Expression of CD11b as an Adhesion Molecule on Neutrophils in Children with Kawasaki Disease

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Received: 16 July 2013; Received in revised form: 26 September 2013; Accepted: 15 October 2013

ABSTRACT

Inflammation of blood vessels is a characteristic feature of Kawasaki disease. Neutrophils play a key role in the inflammatory responses where movement of neutrophils toward the site of inflammation depends on CD11b/CD18 expression as adhesion molecules on these cells. The purpose of this study was to investigate CD11b/CD18 expression in patients with Kawasaki disease upon diagnosis and after treatment.

The study included 20 children with Kawasaki disease aged from 3 months to 8 years. Mean fluorescence intensity of CD11b levels on diagnosis and at 1-2 and 6 weeks after intravenous immunoglobulin (IVIG) therapy was measured in these patients. Level of CD11b was measured in age-matched healthy children and febrile children (each 21) as negative and positive controls, respectively.

Mean fluorescence intensity of CD11b in Kawasaki patients was lower than that of the control groups before and after 1-2 weeks of IVIG therapy. There were no significant differences in CD11b in Kawasaki patients either with aneurysm or without aneurysm.

The CD11b levels at the diagnosis time and after treatment with IVIG in our patients with Kawasaki were lower than the control groups.

Keywords: Adhesion molecules; CD11b; Flowcytometry; Integrin; IVIG; Kawasaki disease; Vascular aneurysm

INTRODUCTION

Kawasaki disease (KD) is an acute self-limited vasculitis that predominantly affects children younger than 5 years of age. KD is characterized by fever, bilateral non-exudative conjunctivitis, erythema of the lips and oral mucosa, changes in the extremities, polymorphous rash, and cervical lymphadenopathy. Coronary artery aneurysms develop in approximately 15% to 25% of untreated children and may lead to ischemic heart disease or sudden death.¹

Etiology of KD remains unknown but epidemiologic and clinical data are in favor of an
infectious origin. A high number of polymorphonuclear leucocytes (PMN) is seen in the peripheral blood during the acute phase of KD which is involved in modulation and initiation of immunological response against infection. The main mechanism which influences on PMN migration from the blood to an inflammatory site increases the expression of adhesion molecules and integrin. Firm adhesion of PMN to the endothelial cells is mediated via interaction between β2-integrins such as CD11a/CD18 and CD11b/CD18 on neutrophils and intracellular adhesion molecules (ICAM) on vascular endothelial cells. CD11b/CD18 is a heterodimer receptor with α chain of 155000 daltons and β chain of 95000 daltons, each chain containing transmembrane and cytoplasmic domains. During inflammation, activation of CD11b/CD18 leads to a conformational change from an inactive, low affinity state to an active, high-affinity state. Interaction between activated β2-integrin and ICAM results in PMN migration across the endothelial barrier. Extravasations of activated PMN to affected tissue results in excessive release of inflammatory cytokines. The inflammations involve the layers of vascular wall and destruction of the internal elastic lamina, and weakness in the structural integrity of the vessel wall results in formation of aneurysm.

This study aimed to investigate expression of CD11b/CD18 on neutrophils of patients with KD before treatment and after intravenous immunoglobulin (IVIG) therapy.

**MATERIALS AND METHODS**

This cross-sectional study was conducted on children with KD who were admitted to pediatric ward of Namazee hospital affiliated to Shiraz University of Medical Sciences, Iran, during one year period from March 2011 to March 2012. KD was diagnosed according to the characteristic signs of American Heart Association Guidelines. Patients with atypical KD and any other diseases known to mimic KD such as viral infections, scarlet fever and drug reactions were excluded.

Following approval of the study protocol by Ethics Committee of Shiraz University of Medical Sciences, informed consent was obtained from patients’ parents. Demographic information, clinical symptoms and results of laboratory analysis were collected in patients with KD before administration of IVIG. The patients with KD received IVIG 2gr/kg over 10-12 hours and high dose aspirin immediately after diagnosis. Another dose of IVIG was given to KD patients with persistent or recrudescent fever.

The controls were two groups: twenty-one age-matched healthy subjects were selected by simple random sampling as a negative control and twenty-one age-matched hospitalized subjects with fever and pneumonia as positive control. Both control groups had the same ethnicity and were from the same geographical region.

Serial two-dimensional echocardiography was performed at the time of diagnosis, at 1-2 weeks and 6 weeks after the onset of illness by the same pediatric cardiologist. Coronary artery lesions were defined when either the right or left coronary arteries showed a diameter of ≥3 mm in children younger than 5 years or ≥4 mm in children older than 5 years, or a diameter of >1.5 times that of an adjacent vessel or if luminal contour was clearly irregular.

**Immunostaining and Flowcytometric Analysis**

Blood samples of all patients were stored at room temperature and processed for flowcytometry within one hour in 3 phases: on admission, within 1-2 and 6 weeks after IVIG. Staining of the blood sample was done using saturating concentrations of fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD11b and phycoerythrin (PE)-conjugated mouse anti-human CD62L (Becton Dickinson, USA) according to the procedure as described by Kobayashi et al. After incubation with antibodies, red cell lysing solution (Optilyse C; Beckman Coulter) was added and incubated at room temperature, washed and analyzed with a FACS caliber (BD USA). Isotope-matched control antibodies, PE-conjugated IgG2 and FITC-conjugated IgG2 (Becton Dickinson, USA) were used to define the cut-off point for fluorescence positively as the 99th percentile of the distribution of the cells labeled with control antibody. PMNs were gated based on forward- and side-scatter on the display. Antigen expression was determined as the mean fluorescence intensity.

**Statistical Analysis**

Results were reported as mean ± standard deviation (SD) and range. Analysis of independent variables was carried out using Kruskal Wallis and Mann–
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Whitney U-test. Comparison of independent and dependent variables was performed through Friedman test. All the statistical analyses were performed using SPSS version 17.0. P values below 0.05 were considered significant.

RESULTS

The study involved twenty patients (13 boys, 7 girls: Mean age= 3.6±1.4 yrs, range: 3- 96 months) admitted to the hospital with typical clinical presentation of KD. The two control groups included 21 febrile patients (10 boys, 11 girls: Mean age= 2.9±1.4 years, range: 12-60 months) as positive control and 21 healthy subjects (12 boys, 9 girls: Mean age=4.7±1.2 years, range: 12-60 months) as negative control. There were no significant differences for sex and age between KD patients and control groups. Clinical features and laboratory results of KD patients are shown in Table 1.

Aneurysm of coronary artery was detected in 8 (40%) patients with KD during serial echocardiography in different times. Abnormalities of echocardiography in KD patients in different times are displayed in Table 2.

Eighteen (90%) patients with KD were treated with single dose of IVIG and the time of receiving of IVIG in all KD patients was five days after the initiation of symptoms. IVIG-resistant KD occurred in two patients (10%) and both developed coronary aneurysms in the follow-up.

Mean fluorescence intensity of CD11b in KD patients was lower than the two control groups at the baseline (p=0.2, 0.4) whereas PMN from five patients with KD showed increased CD11b expression at this time.

Table 3 depicts serial changes of mean fluorescence intensity of CD11b on PMN in the baseline, 1-2 and after 6 weeks IVIG therapy in KD patients compared with control groups.

There was a significant difference between decreased mean level of CD11b in KD patients and normal controls in the sixth week after administering IVIG (p=0.02). Mean levels of CD11b in three stages in patients with KD and two control groups are shown in Table 3 and Figure 1.

Mean fluorescence intensity of CD11b in 8 patients with KD and coronary aneurysm did not differ significantly from 12 patients with KD without aneurysm before IVIG and after IVIG treatment.

Table 1. Clinical features and laboratory findings of 20 patients with Kawasaki disease at the time of diagnosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>19(95)</td>
</tr>
<tr>
<td>Polymorphous exanthema</td>
<td>17(85)</td>
</tr>
<tr>
<td>Cervical lymphadenopathy</td>
<td>13(65)</td>
</tr>
<tr>
<td>Bilateral conjunctiva injection</td>
<td>20(100)</td>
</tr>
<tr>
<td>Changes in lips and oral cavity</td>
<td>20(100)</td>
</tr>
<tr>
<td>Changes in extremities</td>
<td>18(90)</td>
</tr>
<tr>
<td>Leukocytosis&gt;15000/ mm³</td>
<td>7(35)</td>
</tr>
<tr>
<td>Hemoglobin &lt;11g/dl</td>
<td>11(55)</td>
</tr>
<tr>
<td>Elevated sedimentation rate &gt;20mm/h</td>
<td>16(80)</td>
</tr>
<tr>
<td>Elevated C-reactive protein</td>
<td>18(90)</td>
</tr>
<tr>
<td>Elevated serum transaminase</td>
<td>7(35)</td>
</tr>
</tbody>
</table>

Table 2. Abnormal findings of echocardiography in 8 patients with KD

<table>
<thead>
<tr>
<th>Number of patient</th>
<th>At diagnosis</th>
<th>1-2 weeks after IVIG</th>
<th>6 weeks after IVIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>moderate TR- LV dysfunction- mild PI</td>
<td>small aneurysm LCA</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>mild dilatation of LCA</td>
<td>aneurysm LCA</td>
<td>aneurysm LCA</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>Normal</td>
<td>aneurysm LCA</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>Normal</td>
<td>aneurysm LCA</td>
</tr>
<tr>
<td>5</td>
<td>Normal</td>
<td>aneurysm RCA</td>
<td>aneurysm RCA</td>
</tr>
<tr>
<td>6</td>
<td>aneurysm LCA</td>
<td>aneurysm LCA</td>
<td>aneurysm LCA</td>
</tr>
<tr>
<td>7</td>
<td>aneurysm LCA</td>
<td>aneurysm LCA</td>
<td>aneurysm LCA</td>
</tr>
<tr>
<td>8</td>
<td>mild TR</td>
<td>small aneurysm LCA</td>
<td>Normal</td>
</tr>
</tbody>
</table>

TR, Tricuspid regurgitation; LV, Left ventricle; PI, Pulmonary insufficiency; LCA, Left coronary artery; RCA, Right coronary artery
Table 3. Mean of CD11b expression on neutrophils in 20 KD patients and two control groups

<table>
<thead>
<tr>
<th>Individuals</th>
<th>The baseline</th>
<th>1-2 weeks after IVIG</th>
<th>6 weeks after IVIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>KD patients (n=20)</td>
<td>49.8±42.18</td>
<td>54.58±24.67</td>
<td>44.18±25.77</td>
</tr>
<tr>
<td>Normal control *</td>
<td>62.09±32.13</td>
<td>----</td>
<td>---</td>
</tr>
<tr>
<td>Febrile control *</td>
<td>56.83±14.97</td>
<td>----</td>
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</tbody>
</table>

* Mean fluorescence intensity of CD11b only was measured at the baseline

Figure 1. Serial changes of mean fluorescence intensity of CD11b in patients with Kawasaki and controls groups

**DISCUSSION**

This is the second study which reports the level of CD11b/CD18 expression on neutrophils in KD patients; it has been associated with conflicting results. This study showed decreased mean fluorescence intensity of CD11b in 15 (75%) KD patients at the time of diagnosis compared to control groups, it was completely different from those reported by Kobayashi et al.’s study. Although β2 integrin CD11b/CD18 plays an important role in the innate immune response, this different finding gives us an idea about the probable role of other unknown adhesion molecules; however, inflammatory cytokines in early stages of KD might also be produced by other cells such as macrophages. It is noticeable that using CD11b/CD18 blockade in humans has shown limited success against inflammation. The other hypothesis is increasing anti-inflammatory cytokines such as IL-10 in inhibition of CD11b expression on neutrophils during the early phase of KD.

In Kobayashi et al.’s study, KD patients immediately after treatment developed reduction of the level of CD11b unlike our result that showed increased CD11b in comparison with the baseline after 1-2 weeks of therapy. We could not assess whether the increased level was due to the use of IVIG or the course of the disease. Moreover, other molecules except CD11b might be related to anti-inflammatory properties of IVIG within early weeks after administration and it needs to be followed.

In the present study, mean CD11b significantly decreased after 6 weeks of IVIG therapy in patients with KD compared to baseline. Previous studies showed that treatment had a significant reducing effect in mean fluorescence intensity of CD11b in comparison to the level before the treatment. Some mechanisms have been proposed to explain the beneficial effect of IVIG on neutrophils in inflammatory disorders. First, the presence of neutralizing IgG at high dose of IVIG blocked activation of IL-1α on the endothelium and decreased adhesion of PMN to endothelium; then, binding of IVIG to FcγIIIB receptors on neutrophils induces reactive oxygen species (ROS) emission from TNF-α-primed neutrophils. Next, IVIG infusion might modulate TGF-β1 and IL-10 on neutrophils by
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activation of soluble HLA Class I and soluble Fas ligand.\textsuperscript{16,17} Clinical studies have shown high levels of PMN produced oxygen radicals, proteolytic enzymes and resulted coronary artery damage.\textsuperscript{18} CD11b as a strong adhesion molecule can play a part in this event. Mean level of CD11b was not different between eight KD patients with coronary involvement and 12 patients without aneurysm. It is known that various vasoactive substances in addition to adhesion molecules can induce migration and proliferation of the intimal smooth muscle, causing coronary dilatation.\textsuperscript{19} However, the small size of KD patients with coronary dilatation is a limitation of this result.

Coronary artery aneurysm develops in up to 25% of untreated KD patients and is estimated to decline to 5% with IVIG treatment. Our patients with KD developed coronary aneurysm more than 5% after IVIG therapy; it might be explained by Iranian genetic background and the effect of environmental factors.

Incidence of KD in infants younger than 3 months is infrequent where existence of maternal antibody in infant is protective against infection. In this study, the youngest patient with KD was 3 months old who was an interesting case.

In this study, we showed a decrease in the CD11b level at the time of diagnosis and until six weeks after treatment with IVIG in patients with Kawasaki compared to control groups. This finding was in contrast with the first report related to CD11b level in KD patients. Therefore, further studies on KD patients are required for evaluation of adhesion molecules in KD.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Nasrin Shokrpour at Center for Development of Clinical Research of Nemazee Hospital for editorial assistance.

REFERENCES

B. Heidari, et al.


