Assessment of Role s100A7 and Eotaxin (CCL24) in Patients with Atopic Dermatitis in Babylon City

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Abstract

Background Atopic dermatitis (AD) is a chronic, diverse, inflammatory skin disease that generally starts in infancy and lasts into adulthood. T cell-driven inflammation, mostly through T helper (Th) 2- and Th17-derived cytokines, which are controlled by the Janus kinase (JAK) signaling pathway, is critical to AD. S100a7 and eotaxin(ccl24) were measured in serum samples.

Objective Finding diagnostic, prognostic, and predictive biomarkers that can be used to diagnose atopic dermatitis patients early in the course of the disease is the goal of the current study. It also aims to determine changes in s100a7 and eotaxin as a biomarker in atopic dermatitis patients and the study of these markers to give advice on how to treat AD.

Material and methods The samples were obtained from the dermatology clinic’s registered patients at Al-Musayyib General Hospital, Al-Imam Al-Sadiq Hospital, and Murjan Teaching Hospital between October 1 and November 1, 2022. Blood samples were utilized to analyze the biochemical properties of the serum, such as s100a7 and eotaxin(ccl24), were assessed for patients using the ELISA technique. The statistical analysis was carried out using SPSS software.

Results Results of the examination of the eotaxin biomarker in the patient group with atopic dermatitis were substantially better than those in the control group (p ≤ 0.05), and the s100a7 biomarker increased in the patient groups compared to the control group (p ≤ 0.05).

Conclusion Patients with atopic dermatitis had considerably higher levels of every parameter compared to healthy persons.

Keywords: Atopic dermatitis major; Eotaxin(ccl24); s100a7

1 Introduction

The inflammatory skin condition known as atopic dermatitis (AD) is long-lasting, diverse, and characterized by unexpected, recurrent flare-ups of very itchy, eczematous lesions. Has a lifetime frequency in high-income nations of >15% [1]. The most frequent cause of skin-related health burden is atopic dermatitis. The majority of persons begin to exhibit symptoms before the age of six, with 60% beginning to do so during the first year [2]. The new data point to a significant frequency of moderate-to-severe diseases in adults, particularly those with adult-onset or persistent from infancy [3]. Atopic dermatitis has poor social, scholastic, occupational, and economic consequences in addition to a decreased quality of life (QoL) [4]. Physically debilitating symptoms like pain and itching may limit physical activity, interfere with daily routines, and exacerbate mental anguish. Even when atopic dermatitis is mild to severe [5,6], patient and family/caregiver QoL is affected, even if the degree of burden is proportional to severity [7].

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S100a7, often referred to as S100 calcium-binding protein A7 (S100A7), is a kind of protein that is produced by humans and is encoded by the S100A7 gene. [8] S100A7, belongs to the S100 family of proteins, which include two EF-hand motifs that allow them to bind calcium. S100 proteins are connected to the regulation of several cellular processes, including the progression of the cell cycle and differentiation mechanisms. Numerous different types of cells have these proteins in either their cytoplasm or their nuclei [9]. One of the most essential antimicrobial proteins is Psoriasin (S100A7), which is released by skin epithelial cells and disrupts the cell membranes of Escherichia coli. This is why individuals in countries with inadequate sanitation may come into contact with E. coli strains from feculent matter through the skin; this contact frequently does not result in infection [10]. Protein expression of psoriasin in the epidermis was initially analyzed by immunostaining utilizing a specialized antibody. Psoriasin secretion was found to be considerably elevated in both lesional and nonlesional atopic dermatitis skin, which might be due to the overall compromised barrier and accelerated microbial colonization tendency in these cases [11].

Eotaxins belong to the CC chemokine subfamily and are a type of eosinophil chemotactic protein; humans have three family members known as: CCL11 (eotaxin-1), CCL24 (eotaxin-2) and CCL26 (eotaxin-3) [12].

Eotaxin belongs to the chemokine subfamily and is a selective eosinophil chemoattractant. Additionally, as is well known, chemokines encourage leukocyte migration to areas of inflammation and malignancy. Further, specific chemokines may stimulate and control angiogenesis and metastasis [13]. Monocytes and activated lymphocytes do not exhibit any CCL24 chemotactic activity, but neutrophils do [14]. A multipotent hematopoietic progenitor cell line’s ability to form colonies is strongly suppressed by CCL24, which binds to CCR3 [15]. According to research, individuals with AD had T-helper (Th) 2 skewing in both lesional and nonlesional skin, and there was a substantial rise in Th2-related markers (CCL24/eotaxin-2) [16].

2 Materials and Methods

This research sample was obtained from Al-Musayyib General Hospital, Al-Imam Al-Sadiq Hospital, and Murjan Teaching Hospital between October 1 and November 1, 2022. The study was conducted in private labs. Questionnaires were created to collect data from the control and patient groups. In this case-control study, there are two groups: the first includes patients with beta atopic dermatitis major, and the second includes those who appear to be healthy.

2.1 Subjects

In this case-control study, there are two groups: the first includes patients with atopic dermatitis major, and the second includes those who appear to be healthy. The sample size was determined according to the Daniel formula for sample size; this formula is:

\[ n = \frac{z^2(1 - p)d^2}{2} \]  

2.2 Inclusion Criteria

Persons were proven with atopic dermatitis within the age of (4 months-45) years, diagnosed by a dermatologist.

2.3 Exclusion Criteria

1. Every patient who takes systemic steroids or other immune suppressant agents was excluded from the study
2. patient chronic diseases

2.4 Measurement Human Eotaxin, CCL24 ELISA Kit

2.4.1 Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human CCL24 antibody. CCL24 present in the sample is added and binds to antibodies coated on the wells. Then, a biotinylated Human CCL24 Antibody is added and binds to CCL24 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated CCL24 antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added, and color develops in proportion to the amount of Human CCL24. The reaction is terminated by the addition of an acidic stop solution, and absorbance is measured at 450 nm.
Table 1: Components of human eotaxin, CCL24 ELISA kit.

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity (96T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard solution (9600ng/L)</td>
<td>0.5ml×1</td>
</tr>
<tr>
<td>Pre-coated ELISA plate</td>
<td>12 * 8 well strips x1</td>
</tr>
<tr>
<td>Standard diluent</td>
<td>3ml×1</td>
</tr>
<tr>
<td>Streptavidin-HRP</td>
<td>6ml×1</td>
</tr>
<tr>
<td>Stop solution</td>
<td>6ml×1</td>
</tr>
<tr>
<td>Substrate solution A</td>
<td>6ml×1</td>
</tr>
<tr>
<td>Substrate solution B</td>
<td>6ml×1</td>
</tr>
<tr>
<td>Wash buffer Concentrate (25x)</td>
<td>20ml×1</td>
</tr>
<tr>
<td>Biotinylated Human CCL24 antibody</td>
<td>1ml×1</td>
</tr>
<tr>
<td>User instruction</td>
<td>1</td>
</tr>
<tr>
<td>Plate sealer</td>
<td>2 pics</td>
</tr>
</tbody>
</table>

Table 2: Concentration of standards of human Eotaxin, CCL24 ELISA kit.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Standard No.</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>4800ng/L</td>
<td>5</td>
<td>120ul Original standard + 120ul Standard diluent</td>
</tr>
<tr>
<td>2400ng/L</td>
<td>4</td>
<td>120ul Standard No.5 + 120ul Standard diluent</td>
</tr>
<tr>
<td>1200ng/L</td>
<td>3</td>
<td>120ul Standard No.4 + 120ul Standard diluent</td>
</tr>
<tr>
<td>600ng/L</td>
<td>2</td>
<td>120ul Standard No.3 + 120ul Standard diluent</td>
</tr>
<tr>
<td>300ng/L</td>
<td>1</td>
<td>120ul Standard No.2 + 120ul Standard diluent</td>
</tr>
</tbody>
</table>

2.4.3 Steps of Eotaxin Evaluation

Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

1. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

2. Add 50ul standard to standard well. Note: Don’t add antibodies to the standard well because the standard solution contains biotinylated antibodies.

3. Add 40ul sample to sample wells and then add 10ul Human CCL24 antibody to sample wells, then add 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

4. Remove the sealer and wash the plate five times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash five times with wash buffer. Blot the plate onto paper towels or other absorbent material.

5. Add 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
6. Add 50ul Stop Solution to each well; the blue color will change into yellow immediately.

7. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

2.5 Data Analysis

SPSS software version 21 was used for the statistical analysis. Categorical variables were described using frequency and percentages. Means SD was used to describe continuous variables. Using a student t-test, the means of the two groups were compared. The relationship between categorical variables was examined using the Fisher-exact and Pearson chi-square tests. Two continuous variables were compared using the Pearson correlation coefficient. A p-value of 0.05 or less was considered significant. A p-value larger than 0.05 was considered non-significant.

2.6 Ethical approval

Before collecting samples, all research participants were informed, and verbal consent was acquired from each of them. According to document number 14, a local ethics committee evaluated and approved the research protocol, subject information, and consent form on 06/07/2022 to get this permission.

3 Results

Assessment of Eotaxin (ccl24) was done in atopic dermatitis patients healthy control group, and the result revealed that the Mean and SD for the patient was \((2339.63 \pm 494\ ng/L)\) and for healthy control was \((1093.42 \pm 354.92\ ng/L)\) respectively. The findings demonstrated a significant difference in eotaxin levels between patients and their control group (P 0.002), as shown in Table 3 and Figure 2.

Table 3: Independent sample t-test to test differences of Eotaxin according to study group, including (patients and control group).

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Study group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>T-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eotaxin(ccl24)</td>
<td>Patients</td>
<td>45</td>
<td>2339.63 ± 494</td>
<td>3.485</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>45</td>
<td>1093.42 ± 354.92</td>
<td>3.485</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

*P value ≤ 0.05 (significant), N: sample size, SD: standard deviation.

Figure 2: Bar chart for EOTAXIN to compare between patient and control groups.

Table 4: Independent sample t-test to test differences of Eotaxin according to study group, including (patients and control group).

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Study group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>T-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S100-A7(ng/mL)</td>
<td>patients</td>
<td>45</td>
<td>22.81 ± 2.45</td>
<td>5.485</td>
<td>0.0004*</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>45</td>
<td>10.41 ± 1.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P value ≤ 0.05 (significant), N: sample size, SD: standard deviation.
3.1 S100-A7

Assessment of S100A7 in atopic dermatitis patient showed that the Mean and SD for the patient was (22.81 ± 2.45 ng/mL) and for healthy control was (10.41 ± 1.05 ng/mL) respectively. The findings demonstrated a significant difference in S100A7 levels between patients and their control group (P 0.0004), as shown in Table 4 and Figure 3.

Figure 3: Bar chart for S100-A7 to compare between patient and control groups.

Pearson Correlation coefficient between markers there was a significant positive correlation between EOTAXIN (P≤0.01) and between S100 where (P≤0.01). The information exposed in Table 5

Table 5: Correlation between parameters.

<table>
<thead>
<tr>
<th></th>
<th>EOTAXIN</th>
<th>S100A7</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOTAXIN Sig.</td>
<td>1</td>
<td>0.324*</td>
</tr>
<tr>
<td>N</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>R</td>
<td>0.324*</td>
<td>1</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (2-tailed).

Figure 4: The correlation between EOTAXIN and S100.

Table 6: Comparison between Mild and Moderate in both Children and adult groups.

<table>
<thead>
<tr>
<th>age</th>
<th>Severity</th>
<th>EOTAXIN Mean</th>
<th>EOTAXIN S.D.</th>
<th>T-test</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>mild</td>
<td>2263.77</td>
<td>40.81</td>
<td>4.573</td>
<td>0.012*</td>
</tr>
<tr>
<td></td>
<td>Moderate and sevier</td>
<td>2369.25</td>
<td>88.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>mild</td>
<td>1885.31</td>
<td>58.58</td>
<td>5.372</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Moderate and sevier</td>
<td>2474.65</td>
<td>62.68</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference between groups with p-value <0.05.

Figure 5: Comparison between Mild and Moderate in both Children and adult groups and eotaxin.

Figure 6: Comparison between Mild and Moderate in both Children and adults' groups and s100-A7.
3.2 Receiver Operating Characteristic (ROC) for Markers

Plotting the true positive rate (TPR) against the false positive rate (FPR) at different threshold levels yields the ROC curve. Other names for the true-positive rate include sensitivity, recall, and likelihood of detection. One may compute the false-positive rate as (1 - specificity).

3.3 ROC Curve of Eotaxin (CCL24)

ROC curve for the sensitivity and specificity of Eotaxin (ccl24) (ng/l) for diagnosis of atopic dermatitis major, (Cut-off point was ≥ 198.1(ng/l))), AUC=0.95, P= 0.001, the sensitivity and the specificity was 92.3%, 89.2% respectively, as shown in Table 7.

Table 7: ROC for marker to check Sensitivity, Specificity, and Accuracy.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOTAXIN</td>
<td>0.923</td>
<td>0.892</td>
<td>0.917</td>
</tr>
<tr>
<td>S100a7</td>
<td>0.942</td>
<td>0.939</td>
<td>0.933</td>
</tr>
</tbody>
</table>

Figure 7: ROC plot for the marker EXTAXIN (ccl24).

3.4 ROC Curve of S100A7

ROC curve for the sensitivity and specificity of S100A7 (ng/l) for diagnosis of atopic dermatitis major, (Cut-off point was≥ 198.1(ng/l)), AUC=0.95, P= 0.001, the sensitivity and the specificity was 94.2%, 93.9% respectively.

Figure 8: ROC plot for the marker S100-A7.

4 Discussion

Serum levels of Eotaxin (CCL24) were shown to be considerably greater in patients with AD compared to those in healthy controls, and this difference was found to be strongly linked with the severity of AD. Allergic disorders such as asthma, rhinitis, and AD include eosinophils [17]. More recent studies have shown that this protein serves as a powerful eosinophil chemoattractant cytokine. These scientists have also characterized the primary receptor for this chemokine, known as CCR3 (CC chemokine receptor 3)[18–20]. The development and progression of various eosinophilic disorders may be better understood if the processes causing the buildup of eosinophils in inflamed tissues are clarified. Immunoreactivity and transcripts of eotaxin/CCL24 and CCR3 were dramatically enhanced in leisional skin from AD [21], and serum eotaxin/CCL24 levels were shown to be strongly linked with disease activity of AD [22]. The findings of other studies support our finding on Eotaxin. The levels of Eotaxin in patients with atopic dermatitis were significantly higher than the levels in healthy control participants, according to a study done in 2010 and published in the Journal of Allergy and Clinical Immunology. It was also shown that eotaxin concentrations are related to disease severity [23]. In yet another study that was published in the Journal of Investigative Dermatology in 2018, researchers showed that the levels of Eotaxin in the skin of patients with atopic dermatitis were much higher than those seen in healthy control subjects. In addition, the researchers discovered that treatment with a topical steroid reduced the amount of eotaxin present in the skin [24].

In this study, we clearly showed that the serum levels of s100a7 in patients with atopic dermatitis were significantly higher and significantly correlated with the disease activity of atopic dermatitis than those in...
with healthy controls; when the skin barrier is compromised, as in hyperproliferative skin disorders including atopic dermatitis (AD), psoriasin (S100A7) is extensively produced as an antimicrobial peptide and a signaling protein that modulates cellular function [25].

One of the most important antimicrobial proteins is Psoriasin (S100A7), which is released by skin epithelial cells and disrupts the cell membranes of Escherichia coli. This is why individuals in countries with inadequate sanitation may come into contact with E. coli strains from feculent matter through the skin; this contact frequently does not result in infection [26]. There are other studies that agree with our result about s100a7; only one previous research has shown an increase in S100A7 in acute AD, and that work lacked both a clear diagnosis of acute AD and a comparison to chronic lesions from the same individuals [27] Chronic hyperproliferative epithelium has been linked to an alternate differentiation route and has been proven to exhibit high levels of the S100A7 protein [28,29]. Their chemotactic effects on T cells, monocytes, and neutrophils, as well as their proinflammatory roles in a wide variety of inflammatory illnesses, are crucial to the inflammatory process [30,31]. However, because S. aureus skin infections are more common in atopic dermatitis, upregulated S100A7 expression may be less relevant as a proinflammatory mediator in the development of the condition. These proteins may have a role in the chemotaxis of immune cells, especially T-cells, which are dramatically elevated in the early stages of AD [32].

5 Recommendations

Other early markers of atopic dermatitis (such as eotaxin -1, Eotaxin-3) are needed to be measured. In addition to measuring s100a8 and s100a9 because of their importance in this atopic course.

6 Conclusion

Patients with atopic dermatitis had higher levels of Eotaxin (ccl24) and s100a7 compared to healthy individuals; this suggests that Eotaxin may be used as a prognostic marker in people with atopic dermatitis to predict the beginning of atopic dermatitis. Our results unequivocally demonstrate that young patients with atopic dermatitis have elevated levels of the protein eotaxin. To develop new drugs that can counteract or change the effects of Eotaxin on the atopic march and better treat, prevent, or manage various atopic illnesses, particularly in pediatric patients, longitudinal studies in atopic pediatric patients are required. In conclusion, we discovered that newborns with more severe atopic dermatitis have higher s100a7 levels. For the creation of individualized treatment plans, the clinical linkage of AD pathogenesis-related biomarkers and their differences in age groups should be demonstrated.

Conflict of Interest: No conflicts of interest exist between the authors and the publication of this work. Ethical consideration: The ethical committee approved the study at Department of Biochemistry, College of Medicine, University of Babylon, Hilla 51001, Iraq.

References


A review of the role of s100A7 and eotaxin (CCL24) in patients with atopic dermatitis in Babylon City.


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