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Diagnostic value of miRNA-122 in Kawasaki disease

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Abstract. – OBJECTIVE: To explore the expression pattern and diagnostic value of microRNA-122 (miRNA-122) in childhood Kawasaki disease (KD).

PATIENTS AND METHODS: A total of 150 children with KD were included in the KD group. During the same period, 150 children with respiratory infection complicated with fever and without myocardial involvement were included in the control group. Serum level of miRNA-122 in children with acute phase of KD and those in the control group was detected. The relationship between serum level of miRNA-122 and clinical features of KD was analyzed by Pearson correlation test. ROC curves were depicted to assess the diagnostic value of miRNA-122 in KD.

RESULTS: Serum level of miRNA-122 was higher in the KD group than controls. In the acute phase of KD, the serum level of miRNA-122 was positively correlated to CRP and NT-proBNP, while negatively correlated to the sodium level. The specificity and sensitivity of miRNA-122 in diagnosing KD was 78.67% and 84.67%, respectively (AUC=0.8861, cut-off value=2.905).

CONCLUSIONS: Serum level of miRNA-122 is significantly enhanced in the acute phase of KD, and highly expressed miRNA-122 is related to systematic inflammation. MiRNA-122 may be used as a diagnostic hallmark of KD.

Key Words:
MiRNA-122, Kawasaki disease, Diagnostic value, Hallmark.

Introduction

Kawasaki disease (KD), also known as mucocutaneous lymph node syndrome, is an acute, systemic, self-limiting vasculitis. It mainly affects children under 5 years of age and has become one of the major pediatric acquired heart diseases instead of rheumatic heart disease. KD results in permanent coronary artery damage, and it is a risk factor for adult ischemic heart disease[1]. At present, specific diagnostic indicators of KD are lacked. It can only be diagnosed based on clinical manifestations[2]. It is necessary to seek for highly specific biomarkers to assist the diagnosis of KD.

MicroRNAs (miRNAs) are non-coding RNAs with 18-25 nucleotides. They are involved in multiple biological activities through post-transcriptional regulation[3]. Abnormally expressed miRNAs lead to changes in cell phenotypes and genotypes, thus causing inflammation, tumorigenesis, and other pathological behaviors[4,5]. The role of miRNAs in diagnosis and treatment of KD has been well concerned[6,7]. Shimizu et al[8] demonstrated six upregulated miRNAs (miRNA-143, miRNA-199b-5p, miRNA-618, miRNA-223, miRNA-145 and its complementary strand) in blood samples collected from patients with acute phase of KD.

MiRNA-122 is derived from a single genomic locus on chromosome 18 and labeled 18q21.31[9]. Mammalian miRNAs are found as a single or clustered transcription units that are located in the introns of protein-coding mRNAs, introns or exons of non-coding mRNAs. They may have their own independent transcription units as well[10,11]. The human miRNA-122 locus is located in exons of non-coding RNAs and independent of the cluster[10]. Previous studies have shown the involvement of miRNA-122 in many human diseases. The knockdown of miRNA-122 suppresses apoptosis in nasopharyngeal cancer cells[12]. By inhibiting P4HA1 level, miRNA-122 attenuates proliferative and metastatic abilities in ovarian cancer by affecting EMT[13]. Serum level of miRNA-122 is upregulated in patients with cardiovascular diseases, serving as a potential diagnostic hallmark[14]. In this paper, we explored the clinical significance of miRNA-122 in KD and its potential influence on clinical features of children with KD.

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Patients and Methods

Baseline Characteristics

A total of 150 children with KD treated in the Taizhou Jiangyan Hospital of Traditional Chinese Medicine from May 2017 to December 2018 were included, involving 75 males and 75 females. The age of them was 1 month to 7.5 years (median age: 24 months). KD was diagnosed according to the *Revised Diagnostic Guidelines for Kawasaki Disease* (the 5th revised edition)[15]. During the same period, 150 children with respiratory infection complicated with fever and without myocardial involvement were included as controls, involving 73 males and 77 females. Their age ranged from 1.2 month to 8.5 years (median age: 32 months). Children with infection of respiratory syncytial virus (has caused pulmonary hypertension and right heart failure), organic heart disease, history of KD or other chronic systemic diseases were excluded. No significant differences in age and sex between two groups ($p>0.05$). This study was performed after approval of Hospital Ethic Committee and informed consent from families of each subject.

Blood Sample Collection

Venous blood (3 mL) was collected in each subject under the fasting state in the morning and placed at 4°C for 30 min. After centrifugation at 3000 r/min for 15 min, the serum was collected and stored at -80°C.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Serum miRNAs were extracted using the miRNeasy Mini Kit (Qiagen, Hilden, Germany), which were reversely transcribed using the TaqMan microRNA reverse Transcription Kit (Thermo Fisher, Waltham, MA, USA). QRT-PCR was performed using the 2×SYBR Green PCR Master Mix (Thermo Fisher, Waltham, MA, USA). Relative level of miRNA-122 was normalized to that of U6. MiRNA-122: Forward: 5-CGTGTGTAGTCGTAGTCGTGTGACGAT-3'; Reverse: 5'-GGCTGTCGATGTAAATGCGCTGATCGA-3'; U6: Forward: 5'-GGTCGGGCAGGAAAGAGGGC-3'; Reverse: 5'-CTAATCTTCTCTGTATCGTTCC-3'.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. Data were expressed as mean ± SD (standard deviation). The *t*-test was performed for analyzing differences between the groups. The relationship between serum level of miRNA-122 and clinical features of KD was analyzed by Pearson correlation test. Receiver operating characteristic (ROC) curves were depicted to assess the diagnostic value of miRNA-122 in KD. $p<0.05$ indicated the significant difference.

Results

Comparison of Serological Indexes

We determined serological indexes in both groups. It is shown that hematocrit and sodium level were lower in the KD group than the control group ($p<0.05$). AST was similar between the groups ($p>0.05$). In addition, higher levels of WBC, platelets, CRP, ALT, and NT-proBNP were seen in the KD group than controls ($p<0.05$) (Table I).

Table I. Comparison of serological indexes.

Variable	Control (n = 150)	KD (n = 150)	*t*	*p*
WBC (10^9/L)	11.59 ± 5.21	15.33 ± 6.29	5.608	< 0.001
Platelets (10^9/L)	331.69 ± 63.84	369.52 ± 68.71	4.94	< 0.001
Hematocrit (%)	31.68 ± 8.22	29.85 ± 7.64	1.997	0.047
CRP (mg/L)	70.58 ± 10.27	123.47 ± 25.66	23.437	< 0.001
ALT (U/L)	45.95 ± 3.64	51.87 ± 5.24	11.364	< 0.001
AST (U/L)	47.33 ± 5.21	46.44 ± 5.07	1.499	0.135
Sodium (mEq/L)	146.03 ± 24.38	140.11 ± 21.58	2.227	0.027
NT-proBNP (pg/mL)	452.25 ± 62.41	685.11 ± 70.37	30.321	< 0.001

WBC: While blood cells; CRP: C-reactive protein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NT-proBNP: N-terminal prohormone of brain natriuretic peptide.

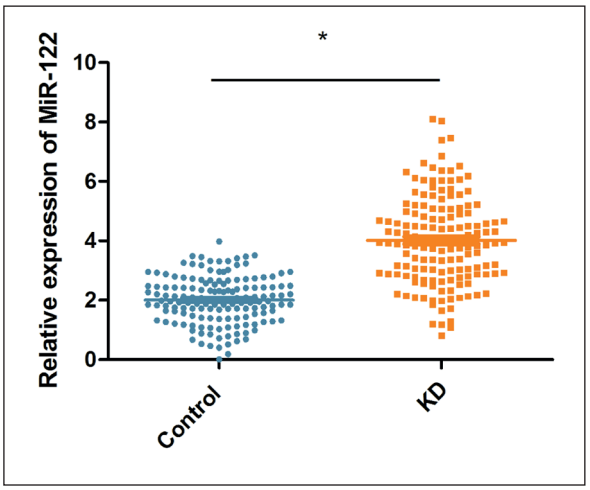

Figure 1. Serum level of miR-122 in children with Kawasaki disease. Serum level of miR-122 was upregulated in 150 children with Kawasaki disease in the acute phase.

Serum Level of MiRNA-122 in Children With KD Was Upregulated

Compared with control group, the serum level of miRNA-122 was upregulated in 150 children with acute phase of KD (p<0.05) (Figure 1). It is indicated that miRNA-122 may be involved in the development of KD.

Relationship Between MiRNA-122 Level and Clinical Features of KD

The relationship between serum level of miRNA-122 and clinical features of KD was analyzed by the Pearson correlation test. As shown in Table II, miRNA-122 level was positively correlated to CRP (r=0.806) and NT-proBNP (r=0.733), and negatively correlated to sodium level (r=-0.601) in children with KD (p<0.05).

Diagnostic Value of MiRNA-122

ROC curves were depicted to assess the diagnostic value of miRNA-122 in KD. Once the cut-off value was 2.905, the specificity and sensitivity of miRNA-122 in diagnosing KD was 78.67% and 84.67%, respectively (AUC=0.8861, Youden=0.6334) (Figure 2). MiRNA-122 may be utilized as a biomarker for diagnosing KD.

Discussion

MiRNAs are stably expressed in body fluids, such as blood and urine[16]. Extracellular miRNAs are resistant to endogenous ribonucleases by interacting with particles[17]. After translocating in other tissues or cells, extracellular miRNAs transmit cell-to-cell information, which provide new ideas for disease diagnosis and treatment. Free miRNAs in the blood display strong biological functions in the cardiovascular system[18,19].

KD is featured by dysfunctions of coronary artery, endothelial cells, and smooth muscle cells in moderate muscular blood vessels[18]. Eventually, endothelial damage and elastic fiber damage aggravate into vasculitis, thus involving other arteries. The cause of KD remains largely unknown, and it is currently believed that innate immune abnormality is the possible reason[20]. Multiple pathways[21,22] are involved in immune regulation in the acute phase of KD. Saito et al[23] showed that miRNA-145-5p and miRNA-145-3p are upregulated in patients with refractory KD. Rong et al[24] uncovered that the serum level of miRNA-92a-3p is upregulated in children with KD than those with fever. During the recovery period of KD, its level is reduced to the baseline. Shimizu et al[8] identified upregulated miRNA-145 in serum of patients with the acute phase of KD. Similarly, our findings showed that serum level of miRNA-122 was upregulated in children with KD.

Table II. Correlation between miR-122 and clinical indexes of Kawasaki disease.

Variable	*r*	95% CI	*p*
WBC (10^9/L)	0.228	0.033 – 1.587	0.362
Platelets (10^9/L)	0.527	0.251 – 2.374	0.438
Hematocrit (%)	-0.608	-0.782 – 1.052	0.257
CRP (mg/L)	0.806	0.622 – 0.961	< 0.001
ALT (U/L)	0.528	0.211 – 2.642	0.593
Sodium (mEq/L)	-0.601	-0.857 – -0.282	0.037
NT-proBNP (pg/mL)	0.733	0.298 – 0.851	0.018

WBC: While blood cells; CRP: C-reactive protein; ALT: Alanine aminotransferase; NT-proBNP: N-terminal prohormone of brain natriuretic peptide.

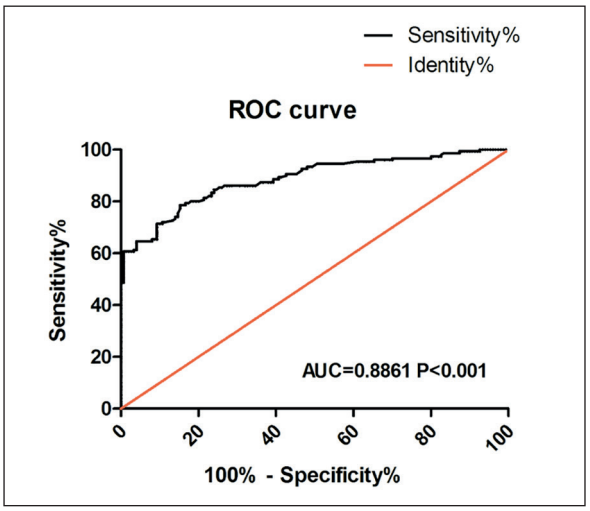

Figure 2. Diagnostic value of miR-122 in Kawasaki disease. AUC of miR-122 in diagnosing Kawasaki disease was 0.8861 (p<0.001). Once the cut-off value was 2.905, the specificity and sensitivity of miR-122 in diagnosing Kawasaki disease was 78.67% and 84.67%, respectively.

We found CRP elevation and sodium level reduction in the acute phase of KD, which may be attributed to fever, immune inflammatory response, inflammatory changes in blood vessels, and hypercoagulability in the body. KD is an acute febrile eruptive disease featured by systemic vasculitis, while CRP is an objective indicator of inflammatory activity in the body[25]. A relevant study[26] demonstrated that serum level of hs-CRP increases in children with KD. In the acute phase of KD, plasma albumin level is associated with myocardial swelling due to increased vascular permeability[27]. In addition, NT-proBNP is reported to be pronouncedly elevated in the acute phase of KD, which is a reliable diagnostic indicator[28,29]. KD leads to highly activated immune system. Inflammatory myocardium and abundant inflammatory factors stimulate the synthesis and secretion of NT-proBNP in ventricular myocytes[26]. In this paper, the serum level of miRNA-122 in children with KD were correlated to CRP, sodium level and NT-proBNP. Notably, the diagnostic value of miRNA-122 in KD was verified. We believed that miRNA-122 may be applied in the auxiliary diagnosis of KD.

Conclusions

Serum level of miRNA-122 is significantly enhanced in the acute phase of KD. MiRNA-122 may be used as a diagnostic hallmark of KD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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