New markers of disease activity in children with atopic dermatitis

Abdel hakeem Abdel mohsen,¹ Hosam  Abdel wahab.,² and Emad Allam ³

From the Departments of Pediatrics,¹ Dermatology and Andrology,² and Clinical Pathology,³ Faculty of Medicine, El-Minia University, Egypt

Abstract: Atopic dermatitis (AD) is a chronic inflammatory skin disease affecting about 15% of children, with consequent costs and morbidity. The AD has its onset during the first 6 months of life in 45%, in the first year of life in 60% and before 5 years in at least 85% of affected children (1, 2). The criteria proposed by Hanifin more than 20 years ago, still maintain their validity for diagnosing the disease (3). In addition, the severity score atopic dermatitis (SCORAD) index provides a standardized and reproducible method to quantify the disease’s severity (4). Nowadays, the exact role of allergy/atopy in the pathogenesis of AD is still controversial, but it is true that the immunological mechanisms underlying the disease (5) somewhere resemble those of other allergic disorders. It is also well-known that many children with AD are sensitized to inhalant/food allergens, so that AD is called extrinsic. (6)

Nitric oxide (NO) has been found to be involved in inflammation, vasodilatation and oxidative damage to cells and tissues of atopic dermatitis skin.(7,8) The direct measurement of NO is difficult due to its very short half-life in plasma,(9) NO reacts readily with molecular oxygen to yield nitrite and nitrate. NO, like nitrite is oxidized very rapidly to nitrate by oxyhemoglobin.(10) Fortunately, nitrate is stable and detectable in the serum.(11) Recent studies about AD and serum nitrate levels have been reported.(12,13)

Adhesion molecules and their ligands play a major role in endothelial–leukocyte interactions which affect the binding, transmigration and infiltration of lymphocytes and mononuclear cells during inflammation, injury, or immunological stimulation.(14) E-selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) are such adhesion molecules. After they are expressed on endothelial cells, they are released into the circulation through shedding or by proteolytic mechanisms (15). Consequently, soluble forms of these adhesion molecules can be detected in the systemic circulation. In vitro studies show that the level of soluble adhesion molecules correlates with the concentration of adhesion molecules expressed on the endothelial cells. (16) These data suggested that the level of systemic inflammation could be measured by the level of soluble adhesion molecules in the blood. (17, 18)

This study was aimed to evaluate serum nitrate and sE-selectin levels for assessment of disease severity and clinical course atopic dermatitis in children.

Key words; Atopic dermatitis, sE-selectin and Nitric oxide
Subjects and Methods:
This study was conducted in the departments of Pediatrics, Dermatology & Andrology and Clinical Pathology, EL-Minia University Hospital, during the period from January 2010 to December 2010. Fifty-six infants and children were included in this study. They were 40 patients with AD (aged 8-84 months: 24 boys and 16 girls) and 16 healthy non-atopic child (aged 6-60 months: 9 boys and 7 girls). The diagnosis was based on the criteria of Hanifin. None of the studied patients was febrile, had an infection or was receiving systemic steroids, anti-inflammatory medications, anti-allergic medications or anti-histamine at the time of sampling of blood (baseline). The patients with atopic dermatitis were divided into three subgroups depending upon the severity of the atopic dermatitis according to Rajka’s grading (19) and Kiamata scoring system, that is mild (16 patients, skin score 0-8), moderate (14 patients, skin score 9-16), and severe (10 patients, skin score 17-24). (20)

Patients with mild and moderate disease were received topical corticosteroids ointment and oral antihistaminic (citrizine dihydrochloride, 0.5-1mg/kg/day), While severe cases received systemic corticosteroids (prednisone, 1-2mg/kg/day) in addition. A specimen of peripheral venous blood (3ml) was collected to determine peripheral eosinophilic counts, serum nitrate and sE-selectin levels. After centrifugation, the serum was stored immediately at -70°C until analysis.

The following investigations were done for all patients and controls:
1. Peripheral eosinophilic count: peripheral blood leukocytes were counted in a coulter counter, and the eosinophilic percentage was evaluated after May-Grunwald staining. Data were expressed as range and median (x10^9/L).
2. Serum nitrate was measured by an enzymatic one step assay with nitrate reductase (Boehranger Manheim) as described by Bories. (21)
3. Serum sE-selectin was measured by sandwich ELISA according to the instructions provided by the manufacturer (R&D Systems Inc., Minneapolis, Minn.). (22)

Statistical Methods:
After collection of data, they were added and entered into a personal computer. Analysis of the data was done using SPSS (Statistical Package for the Social Sciences). The following statistical tests were used:
1. Mean and standard deviation (SD) to describe quantitative data’s
2. Student t test was used to compare between two groups as regards parametric data.
3. Chi-square test was used to compare between two groups as regards non-parametric data.
4. Pearson correlation was used to correlate two quantitative variables.
For all tests, a probability (p) of less than 0.05 was considered significant.

Results:
Fifty-six infants and children were included in the study and the results were shown in the following tables;

Table (I); showed the characteristics of studied subjects. Patients with mild disease (skin score 0-8) were 16 (40%) child, aged from 8-72 months and were 10 males and 6 females, while patients with moderate disease (skin score 9-16) were 14 child (35% of patients), age from 10-66 months and were 6 females and 8 males, and 10 patients with severe disease (skin score 17-24) aged from 9-84 months and were 7 females and 3 males. As regard serum Nitrate, sE-selectin and peripheral blood eosinophils levels in the patients were ranged from (14-225 mmol/L), (122-2722 ng/ml) and (0.39-2.78x10^6/L) respectively while that of the control were ranged (8-40 mmol/L), (25-512 ng/L) and (0.05-0.3x10^6/L).

Table II; showed a significant increase of serum nitrate and sE-selectin concentrations in patients with AD as compared to non-atopic controls {85.62 ± 20.12 vs 20.2 ± 7.61} for serum nitrate and 650.43 ± 153.1 vs 122.65 ± 70.16 for sE-selectin}. (P < 0.001)

Table III; showed a highly significant increase in serum nitrate and sE-selectin levels especially in moderate and severe cases compared to control group (p value were <0.001 and 0.000) and also among the three grades themselves (P < 0.001 for both markers).

Table IV shows a significant decrease of serum nitrate and sE-selectin levels after treatment of 10 cases with severe AD {36.2 ± 15.6 vs 130.3 ± 60.7} (P <0.002) and (350.65 ± 112.4 vs 1180.46 ± 515.2) (P <0.001) respectively.

Table V shows a positive correlation between serum nitrate levels and grades of skin scores (disease activity) (r=0.69, P<0.05), and also with eosinophilic count (r=0.71, P<0.001). There was a positive correlation between sE-selectin levels and disease activity (r=0.73, P<0.01), and also with eosinophilic count (r=0.63, P<0.05). A positive correlation was reported between eosinophilic count and disease activity (r=0.721, P<0.01), but no correlation was reported between serum nitrate and sE-selectin levels (r=0.353, P>0.05).
Table I: Characteristics of studied subjects

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Age (months)</th>
<th>Sex (F/M)</th>
<th>Serum nitrate (mmol/L)</th>
<th>Serum E-selectin (ng/ml)</th>
<th>Eosinophils (x10⁶/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Controls</td>
<td>16</td>
<td>6 – 60*</td>
<td>7/9</td>
<td>8-40*(20)**</td>
<td>25-512* (123)**</td>
<td>0.05-0.3* (0.12)**</td>
</tr>
<tr>
<td>2- Cases of AD</td>
<td>40</td>
<td>8-84 (17)</td>
<td>24/16</td>
<td>14-225 (95)</td>
<td>122-2722 (816)</td>
<td>0.39-2.78 (0.97)</td>
</tr>
<tr>
<td>a-Mild AD (score 0-8)</td>
<td>16(40%)</td>
<td>8-72 (16)</td>
<td>6/10</td>
<td>14-72 (46)</td>
<td>122-826 (495)</td>
<td>0.39-1.85 (0.84)</td>
</tr>
<tr>
<td>b-Moderate AD (score 9-16)</td>
<td>14(35%)</td>
<td>8-72 (16)</td>
<td>6/8</td>
<td>28-195 (93)</td>
<td>136-1050 (635)</td>
<td>0.43-2.20 (0.96)</td>
</tr>
<tr>
<td>c-Severe AD (score 17-24)</td>
<td>10(25%)</td>
<td>9-84 (20)</td>
<td>7/3</td>
<td>67-225 (146)</td>
<td>216-2722 (1317)</td>
<td>0.49-2.78 (1.12)</td>
</tr>
</tbody>
</table>

* Range ** Median

Table II: comparison of serum nitrate & s E-selectin concentrations among cases and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n =16)</th>
<th>Cases of AD (n =40)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Nitrate (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean + SD</td>
<td>20.02 ± 7.61</td>
<td>85.62 ± 20.12</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>2. sE-selectin (ng/ml)</td>
<td>122.65 ± 70.16</td>
<td>650.43 ± 153.1</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Mean + SD</td>
<td>650.43 ± 153.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Highly significant

Table III: comparison of serum nitrate and sE-selectin and eosinophil levels according to severity of disease

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mild cases N=16</th>
<th>Moderate cases N=14</th>
<th>Severe cases N=10</th>
<th>Controls N=16</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Nitrate (mmol/L)</td>
<td>Mean ± S.D</td>
<td>46.62 ± 9.16</td>
<td>85.62 ± 6.41</td>
<td>130.3 ± 60.7</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>2. sE-selectin (ng/ml)</td>
<td>Mean ± ss.S.D</td>
<td>426.1 ± 210.3</td>
<td>755.14 ± 362.35</td>
<td>1180.46 ± 515.2</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>3. Eosinophils (x10⁶/L)</td>
<td>Mean ± S.D</td>
<td>0.39-1.85</td>
<td>0.43-2.20</td>
<td>0.49-2.78</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

* P<0.05: significant ** highly significant p>0.5 non significant

Table IV: comparison of serum nitrate and sE-selectin levels in severe cases of AD before and after treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Severe AD before treatment</th>
<th>Severe AD after treatment</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Nitrate (mmol/L)</td>
<td>Mean ± S.D</td>
<td>130.3 ± 60.87</td>
<td>36.2 ± 15.64</td>
</tr>
<tr>
<td>2. sE-selectin (ng/ml)</td>
<td>Mean ± S.D</td>
<td>1180.46 ± 515.2</td>
<td>350.65 ± 112.4</td>
</tr>
</tbody>
</table>

** highly significant

Table V: Correlations between serum nitrate levels, sE-selectin levels, disease activity and peripheral eosinophil count

<table>
<thead>
<tr>
<th>Variables correlated</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Serum nitrate and sE-selectin levels</td>
<td>0.345</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>2. Serum nitrate and disease activity</td>
<td>0.68</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>3. sE-selectin and disease activity</td>
<td>0.73</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>4. Serum nitrate and eosinophilic count</td>
<td>0.71</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>5. sE-selectin and eosinophilic count</td>
<td>0.65</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>6. Eosinophilic count and disease activity</td>
<td>0.72</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

* P<0.05: significant

Grades of r: 0.00 to 0.24(weak or no association), 0.25 to 0.49 (fair association), 0.50 to 0.74 (moderate association), 0.75 and more (strong association).

Discussion:

Atopic dermatitis is a multifactorial genetically based disease that affects 10 to 15% of children in many parts of the world and constitutes a significant burden to patients and their families. The pathogenesis of AD is considered a combination of both Ig-E mediated and delayed type hypersensitivities (23).

In this study, we measured serum level of nitrate and sE-selectin in 40 infants and children with AD and 16 healthy controls in order to evaluate their roles in the pathogenesis, assessment of severity and clinical course of atopic patients.

The study showed that serum level of nitrate in AD children were significantly increased as compared to controls and also there were significant
Nitric oxide synthetase (iNOS) immunoactivity was found to be closely associated with upper dermal microvasculature in all inflammatory skin lesions of AD patients, but not in the non-inflammatory area. The increased nitrate levels of AD patients' serum suggest that iNOS of endothelial cells in the atopic skin are stimulated and endothelial cells may yield the endothelial nitrate.

Inducible nitric oxide synthetase (iNOS) immunoactivity was found to be closely associated with upper dermal microvasculature in all inflammatory skin lesions of AD patients, but not in the non-inflammatory area. The increased nitrate levels of AD patients' serum suggest that iNOS of endothelial cells in the atopic skin are stimulated and produce nitric oxide, which causes vasodilatation associated with skin erythema or edema. Therefore, vascular injury and/or stimulated endothelial cells may yield the endothelial nitrate.

Soluble E-selectin levels after treatment of severe AD in our study and previous reports may support this hypothesis.

In the present study, there were a significant correlations between serum nitrate levels and both the disease activity and eosinophilic count. These results are in agreement with those of Eererugi et al., and Taniuchi et al., who reported that serum nitrate levels of patients with AD were significantly correlated with skin scores (disease activity) as well as peripheral eosinophil counts. These results suggest that nitric oxide product bears an important function in the allergic inflammation, which is concerned with the lesion expansion of the skin and correlated well to skin scores of AD patient.

As regards soluble E-selectin, there was a significant increase in their levels in AD patients as compared to control group. There were also significant differences among subgroups of AD. Our findings are in agreement with previous recent studies by Loan et al., and Kagi, in agreement with our results which demonstrated a significant reduction of soluble E-selectin levels after treatment.

Our results showed significant correlations between sE-selectin concentrations and disease activity as well as eosinophilic count. These findings are in accordance with Wolberstofer et al., and Loan et al., reported that disease activity which is measured by objective SCORAD (scoring Atopic Dermatitis), as well as eosinophilic count were significantly correlated with the serum levels of sE-selectin. The expression of adhesion molecules on endothelial cells regulates leukocyte migration. The level of soluble adhesion molecules which are shed into the circulation is known to reflect the degree of inflammation, and this level can therefore be used as an indicator of disease activity.

Lewis suggested that the atopic's monocytes/T cell interaction enhances development of eosinophil infiltration in skin lesions of AD and also in the peripheral circulation, which is related to the skin expansion and disease activity. This supports our results which demonstrated a positive correlation between eosinophil count and disease activity.

Conclusion:

From this work we can conclude that nitric oxide and sE selectin are involved in the pathogenesis of atopic dermatitis in children and their level was highly increased in acute and severe disease compared to those in remission, therefore NO and sE-selectin are good and useful markers for assessment of severity and clinical course of atopic dermatitis. New drugs that selectively inhibit NO and selectin production may help in treatment and maintenance of remission of the disease instead of long term corticosteroid therapy which has serious side effects.

Acknowledgement

We thank all members of Pediatrics, Dermatology and Andrology, and Clinical Pathology departments also the parents and their children for their support and compliance in...
sampling and receiving treatment properly to complete this works.

References: