SERUM INTERCELLULAR ADHESIONS MOLECULE-1 (cICAM-1) IN CHILDREN WITH JUVENILE CHRONIC ARTHRITIS

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ABSTRACT

The intercellular adhesion molecule-1 (ICAM-1) is a cytokine induced glycoprotein involved in recruitment of cells into tissues undergoing inflammatory responses. The aim of this study was to determine the level of soluble circulating ICAM-1 (cICAM-1) in children with Juvenile chronic arthritis (JCA) and to find whether it has a correlation with the disease activity or not. Levels of cICAM-1 were measured in sera using a monoclonal antibody sandwich enzyme linked immunoassay. The serum level of cICAM-1 was determined in 20 children with active Juvenile chronic arthritis. The serum level of cICAM-1 was significantly increased in patients with Juvenile chronic arthritis in comparison to the control group (P<0.05). Higher levels of cICAM-1 were recorded in JCA children showing systemic features than in patients with pauciarticular or polyarticular arthritis. Positive correlation was observed between cICAM-1 level and determinants of disease activity. We concluded that cICAM-1 levels are increased in children with JCA and this increment reflects the disease status and activity.

Introduction and Aim of the Work

Rheumatic diseases are characterized by inflammation of connective tissue involving different organs in a multisystemic way. The diagnosis of these disorders and the evaluation of disease activity was based on clinical features and some laboratory tests indicating the presence of autoantibodies and acute phase reactant proteins (Pemi et al, 1991).

The intercellular adhesion molecule (ICAM-1) is a cytokine induced glycoprotein expressed on a multitude of cell lineages and has several vital roles in the normal
function of the immune system. Lymphocytes and other leukocytes interact with ICAM-1 expressed on endothelial cells during recruitment into tissues undergoing inflammatory responses (Springer, 1990).

A soluble form of ICAM-1 (sICAM-1) lacking the transmembranous and intracytoplasmic domains, had also been described. Although the possibility that sICAM-1 inhibit T-cell target cell interaction has been suggested, the physiologic function of sICAM-1 is still not clear. The release of sICAM-1 from cells is affected by the same stimuli that up-regulate cellular ICAM-1 (Rothlein et al, 1991 and Becker et al., 1993). sICAM-1 levels have been reported to be elevated in inflammation, infection and cancer (Gearing & Mewman, 1993).

The aim of this study was to measure the serum level of sICAM-1 in pediatric patients suffering from active Juvenile chronic arthritis (JCA) and to find whether it has a correlation with disease activity or not.

Patients and Methods

This study was carried out in Benha University Hospital (Pediatric & Rheumatology departments) from October 1998 to December 1999.

Twenty children aging 6-16 years with active Juvenile chronic arthritis were submitted to good clinical examination for evaluation of disease activity. The evaluation of disease activity based on the number of affected joints, duration of morning stiffness, swelling and painful joints and presence of systemic features. In addition to 10 age and sex matched healthy children were included as a control group.

The patients were classified according to the criteria established by the American College of Rheumatology (1986) into two groups (Casidy et al. 1986):

- Group I (n = 9) patients with systemic features (Still's disease).
- Group II (n =11) patients with pauciarticular (n =6) and polyarticular arthritis (n=5).

All patients & controls were submitted to the following investigations:

1. Complete blood picture, using the Coulter counter.

2. Erythrocyte sedimentation rate (ESR).

3. C-reactive protein (CRP) (Singer et al., 1957).

4. Serum intercellular adhesion molecule-1 (sICAM-1) (Dustin et al., 1986).
Serum samples were collected from the patients with JCA and controls. The concentration of cICAM-1 was determined by means, of an enzyme linked immunoassay, using mouse monoclonal antibody (Genzyme Diagnostic Comp., Cambridge). The principles of the test depend on using microtiter plate with immobilized mouse monoclonal antibody to human ICAM-1. A measured volume of standards or samples is added to each test well followed immediately by the addition of anti-ICAM-1 HRP-conjugate. Test wells are incubated to allow anti ICAM-1 present to be bound by antibodies on the microtiter plate. The wells are then washed and TMB substrate is added to the wells producing a blue color in the presence of peroxidase enzyme. The color reaction is then stopped by the addition of acid, which changes the blue color to yellow. The intensity of the yellow color is in direct proportion to the amount of ICAM-1 present in the standards or samples. The absorbance of each well is read at 450 nm and a standard curve is constructed to quantitate ICAM-1 concentrations in the samples.

All statistical tests were performed using microstate program. One way analysis of variance were applied to detect difference in group means. Simple regression analysis was used to correlate cICAM-1 with the different studied clinical and biochemical parameters.

RESULTS

Table (1): The cICAM-1 level in patients with juvenile chronic arthritis (JCA) and control group, in ng/ml.

<table>
<thead>
<tr>
<th>Group</th>
<th>JCA (n=20)</th>
<th>Control (n=10)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>625</td>
<td>215</td>
<td>4.47</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SD</td>
<td>±193</td>
<td>±85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2): The cICAM-1 in patients with systemic manifestations (group I) and patients with pauciarticular and polyarticular arthritis (group II).

<table>
<thead>
<tr>
<th>cICAM-1</th>
<th>group I</th>
<th>group II</th>
<th>Control group</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>989</td>
<td>395</td>
<td>215</td>
<td>8.96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SD</td>
<td>±290</td>
<td>±49</td>
<td>±85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group I vs control P < 0.01
Group II vs control P < 0.05
Group I vs Group II P < 0.01
Table (3): The number and percentage of JCA children with cICAM-1 above the cut off value (385 ng/ml)

<table>
<thead>
<tr>
<th>JCA children (n = 20)</th>
<th>Group (I) (n = 9)</th>
<th>Group II (n = 11)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pauciarticular (n=6)</td>
<td>Polyarticular (n = 5 )</td>
<td></td>
</tr>
<tr>
<td>9/9 (100%)</td>
<td>4/6 (67%)</td>
<td>5/5 (100%)</td>
<td>18/20 (90%)</td>
</tr>
</tbody>
</table>

Cut off value 385 ng/ml = mean + 2 SD of the control group.

Table (4): Correlation between cICAM-1 and the studied parameters.

<table>
<thead>
<tr>
<th>° Variable</th>
<th>r</th>
<th>P</th>
<th>Strength of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic manifestations</td>
<td>0.931</td>
<td>&lt;0.01</td>
<td>Strong positive correlation</td>
</tr>
<tr>
<td>Number of affected joints</td>
<td>0.863</td>
<td>&lt;0.01</td>
<td>Strong positive correlation</td>
</tr>
<tr>
<td>Joint swelling and pain</td>
<td>0.781</td>
<td>&lt;0.01</td>
<td>Strong positive correlation</td>
</tr>
<tr>
<td>Duration of morning stiffness</td>
<td>0.621</td>
<td>&lt;0.05</td>
<td>Moderate positive correlation</td>
</tr>
<tr>
<td>ESR</td>
<td>0.91</td>
<td>&lt;0.01</td>
<td>Strong positive correlation</td>
</tr>
<tr>
<td>CRP</td>
<td>0.7</td>
<td>&lt;0.05</td>
<td>Moderate positive correlation</td>
</tr>
<tr>
<td>Hemoglobin concentration</td>
<td>0.561</td>
<td>&lt;0.05</td>
<td>Moderate positive correlation</td>
</tr>
<tr>
<td>Total leucocytic count</td>
<td>0.635</td>
<td>&lt;0.05</td>
<td>Moderate positive correlation</td>
</tr>
</tbody>
</table>

Our results are summarized, statistically analysed and tabulated in (4) tables.

Table (1) shows the serum (cICAM-1) level (ng/ml) in the patients with JCA and control group there was a statistical significant difference between the JCA children and control group (P < 0.05)

Table (2) shows the comparison of cICAM-1 level in the JCA patients with systemic manifestation (group I); patients with pauciarticular and polyarticular arthritis (group II), and control group. There was a statistical significant difference between the studied groups & control group (P < 0.01) with highest values in JCA patients with systemic manifestations.

Table (3) shows the number of patients with cICAM-1 above the cut off value of control group (mean + 2 SD). All patients (100%) with systemic manifestations and polyarthritis had cICAM-1 level above the cut off value, while (67%) of pa-
tients with pauciarticular arthritis had cICAM-1 above the cut off value.

Table (4) shows the correlation between cICAM-1 and different studied clinical and biochemical parameters. There were positive correlations between cICAM-1 level in JCA patients and systemic manifestation, number of affected joints, joint swelling and pain, duration of morning stiffness, ESR, CRP total leukocytic count and hemoglobin concentration.

**Discussion**

In our study the level of cICAM-1 is significantly increased in children with juvenile chronic arthritis in comparison to the control group; and this agree with Aoki et al. (1993) who reported high cICAM-1 levels in adult patients with rheumatoid arthritis and systemic lupus erythematosis. (Oppenheimer and Lipsky, 1996).

The high levels of cICAM-1 may contribute in the immunological abnormalities seen in this disease. cICAM-1 may play its physiological effects by competing in T-cell target cell interaction, by blocking lymphocyte associated factor-1, the co-receptor of ICAM-1 and prevents them from binding to membrane bound inflammation; and by triggering a response in ligand bearing cells to facilitate adhesion of leukocytes bound to endothelium, so that transendothelial migration can occur to the site of the inflammation (Becker et al., 1993; Gearing & Newman, 1993). Schulz et al (1995) found that the abnormal elevation of Th0 and Th1 In peripheral circulation of patients with rheumatoid arthritis could be modulated with anti ICAM-1 and this modulation correlate with the clinical benefit of the patients.

Also we found significant higher level of cICAM-1 in JCA-children with systemic features than those with polyarticular or pauciarticular arthritis. This agrees with Loucella et al. (1999) who reported similar results. Elevated levels of cICAM-1 would be expected to reflect the overall level of inflammatory response through the broad distribution of this molecule. Thus the higher level of cICAM-1 in JCA children with systemic features might be related to the associated intense cytokine release observed in
the former group.

Dastin et al. (1986) found higher levels of interferon-γ, tumour necrosis factor-α, interleukin-1 α, and interleukin-6 in JCA patients with systemic features than in JCA patients with polyarthritiarcular or pauciarticular arthritis. Prieto et al. (1992) also reported that cICAM-1 is induced on target cells by proinflammatory cytokines such as interferon-γ, tumour necrosis factor-α, interleukin-1α.

Our results showed that 90% of patients with JCA had high levels of cICAM-1 (above the cut-off value 385 ng/ml) while Laucella et al. (1999) found only 57% of patients suffering from JCA had cICAM-1 level above the cut-off value. The higher levels in our study may be attributed to that all our patients were active while Laucella patients were mixed (20% in remission and 80% active).

In this study the cICAM-1 levels in children with JCA were positively correlated with the presence of systemic manifestations, number of affected joints, joint swelling and pain, duration of joint stiffness, ESR total leukocytic count, and hemoglobin concentration. Bullard et al. (1996) also observed a correlation between the level of ICAM-1 in serum and synovial fluid and disease activity, as well as a reduced incidence of arthritis in ICAM-1 homozygous mutant mice.

Collectively, these results suggested that cICAM plays a crucial role in the immunological abnormalities seen in the disease and the high cICAM levels in children with JCA might reflect the disease status and activity so it may be used as a useful tool to assess the disease activity. Also, we recommend further study with pharmacological approaches for reduction in the expression or function of cICAM by using anti-cICAM monoclonal antibodies which may be of therapeutic value.

REFERENCES


4. Cassidy, J.T.; Levinson, J.E.
Serum Intercellular Adhesions


