Correlations of complication with coronary arterial lesion with VEGF, PLT, D-dimer and inflammatory factor in child patients with Kawasaki disease

Y. ZHOU, S. WANG, J. ZHAO, P. FANG

Department of Pediatrics, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, China

Abstract. – OBJECTIVE: To investigate the correlations of complication with coronary arterial lesion (CAL) or not with vascular endothelial growth factor (VEGF), platelet (PLT), D-dimer, and inflammatory factor in child patients with Kawasaki disease (KD).

PATIENTS AND METHODS: A total of 60 KD child patients meeting the inclusion criteria diagnosed and treated from January 2016 to October 2017 were collected. There were 27 child patients complicated with CAL enrolled as observation group and 33 child patients not complicated with CAL selected as control group. The venous blood of the two groups of patients was acquired after admission to hospital. Enzyme-linked immunosorbent assay (ELISA) was utilized to detect the content of serum VEGF and interleukin-6 (IL-6); the content of serum PLT and D-dimer was measured using a fully automatic biochemistry analyzer, and the coronary artery diameter was determined through color Doppler ultrasound.

RESULTS: Compared with those in control group, the content of VEGF, IL-6, PLT, and D-dimer in the serum was increased remarkably in the observation group, and the differences were statistically significant (p<0.05). The thickness of the coronary artery in the observation group was markedly greater than that in the control group, with a statistically significant difference (p<0.05). The content of VEGF, IL-6, PLT, and D-dimer in the serum was positively correlated with the thickness of the coronary artery.

CONCLUSIONS: For KD child patients, the complication with CAL or not has a close correlation with VEGF, PLT, D-dimer, and inflammatory factor; and VEGF, IL-6, PLT, and D-dimer are the important risk factors for KD complicated with CAL.

Key Words: Kawasaki disease, Coronary arterial lesion, Correlation.

Introduction

Kawasaki disease (KD), also known as mucocutaneous lymph node syndrome, is a type of self-limited, suppurative systemic vasculitis. The vascular inflammation all over the body is the primary pathological response of KD. So far, it has been discovered that coronary arterial lesion (CAL) is the most serious complication of KD. Coronary artery ectasia and coronary arteritis are the major pathological changes of KD-induced CAL in the early stage, while coronary arterial aneurysm, coronary artery stenosis and even sudden death of the patients are the major pathological changes in the later stage. According to epidemiologic statistics, nearly 5% of KD patients have severe CAL in the end. Therefore, KD is regarded as one of the important causes of acquired heart diseases in child patients. However, the pathologic cause of KD-induced CAL still remains unclear, and numerous factors are considered as important risk factors for KD complicated with CAL. This research aims to investigate the relations of KD complicated with CAL with vascular endothelial growth factor (VEGF), platelet (PLT), D-dimer and interleukin-6 (IL-6), and clarify the risk factors for KD complicated with CAL.

Patients and Methods

Patients

A total of 60 KD child patients admitted and treated in Affiliated Hospital of North Sichuan Medical College from January 2017 to July 2017 were collected. 27 child patients complicated with CAL were enrolled as observation group, and 33 child patients not complicated with CAL were se-
lected as control group. There were 15 males and 12 females aged (4.2±1.5) years old in the observation group. Control group included 18 males and 15 females aged (4.2±1.2) years old. There were no differences in the age and gender between the two groups of patients, which were comparable.

**Diagnostic Criteria**

Diagnostic criteria of KD are as follows: 1) child patients with fever symptoms for over 5 consecutive days, 2) symptoms of conjunctiva hyperemia in both eyes, 3) chapped lips, diffuse congestion of the oral mucosa and typical strawberry tongue, 4) erythema multiforme and rashes on the skin, 5) changes at the ends of the four limbs, hard edemas of the hands and feet in the acute stage and molting changes at the position of nail bed skin migration in the remission stage, and 6) acute non-suppurative lymphadenitis in the neck.

Diagnostic criteria of CAL are as follows: 1) normal (thickened coronary artery, with a coronary artery diameter <3.5 mm for child patients aged 1-3 years old), 2) mild (coronary artery diameter <4 mm for child patients, with apparent but limited dilatation), 3) moderate (a certain degree of diffuse change visible in the coronary artery of the child patients, with a coronary artery diameter of 4-7 mm) and 4) severe (giant arterial aneurysms in the coronary artery of the child patients, with a coronary artery diameter ≥8 mm).

**Methods**

**Specimen Acquisition**

The venous blood of the patients in both observation group and control group was acquired immediately after admission to hospital, and was centrifuged to collect the supernatant. The expression levels of VEGF and IL-6 in the venous blood were detected in accordance with the operation steps in the kit instruction of enzyme-linked immunosorbent assay (ELISA). A fully automatic biochemistry analyzer was utilized to measure the content of PLT and D-dimer in the venous blood, and the color Doppler ultrasound was applied to determine the coronary artery diameter of the child patients.

**Examination via ELISA**

1) Loading: 100 μL standard substance or serum was added to each well to be detected, and the reaction plate was placed at 37°C for reaction for 40 min after being adequately mixed. 2) Plate washing: the reaction plate was fully washed with washing solution for 4-6 times and then dried on a piece of filter paper. 3) 50 μL distilled water and 50 μL primary antibody working solution in the kit were added into each well (except for blank control group), and then, the reaction plate was reacted at 37°C for 20 min after sufficient mixing. 4) Plate washing: the reaction plate was thoroughly washed with the washing solution for 4-6 times and then dried on a piece of filter paper. 5) 100 μL enzyme-labeled antibody working solution in the kit was added into each well. Next, the reaction plate was placed at 37°C for reaction for 10 min. 6) Plate washing: the reaction plate was fully washed with the washing solution for 4-6 times, followed by drying on a piece of filter paper. 7) 100 μL substrate working solution in the kit was added into each well, then the reaction plate was put in the dark at 37°C for reaction for 15 min. 8) 100 μL stop buffer in the kit was added and evenly mixed in each well. 9) The absorbance at the wavelength of 450 nm was measured using a microplate reader.

**Color Doppler Ultrasonography**

Every child patient was examined by the color Doppler ultrasonic diagnostic apparatus, and the detailed methods are as follows: the subjects were in the supine position with hands placed at both sides of the body. The probe frequency of the color Doppler ultrasonic diagnostic apparatus was set at 5-12 MHz. The detection was performed carefully from the aortic root to the proximal part, and the probe was moved towards the head along with the blood vessels. The internal diameter of the coronary artery was measured carefully.

**Statistical Analysis**

In this research, Statistical Product and Service Solutions (SPSS; IBM Corp., IBM SPSS Statistics for Windows, Version 20.0., Armonk, NY, USA) was used for statistical analysis. Enumeration data was presented as mean ± standard deviation. The t-test was adopted for data in line with normal distribution and homogeneity of variance, corrected t-test was applied for data conforming to normal distribution and heterogeneity of variance, and non-parametric test was performed for data that was not in line with normal distribution and homogeneity of variance. Rank sum test was adopted for ranked data, and chi-square test was conducted for enumeration data. Pearson corre-
Correlation analysis was performed for correlation analysis. \( p < 0.05 \) suggested that the difference was statistically significant.

**Results**

**Expression Levels of Serum VEGF and IL-6 Detected Via ELISA**

As shown in Figure 1, the expression levels of serum VEGF and IL-6 were low in the control group and higher in the observation group. Compared with those in the control group, the expression levels of VEGF and IL-6 in the serum were elevated remarkably in the observation group, and the differences were statistically significant \( (p < 0.05) \), suggesting that the expression levels of serum VEGF and IL-6 were markedly increased in KD child patients complicated with CAL.

**PLT and D-Dimer Content Detected Via the Fully Automatic Biochemistry Analyzer**

The content of PLT and D-dimer in the venous blood was relatively low in the control group and higher in the observation group. In the observation group, the content of PLT and D-dimer in the venous blood was notably higher than those in the control group, displaying statistically significant differences \( (p < 0.05) \) (Figures 2-3). It indicated that KD child patients complicated with CAL have markedly increased PLT and D-dimer content in the serum.

**Coronary Artery Diameter of Child Patients Detected Via Color Doppler Ultrasound**

Child patients in the control group had smaller coronary artery diameter, while those in the observation group had larger coronary artery diameter. The thickness of the coronary artery in the child patients in the observation group was significantly greater than that in the control group, with a statistically significant difference \( (p < 0.05) \). It implied that the coronary arteries of the KD child patients complicated with CAL are thickened markedly (Figure 4).

**Correlation Analysis**

According to calculations, the \( r \)-value was 0.95 in the correlation analysis between VEGF expression level in the venous blood and the coronary artery diameter of the KD child patients, indicating that the two indexes were positively correlated with each other (Figure 5). The correlation analy-
ses manifested that PLT, IL-6, and D-dimer content in the venous blood had positive correlations with the coronary artery diameter of the KD child patients ($r=0.88$, $r=0.81$, $r=0.88$, Figures 6, 7, 8).

**Discussion**

The severity of CAL, as the most serious complication of KD, can intensively affect the prognosis of KD. The pathogenesis of KD and KD complicated with CAL is not very clear at present. Studies have indicated that KD can activate large quantities of monocytes/macrophages in the body of the child patients, release massive pro-inflammatory cytokines, directly invade the vascular endothelial cells, and trigger vascular injury and reconstruction of the vascular structure, ultimately resulting in inflammation and dilatation of the coronary artery in the early stage as well as stenosis and arterial aneurysm in the late stage in the KD child patients. VEGF possesses the functions of promoting the proliferation and division of the vascular endothelial cells and ameliorating microvascular permeability. It plays crucial roles in revascularization, reconstruction of the vascular structure, and vascular-associated diseases. It is revealed in studies that VEGF is abnormally highly expressed in the serum of the KD child patients in the early stage of the vascular inflammation, and the expression level is much higher in the KD child patients complicated with CAL. The results of this research manifested that the VEGF expression level in the serum of the KD child patients complicated with CAL was significantly elevated compared with that in the child patients with simple KD, which is consistent with...
Correlations of coronary arterial lesion with VEGF, PLT, D-dimer and inflammatory factor

the finding of previous studies. As a product of acute reactions in the cells, PLT is regarded as a major participant of the vascular inflammation\(^{10,11}\). As a result, PLT count elevation is considered to be one of the key risk factors of CAL. PLT can interact with the immune complexes in the blood, thereby leading to the vascular inflammation after KD, invading the coronary artery and finally influencing the CAL onset\(^{12,13}\). It was discovered in this research that the PLT content in the blood of the KD child patients complicated with CAL was remarkably higher than that in the child patients with simple KD, indicating that severe vascular inflammations exist in the coronary arteries of the KD child patients complicated with CAL, which may be an important cause of CAL in the KD child patients. D-dimer is a kind of degradation product that is formed by complexes generated from the thrombin-activated factors and fibrin monomers hydrolyzed by fibrinolytic enzyme\(^{14,15}\). Since the content of D-dimer can reflect the tendency of hypercoagulable state and hemorrhage, it is regarded as one of the primary markers of the body’s coagulation-fibrinolysis system. Studies have demonstrated that\(^{16}\) the expression level of D-dimer is increased abnormally in KD child patients, and that the content of D-dimer is of prominent significance for diagnosis and judgment of KD. It was revealed in this research that the D-dimer content in the blood of the KD child patients complicated with CAL was notably higher than that in the child patients with simple KD, manifesting that a high tendency of blood coagulation is presented in the KD child patients complicated with CAL. IL-6, a vital pro-inflammatory cyto-

Conclusions

We elaborated that VEGF, PLT, D-dimer, and IL-6 have close relations with the complication of KD. The higher the content is, the greater the possibility of complication with CAL in the KD child patients will be. Therefore, they are important risk factors for KD complicated with CAL.

Funding

The work was funded by Natural Science Foundation of China (No.: 81300528).

Availability of Data and Material

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

Authors' Contributions

YZ wrote the manuscript. YZ and SW collected Specimen, JZ was responsible for ELISA, PF interpreted color Doppler ultrasonography. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Affiliated Hospital of North Sichuan Medical College and informed consents were signed by the patients and/or guardians.
Confidentiality of Publication
Informed consents were signed by the patients and/or guardians.

Conflict of Interest
The Authors declare that they have no conflict of interest.

References