Difference in serum miRNA expression between immunoglobulin-sensitive and -insensitive incomplete Kawasaki disease patients

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Abstract. The present study aimed to investigate the expression of microRNAs (miRNAs/miRs) and inflammatory factors in patients with immunoglobulin-sensitive and IVIG-insensitive incomplete Kawasaki disease (KD). One hundred and eighty-five patients with incomplete KD were included as the study group (KD group), and 182 patients with respiratory infection as the control group. Neutrophil to lymphocyte ratio (NLR), C-reactive protein (CRP) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), white blood cell count (WBC), hemoglobin level (Hb), platelet count (PLT) and T cell subsets ($CD3^+$, $CD3^+$ $CD4^+$) were compared. Patients in the KD group received aspirin (30 mg/kg orally daily) and gamma globulin (IVIG, 1 g/kg intravenously daily). According to the sensitivity to IVIG, patients were divided into IVIG-sensitive group and IVIG-insensitive KD group. The relative expression levels of miRNA-21, miRNA-145, miRNA-155 and miRNA-199b-5p in the serum were detected by RT-qPCR. Serum TNF-α, IL-6 and IL-1β levels were assessed using ELISA. Before treatment, the neutrophil to lymphocyte ratio (NLR), levels C-reactive protein, and leukocytes in the KD group were significantly higher compared with the control group (P<0.05). After medical intervention, the relative expression of miRNA-21, miRNA-145 and miRNA-155 in the serum of patients in IVIG-sensitive and IVIG-insensitive KD groups were increased when compared with these levels in the control group (P<0.05). Meanwhile, the relative expression of miRNA-199b-5p was decreased (P<0.05). Compared with the IVIG-sensitive KD group, the relative expression levels of miRNA-145 and miRNA-155 were increased in the serum of patients in the IVIG-insensitive KD group (P<0.05). Compared with the control group, the levels of TNF-α, IL-6 and IL-1β were increased in the serum of patients in the IVIG-sensitive and IVIG-insensitive KD groups (P<0.05). Compared with the IVIG-sensitive KD group, the serum levels of TNF-α and IL-6 were increased in patients of the IVIG-insensitive KD group (P<0.05). Except for NLR and CRP, there were differences in the expression of peripheral blood miRNA-145, miRNA-155 and serum TNF-α and IL-6 in patients with immunoglobulin-sensitive and -insensitive incomplete KD.

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Introduction

Kawasaki disease (KD) is an acute febrile rash disease in children, which is a type of non-specific vasculitis. The main cause of the disease is systemic inflammatory response (1). Arteries, veins and capillaries may be involved. Arteries are the most vulnerable, which is currently the leading cause of acquired heart disease in children (2,3). KD can be divided into complete KD and incomplete KD. Incomplete KD has fewer types of symptoms than complete KD. However, the diagnosis and treatment of patients with incomplete symptoms are often delayed, and patients with incomplete KD have a similar risk of coronary artery abnormalities as those with complete KD (4,5). Therefore, improving the rate of a correct diagnosis and timely treatment is valuable to reduce the incidence of KD and its risk of coronary artery damage. KD in children under 1 year of age is difficult to be diagnosed. Infants show less classic clinical features; thus it is difficult to meet the typical KD diagnostic criteria (6), which increases the possibility of misdiagnosis. At present, apart from clinical manifestations, the diagnosis of KD also depends on the support of laboratory tests. KD biomarkers may facilitate the early diagnosis of KD (7). Typical KD biomarkers include N-terminal brain natriuretic peptide, neutrophil to lymphocyte ratio (NLR), C-reactive protein (CRP), D-dimer (DD), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (8-11). However, it is still unknown whether the above-mentioned biomarkers can be used as judgment criteria for gamma globulin (IVIG)-sensitive and -insensitive incomplete KD. miRNAs are a type of non-coding single-strand RNA molecules

approximately 22 nucleotides long. They can bind to target mRNAs and participate in post-transcriptional gene expression regulation. A variety of miRNAs are related to inflammatory cell differentiation and development, regulation of inflammatory factors, and vascular inflammatory damage (12). Studies have shown that miRNA-21, miRNA-145, miRNA-155 and miRNA-199b-5p are closely related to the occurrence and development of KD (13-15). However, there are few studies concerning the expression of the above-mentioned miRNAs in patients with IVIG-sensitive and -insensitive incomplete KD. Therefore, the present study provides a basis for the clinical diagnosis of incomplete KD, and for drug selection and prognostic evaluation of these patients.

Patients and methods

Patient information. One hundred and eighty-five patients with incomplete KD were collected as the KD group. They were admitted to Xuzhou Children's Hospital Affiliated to Xuzhou Medical University from March 2016 to December 2018. There were 92 males and 93 females, with a mean age of 4.3±3.1 years. According to the American Heart Association (AHA), inclusion criteria were as follows: Patients aged 2 months to 12 years, with fever that lasts for at least 5 days without any other explanation (>38˚C). Patients had at least 2 symptoms, including: polymorphous rash (rash in any form), bilateral bulbar conjunctival injection without exudate, erythema of the oral mucosa (including lips, pharynx or tongue), changes in peripheral limbs (including erythema on the palms or soles and/or swelling of the hands or feet) and cervical lymphadenopathy (at least the diameter of 1 lymph node ≤1.5 cm) (4,16). Exclusion criteria: Patients with severe hematological diseases; patients with combined liver and kidney dysfunction; patients allergic to treatment drugs. One hundred and eighty-two patients with respiratory infections were taken as the control group. They had a mean age of 4.2±3.0) years, and included 90 males and 92 females. Inclusion criteria consisted of patients with respiratory infections aged 2 months to 11 years. Exclusion criteria included patients with severe blood system diseases; patients with combined liver and kidney dysfunction; patients with previous history of KD. The study was approved by the Ethics Committee of Xuzhou Children's Hospital. Signed written informed consents were obtained from the patients and/or guardians.

All patients in the KD group received aspirin (30 mg/kg orally daily) and gamma globulin (IVIG, 1 g/kg intravenously daily). The patients were divided into the IVIG-sensitive KD group (n=104) and IVIG-insensitive KD group (n=81). The criteria for the IVIG-insensitive KD group included patients for whom the first treatment of IVIG was ineffective, the body temperature remained over 38˚C after 48 h, or the body temperature rose again within 2-7 days after administration, and who had at least 1 symptom in the inclusion criteria of incomplete KD as mentioned above.

Methods

Neutrophil to lymphocyte ratio (NLR) and C-reactive protein (CRP) testing. Five milliliters of fasting venous blood was collected from the control group, IVIG-sensitive KD group and IVIG-insensitive KD group in the morning. The Siemens BN-II automatic protein analyzer was used to determine CRP before treatment and 2 days after IVIG was administered. The blood cell meter was used to determine the neutrophil to lymphocyte ratio (NLR), C-reactive protein (CRP) levels, white blood cell count (WBC), hemoglobin level (Hb), platelet count (PLT) levels before treatment and 2 days after IVIG was used. Au5400 automatic biochemical analyzer (Beckman Coulter, Inc.) was used to test the indicators of liver function [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] in patients before treatment. Beckman Coulter FC500 flow cytometer (Beckman Coulter, Inc.) was used to test treatment pre-T cell subset index ($CD3^+$, $CD3^+CD4^+$).

miRNA relative expression levels. Peripheral blood mononuclear cells were obtained from the control group, IVIG-sensitive KD group and IVIG-insensitive KD group, and a certain amount of cell lysate was added. Total RNA extraction kit was used to extract total RNA according to the TRIzol method. RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas) was used to prepare the mRNA reverse transcription system to synthesize cDNA by reverse transcription. The primer sequences of miRNA-21, miRNA-145, miRNA-155, and miRNA-199b-5p are as previously documented (17-20).

The reaction conditions of PCR consisted of pre-denaturation at 94˚C for 3 min, for a total of 40 cycles (at 94˚C for 30 sec at 60˚C for 30 sec, and at 72˚C for 45 sec). Each samples was set up with 3 parallel duplicate wells. U6 was taken as an internal reference, and $2^{-\Delta\Delta Cq}$ (21) was used to calculate the relative expression of miRNA-21, miRNA-145, miRNA-155, and miRNA-199b-5p in peripheral blood. The primers are documented in Table I.

Inflammatory factor expression. The fasting venous blood of the study subjects in the above three groups was collected from 9:00-10:00 Beijing time in the morning. After centrifugation, the samples were marked with the date, group and name. They were tested in strict accordance with the instructions contained in the TNF-α, IL-6 and IL-1β kits (Shanghai Yubo Biological Technology Co., Ltd., product nos. YBC102g, YBA079Ov01 and YBA056Bo01). The blank holes were adjusted to zero, the absorbance (OD value) of each well samples was tested sequentially at 450 nm wavelength. The concentration of the standard was taken as the abscissa, and the OD value as the ordinate to draw the standard curve. According to the OD value of samples, the corresponding concentration was determined from the standard curve, and then timed by the dilution factor to obtain the values of TNF-α, IL-6 and IL-1β.

Statistical processing. All data were analyzed using SPSS 20.0 software (IBM Corp.). Measurement data are expressed as mean ± standard deviation (SD). The data of experimental indicators in each group conformed to the normal distribution and the variances are equal. The comparison between multiple groups was conducted using one-way analysis of variance (ANOVA), followed by the Student-Newmnan-Keuls test. The sample comparison between two groups was conducted using the independent sample t-test (unpaired). Receiver operating

Table I. Primer sequences of miRNA-21, miRNA-145, miRNA-155 and miRNA-199b-5p.

Name	Upstream	Downstream	(Refs.)
miRNA-21	5'-ACACTCCAGCTGGGTAGCTTATCAGACTGATG-3'	5'-CTCAACTGGTGTCGTGGA-3'	(17)
miRNA-145	5'-GTCCAGTTTTCCCAGGAATCCCT-3'	5'-TCCAGTCCTATTGAATGTGGGA-3'	(18)
miRNA-155	5'-TTAATGCTAATCGTGACT-3'	5'-ACCTGAGAGTAGACCAGA-3'	(19)
miRNA-199b-5p	5'-CAGCCCAGTGTTTAGACTATC-3'	5'-CAGTGCAGGGTCCGAGGT-3'	(20)
U6	5'-CTCGCTTCGGCAGCACATATACT-3'	5'-ACGCTTCACGAATTTGCGTGTC-3'	(20)

Table II. Comparison of the related indicators before treatment in patients in the control and KD groups.

Basic indicators	KD group (N=185)	Control group (N=182)	t	P-value
NLR	5.91±1.24	1.01±0.81	44.888	<0.001
CRP (mg/l)	78.07±59.03	19.22±28.83	12.165	<0.001
ALT (U/l)	41.21±70.04	39.71±14.44	0.285	0.776
AST (U/l)	39.08±67.54	38.61±16.64	0.092	0.927
WBC ($x10^9$/l)	17.61±3.85	10.21±2.64	21.504	<0.001
Hb (g/l)	102.26±10.85	104.08±10.65	1.621	0.106
PLT ($x10^9$/l)	397.61±47.85	405.01±87.65	1.002	0.317
$CD3^+$ (%)	64.51±9.25	65.31±7.45	0.913	0.362
$CD3^+CD4^+$ (%)	42.71±8.95	41.69±8.79	1.101	0.271

Data are expressed as the mean ± SD. KD, Kawasaki disease; NLR, neutrophil to lymphocyte ratio; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cells; Hb, hemoglobin level; PLT, platelet count.

characteristic (ROC) curve was used to determine the best predictive value. P<0.05 was considered as indicative of a statistically significant result.

Results

General results. There was no significant difference in age and sex between the two groups (P>0.05; P>0.05). Before treatment, the levels of NLR, C-reactive protein (CRP), and white blood cells (WBC) in the KD group were significantly higher compared with the control group, and the differences were statistically significant (P<0.05, P<0.05, P<0.05). There were no changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST), hemoglobin (Hb), platelets (PLT), $CD3^+$ and $CD3^+CD4^+$ in patients of the KD group; the differences were not statistically significant (P>0.05, P>0.05, P>0.05, P>0.05, P>0.05, P> 0.05). The results are shown in Table II.

Comparison of the levels of NLR and CRP in IVIG-sensitive and IVIG-insensitive KC groups. The results showed that the levels of NLR and CRP of patients in the IVIG-insensitive KD group were higher than those in the IVIG-sensitive KD group before IVIG treatment, and the differences were statistically significant (P<0.05, P<0.05). Compared with the IVIG-sensitive KD group, the levels of NLR and CRP in the IVIG-insensitive KD group were increased after IVIG treatment, and the difference was statistically significant (P<0.05, P<0.05) (Fig. 1).

Predictive value of NLR and CRP to the sensitivity of IVIG of incomplete KD. Before IVIG treatment, the AUC indicated that the predictive value of NLR for IVIG-insensitive patients was 0.72 (95% CI, 0.64-0.80), the sensitivity was 0.74, and the specificity was 0.67. AUC indicated that the predictive value of CRP for IVIG-insensitive patients was 0.72 (95% CI, 0.64-0.79), the sensitivity was 0.77, and the specificity was 0.61. After IVIG treatment, AUC indicated that the predictive value of NLR for IVIG-insensitive patients was 0.74 (95% CI, 0.68-0.81), the sensitivity was 0.64, and the specificity was 0.73. AUC indicated that the predictive value of CRP for IVIG-insensitive patients was 0.82 (95% CI, 0.76-0.88), the sensitivity was 0.68, and the specificity was 0.82 (Fig. 2).

miRNA relative expression. The results showed that compared with the control group, the relative expression of miRNA-21, miRNA-145 and miRNA-155 in the serum of patients in the IVIG-sensitive and IVIG-insensitive KD groups was increased, and the difference was statistically significant (P<0.05, P<0.05, P<0.05, P<0.05). Meanwhile, the relative expression of miRNA-199b-5p was decreased compared to the control group, and the difference was statistically significant (P<0.05). Compared with the IVIG-sensitive KD group, the relative expression of miRNA-145 and miRNA-155 was increased, and the difference was statistically significant (P<0.05, P<0.05) (Fig. 3).

Inflammatory factor expression. The results showed that compared with the control group, the levels of TNF-α, IL-6

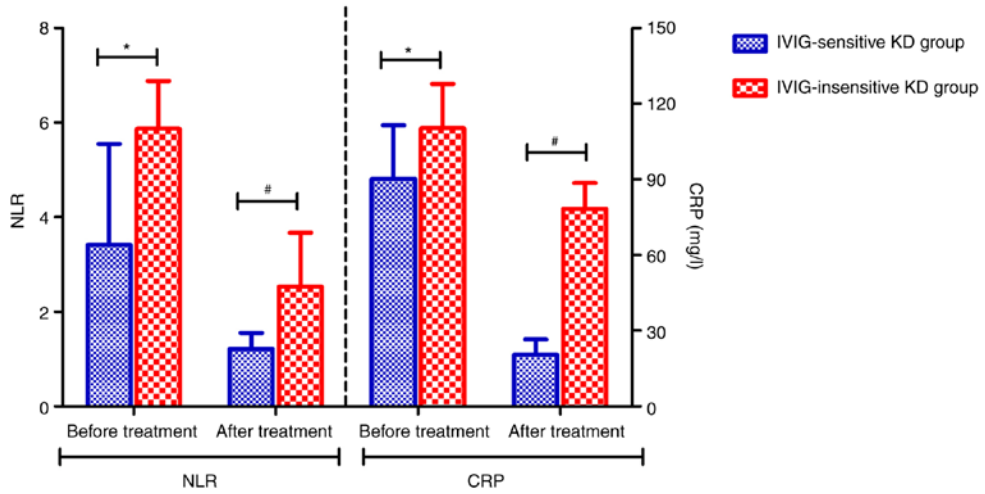

Figure 1. Comparison of the NLR and CRP level in the IVIG-sensitive and IVIG-insensitive groups. *P<0.05, compared with the IVIG-sensitive KD group before treatment; #P<0.05 compared with the IVIG-sensitive KD group after treatment. KD, Kawasaki disease; IVIG, gamma globulin; NLR, neutrophil to lymphocyte ratio; CRP, C-reactive protein.

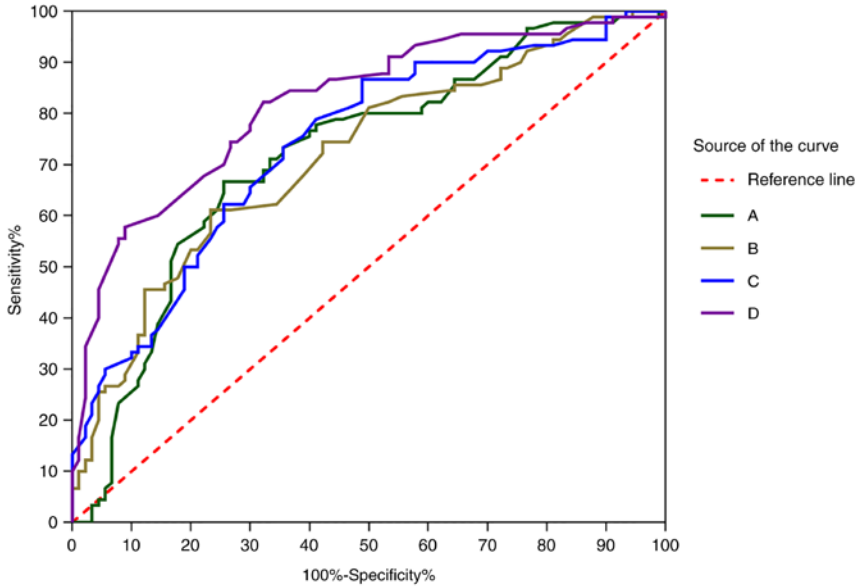

Figure 2. ROC curve of NLR and CRP prediction of IVIG sensitivity of incomplete KD before and after IVIG treatment. A: ROC curve of NLR before IVIG treatment; B: ROC curve of CRP before IVIG treatment; C: ROC curve of NLR after IVIG treatment; D: ROC curve of CRP after IVIG treatment. ROC, receiver operating curve; KD, Kawasaki disease; IVIG, gamma globulin; NLR, neutrophil to lymphocyte ratio; CRP, C-reactive protein.

and IL-1β in the serum of patients in the IVIG-sensitive and IVIG-insensitive KD group were increased, and the differences were statistically significant (P<0.05, P<0.05, P<0.05). Compared with the IVIG-sensitive KD group, the levels of TNF-α and IL-6 in the serum of the patients in the IVIG-insensitive KD group were increased, and the differences were statistically significant (P<0.05, P<0.05) (Fig. 4).

Predictive value of miRNA and inflammatory factors to IVIG sensitivity. We predicted whether patients with incomplete KD are sensitive to IVIG. The area under the ROC curve (AUC), the sensitivity and the specificity of miRNA-145 were 0.86 (95% CI, 0.80-0.92), 0.81 and 0.81, respectively. The AUC, the sensitivity and the specificity of miRNA-155 were 0.79 (95% CI, 0.72-0.87), 0.80 and 0.69, respectively. The AUC, the sensitivity and the specificity of TNF-α were 0.87 (95% CI, 0.82-0.93), 0.80 and 0.85, respectively. The AUC, the sensitivity and the specificity of IL-6 were 0.82 (95% CI, 0.75-0.89), 0.80 and 0.72, respectively. (Fig. 5).

Discussion

In most children with Kawasaki disease (KD), intravenous IVIG and oral aspirin therapy can quickly reduce inflammatory factor levels, fever, and other clinical symptoms. But approximately 15-20% of children receiving the initial gamma globulin (IVIG) infusion show persistent or recurrent fever, which is classified as IVIG-insensitive KD (11). Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are related to the onset of KD, which can be used as

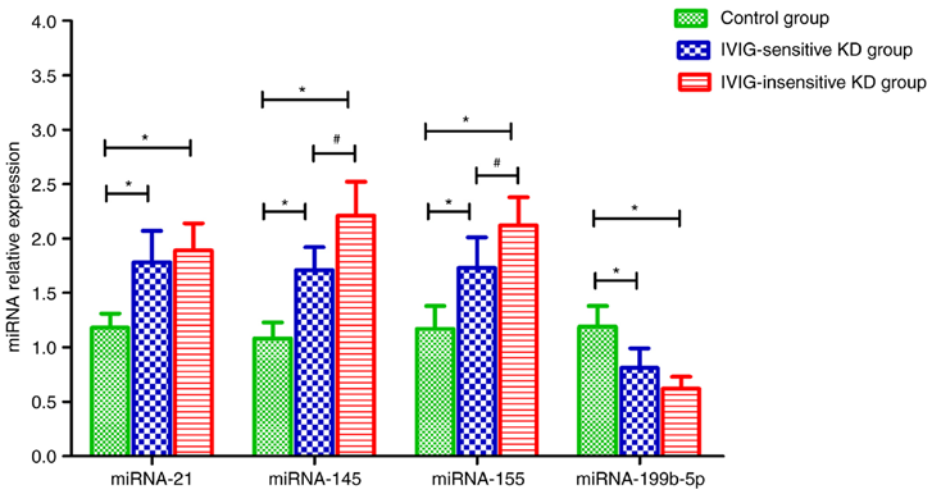

Figure 3. Relative expression of serum miRNAs of patients in the IVIG-sensitive and IVIG-insensitive group. *P<0.05, compared with the control group; #P<0.05, compared with the IVIG-sensitive KD group. KD, Kawasaki disease; IVIG, gamma globulin.

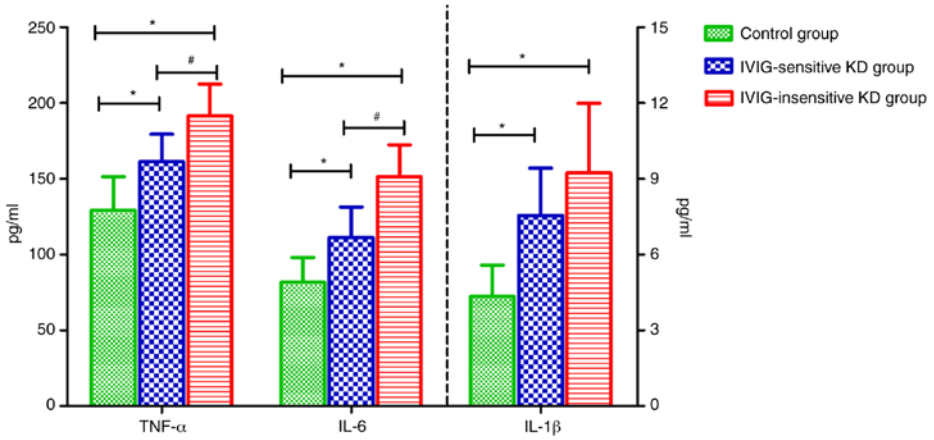

Figure 4. Expression levels of inflammatory factors, TNF-α, IL-6 and IL-1β, in the serum of patients in the IVIG-sensitive and IVIG-insensitive group. *P<0.05, compared with the control group; #P<0.05, compared with the IVIG-sensitive KD group. KD, Kawasaki disease; IVIG, gamma globulin; TNF, tumor necrosis factor; IL, interleukin.

a basis for early diagnosis of KD. NLR is a predictive factor of IVIG-insensitive KD (22). The present study demonstrated that NLR and CRP were significantly increased in the KD group compared with those in the control group, which was statistically significant. The results were consistent with the reports in the literature, indicating that NLR and CRP can be used as reference indicators for the diagnosis of incomplete KD. In addition, this study also found that before and after IVIG treatment, the levels of NLR and CRP in patients in the IVIG-insensitive KD group were significantly higher than those in the IVIG-sensitive KD group. The results suggested that NLR and CRP can also be used as indicators to predict whether patients with incomplete KD are sensitive to IVIG, to provide a basis for drug selection and prognosis evaluation.

IL-6 is an inflammatory cytokine produced in response to the activation of monocytes and macrophages during the acute phase of KD. It can stimulate other inflammatory markers, for example, CRP. Higher levels of serum IL-6 and CRP are related to coronary artery damage and insensitivity to IVIG. Therefore, serum IL-6 can be used as a novel marker (23) to predict coronary artery involvement and resistance to IVIG. TNF-α is an inflammatory cytokine, which plays an important role in the defense against infection and immune response. Compared with other cases without treatment or without IVIG, TNF-α blockers have a beneficial effect on treatment resistance after the start of KD treatment (11). The present study found that compared with the control group, the levels of TNF-α and IL-6 in the serum of patients with IVIG-sensitive and IVIG-insensitive KD group were increased, which were basically consistent with those reported in the literature (22,23). In addition, compared with the IVIG-sensitive KD group, the levels of TNF-α and IL-6 in the serum of patients in the IVIG-insensitive KD group were higher. In this study, the levels of TNF-α and IL-6 in the three groups were consistent with the trends of NLR and CRP levels. The results confirmed that there may be a close positive correlation between incomplete KD and inflammation, but its mechanism needs to be further studied.

Inflammatory response is a key mechanism of KD pathogenesis, and miRNAs may be the main regulators of this inflammatory response (24). Multiple studies (18-21) have shown that compared with the control group, the expression

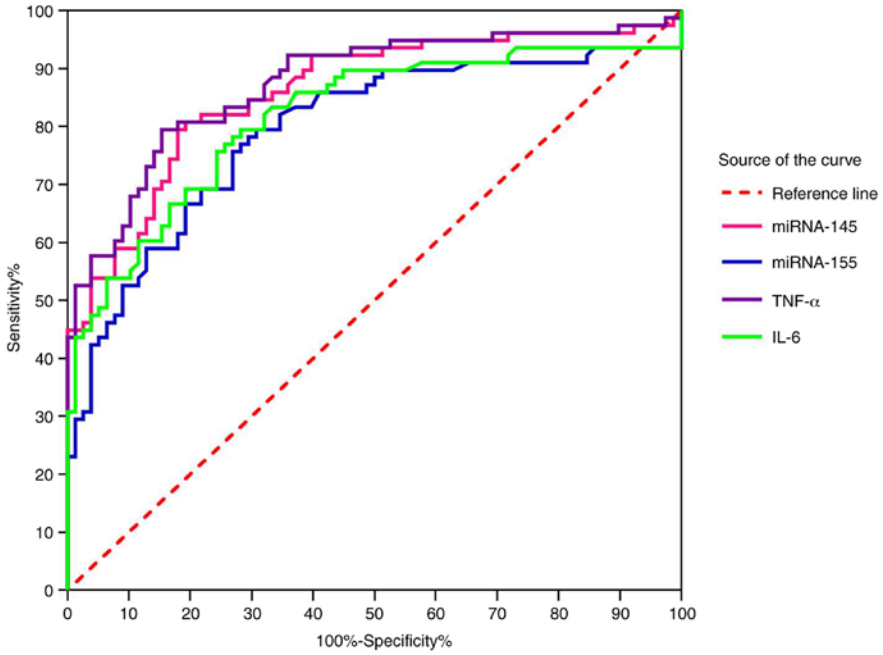

Figure 5. ROC curve of miRNAs and inflammatory factors predicting the sensitivity of incomplete KD patients to IVIG. ROC, receiver operating curve; KD, Kawasaki disease; IVIG, gamma globulin; TNF, tumor necrosis factor; IL, interleukin.

levels of serum miRNA-21, miRNA-145 and miRNA-155 in KD are relatively increased. In comparison, the relative expression of miRNA-199b-5p is reduced. miRNA-199b-5p can be used as an auxiliary indicator for KD diagnosis. The present study found that compared with the control group, the relative expression of serum miRNA-21, miRNA-145 and miRNA-155 of patients in the IVIG-sensitive and IVIG-insensitive KD groups were all increased, while the relative expression of miRNA-199b-5p was decreased. The results were basically consistent with the reports in the literature. The difference is that there was no reclassification of patient coronary artery lesions, which is a deficiency of this study. In addition, the present study also found that the relative expression levels of miRNA-145 and miRNA-155 in the serum of patients with IVIG-insensitive KD group were higher than those in the IVIG-sensitive KD group; the difference was statistically significant. The results suggest that the relative expression levels of serum miRNA-145 and miRNA-155 can also be used as indicators to predict whether patients with incomplete KD are sensitive to IVIG. No similar report has been reported at present. The sample size of the IVIG-sensitive KD group (N=104) and the IVIG-insensitive KD group (N=81) in this study was small. To further confirm whether miRNA-145 and miRNA-155 can be sensitive indicators of IVIG, it is necessary to further expand the sample size.

Studies have reported that plasma miRNA-155 can show the calculation ability of atrial fibrillation recurrence after cardioversion. It is positively correlated with serum B-type natriuretic peptide (BNP), TNF-α, CRP and IL-6 (25). IL-6 can stimulate inflammation marker CRP. In addition, there have been studies that have reported that miRNA-217 can regulate the Toll-like receptor 4/nuclear factor-κB (TLR4/NF-κB) signaling transduction pathway, block inflammatory response, and improve lung injury caused by lung tissue protection (26). Lipopolysaccharide (LPS) of TLR4/NF-κB signaling pathway can increase the expression of TNF-α and IL-6 in hippocampus tissue of rats, induce brain neuroinflammation, and ultimately lead to cognitive memory impairment (27). The above studies have shown that miRNAs can mediate the TLR4/NF-κB signaling pathway and affect the expression of inflammatory factors. In this study, it was found that the relative expression of miRNAs and the levels of inflammatory factors are consistent. Therefore, we speculated that changes in serum-related miRNAs in patients with incomplete KD may activate the TLR4/NF-κB signal transduction pathway, promote inflammatory responses, leading to changes in the inflammatory factors of IL-6 and TNF-α, and stimulating the increase in NLR and CRP levels. Eventually they lead to systemic inflammation, KD and related complications, which needs to be further studied.

To summarize, the expression of miRNAs and inflammatory factors in serum of patients with IVIG-sensitive and -insensitive incomplete KD differ. It is necessary to further verify whether the levels of NLR, CRP, TNF-α and IL-6 and the expression of miRNA-145 and miRNA-155 in serum can be used as potential predictors of the sensitivity of patients with incomplete KD to IVIG.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YW, CL, LN and XA conceived and designed this study. YW, CL, MF, JT and XA helped with data collection and summary. CL, LN, MF and JT were responsible for data analysis and interpretation. XA made contributions to manuscript writing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Xuzhou Children's Hospital. Signed written informed consents were obtained from the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Lazea C, Man O, Sur LM, Serban R and Lazar C: Unusual presentation of kawasaki disease with gastrointestinal and renal manifestations. Ther Clin Risk Manag 49: 1411-1416, 2019.
2. Eleftheriou D, Levin M, Shingadia D, Tulloh R, Klein NJ and Brogan PA: Management of Kawasaki disease. Arch Dis Child 99: 74-83, 2014.
3. Kowalczyk M, Turska-Kmieć A, Ziółkowska L, Raszek M and Kawalec W: Symptoms, diagnosis and characteristic abnormalities in the coronary arteries in Kawasaki disease in children. Med Wieku Rozwoj 14: 344-349, 2010 (In Polish).
4. Pacheco DA, Miller CR, Boor PJ and Mambo NC: Incomplete Kawasaki disease with development of fatal coronary artery thrombosis in a 13-year-old male. Cardiovasc Pathol 42: 54-58, 2019.
5. Shi H, Qiu H, Jin Z, Li C, Yang X, Huang C, Wu R, Zhuang G and Chu M: Coronary artery lesion risk and mediating mechanism in children with complete and incomplete Kawasaki disease. J Investig Med 67: 950-956, 2019.
6. Ellepola C, Borsheim K, James J, Mitchell M, Gudausky T and Frommelt PC: Giant thrombotic right coronary aneurysm in an infant with undiagnosed incomplete Kawasaki disease and rapidly progressive cardiovascular collapse. CASE (Phila) 3: 250-254, 2019.
7. Ha KS, Lee J, Jang GY, Lee JB, Lee KC, Son CS and Lee JW: Value of neutrophil-lymphocyteratio in predicting outcomes in Kawasaki disease. Am J Cardiol 116: 301-306, 2015.
8. Tazón-Varela MA, Alonso-Valle H and Muñoz-Cacho P: On the usefulness of N-terminal prohormone of brain natriuretic peptide for the diagnosis of incomplete Kawasaki disease. Emergencias 31: 366-367, 2019.
9. Hwang SY, Shin TG, Jo IJ, Jeon K, Suh GY, Lee TR, Yoon H, Cha WC and Sim MS: Neutrophil-to-lymphocyte ratio as a prognostic marker in critically-ill septic patients. Am J Emerg Med 35: 234-239, 2017.
10. Azab B, Zaher M, Weiserbs KF, Torbey E, Lacossiere K, Gaddam S, Gobunsuy R, Jadonath S, Baldari D, McCord D and Lafferty J: Usefulness of neutrophilto lymphocyte ratio in predicting short- and long-term mortalityafter non-ST-elevation myocardial infarction. Am J Cardiol 106: 470-476, 2010.
11. Yamaji N, da Silva Lopes K, Shoda T, Ishitsuka K, Kobayashi T, Ota E and Mori R: TNF-α blockers for the treatment of Kawasaki disease in children. Cochrane Database Syst Rev 8: CD012448, 2019.
12. Kyuno D, Zhao K, Bauer N, Ryschich E and Zöller M: Therapeutic targeting cancer-initiating cell markers by exosome mirna: Efficacy and functional consequences exemplified for claudin7 and EpCAM. Transl Oncol 12: 191-199, 2019.
13. Sheng W, Gao C and Guan H: Expression and its significance of MicroRNA-1 4 5 and MicroRNA-1 4 3 in plasma in children with Kawasaki disease. J Mod Lab Med 31: 34-37, 2016.
14. Xie Y, Li W, Feng J, Wu T and Li J: MicroRNA-363 and GATA-1 are regulated by HIF-1α in K562 cells under hypoxia. Mol Med Rep 14: 2503-2510, 2016.
15. Busch A, Eken SM and Maegdefessel L: Prospective and therapeutic screening value of non-coding RNA as biomarkers in cardiovascular disease. Ann Transl Med 4: 236, 2016.
16. Yim D, Curtis N, Cheung M and Burgner D: An update on Kawasaki disease II: Clinical features, diagnosis, treatment andoutcomes. J Paediatr Child Health 49: 614-623, 2013.
17. Zou YC, Yan LM, Gao YP, Wang ZY and Liu G: miR-21 may act as a potential mediator between inflammation and abnormal bone formation in ankylosing spondylitis based on TNF-α concentration-dependent manner through the JAK2/STAT3 pathway. Dose Response 18: 1559325819901239, 2020.
18. Yong Q, Nan Y, Lei Z, Li P, Liu K, Wu HW, Shen Y and Li ZD: Expression of serum microRNA-145 in patients with non-small cell lung cancer and its clinical significance. J Med Postgra 29: 62-65, 2016.
19. Zhang X, Wang PH and Liao L: The test of miRNA-155 and miRNA-26a for the children with bronchial asthma in airway remodeling in the clinic application. J Chin Phys 18: 1506-1510, 2016.
20. LI WH, LI YX, Guo JK, Pan HG, Zhang YL and Wang X: Overexpression of miR-199b-5p inhibits Ewing's sarcoma cell lines by targeting CCNL1. Mol Med Rep 12: 3359-3364, 2015.
21. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
22. Chen Y, Hua Y, Zhang C, Chen S, Zhang Q, Liao Y, Yan H, Wang Y, Liu P, Qi J, *et al*: Neutrophil-to-lymphocyte ratio predicts intravenous immunoglobulin-resistance in infants under 12-months old with Kawasaki disease. Front Pediatr 7: 81, 2019.
23. Nandi A, Pal P and Basu S: A comparison of serum IL6 and CRP levels with respect to coronary changes and treatment response in Kawasaki disease patients: A prospective study. Rheumatol Int 39: 1797-1801, 2019.
24. Yun KW, Lee JY, Yun SW, Lim IS and Choi ES: Elevated serum level of MicroRNA (miRNA)-200c and miRNA-371-5p in children with Kawasaki disease. Pediatr Cardiol 35: 745-752, 2014.
25. Zhang X, Xiao XP, Ren XA and Cui T: Plasma miRNA-155 levels predict atrial fibrillation recurrence after cardioversion. Heart Surg Forum 22: E140-E148, 2019.
26. Li J, Feng Shao F, Xu J, *et al*: Inhibition effects of miR-217 on sepsis induced lung injury in rats based on TLR4 signaling pathway. J Hainan Med University 26: 6-11, 2020.
27. Tang S, Yan LR, Ma ZG and Ji C: Influences of the TLR4/NF-κB pathway on memory function and inflammatory factors in rats with cerebral small vessel disease. Eur Rev Med Pharmacol Sci 23: 6264-6271, 2019.