Assessment of Soluble ICAM-1 and Soluble E-SELECTIN in Juvenile Idiopathic Arthritis: Clinical and Laboratory Correlation

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Juvenile idiopathic arthritis (JIA) is a systemic inflammatory disease with dysregulation of normal immune responses that lead to chronic tissue inflammation and damage. Synovial neoangiogenesis, one of the pathologic hallmarks of JIA, was recently found to be caused mainly by vascular endothelial cell growth factor and was associated with increased infiltration of inflammatory cells. The activation, migration and penetration of leukocytes into local inflammatory tissues are dependent on attachment to adhesion molecules on endothelial cells. For that reason, adhesion molecules are believed to play part in initiation and propagation of autoimmune diseases. So the aim of this work is to measure serum and synovial fluid (SF) concentrations of soluble adhesion molecule-1 (ICAM-1) and E-selectin in juvenile idiopathic arthritis patients and to correlate them with clinical and laboratory variables. A Sandwich ELISA technique was used to estimate the serum and synovial levels of soluble intercellular adhesion molecule-1 (ICAM-1) and E-selectin in 38 patients with JIA, 12 systemic (JIA-sys), 13 polyarticular (JIA-poly) and 13 oligoarticular (JIA-oligo.), who had active disease or were in clinical remission. Fifteen healthy subjects matched in sex and age with the patients group were studied as control group. The total leukocytic (blood & synovial) and platelet counts, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were recorded. A significantly higher level of sICAM-1 and E-selectin (p < 0.001 & < 0.05, respectively) were found in (JIA-sys) & (JIA-poly) than in (JIA-oligo) or in control group. The level of both molecules in the three JIA subtypes, in the active stages and clinical remission, were still higher than in control group. A significant negative correlation with age was observed for the group as a whole (p < 0.001 for both, sICAM-1: r = -0.581 & sE-selectin: r = -0.497) while no correlation was found with disease duration or ESR. sE-selectin was correlated with total blood leukocytic and platelet counts (p < 0.05 for both, r = 0.37 & 0.34, respectively) and both molecules with CRP (p < 0.05, ICAM-1: r = 0.33 & E-selectin: r = 0.35) and with each other (p < 0.01, r = 0.600). No correlation was found between serum and synovial adhesion molecules (p > 0.05) or between SF adhesion molecules concentration and total SF leukocytic count (p > 0.05).

Conclusion: Increased level of soluble adhesion molecules in both (JIA-sys) & (JIA-poly) may be due to endothelial cell activation which is the key to the pathogenesis of JIA especially in systemic subtype and its persistence in spite of clinical remission could be used as a marker of aggressive disease.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is one of the most common rheumatic diseases of children and a major cause of chronic disability, especially the polyarticular and systemic subtypes. The diagnosis of this disorder and evaluation of disease activity is based on the clinical findings and laboratory tests that show the presence of autoantibodies, changes in serum immunoglobulins and acute phase proteins. This systemic inflammation is characterized by activation of the vascular endothelium. Endothelial activation is the key to the pathogenesis of JIA, especially in the systemic subtype. Pro-inflammatory cytokines stimulate endothelial cells to express E-selectin and intercellular adhesion molecule-1 (ICAM-1). Soluble adhesion molecules (SAMs) are commonly formed as the result of cell surface adhesion molecules shedding due to cell stimulation. Soluble adhesion molecules has been shown to enhance and inhibit different aspects of the inflammatory process. SAMs has been shown to be important regulators of leukocyte recruitment into the synovial tissue.

Cell adhesion molecules are large groups of cell surface proteins. These molecules (especially selectin) play an important role in the pathogenesis of endothelial–leukocyte interaction angiogenesis and lymphocyte activation leading to the progression of the disease. Up-regulation of adhesion molecule production and or cell surface expression on endothelial cells and leukocytes depends upon stimulation with inflammatory cells in the

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tissues. E-selectin is exclusively expressed by activated endothelial cells. As the selectin adhesion molecules are shed from the activated cells the concentration of the soluble form of these proteins can be used as activation markers of endothelium. The measurement of circulating soluble ICAM-1 is considered to be less specific for endothelial cell activation as ICAM-1 is present and inducible on a variety of cell types including endothelial cells, leukocytes and fibroblasts. De Benedetti et al. studied the concentration of soluble adhesion molecules in different types of juvenile idiopathic arthritis (JIA) and found a high concentration of soluble ICAM-1 and E-selectin in patients with systemic disease.

So this work aims to study the serum and synovial fluid levels of soluble E-selectin and ICAM-1 among different subtypes of JIA and their possible correlation with clinical and laboratory variables of disease activity.

SUBJECTS AND METHODS

The present study was carried out at the Microbiology & Immunology and Rheumatology & Rehabilitation Departments, Faculty of Medicine Zagazig University in the period from December 2004 to September 2005.

Subjects:

Thirty eight patients from the Outpatient Clinic of Rheumatology & Rehabilitation Department of Zagazig University Hospitals, who fulfilled the criteria for classification of juvenile idiopathic arthritis, were investigated and divided according to their disease course into three groups:

Group I: consisted of 12 patients with systemic onset type (JIA-sys).
Group II: consisted of 13 patients with polyarticular type (JIA-poly).
Group III: consisted of 13 patients with oligoarticular type (JIA-oligo).

Active synovitis was defined as the presence of intra-articular swelling or limitation in the range of joint movement with pain or tenderness.

Patients were considered to be in clinical remission when they had morning stiffness not exceeding 15 minutes, no fatigue, no joint pain, no joint tenderness, no joint or tendon sheath swelling and or erythrocytes sedimentation rate of less than 20 mm/1st hour based on American college of rheumatology (ACR) criteria for remission of adult rheumatoid arthritis (RA). The patients received NSAIDs with methotrexate with or without concomitant corticosteroids administration. 15 apparently healthy subjects matched in age and sex with patients group were used as control group.

Samples:

Patients group: serum samples were taken from the 38 patients for estimation of soluble adhesion molecules level (ICAM-1 & E-selectin) and CRP. Heparinized synovial fluid samples were taken for measuring the level of synovial soluble adhesion molecules (ICAM-1 & E-selectin) and the total synovial leukocytic count. EDTA blood was used for routine measuring of total blood leukocytic & platelet counts and blood on citrate was taken. Control group: serum samples were taken from 15 health subjects for determining the level of soluble adhesion molecules (ICAM-1 & E-selectin).

Methods:

Serum and heparinized synovial fluid supernatant samples were separated following synovial fluid centrifugation within 3 hours from venepuncture joint aspiration and stored at −70 °C until tested. ICAM-1 and E-selectin concentration were estimated using sandwich ELISA kit according to the manufacturer’s instructions (Diacrone Research, France) and (Barton lane, Abingdon Oxon, United Kingdom). The colour intensity that was measured at 450 nm and reference wave length 620-630 nm is proportional to the amount of the soluble adhesion molecules in the samples. Values were determined by interpretation of values from standard curve determined by spectrophotometric readings from a series of dilution of a positive control with a known concentration of each molecule. Total leukocytic count (blood & synovial), platelet count and ESR were routinely obtained. CRP was estimated by latex agglutination test (Stanbio, Lab, Texas).

Statistical analysis:

For comparison of several means, F test was used and for multiple comparison,
Least significant difference (LSD) were computed. Testing association between two variables, correlation coefficient (r) were computed. P value is considered significant if $\leq 0.05$.

**RESULTS**

**Clinical data:**

The mean age at evaluation was 7.49 ± 4.16 years (range 1-16) for the group as a whole. 8.28 ± 4.03 years (range 3-16) for the children with systemic disease, 8.23 ± 3.81 years (range 3-16) for children with polyarticular disease and 6.57 ± 4.71 years (range 1-15) for children with oligoarticular disease. The duration of disease until evaluation was 24.02 ± 14.79 months (range 2-96) for the whole group, 25.33 ± 14.87 months (range 2-96) for the children with systemic disease, 23.76 ± 16.15 months (range 6-84) for children with polyarticular disease and 23.07 ± 14.43 months (range 2-96) for children with oligoarticular disease. Age and duration of disease were not significantly different among subtypes groups (Table: 1).

A significant negative correlation of serum ICAM-1 & E-selectin with age was observed for the group as a whole ($p < 0.001$ for both, $r = -0.581$ & $r = -0.497$, respectively) (Table: 2).

**Serum concentrations of soluble ICAM-1 and E-selectin:**

The mean serum concentration of soluble ICAM-1 in the JIA-sys group & JIA-poly group (469.80 ±173.11 & 446.46 ± 169.96 ng / ml respectively) were significantly higher ($F = 4.92$, $p < 0.001$) than that concentration seen in the control group (288.93 ± 20.6 ng / ml). Also E-selectin mean concentration in the JIA-sys group & JIA-poly group (68.16 ±13.68 & 64.53 ± 16.23 ng / ml, respectively) were significantly higher ($F = 3.39$, $p < 0.05$) than that concentration seen in the control group (49.04 ± 13.97 ng / ml). In comparison, when we examind JIA-oligo group we did not find any significant difference ($p > 0.05$) in the mean concentration of soluble ICAM-1 & E-selectin (381.38 ± 135.54 & 60.55 ± 22.10 ng /ml respectively) as compared with the control group (288.93 ± 20.65 & 49.04 ± 13.97 ng /ml, respectively) (Table: 1). Of note serum levels of soluble ICAM-1 and E-selectin were significantly correlated with each other ($p < 0.01$, $r = 0.600$) (Table: 2).

**Serum versus synovial fluid (SF) concentrations:**

The mean concentration of serum soluble ICAM-1 & E-selectin levels (431.57±160.22 & 64.32±17.59 ng/ml, respectively) appeared to be higher than that concentration in the SF (414.54 ± 159.31 & 54.81 ± 13.46, respectively ng / ml) but the correlation was not statistically significant ($p > 0.05$ for both, $r = 0.172 & r = -0.086$, respectively) (Table: 2).

**Correlations between clinical and laboratory variables:**

We next correlated the level of soluble adhesion molecules with parameters of disease activity (Table: 3). We did not found any correlation between soluble adhesion molecules and disease duration or ESR ($p > 0.05$). There was a statistically significant correlation of serum soluble ICAM-1 and E-selectin levels with CRP in all JIA groups ($p < 0.05$). There was a statistically significant correlation of serum soluble ICAM-1 and E-selectin levels with total blood leukocytic and platelet counts ($p < 0.05$ for both, $r = 0.37 & 0.34$, respectively) while ICAM-1 did not. Neither of the SF adhesion molecules concentration correlated with the total SF leucocytic count ($p > 0.05$, ICAM-1: $r = 0.17$ & E-selectin: $r = 0.25$).
Table (1): Comparison of age, duration and adhesion molecules (ICAM-1 & E-selectin in serum & Synovial fluid) among patients and Control group.

<table>
<thead>
<tr>
<th></th>
<th>JIA-SYST. (n=12)</th>
<th>JIA-poly. (n=13)</th>
<th>JIA-oligo. (n=13)</th>
<th>Control group (n=15)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>8.28</td>
<td>8.23</td>
<td>6.57</td>
<td>7.01</td>
<td>4.24</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>± 4.03</td>
<td>± 3.81</td>
<td>± 4.71</td>
<td>± 4.24</td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>DURATION</td>
<td>25.33</td>
<td>23.76</td>
<td>23.07</td>
<td>14.43</td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>± 14.87</td>
<td>± 16.15</td>
<td>± 14.43</td>
<td>±</td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S ICAM-1</td>
<td>469.80</td>
<td>446.46</td>
<td>381.38</td>
<td>288.93</td>
<td>4.92</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>± 173.11**</td>
<td>± 169.96**</td>
<td>± 135.54</td>
<td>± 20.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S E-selectin</td>
<td>68.16</td>
<td>64.53</td>
<td>60.55</td>
<td>49.04</td>
<td>3.39</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td></td>
<td>± 13.68*</td>
<td>± 16.23*</td>
<td>± 22.11</td>
<td>± 13.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF ICAM-1</td>
<td>435.97</td>
<td>443.23</td>
<td>366.07</td>
<td>136.52</td>
<td></td>
<td>0.917</td>
</tr>
<tr>
<td></td>
<td>± 172.27</td>
<td>± 168.89</td>
<td>± ± 136.52</td>
<td>± ± 0.917</td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>SF E-selectin</td>
<td>59.22</td>
<td>54.63</td>
<td>50.91</td>
<td>15.21</td>
<td>1.20</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>± 14.41</td>
<td>± 10.05</td>
<td>± 15.21</td>
<td>± ± 1.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X = Mean  
SD = standard deviation  
* P < 0.05 is significant  
** P < 0.001 is highly significant  
* Least significant difference (LSD) of serum ICAM-1 & E-selectin with (JIA-SYS & JIA-poly groups).

Table (2): Correlation (r) of soluble adhesion molecules with age and with each other.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>SF ICAM-1</th>
<th>S E-selectin</th>
<th>SF E-selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>S ICAM-1</td>
<td>-0.581**</td>
<td>0.172</td>
<td>0.600**</td>
<td>-0.017</td>
</tr>
<tr>
<td>SF ICAM-1</td>
<td>0.181</td>
<td>--</td>
<td>-0.044</td>
<td>0.447**</td>
</tr>
<tr>
<td>S E-selectin</td>
<td>0.497**</td>
<td>-0.044</td>
<td>--</td>
<td>-0.086</td>
</tr>
<tr>
<td>SF E-selectin</td>
<td>0.185</td>
<td>0.447**</td>
<td>-0.086</td>
<td>--</td>
</tr>
</tbody>
</table>

*Correlation (r) is significant at the 0.01 level.

Table (3): Correlation (r) of soluble adhesion molecules with some laboratory variable (ESR, CRP, total Leukocytic & Platelet counts).

<table>
<thead>
<tr>
<th></th>
<th>ESR</th>
<th>CRP</th>
<th>Leukocytes</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>S ICAM-1</td>
<td>0.27</td>
<td>0.33*</td>
<td>0.21</td>
<td>0.23</td>
</tr>
<tr>
<td>S E-selectin</td>
<td>0.19</td>
<td>0.35*</td>
<td>0.37*</td>
<td>0.34*</td>
</tr>
<tr>
<td>SF ICAM-1</td>
<td>0.18</td>
<td>0.23</td>
<td>0.17</td>
<td>0.25</td>
</tr>
<tr>
<td>SF E-selectin</td>
<td>0.17</td>
<td>0.24</td>
<td>0.25</td>
<td>0.18</td>
</tr>
</tbody>
</table>

* Correlation (r) is significant at the 0.05 level.

**DISCUSSION**

E-selectin and intercellular adhesion molecule (ICAM)-1 are crucial to the inflammatory response in chronic inflammatory arthritis. The level of these soluble adhesion molecules in sera and synovial fluid (SF) correlate with some clinical parameters and synovial tissue expression of the same molecules in rheumatoid arthritis (Bloom et al., 2002). In our study we found a significant increase in serum concentration of
soluble adhesion molecules, ICAM-1 and E-selectin in (JIA-sys) and (JIA poly) than in (JIA-oligo) and control group. This was in agreement with (De Benedetti et al., 2000)^{(6)} & (Bloom et al., 2005)^{(7)} both found higher level of E-selectin in (JIA sys) than any other group. This finding may explain why systemic JIA is associated with greater morbidity and is more refractory to medical treatment than the oligoarticular group, probably (JIA-sys) causes more inflammation through reaction of adhesion molecules.

Previously mentioned studies (De Benedetti et al., 2000)^{(8)} & (Bloom et al., 2005)^{(9)} observed no significant increase of (sICAM) and (sE-selectin) in (JIA-poly) type in contrast to our findings. But our findings were in agreement with Dolezalova et al., 2002^{(9)} who found a significant increase of soluble adhesion molecules (SAMs) in (JIA-pol.) and (Chen et al., 2002)^{(10)} who found a significant increase of (SAMs) in both (JIA-sys) & (JIA-poly).

A significant negative correlation with age was found for both soluble adhesion molecules. This observation was also found by Nash et al., 1995, Boolum et al., 1999 and Dolezalova et al., 2002^{(11)} A negative correlation between SAMs and age may be explained by reduction of its concentration due to the therapeutic effect of drugs, the significant increase of SAMs was observed in the systemic type that starts in young ages.

We did not find any correlation between (SAMs) level and disease duration or ESR and this was in agreement with previous studies (Chen et al., 2002 and Dolezalova et al., 2002).^{(6,9)} On the other hand there was a statistically significant correlation of serum ICAM-1 and E-selectin with CRP in all patient groups which was in agreement with (De Benedetti et al., 2002).^{(8)}

Interpretation of the comparison of serum ICAM-1 and E-selectin levels versus synovial fluid adhesion molecules was even more difficult. Carson et al. (1994)^{(4)} found higher synovial fluid than serum concentration of E-selectin, Mason et al.(1993)^{(12)} reported increased synovial ICAM-1 level as compared to serum level while the reverse was observed by Cush et al.(1993)^{(13)}.

Dolezalova, et al.(2002)^{(9)} find a tendency for SF ICAM-1 to be higher than in paried sera, but without statistically significant difference. Our result found that serum adhesion molecules (ICAM-1 & E-selectin) level were higher than those of SF adhesion molecules level but without statistically significant correlation. There were many possible explanations for these unexpected findings. Frist, due to non-homogenous groups (polyarticular and oligoarticular) as seen in the adult, a wide variability in disease duration, the small number of patients in each group did not allow us to make comparisons (Dolezalova, et al. 2002)^{(9)}. The last one was that the difference in adhesion molecules concentration both in serum and synovial fluid could be explained by the different passage of the shed molecules from the synovial interstitium into the blood and synovial fluid (Krenn et al. 1997).^{(12)}

Our findings showed additionally that sE-selectin correlates with total blood leukocytic and platelet counts while ICAM-1 did not. This was in agreement with Dolezalova, et al. 2002^{(9)}. Neither of synovial adhesion molecules concentration correlated with the SF total leukocyte count. This was in contrast to (Mason. et. al., 1993 & Carson et al., 1994)^{(14,15)} but coincided with Dolezalova, et al. 2002^{(9)}. We think a further large cohort study is needed to provide more information about the value of SAMs for monitoring inflammatory arthritis.

Perhaps, the most interesting observation of this study was the persistent increase of SAMs in spite of remission. This was occur in JIA especially polyarticular and systemic onset types which was in agreement with Chen et al, 2002^{(6)}. A possible explanation may be that although we clinically define patients as in remission, increased production and endothelial cell activation, which causes tissues inflammation and damage may still continue insidiously and this is expressed by an increase of SAMs, resulting in recurrence.

**Conclusion:**

Increased levels of soluble adhesion molecules in both (JIA-sys) & (JIA-poly) may be due to endothelial cell activation which is the key to the pathogenesis of JIA especially in systemic subtype and its persistence in spite of clinical remission could be used as markers of aggressive disease, but their predictive value needs to be further studied. AS significantly higher level of sICAM-1 and sE-selectin were found in the active
stages and clinical remission of the three JIA subtypes compared with those in the control group, JIA may recur even when clinical remission has been achieved.

REFERENCES

18. Pinals RS, Masi AT, Larsen RA and the subcommittee for criteria of remission in rheumatoid arthritis of the American Association Diagnostic and Therapeutic Criteria Committee.


تقييم الجزيئات اللاصقة الذانية في مرض الرثيان المفصلي الحديث وربطها بنشاط المرض

(ألكلينيكي وعمليا)

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قسم الميكروبولوجي ولفقار وروماتزم والتاهيل

إن أسباب المظهر المبكر في مرضي الرثيان المفصلي الحديث مازالت غير واضحة حتى الآن، ولم يحدد سبب واضح لحدث الريثيان المفصلي الحديث و إن هناك آليات مختلفة يمكنها أن تؤدي إلى ضرر في الأنسجة و تسبب التهاب في الغشاء الميسيفي.

الهدف: وقد استهدفت هذه الدراسة تحديد مستوى الجزيئات اللاصقة الذانية (إيكام-1، إي سلكاتين) في المصل والسائل السينوفي في مرضى الرثيان المفصلي الحديث و أنواعه الثلاثة مع دراسة علاقة هذه الجزيئات اللاصقة ببعض ظواهر نشاط المرض.

الطريقة: تمتد الدراسة على 38 حالة يعانون من مرض الرثيان المفصلي الحديث و 15 أحياناً أصحاء كمجموعة ضبطة، تم تقسيم المرضى على حسب بداية ظهور المرض إلى ثلاثة مجموعات (12 نوع التهاب مفصلي جاهزي و 13 نوع التهاب مفصلي متعدد) حيث أخذت عينات من المصل و السائل السينوفي من مجموعة المرضى وعينات من المصل فقط من المجموعة الضبطة. وتم قياس مستوى الجزيئات اللاصقة الذانية بطريقة الإلإزا. و أيضاً تم قياس روتيني لكل من سرعة الترسيب، البروتون النشط، والعدد الكلي لكل من الكرات البيضاء (في الدم و السائل السينوفي) والصفيحة الدموية.

النتائج: وقد أظهرت نتائج هذا البحث:

1- إن هناك زيادة ذو دلالة إحصائية في مستوى الجزيئات اللاصقة الذانية في المصل في مجموعتي المرضى (نوع التهاب المفصلي الجاهزي و مجموعة التهاب المفصل المتعدد) عنها في المجموعة الثالثة غير متعدد المفاصل أو المجموعة الضبطة.

2- إن هناك أيضاً زيادة في مستوى الجزيئات اللاصقة الذانية في المصل لمرضى الرثيان المفصلي في المجموعات الثلاثة مقارنة بالمجموعة الضبطة في حالتي (النشاط والتراجع الإكليكتي للمرض).

3- كما وجدت علاقة عكسية بين مستوى الجزيئات اللاصقة الذانية في المصل و سن المرض.

4- العلاقة بين مستوى الجزيئات اللاصقة الذانية وبعض ظواهر النشاط المصاحب للمرض:

- هناك علاقة بين معدل إي سلكاتين الذانيّ في المصل و العدد الكلي لكل من كرات الدم البيضاء و الصفيحة الدموية.

- هناك زيادة ذو دلالة إحصائية في مستوى الجزيئات اللاصقة الذانية في المصل والبروتين النشط.

- لا يوجد علاقة ذو دلالة إحصائية في مستوي الجزيئات اللاصقة الذانية في السائل السينوفي وذلك الذانية في المصل أو مع العدد الكلي للكرات البيضاء في السائل السينوفي.

الاستنتاج: و تستلزم من هذه الدراسة أن مستوى (إيكام-1) الفي للذئاب و إي سلكاتين الفي للذئاب يعكس نشاط المرض والآلام الإكليكتية لمرضي الرثيان المفصلي حيث أنه كان زائداً في مجموعات التهابات المفصل المتعدد و التهابات المفاصل الجهازية و بدلاً على هذا يمكن استخدام قياس مستوى الجزيئات اللاصقة كمؤشر على نشاط المرض كما أنه يمكن أن يكون للعلاج الدوائي الذي يعمل على هذه الجزيئات الملتصفة دور في التحكم في الأعراض الإكليكتية لهذا المرض.

المصادر: 
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