cardiac events (MACE) rates between patients in high and low plasma miR-16 group using the log-rank test. Multivariate logistic regression analysis was used to determine the independent predictors of the AMI. A *P*-value less than 0.05 was considered statistically significant.

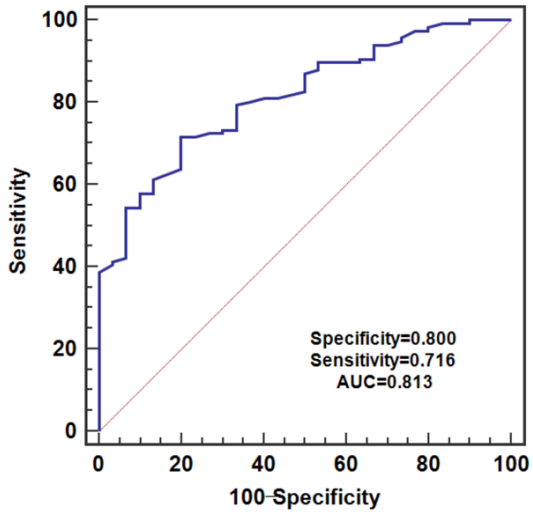

Figure 2. The diagnostic value of plasma miR-16 for AMI.

Results

Plasma miR-16 was upregulated in patients with AMI

We first compared the expression level of plasma miR-16 between AMI patients and healthy controls using real-time PCR. Our results showed that plasma miR-16 level was significantly upregulated in both STEMI and non-STEMI patients compared with healthy controls ($P<0.001$) (**Figure 1A**). However, no significant difference in plasma miR-16 expression was found between STEMI patients and non-STEMI patients ($P>0.05$) (**Figure 1B**). In addition, our ROC analysis revealed that plasma miR-16 was able to accurately discriminate AMI patients from healthy controls, with the AUC value of 0.813 (cutoff value = 1.82 fold, specificity = 0.800, sensitivity = 0.716) (**Figure 2**).

The association between plasma miR-16 and clinical variables of AMI

All the 116 AMI patients were divided into high plasma miR-16 group and low plasma miR-16 group by the median value of plasma miR-16 (cutoff value = 2.40 fold). Sixty patients were in the high plasma miR-16 group while 56 patients were in the low plasma miR-16 group. Our data showed that plasma miR-16 was significantly associated with left ventricular ejection fraction ($P<0.001$) and GRACE score ($P = 0.006$).

Table 2. The association between plasma miR-16 levels and clinical variables of AMI

Variables	High miR-16 (n = 60)	Low miR-16 (n = 56)	*P*
Gender (male)	38 (63.3%)	36 (64.3%)	0.915 (z = 0.107)
Age	56.58±7.32	58.18±6.56	0.643 (t = 0.465)
Obesity	16 (26.7%)	12 (21.4%)	0.510 (z = 0.659)
Hypertension	33 (55.0%)	36 (64.3%)	0.309 (z = 1.108)
Hyperlipidemia	19 (31.7%)	17 (30.4%)	0.879 (z = 0.152)
DM	18 (30.0%)	14 (25.0%)	0.547 (z = 0.602)
Smoking	29 (48.3%)	24 (42.8%)	0.554 (z = 0.592)
Stroke	3 (5.0%)	2 (3.6%)	0.705 (z = 0.379)
Prior MI	8 (13.3%)	5 (8.9%)	0.452 (z = 0.752)
TC, mmol/L	4.34 (1.74-6.41)	4.60 (1.85-6.81)	0.219 (t = 1.326)
LDL, mmol/L	3.65(0.52-5.66)	3.36 (0.39-5.48)	0.240 (t = 1.182)
HDL, mmol/L	1.07 (0.34-2.13)	1.05 (0.36-2.10)	0.765 (t = 0.299)
TG, mmol/L	1.52 (0.41-7.53)	1.44 (0.48-7.47)	0.411 (t = 0.825)
Creatinine, μmol/L	67 (39-205)	67 (37-203)	0.953 (t = 0.059)
LVEF %	36 (23-44)	54 (28-58)	<0.001 (t = 3.530)
eGFR	103 (34-201)	107 (38-206)	0.530 (t = 0.630)
cTnI, ng/mL	4.56 (0.018-116.800)	4.27 (0.005-108.400)	0.062 (t = 1.882)
GRACE score	172.00±30.54	118.60±19.39	0.006 (t = 2.783)

DM: diabetes mellitus; MI: myocardial infarction; TC: total cholesterol; TG: trigliserid; HDL: high density lipoprotein; LDL: low density lipoprotein; LVEF: left ventricular ejection fraction; eGFR: estimated glomerular filtration rate; cTnI; cardiac troponin I.

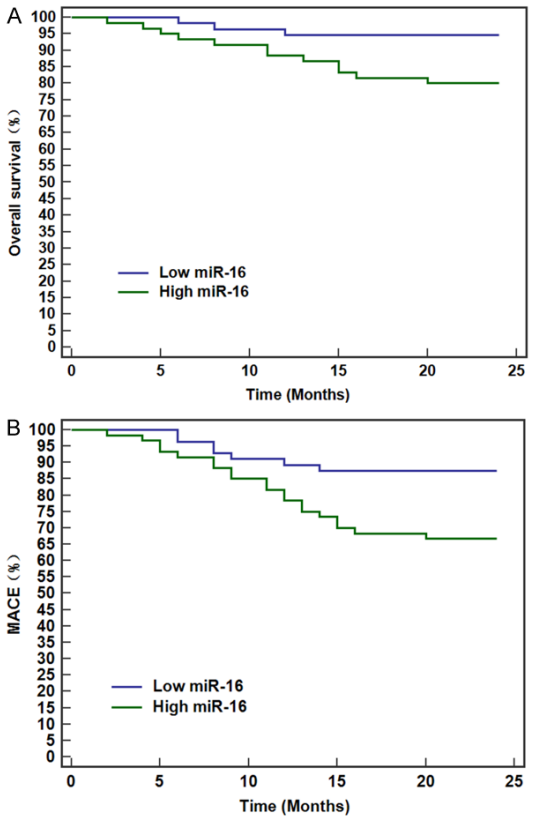

Figure 3. The prognostic value of plasma miR-16 for AMI.

However, it was not correlated with other clinical variables listed in **Table 2**.

The prognostic value of plasma miR-16

As shown in **Figure 3A**, a Kaplan-Meier survival curve showed that the AMI patients in the high plasma miR-16 group had a significantly shorter survival when compared with those in the low plasma miR-16 group ($P<0.01$). Moreover, patients above the median levels of plasma miR-16 suffered a significantly high risk of MACE compared to the patients below the median value ($P<0.01$) (**Figure 3B**).

Multivariate logistic regression analysis showed that STEMI (OR = 2.40, 95% CI = 1.31-4.03, $P = 0.005$), Grace score (OR = 2.86, 95% CI = 1.65-5.46, $P = 0.003$) and high plasma miR-16 (OR = 1.87, 95% CI = 1.12-3.18, $P = 0.029$) were independent prognostic factors for AMI.

Discussion

AMI is the most serious cardiovascular disease and remains to be a significant public health problem around the world [11]. The use of laboratory markers for diagnosis and prognosis of AMI has saved many lives in the past few

decades. However, these biomarkers have changed dramatically, indicating that they are not sensitive and specific enough [12]. Currently cardiac troponins represent the biochemical gold standard for diagnosing AMI, but it still has many shortcomings. Therefore, there is a clinical need for exploring novel marker.

In the current study, our data demonstrated that miR-16 was significantly increased in the plasma samples from AMI patients compared with those from healthy controls, and plasma miR-16 had good discriminatory capacity. In addition, plasma miR-16 levels were associated with left ventricular ejection fraction, GRACE score and Killip class. Moreover, patients with higher plasma miR-16 had more unfavorable overall survival and higher risk of MACE compared to the patients with lower plasma miR-16. Finally, high plasma miR-16 was demonstrated to be an independent risk factor for AMI. These findings suggest that miR-16 might be a key regulator during the pathogenesis of AMI. Consistent with the findings in previous studies, AMI patients with elevated plasma miR-16 had a higher risk of suffering impaired left ventricular (LV) contractility, indicating miR-16 was associated with adverse LV remodeling [10]. One limitation of this study was the small sample size, further investigation with larger number of patients should be carried out to validate the clinical significance of plasma miR-16 in AMI. Also, the molecular mechanisms responsible for the role of miR-16 in AMI should also investigated in animal models.

MiR-16 maps to the human chromosome 13q14 region, a chromosomal region that is frequently deleted in human cancers [13]. miR-16 has been reported to be downregulated in many types of cancers and regards as a tumor suppressor [14-16]. It is probably that plasma miR-16 is reduced in cancer patients, which is the reason why we exclude the patients with malignancy from this study. However, the molecular function of miR-16 remains poorly understood. Various studies have suggested that miR-16 was closely associated with cardiac function. Kuosmanen et al globally profiled the miRNAs in the pericardial fluid from patients with heart failure. They reported that miR-21-5p, miR-451a, miR-125b-5p, let-7b-5p and miR-16-5p were abundantly expressed in the pericardial fluid samples, indicating that the cardiac cells might secret these miRNAs into the pericardial fluid [17]. miR-16 levels were found to be robustly upregulated in rat model with hypertension-induced heart failure. In addition, its level increased during disease progression and had a positive correlation with circulating B-type natriuretic peptide, indicating miR-16 might be closely correlated with the severity of heart failure [9]. The expression level of miR-16 was upregulated in human bone marrow mesenchymal stem cells cultured in cardiac niche. In addition, ectopic overexpression of miR-16 promoted the differentiation of hMSCs toward myogenic phenotypes [18].

Taken together, our results proved that plasma miR-16 was remarkably elevated in AMI patients and its levels was significantly correlated with the prognosis of AMI, indicating plasma miR-16 might be a promising diagnostic and prognostic biomarker for this deadly cardiovascular disease.

Disclosure of conflict of interest

None.

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References

[1] Jenab Y, Pourjafari M, Darabi F, Boroumand MA, Zoroufian A, Jalali A. Prevalence and determinants of elevated high sensitivity cardiac troponin T in hypertrophic cardiomyopathy. J Cardiol 2014; 63: 140-4.

[2] Mendell JT. MicroRNAs: critical regulators of development, cellular physiology and malignancy. Cell Cycle 2005; 4: 1179-84.

[3] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-97.

[4] Alvarez-Garcia I, Miska EA. MicroRNA functions in animal development and human disease. Development 2005; 132: 4653-62.

[5] Romaine SP, Tomaszewski M, Condorelli G, Samani NJ. MicroRNAs in cardiovascular disease: an introduction for clinicians. Heart 2015; 101: 921-8.

[6] Cavarretta E, Frati G. MicroRNAs in coronary heart disease: ready to enter the clinical arena? Biomed Res Int 2016; 2016: 2150763.

[7] Liu X, Dong Y, Chen S, Zhang G, Zhang M, Gong Y, Li X. Circulating microRNA-146a and microRNA-21 predict left ventricular remodeling after ST-elevation myocardial infarction. Cardiology 2015; 132: 233-41.
[8] O Sullivan JF, Neylon A, McGorrian C, Blake GJ. miRNA-93-5p and other miRNAs as predictors of coronary artery disease and STEMI. Int J Cardiol 2016; 224: 310-316.
[9] Dickinson BA, Semus HM, Montgomery RL, Stack C, Latimer PA, Lewton SM, Lynch JM, Hullinger TG, Seto AG, van Rooij E. Plasma microRNAs serve as biomarkers of therapeutic efficacy and disease progression in hypertension-induced heart failure. Eur J Heart Fail 2013; 15: 650-9.
[10] Devaux Y, Vausort M, McCann GP, Kelly D, Collignon O, Ng LL, Wagner DR, Squire IB. A panel of 4 microRNAs facilitates the prediction of left ventricular contractility after acute myocardial infarction. PLoS One 2013; 8: e70644.
[11] Sacks NC, Ash AS, Ghosh K, Rosen AK, Wong JB, Rosen AB. Trends in acute myocardiac infaction hospitalization: Are we seeing the whole picture? Am Heart J 2015; 17: 1211-9.
[12] Danese E, Montagnana M. An historical approach to the diagnostic biomarkers of acute coronary syndrome. Ann Transl Med 2016; 4: 194.
[13] Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chroniclymphocytic leukemia. Proc Natl Acad Sci U S A 2002; 99: 15524-9.
[14] Bonci D, De Maria R. miR-15/miR-16 loss, miR-21 upregulation, or deregulation of their target genes predicts poor prognosis in prostate cancer patients. Mol Cell Oncol 2015; 3: e1109744.
[15] Li F, Xu Y, Deng S, Li Z, Zou D, Yi S, Sui W, Hao M, Qiu L. MicroRNA-15a/16-1 cluster located at chromosome 13q14 is down-regulated but displays different expression pattern and prognostic significance in multiple myeloma. Oncotarget 2015; 6: 38270-82.
[16] Lovat F, Fassan M, Gasparini P, Rizzotto L, Cascione L, Pizzi M, Vicentini C, Balatti V, Palmieri D, Costinean S, Croce CM. miR-15b/16-2 deletion promotes B-cell malignancies. Proc Natl Acad Sci U S A 2015; 112: 11636-41.
[17] Kuosmanen SM, Hartikainen J, Hippeläinen M, Kokki H, Levonen AL, Tavi P. MicroRNA profiling of pericardial fluid samples from patients with heart failure. PLoS One 2015; 10: e0119646.
[18] Liu JL, Jiang L, Lin QX, Deng CY, Mai LP, Zhu JN, Li XH, Yu XY, Lin SG, Shan ZX. MicroRNA 16 enhances differentiation of human bone marrow mesenchymal stem cells in a cardiac niche toward myogenic phenotypes in vitro. Life Sci 2012; 90: 1020-6.