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Plasma miR-181a as a Candidate Diagnostic Biomarker for Kawasaki Disease Patients with Coronary Artery lesions

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Abstract

Background: Kawasaki disease (KD) is an acute and systemic vasculitis, and the critical complication in KD patients is coronary artery lesions (CAL). Plasma miR-181a was found dysregulated in a variety of cardiovascular disease. The aim of this study was to define the relationship between the plasma miR-181a levels and CAL in KD. **Methods:** Plasma miR-181a levels were analyzed by quantitative reverse transcriptase-polymerase chain reaction in 121 patients with KD. **Results:** We found that plasma miR-181a levels at the acute phase were significantly elevated in KD patients with CAL than those without CAL. Correlation analysis showed that plasma miR-181a levels were positively correlated with the concentrations of CRP ($r=0.363$, $P < 0.05$) and NT-proBNP ($r=0.389$, $P < 0.05$). Receiver operating characteristic curve analyses showed that plasma miR-181a was of significant prediction value for CAL in KD, the area under receiver operating characteristic curve value for plasma miR-181a in prediction of CAL was 0.747, and the estimated sensitivity and specificity were 75.0% and 68.8%, respectively. **Conclusions:** Plasma miR-181a is prone to be a candidate biomarker for predicting CAL in KD. Therefore, further investigations are warranted to fully elucidate its role in KD.

Keywords: Kawasaki disease; miR-181a; biomarker; coronary artery lesions

Background

Kawasaki disease (KD) is an acute febrile illness characterized by systemic vasculitis mainly affecting small and medium sized arteries, particularly the coronary artery. The prevalence of KD has been increasing dramatically over the past few years, it occurs predominantly in children younger than 5 years old[1]. Coronary artery lesions (CAL) are the most common and serious complication of KD, developing in 25% of untreated patients and 5% of patients treated with intravenous immunoglobulin (IVIG) and aspirin[2]. Therefore, KD has become the leading cause of acquired heart disease in children and is also an important cause of cardiovascular disease in young adults. According to the medical records of 261 young adults with suspected myocardial infarction (MI), Daniels et al.[3] found that 5% of them had a KD history in their childhoods.

MicroRNAs (miRNAs) are small, endogenous, noncoding RNAs that are epigenetic regulators of cellular protein levels through translational repression and transcript cleavage by pairing with the 3′-untranslated regions (3′-UTRs) of target mRNAs. Accumulating evidence has shown that miRNAs play crucial roles in various biological processes, such as proliferation, differentiation, angiogenesis and apoptosis[4-6]. Many miRNAs are remarkably stable and readily detectable in biological fluids like plasma, and the levels of plasma miRNAs vary under different physiological and pathological conditions[7], indicating that plasma miRNAs could serve as potential biomarkers for early diagnosis and prediction of prognosis in numerous human diseases. The amounts of research reports have

demonstrated that plasma miRNAs serve as potential biomarkers in diseases including cancer[8], autoimmune disease [9], cardiovascular disease[10] and so on.

MiR-181a is a highly studied miRNA that plays important roles in various biological events, and its levels were shown to change correlating with disease activity and/or disease prognosis[11,12]. There is an increasing amount of evidence that support the importance of miR-181a as regulator in the development of coronary artery disease, Jianbing Zhu et al.[13] reported that miR-181a could be a novel biomarker for diagnosing acute myocardial infarction (AMI), Bailin Liu et al.[14] discovered that miR-181a had an intimate association with exaggerated coronary vascular tone. However, the specific expression patterns of miR-181a in KD patients with CAL remain unknown. The current study was designed to determine if the levels of plasma miR-181a correlate with the development of KD and development of CAL.

Methods

Study subjects

We recruited 121 KD patients hospitalized in Jiangxi Children's Hospital from January 2015 to December 2017. The inclusion criteria for KD patients were based on the scientific statement from American Heart Association (AHA) [15], all patients diagnosed with KD in the acute phase were treated with IVIG and aspirin. Clinical data including demographic, baseline clinical characteristics, laboratory features, and coronary outcome, were recorded for all participants. Exclusion criteria included (1) receipt of immunosuppressive and glucocorticoid therapy before blood collection; (2) initial IVIG treatment started before admission to our hospital; (3) incomplete medical data; and (4) patients not in the acute phase when admission to our hospital. IVIG-resistant is defined as persistent or recrudescent fever ≥ 36 h after completion of the initial IVIG treatment[15]. CAL was diagnosed on the basis of echocardiography and defined as a coronary artery with an internal lumen diameter of at least 3 mm (4 mm if the patient was more than 5 years old), a segment with an internal diameter at least 1.5 fold larger than that of an adjacent segment[16], or a Z score ≧ 2.5[15]. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Medical Ethics Committee of Jiangxi Province Children's Hospital (JXSETYY-YXKY-20190070).

Blood Sampling and quantitative real-time PCR

We drawn whole blood samples (2ml) into EDTA-containing tubes at two time points during the study: before IVIG treatment and two days after IVIG treatment. Samples were separated by centrifugation at 3000 ×g for 5 min at 4℃. After separation, plasma was stored at −80°C until used. The quantitative real-time PCR was used to evaluate the expression levels of plasma miR-181a. Total RNA was extracted from the plasma using a TRIzol reagent (Invitrogen Life Technologies, CA) according to the manufacturer's instructions. cDNA was reverse-transcribed using miScript Reverse Transcription Kit (Qiagen, Germany) according to the manufacturer's directions. Quantitative real-time PCR (qRT-PCR) was performed in triplicate using the ABI Prism 7500 sequence detection system (Applied Biosystems). The data were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to RNA U6 controls.

Statistical analysis

Data were presented as the mean ± SD, median with range or percentage of patients. Independent sample t-tests and Mann-Whitney U tests were performed to compare groups of continuous variables. Categorical data were analyzed using the Chi-squared test. ROC curve analysis and comparison of the derived area under the curve (AUC) were performed to assess the miRNA as a predictor for distinguishing CAL from non-CAL. Correlations between variables were determined by Spearman tests. Analyses were performed using SPSS software (version 24). Statistical significance was set at $P < 0.05$.

Results

Patient's characteristics

Demographic and clinical data were presented in Table 1. A total of 121 KD patients, including 43 females, and 78 males, were enrolled in this study. The median age of KD patients was 19.0 months, with a range from 2 months to144 months. Most of patients (88 patients, 72.7%) were complete KD, and 33 patients (27.3%) were incomplete KD. The majority of patients responded to the initial IVIG treatment (96, 79.3%), and the remaining 25 patients (20.7%) were regarded as non-responders. CAL was found in 26 (21.5 %) of the KD patients. Mucosal changes, conjunctival injection, rash, changes in extremities and cervical lymphadenopathy occurred in 88.4%, 83.5%, 62.8%, 42.1% and 65.3% of patients, respectively.

Circulating miR-181a expression levels

We analyzed the levels of circulating miR-181a in different groups of KD. Circulating miR-181a levels in KD patients at two days after initial IVIG infusion were found to be significantly lower than the levels before IVIG infusion ($P < 0.05$, Fig1A). No significant difference in circulating miR-181a levels was found between complete KD group and incomplete KD group (Figure1B),
and the same was the case with IVIG-response group and IVIG-resistant group (Figure1C). The difference of circulating miR-181a between KD patients with and without CAL was presented in Figure1D. The level of circulating miR-181a expression levels were significantly elevated in KD patients with CAL when compared with their non-CAL counterparts ($p<0.05$).

The demographic and laboratory characteristic in KD with and without CAL

There was no significant difference between the KD patiens with CAL and without CAL in age and sex distribution. We then compared the laboratory data in those patients. As given in Table 2, the patients with CAL had significantly higher levels of neutrophil count ($p =0.043$), C-reactive protein (CRP) ($p=0.012$) and N-terminal pro-brain natriuretic peptide (NT-proBNP) ($p=0.025$). No difference was found in other laboratory parameters such as white blood cell (WBC), hemoglobin, platelet count, erythrocyte sedimentation rate (ESR), procalcitonin (PCT) and serum ferritin (SF) between the KD patients with CAL and without CAL.

Correlations between plasma miR-181a and other laboratory parameters

We investigated the relationships between plasma miR-181a and other laboratory parameters like blood count, hemoglobin, CRP, ESR, PCT, NT-proBNP and serum SF. As shown in Table 3, plasma miR-181a was closely associated with the concentrations of CRP ($r = 0.363$, $P < 0.05$) and NT-proBNP ($r = 0.389$, $P < 0.05$).

Evaluation of plasma miR-181a as novel predictive biomarker of CAL

The receiver operating characteristic curves (ROC) analysis was used to determine the prediction efficacies of plasma miR-181a, CRP and NT-proBNP as biomarkers for CAL in KD patients. The area under receiver operating characteristic curve (AUC) for plasma miR-181a in prediction of CAL was 0.747 (95% CI, 0.652–0.843, $P < 0.05$, Figure 2), which higher than CRP (AUC=0.629, 95% CI, 0.535–0.723, $P < 0.05$) and NT-proBNP (AUC=0.659, 95% CI, 0.552–0.765, $P < 0.05$). The ROC curves revealed that the specificity and sensitivity of miR-181a for the prediction of CAL were 0.750 and 0.688 with a cut-off value of 3.350, respectively.

Discussion

KD is a systemic vasculitis mainly affecting coronary artery, which has become the leading cause of pediatric acquired heart disease. CAL is the major cause of morbidity and mortality related to KD. Currently, the diagnosis of CAL is still highly dependent on echocardiography[15]. However, echocardiography should be performed by experienced echocardiographer with appropriate transducers. In addition, it is usually hard to detect the detail of distal coronary artery by transthoracic echocardiography, especially in uncooperative children[15]. Moreover, echocardiography is valuable in the diagnosis of CAL, but fails to predict CAL. A recently study found that an early and aggressive initial anti-inflammation therapy for high-risk patients may be beneficial to improve coronary outcomes[17]. Predicting CAL before initiating therapy would assist pediatricians in selecting more aggressive and judicious treatment. Thus, serologic markers for the development of CAL are required.

MiRNA is one kind of functional single-stranded small noncoding RNA, ranging in length from 17 nt to 25 nt, and regulating the translation of target genes. It has been proved that miRNA is suitable to be used as biomarker due to low false positives and false negatives[7]. Previous studies have supported an important role for miRNAs in KD, but they were mainly reported serve as biomarkers for early diagnosis or IVIG-resistant[18,19]. Few miRNAs associated with specific diagnostic or predictive test for CAL had been described.

In this study, we shed light on the relationship of circulating miR-181a with clinical classification, IVIG response and CAL in KD. We found that circulating miR-181a markedly increased in KD patients with CAL, and the level of circulating miR-181a significantly declined after initial IVIG infusion. However, no significant difference in circulating miR-181a expression levels was found in those who had incomplete KD or IVIG-resistant KD. These results indicated that high level of circulating miR-181 would be expected to be associated with CAL. We further investigated the relationship between circulating miR-181a and other laboratory parameters. In these laboratory parameters, neutrophil count, CRP and NT-proBNP were significantly elevated in patients with CAL. Our study showed that circulating miR-181a was positively correlated with CRP and NT-proBNP. As a conventional inflammatory mediator, CRP usually reached the summit during the acute phase of KD, and it declined to normal after IVIG therapy[15,20]. Many studies found that CRP level was significantly higher in KD patients with CAL than without CAL, and would be effective predictor for CAL in KD patients[21,22]. NT-proBNP is a physiologically inactive fragment of BNP, which produced and secreted by ventricular

cardiomyocytes, reflecting the severity of hemodynamic overload[23]. NT-proBNP is currently used in the diagnosis and management of heart diseases including heart failure, myocardial ischemia and cardiovascular disease. Several studies have reported measurement of serum NT-proBNP level as biomarker for KD diagnostic[24-26]. Ye et al. showed that the NT-proBNP levels in IVIG-resistant patients were significantly higher than IVIG-response patients[27]. What's more, a recently research found that serum NT-proBNP might be a laboratory marker for detecting early coronary artery dilatation during the hyperacute phase of KD[28]. To clarify the clinical utility and prognostic value of circulating miR-181a in KD patients with CAL, an AUC based on ROC analysis was conducted. Our results suggest that circulating miR-181a is more reliable than CRP and NT-proBNP as a biomarker for predicting CAL in KD.

MiR-181a, widely present in human organs, is a multifaceted miRNA that has been reported to modulate cell proliferation, apoptosis and migration[29,30]. In recent years, the miR-181a family was found dysregulated in a variety of diseases[31], especially in cardiovascular disease[13,14]. Various studies have confirmed that miR-181a expression levels in cardiovascular disease were closely related to oxidative stress[32,33]. Wang et al reported that reactive oxygen species directly increases miR-181a expression levels in cardiomyocytes and even suggested that miR-181a might represent a potential therapeutic target for the treatment of oxidative stress-associated cardiovascular diseases[33]. Oxidative stress is the imbalance in favor of increased generation of reactive oxygen species and/or reduced body's innate anti-oxidant defense systems. Oxidative stress has been demonstrated to play an important role in the pathological progress of cardiovascular diseases[34]. It damages the function of intravascular endothelial cells and weakens the structure of subendothelial layer, reducing vascular elasticity and leading to cardiovascular events. In KD patients, it has been believed that oxidative stress is likely to be involved in the pathogenesis of CAL[35,36]. From those findings, we speculated that oxidative stress might be the underlying mechanism of up-regulation of circulating miR-181a expression in KD patients with CAL.

In summary, our study showed that circulating miR-181a expression levels on admission were significantly higher in KD patients who later developed CAL than in patients who did not. Furthermore, circulating miR-181a levels on admission predicted the development of CAL with high accuracy. These findings suggest that circulating miR-181a may play a crucial role in the development of CAL and be a potential prognostic biomarker for KD with CAL.

Limitations

Due to the relatively small number of patients recruited in present study, additional study is required to validate circulating miR-181a in more clinical samples before clinical application. Moreover, further researches are needed to elucidate the exact mechanism of miR-181a involving in the pathogenesis of CAL.

Conclusion

Plasma miR-181a was highly expressed in KD patients with CAL and closely associated with the concentrations of CRP and NT-proBNP. Moreover, the prediction efficacy of miR-181a in KD patients

with CAL was higher than that of CRP and NT-proBNP.

Author Contributions: All co-authors, listed on the title page, have participated in the planning, execution or analysis of the study and resulting manuscript. All authors read and approved the final manuscript.

Consent for publication: Consent to publish has been obtained from all authors.

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Conflicts of Interest: The authors declare no conflict of interest.

Ethics approval and consent to participate: This study was approved by the Medical Ethics Committee of Jiangxi Province Children's Hospital (JXSETYY-YXKY-20190070). Research was conducted according to all ethical standards, and written informed consent was obtained from all patients.

Availability of data and materials: All data generated and analyzed during the current study are included in this published article.

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Figures

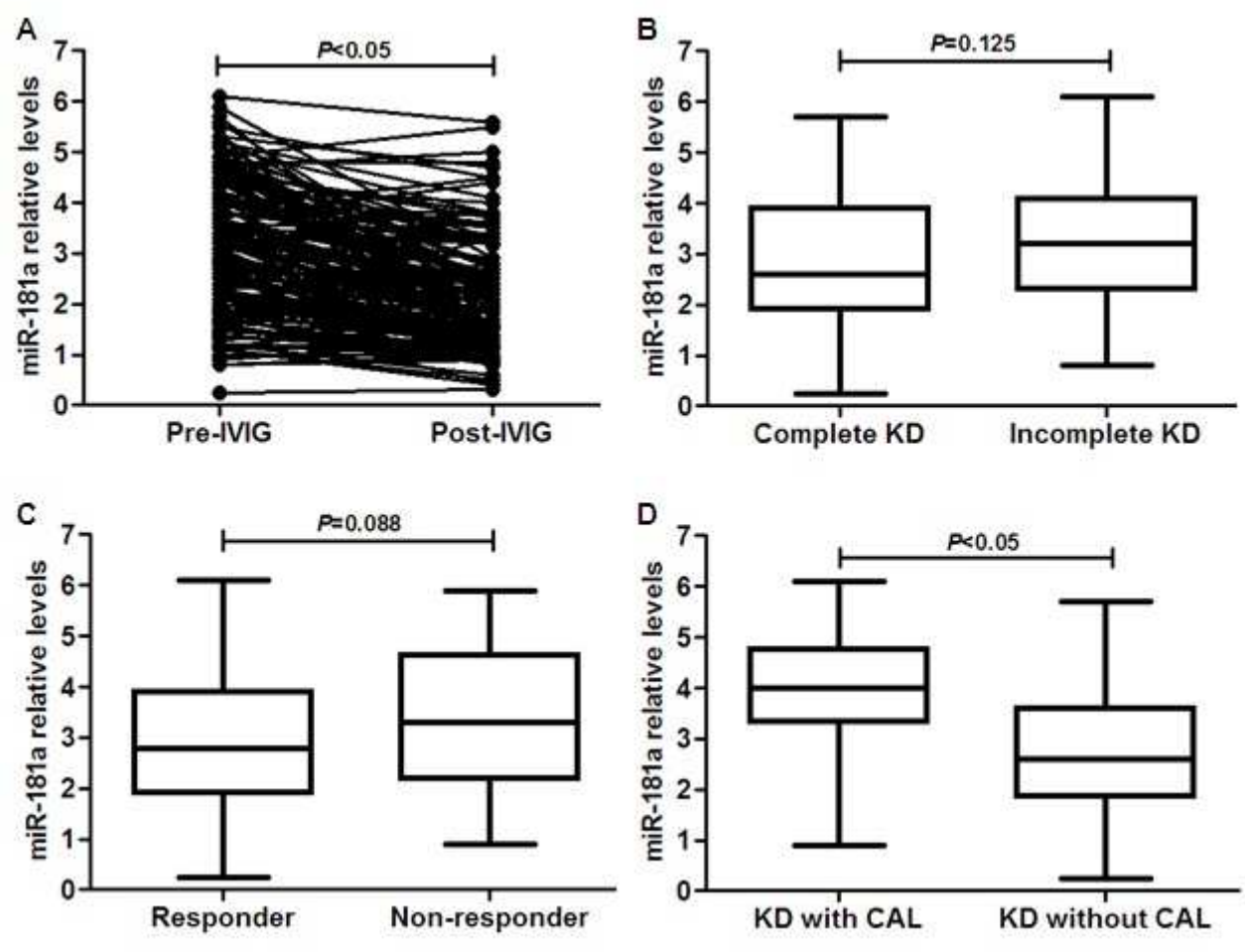

Figure 2

Circulating miR-181a expression levels in KD. A. Circulating miR-181a expression levels in KD before and two days after initial IVIG infusion. B. Circulating miR-181a expression levels in complete KD and incomplete KD. C. Circulating miR-181a expression levels in IVIG-response and IVIG-resistant patients. D. Circulating miR-181a expression levels in KD patients with and without CAL.

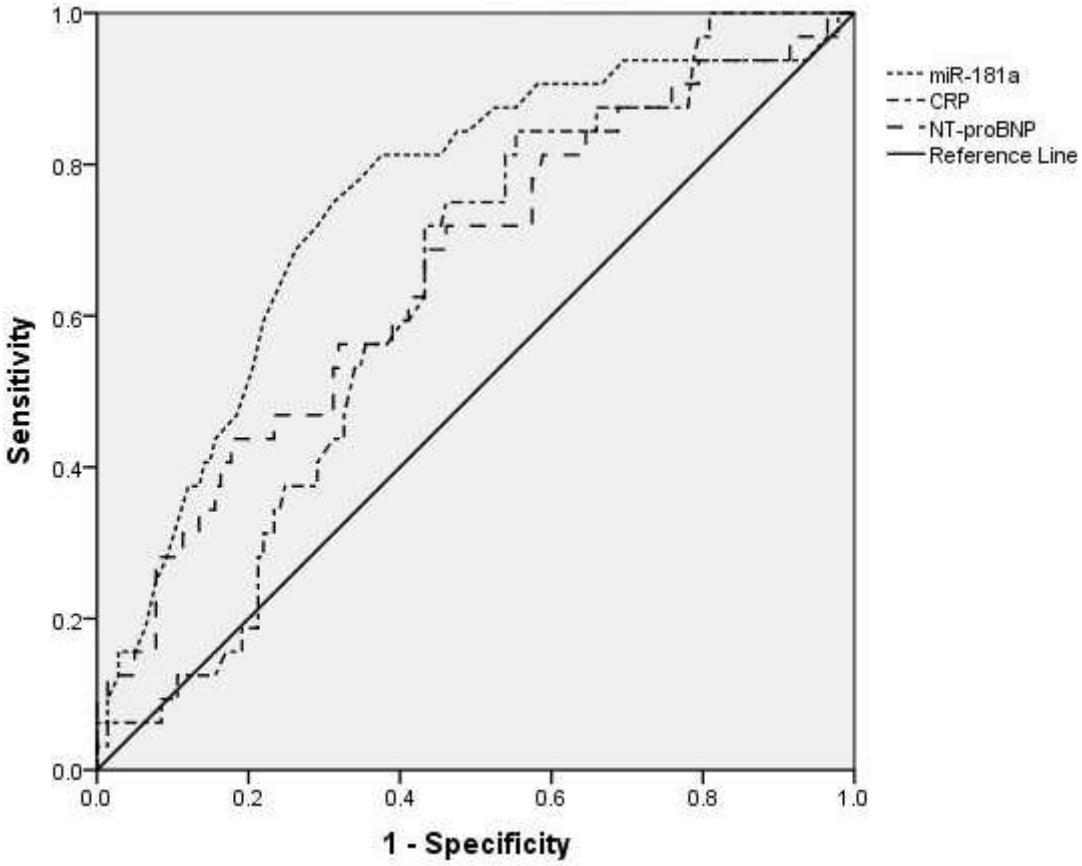

Figure 4

Receiver operating characteristic (ROC) curve analysis of miR-181a, CRP and NT-proBNP as predictors for CAL in KD.