A Study of Interleukin-8 (IL-8), Intercellular Adhesion Molecule-1 (ICAM-1) and Nitric Oxide (NO) in Assessment of Children with Asthma

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Abstract:

To evaluate the possible role of serum ICAM-1, IL-8 and NO in assessment of children with asthma the present study included 45 children with asthma and 15 control children. The asthma cases were 30 males and 15 females (age from 4 to 12 years). According to the severity of asthma, patients were classified as mild (n=19), moderate (n=12) and severe (n=14) asthma. Estimation of serum levels of ICAM-1, IL-8 and NO were done. Serum levels of ICAM-1 were significantly higher in asthmatic than in control children, in severe than in moderate, and in both than in mild asthma cases. Serum levels of IL-8 were significantly higher in asthmatics than in controls. Also serum levels of IL-8 were significantly higher in cases with severe and cases with moderate asthma than in cases with mild asthma. A strong positive correlation was present between the serum levels of ICAM-1 and IL-8. The serum levels of NO showed an insignificant difference between asthmatic children and controls.

In conclusion, the present study supports that IL-8 may participate in bronchial hyperactivity in asthma. The serum level of ICAM-1 can be useful as one of the indices of assessment of the severity of asthma in children. New modalities of therapy including anti-adhesive molecules is worthy study.

Introduction:

Asthma remains a leading cause of chronic illness in children\(^1\). Current theories of the pathogenesis of asthma suggest that airway inflammation is an important determinant of bronchial hyperactivity\(^2\). The interaction of several inflammatory cells, soluble mediators and adhesion molecules may be important determinants of asthma\(^3,4\). Several inflammatory cells may participate in the inflammatory response to an inhaled antigen\(^1,5,6\). Soluble mediators may play a major role in the development of acute and chronic allergic inflammation\(^7\). One of these mediators, IL-8 may play a major role in determining the type of cells found at the site of inflammation\(^8\). ICAM-1 is involved in the recruitment and activation of T-lymphocytes\(^9\) and may serve as the ligand for some respiratory viruses as well as for leukocytes\(^10,11\).

On the other hand, NO affects T lymphocytes\(^12\), neutrophils\(^13\) and macrophages\(^14\) and the increased exposure to NO in car exhaust, gas cookers and cigarette smoke has been blamed, in part, for the increased incidence of asthma over the last few decades\(^15\).

The present study aims to evaluate the possible role of estimation of serum levels of ICAM-1, IL-8 and NO in assessment of asthma in children.

Subjects and Methods:

The present study included 45 children with asthma (30 males and 15 females) aged from 4 to 12 years (mean ± SD 7.38±3.11 years), recruited from the outpatient clinic and emergency unit of Pediatric Department, Assiut University Hospitals. Inclusion criteria included: recurrent episodes of coughing, wheezing and breathlessness, aggravation or triggering of symptoms by exercise, common respiratory viral infection or during spring season, relief of symptoms by the use of bronchodilators, corticosteroids or subcutaneous epinephrine.

Patients included in the study were thoroughly examined clinically and by urine and stool examination for parasites. All children with parasitic infestations, those with suspected tuberculosis, foreign body inhalation, bronchiectasis, pneumonia or any other anatomic or congenital (respiratory, cardiovascular or gastrointestinal) malformations were excluded from the study.

Detailed history from enrolled cases included: age of the patient, consanguinity, family history of atopic disease, number of asthmatic attacks, previous admission to hospitals, other allergic problems, cough, wheezing, exercise tolerance, school attendance, interrupted sleep, use of bronchodilators, corticosteroids or asthma prophylaxis.
According to the severity of asthma patients were classified as follows:

1- Nineteen patients with mild asthma (12 males and 7 females) aged from 4 to 12 years (M±SD 8.10±3.43 years); their asthmatic attacks recurred no more than once each week and responded to bronchodilator treatment within 1 or 2 days, medications were not required in between the attacks, they enjoyed good school attendance, fair exercise tolerance most times and uninterrupted sleep.

2- Twelve patients with moderate asthma (8 males and 4 females), aged from 4 to 11 (M±SD 6.08±2.71 years); they suffered from cough and mild wheezing between the more severe exacerbations, school attendance was to some extent impaired, their exercise tolerance was diminished, they had interrupted sleep at night and required bronchodilator therapy most times.

3- Fourteen patients with severe asthma (10 males and 4 females), aged from 4 to 12 years (M ± SD 7.10±3.34 years); they had daily wheezing and more frequent exacerbations that required recurrent hospitalization, school attendance was greatly affected and their sleep was often interrupted by asthma, they had poor exercise tolerance and continuous therapy especially with steroids was needed.

The controls were 15 apparently healthy children of matchable age and sex free from any allergic disease, chronic disease or any family history of atopy. They attended the hospital for minor surgical problems and they gave verbal consent to participate in the study.

From each child 5 cc of blood were obtained by venipuncture for blood picture and separation of serum. Sera were kept at -70°C till analysis. Both patients and controls were subjected to:

1- Chest x-ray postero-anterior view
2- Complete blood picture including differential leucocytic count
3- Estimation of serum levels of intercellular adhesion molecule-1 (ICAM-1) by ELISA method using kits supplied by Immunotech Company, France, Cat. No. 2169.
4- Estimation of serum level of interleukin-8 (IL-8) by ELISA method (Immunotech, France, Cat. No. 2237).
5- Nitric oxide serum levels were determined by the evaluation of its oxidation products; nitrates and nitrites; where nitrates were reduced to nitrites with cadmium fillings, the total concentration of nitrite was determined by using Griess reaction(16,17).

**Results:**

Blood neutrophil and eosinophil absolute counts, serum levels of ICAM-1, IL-8 and NO in children with asthma and in controls are shown in table I.

Patients with blood eosinophilic count <400/mm³ (n=16), showed no significant difference from patients with blood eosinophilic count >400/mm³ (n=29), regarding: duration of illness, asthma severity, neutrophil count as well as serum levels of ICAM-1, IL-8 and NO. Table II shows blood neutrophil, and eosinophil absolute counts, serum levels of ICAM-1, IL-8 and NO in asthmatic children in relation to the severity of disease. The studied asthmatic patients showed no significant differences by the degree of severity regarding each of: reported age, reported duration of illness, and reported other allergic symptoms. However, the frequency of a positive previous history of hospital admission was significantly higher with severe asthma (100%) than with either moderate (58.3%) or mild (47.4%) (p<0.005 for both) and significantly higher with moderate than mild disease (p<0.05).

Table III shows some clinical and laboratory parameters in children with asthma in relation to the use of steroid.

Figure (1) shows a strong positive correlation between serum levels of ICAM-1 and of IL-8 in the studied children with asthma (r=0.897, p<0.001).

No significant correlations were found between either absolute neutrophil count or absolute eosinophil count on one hand, and each of serum levels of ICAM-1, IL-8 and NO on the other hand.
Table I: Blood neutrophil and eosinophil absolute counts, serum levels of ICAM-1, IL-8 and NO in children with asthma and controls

<table>
<thead>
<tr>
<th></th>
<th>Neutrophils Cell/mm³</th>
<th>Eos. Count Cell/mm³</th>
<th>ICAM-1 Ng/ml</th>
<th>IL-8 Pg/ml</th>
<th>NO µmol/L</th>
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<tbody>
<tr>
<td><strong>Patients (N=45)</strong></td>
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<tr>
<td>5450.33 ± 3582.09</td>
<td>620.86 ± 465.19</td>
<td>461.40 ± 130.60</td>
<td>234.78 ± 69.42</td>
<td>48.97 ± 12.95</td>
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<td><strong>Controls (N=15)</strong></td>
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<tr>
<td>5363.93 ± 1300.84</td>
<td>70.09 ± 92.6</td>
<td>310.09 ± 46.97</td>
<td>103.10 ± 32.86</td>
<td>45.53 ± 8.99</td>
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<tr>
<td><strong>Significance</strong></td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table II: Blood neutrophil and eosinophil absolute counts, serum levels of ICAM-1, IL-8 and NO in children with asthma and controls

<table>
<thead>
<tr>
<th></th>
<th>Neutrophils Cell/mm³</th>
<th>Eos. Count Cell/mm³</th>
<th>ICAM-1 Ng/ml</th>
<th>IL-8 Pg/ml</th>
<th>NO µmol/L</th>
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<tbody>
<tr>
<td><strong>Mild (N=19)</strong></td>
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<tr>
<td>5262.84 ± 3353.20</td>
<td>759.47 ± 582.28</td>
<td>364.01 ± 91.05</td>
<td>188.53 ± 41.57</td>
<td>47.84 ± 12.56</td>
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<tr>
<td><strong>Moderate (N=12)</strong></td>
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<tr>
<td>4388.58 ± 3564.02</td>
<td>502.41 ± 323.58</td>
<td>489.45 ± 66.00</td>
<td>252.70 ± 66.36</td>
<td>46.83 ± 11.59</td>
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<tr>
<td><strong>Severe (N=14)</strong></td>
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<tr>
<td>6614.85 ± 3815.80</td>
<td>533.85 ± 354.02</td>
<td>569.52 ± 124.04</td>
<td>282.19 ± 65.96</td>
<td>52.35 ± 14.73</td>
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<tr>
<td><strong>I versus II</strong></td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>NS</td>
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<tr>
<td><strong>I versus III</strong></td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>NS</td>
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<tr>
<td><strong>II versus III</strong></td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.05</td>
<td>NS</td>
<td>NS</td>
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</table>

Table III: Some clinical and laboratory parameters in children with bronchial asthma in relation to the use of steroid

<table>
<thead>
<tr>
<th></th>
<th>Group A Not on steroids N = 35</th>
<th>Group B On steroids N = 10</th>
<th>P (Significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Audible wheezing</td>
<td>18 (51.5%)</td>
<td>9 (90%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Exercise induced asthma</td>
<td>25 (71.4%)</td>
<td>8 (80%)</td>
<td>NS</td>
</tr>
<tr>
<td>Other allergic symptoms</td>
<td>4 (11.1%)</td>
<td>1 (10%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hospital admission</td>
<td>20 (57.1%)</td>
<td>10 (100%)</td>
<td>P&lt;0.05</td>
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<tr>
<td>Severity</td>
<td></td>
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<tr>
<td>Mild</td>
<td>18 (51.4%)</td>
<td>1 (10%)</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Moderate</td>
<td>12 (34.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>5 (14.3%)</td>
<td>9 (90%)</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (cell/mm³)</td>
<td>4764.54±483.01</td>
<td>7850.60±3750.80</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Eosinophils (cell/mm³)</td>
<td>460.97±483.01</td>
<td>550.50±411.79</td>
<td>NS</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>444.25±123.46</td>
<td>520.47±144.28</td>
<td>NS</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>227.63±17.66</td>
<td>259.81±57.16</td>
<td>NS</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>48.34±11.10</td>
<td>51.20±18.64</td>
<td>NS</td>
</tr>
</tbody>
</table>
Discussion:

Airway inflammation is an important determinant of bronchial hyperactivity in asthma cases. An inflammatory stimulus results in the production of cytokines and/or chemoattractants, the resident airway cells (mast cells, helper T cells) or epithelial cells) may secrete pro-inflammatory mediators such as tumor necrosis factor, interleukin-1, -6, and -8 and platelet activating factor. The cytokines and chemoattractants activate bronchial microvascular endothelial cells and/or circulating leukocytes thereby initiating a leukocyte endothelial adhesion cascade. In the final step leukocytes traverse the basement and pseudostratified columnar epithelium to gain access to the airway lumen. The adhesion receptor ICAM-1 appears important for the final step in leukocyte infiltration, namely, leukocyte-epithelial adhesion.

In the present study, serum levels of ICAM-1 were significantly higher in asthmatics than in control children. This goes with Shiota et al. and Kobayashi et al. who reported increased serum levels of ICAM-1 in asthmatic patients. However, El-Genaidy et al. reported no significant difference in serum levels of ICAM-1 between asthmatic children and controls. In the present series, the serum levels of ICAM-1 were significantly higher in severe than in moderate and in both than in mild asthma cases. In sensitized monkeys, immunohistochemical analysis of airway section taken after multiple antigen challenges clearly demonstrated a marked increase in ICAM-1 in both airway endothelium and epithelium. Cultured human airway epithelial cells produced increased level of ICAM-1 in response to infection with parainfluenza virus type 2. Furthermore, treatment with monoclonal antibodies to ICAM-1 significantly attenuated eosinophil infiltration and inhibited the induction of airway hyperresponsiveness to methacholine challenge. Similarly, monoclonal antibodies may block increased adhesion to both neutrophils and eosinophils after respiratory viral infection. These data may suggest that the serum level of ICAM-1 can be used as one of the indices of the categorization of the severity of asthma in children. Furthermore, it is worthy to conduct studies for trial of monoclonal antibodies against ICAM-1 as a new modality of therapy in severe cases of asthma.

In the present work, serum levels of IL-8 were significantly higher in asthmatic than in control children. Also, serum IL-8 levels were significantly higher in cases with severe or moderate asthma than in cases with mild disease. IL-8 is a neutrophil activating protein secreted by monocytes and other cells in the peripheral blood and may play an important role in the genesis and persistence of bronchial inflammation in some pathologic conditions like asthma. IL-8 belongs to a unique cytokine subfamily called chemokines and has chemotactic activity for neutrophils, lymphocytes, basophils and eosinophils and may also increase the expression of granulocyte adhesion molecules. Kanazawa et al. demonstrated markedly increased serum level of IL-8 during exacerbation of asthma. Also Kono et al. reported increased level of concentration of IL-8 in sputum of symptomatic patients than in asymptomatic subjects. These data may suggest...
that IL-8 may be one of the causal factors in asthma. However, Yousefi et al. demonstrated that IL-8 level was increased in bronchoalveolar lavage fluids but not in the blood plasma. They explained the lack of increased concentration of plasma IL-8, in part, by its high affinity to red cells and thus being not detectable in plasma.

A strong positive correlation was found between the serum levels of IL-8 and ICAM-1 among the studied asthmatic patients. Churchill et al. demonstrated that ICAM-1 expression on cultured respiratory epithelial cells is enhanced by stimulation with various cytokines. The release of cytokines during inflammatory reactions may therefore contribute to the pathophysiology of airway inflammation through the enhanced expression of adhesion molecules.

Regarding the absolute eosinophilic count, it was significantly higher in asthmatic children than in controls. This is in agreement with many previous studies that demonstrated significant increase in the number of eosinophils in asthmatic children. Eosinophil infiltration is a characteristic feature of asthmatic airways and differentiates asthma from other inflammatory conditions of the airways.

In conclusion, the present study supports that IL-8 may participate in bronchial hyperactivity in asthma. Serum level of ICAM-1 can be useful as one of the indices of assessment of the severity of asthma in children. New modalities of therapy as anti adhesive molecules may be searched for. To clarify any possible role of NO in the pathogenesis of bronchial asthma, further study is needed.

References:


20. Shiota Y, Sato T, Ono T: Serum levels of sICAM-1 in asthmatic patients. Aererugi 1993; 42(12):1782-7 [English Abstr].


