Assessment of Serum Level of CXCL9 and CXCL10 in Cutaneous versus Systemic Lupus Erythematosus

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ABSTRACT

Background: Lupus erythematosus has been a chronic inflammatory illness where chemokines play a great role in its pathogenesis. CXCL9 and CXCL10 are chemokines which were researched in a variety of inflammatory skin disorders for their crucial role in immune responses.

Aim of the study: To compare CXCL9 and CXCL10 serum levels in patients suffering from cutaneous versus systemic lupus erythematosus.

Patients and Methods: The forty-five Egyptian patients in this case control research were collected from the University Hospital’s outpatient clinic between October 2018 and June 2019. Two groups of patients were established: DLE Group (Fifteen patients), and SLE group (Thirty patients) and forty control persons. Five ml of blood were obtained from all patients for assessment of serum CXCL9 and CXCL10 by enzyme-linked immunosorbent assay (ELISA) method.

Results: When comparing patients with DLE and SLE to controls, our findings demonstrated highly statistically considerable increases in CXCL9 and CXCL10 serum levels. Moreover, patients having SLE had a statistically significant rise in both CXCL9 and CXCL10 serum levels when compared to DLE patients. Meanwhile, when comparing SLE patients having active disease to those having inactive disease, there was a statistically significant rise in CXCL9 and CXCL10 serum levels.

Conclusion: The chemokines CXCL9 and CXCL10 may have had an etiopathogenesis role in SLE and DLE as they are increased in both DLE and SLE patients. In addition, CXCL10 levels in the serum could be used as a stand-alone biomarker for SLE activity.

Keywords: Systemic lupus erythematosis; discoid lupus erythematosus; CXCL9; CXCL10.

INTRODUCTION

Lupus erythematosus is a chronic autoimmune multisystem disorder. The disease severity ranges from little cutaneous involvement to severe, potentially lethal systemic disease. Skin is the second most frequent organ involved with varying morbidity.

Cutaneous Lupus erythematosus (CLE) is a disfiguring, chronic skin condition that has a great influence on patients’ quality of life. Cutaneous lesions are usually well-defined and present in areas that are exposed to the sun. Ultraviolet light can trigger or exacerbate the disease, which has a remitting and exacerbating course.

DLE is the most prevalent subtype of CCLE. About 25% of SLE patients have DLE-like lesions. They are presented as well-defined, erythematous, discoid plaques, with adherent scales and follicular plugging. Healing occur with thin, white atrophic non-contractile scar with slightly raised or hyperpigmented border.

SLE is a chronic inflammatory autoimmune disease. Females are the ones that are most affected (~90% of cases), and is more prevalent in people of African descent. The etiology of SLE is multifactorial. The main Pathological features are: auto-antibodies overproduction secreted by overactive B lymphocytes, immune complexes deposition in target organs, increased pro-inflammatory cytokine production and T-lymphocyte defect.

CXCL9 and CXCL10, which are members of the CXC chemokine family, were discovered to be...
overexpressed in the sera and/or tissues of patients with autoimmune disorders such as SLE. In patients with SLE, the CXCR3 ligands CXCL9 and CXCL10 were demonstrated to be the most significantly upregulated genes.

**PATIENTS AND METHODS**

This case control study comprised 45 patients who were split into two groups based on clinical characteristics: the DLE group (15 patients) and the SLE group (30 patients). A control group of forty age- and gender-matched, apparently healthy individuals was also included. All patients were recruited from the University Hospital's Dermatology and Venerology outpatient clinic between October 2018 and June 2019. Before participating in this study, the subjects gave their informed written consent. The faculty of medicine's research ethics committee approved the study.

The study participants were of both sexes, aged between 20-50 years. Patients of other co-morbidities (systemic or dermatological diseases), other autoimmune diseases, drug induced lupus or Pregnancy and lactation were excluded.

A full medical history was taken for all patients, a complete general examination to detect systemic manifestations of LE, a dermatological examination including distribution, morphology, extent and severity of skin lesions, and measuring disease activity using CLASI score in the DLE group Figure 1 and SLEDAI score in SLE group.

Two scores make up the CLASI: one that describes the disease's activity and another that measures the disease's damage. Dyspigmentation and scarring, as well as scarring alopecia, are used to assess damage.

CLASI revealed that activity scores were significantly determined by the amount of erythema. The total activity score is composed of the following components: redness degree (0–3) and scale (0–2), involvement of mucous membrane (0–1), recent loss of hair (0–1), and non-scarring alopecia (0–3). The total damage score consists of the following components: the dyspigmentation degree (0–2) and scarring (0–2), the dyspigmentation persistence for more than twelve months, double the dyspigmentation score, and scalp scarring (0, 3, 4, 5, 6).

SLEDAI is a list of 24 elements, 16 of which are clinical elements and 8 are laboratory data. The presence or absence of these symptoms in the preceding 10 days is used to score these items.

Following the evaluation of each item, a score is computed by adding the scores assigned to every item, depending on its individual weights. The involvement of organs has been weighed as follows: pain in joints or renal disease is multiplied by four, whereas neurological involvement of the central nervous system is multiplied by eight. The ultimate score runs from 0 and 105. The greater the score, the more significant the disease activity. Scores of 6 and higher are regarded to be indicative of active disease that necessitates treatment. Scores of more than 20 are, however, extremely rare. Clinically important changes include those with a score of 6 (improving) and 8 (worsening).

Five to seven ml of blood was collected from all studied groups by venipuncture under aseptic condition. Samples were collected into a serum separator tube. Samples were centrifuged for 15 min after clot formation. The serum has been removed, and the samples have been kept at 5 ~20 °C until analysis. CXCL9 and CXCL10 serum concentrations were assayed via ELISA utilizing a commercially accessible kit (R & D Systems, Inc. 614 McKinley *Place NE, Minneapolis, MN 55413, USA. E-MAIL: info@RnDSystems.com).

**Statistical Analysis**

The Statistical Package for the Social Sciences (SPSS/version 2000) program has been utilized to analyze the data.

**RESULTS**

This case control study comprised forty five Egyptian patients who were split into two groups, the DLE group (15 patients) and the SLE group (30 patients). The DLE group age ranged from 20-49 years (mean 38.87±8.42 SD). They were 8 females (53.3%) and 7 males (46.7%). The SLE group age ranged from 20-49 years (mean 34.77±9.95 SD). They were 23 females (76.7%) and 7 males (23.3%). In addition, the study included forty healthy gender and age matched volunteers who acted as controls.
Their ages ranged from 21 – 49 (mean of 32.83±7.41 SD) they were 29 females (71.5%) and 11 males (27.5%).

The mean disease duration in DLE patients was (13.00 ± 6.01 SD), ranging from 4 to 22 years. The mean onset age was 11.7 years (median 12.5 range 2-17). 7 patients (46.7 percent) had a positive family history, while 8 patients had a negative family history (53.3 percent). Five patients (33.3 percent) were DLE active and 10 patients (66.7 percent) were DLE inactive based on CLASI score.

The disease duration for SLE varied from 1 year to 23 years, with a mean of (9.97 ± 6.92 SD). The mean onset age was 16.7 years (median 15.5 range 13.2 – 18 y). 6 patients (20%) had a positive family history, whereas 24 (80%) had a negative one. Twenty patients (66.7 %) were SLE active and 10 patients (33.3 %) were SLE inactive based on SLEDAI score.

When it came to CXCL9 and CXCL10 serum levels, there were highly statistically significant differences between the DLE and control groups (p-value = 0.000) (Table 1).

There were also highly statistically significant differences in serum levels of CXCL9 and CXCL10 between the SLE and control groups (p-value = 0.000) (Table 2).

There were highly statistically significant differences in serum CXCL9 and CXCL10 levels between the DLE and SLE groups (p-value = 0.000) (Table 3).

When it came to CXCL9 and CXCL10 serum levels, there were highly statistically significant differences between the DLE and control groups (p-value = 0.000) (Table 1).

<table>
<thead>
<tr>
<th>DLE group</th>
<th>Control group</th>
<th>Test value</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. = 15</td>
<td>No. = 40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCL9 (pg/ml)</td>
<td>Mean ± SD</td>
<td>4.87 ± 1.03</td>
<td>26.63 ± 6.09</td>
<td>-22.117*</td>
</tr>
<tr>
<td>Range</td>
<td>3.5 – 7.5</td>
<td>18.4 – 36.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCL10 (pg/ml)</td>
<td>Mean ± SD</td>
<td>3.18 ± 1.05</td>
<td>16.95 ± 2.46</td>
<td>-29.252*</td>
</tr>
<tr>
<td>Range</td>
<td>2 – 7</td>
<td>12.6 – 22.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1:** Comparison between the DLE group and the controls in terms of CXCL9 and CXCL10.

<table>
<thead>
<tr>
<th>SLE group</th>
<th>Control group</th>
<th>Test value</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. = 30</td>
<td>No. = 40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCL9 (pg/ml)</td>
<td>Mean ± SD</td>
<td>4.87 ± 1.03</td>
<td>45.03 ± 6.78</td>
<td>37.011*</td>
</tr>
<tr>
<td>Range</td>
<td>3.5 – 7.5</td>
<td>22.2 – 58.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCL10 (pg/ml)</td>
<td>Mean ± SD</td>
<td>3.18 ± 1.05</td>
<td>20.98 ± 3.62</td>
<td>-29.541*</td>
</tr>
<tr>
<td>Range</td>
<td>2 – 7</td>
<td>15.5 – 27.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** Comparison between the SLE group and controls in terms of serum CXCL9 and CXCL10 levels.

<table>
<thead>
<tr>
<th></th>
<th>DLE group</th>
<th>SLE group</th>
<th>Test value</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. = 15</td>
<td>No. = 30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCL9 (pg/ml)</td>
<td>Mean ± SD</td>
<td>26.63 ± 6.09</td>
<td>45.03 ± 6.78</td>
<td>-8.870*</td>
<td>0.000</td>
</tr>
<tr>
<td>Range</td>
<td>18.4 – 36.7</td>
<td>22.2 – 58.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXCL10 (pg/ml)</td>
<td>Mean ± SD</td>
<td>16.95 ± 2.46</td>
<td>20.98 ± 3.62</td>
<td>-3.880*</td>
<td>0.000</td>
</tr>
<tr>
<td>Range</td>
<td>12.6 – 22.4</td>
<td>15.5 – 27.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Comparison between the DLE group and the SLE group in terms of serum CXCL9 and CXCL10 levels.

**Table 4:** Connection between serum CXCL9 and CXCL10 levels with age and duration in all cases.
lar leukocytes, recruited CXCL11 generated by resident cells – cells, which include skin infiltrate constituted primarily of CXCR3 linked to the existence of a large inflammatory family in CLE. The expression of such ligands is commonly expressed members of the chemokine ligands CXCL9, Meller et al. (2005) identified that the CXCR3 expression of various proinflammatory cytokines, notably CXCL9, that drive immune cell recruitment and have been related to the creation of CLE skin infections, according to Zahn et al. (2011) and have been related to the creation of CLE skin notably CXCL9, that drive immune cell recruitment

In human keratinocytes, IFN-γ, promote expression of CXCR3 on lymphocytes and dendritic cells (DC) in lymphoid organs, if an autoimmune response develops in SLE. These cells then gain the ability to move towards inflammatory chemokines (CXCL9, CXCL10, and CXCL11) generated by resident cells in the skin and kidneys (like fibroblasts). These cells (particularly Th1 cells) induce resident cells to boost chemokine synthesis through IFN-γ secretion after being recruited from the blood in tissues which are inflamed

Moreover, Watanabe et al (2008) and Henneken et al (2005) reported an elevation in the ratio of CXCR3+ B cells in SLE patients as well as an elevation in CXCL10 serum levels. However, there was no significant relationship between CXCL9 and CXCL10 in DLE and disease activity, but there was a significant relationship between both CXCL9 and CXCL10 in SLE and disease activity, particularly Th1 cytokines, such as IFN-γ, which recruit cytotoxic type I immune cells to the lesions. T-helper 1 chemokines (CXCL9, CXCL10, CXCL11 and CXCL12) are the most significantly increased chemokines in the whole chemokine family, and they're detected where dermis and epidermis injury is seen in DLE. CXCR3, which promotes lesional keratinocytic cell death, most likely through keratinocyte necroptosis, is activated by these proinflammatory chemokines, which recruit cytotoxic type I immune cells to the lesions.

The goal of this case control study was to evaluate serum CXCL9 and CXCL10 levels in CLE patients versus SLE patients.

Our findings revealed that serum CXCL9 and CXCL10 levels in DLE patients were considerably higher than in controls, significantly higher in SLE patients compared to controls, and significantly higher in SLE patients compared to DLE patients.

These results were consistent with Bauer et al. (2009) and Mikita et al. (2011) who observed an increased serum level of CXCL9 and CXCL10 in both DLE and SLE patients than in healthy control subjects.

In human keratinocytes, IFN-gamma promoted the expression of various proinflammatory cytokines, notably CXCL9, that drive immune cell recruitment and have been related to the creation of CLE cell death, keratinocytic cell death, most likely through keratinocyte necroptosis, is activated by these proinflammatory chemokines, which recruit cytotoxic type I immune cells to the lesions.

DISCUSSION

Systemic lupus erythematosus (SLE) is an autoimmune disease that causes inflammation of the organs. Its pathogenesis includes formation of pathogenic autoantibodies directed towards nucleic acids and the proteins that bind to them, indicating a complete loss of self-antigen recognition. The interaction between genetic susceptibility and environmental triggers results in tolerance loss and subsequent immunological dysregulation. DLE is the most frequent type of (CLE) 10. CXCL9 and CXCL10 have been linked to the development of inflammatory and autoimmune diseases. They are chemokines that belong to the CXC subfamily that attract activated T cells that express the CXCR3 chemokine receptor. CXCL9 and CXCL10 have been shown to be important in Th1 and Tc cell recruitment and activation.

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Moreover, Watanabe et al (2008) and Henneken et al (2005) reported an elevation in the ratio of CXCR3+ B cells in SLE patients as well as an elevation in CXCL10 serum levels. However, there was no significant relationship between CXCL9 and CXCL10 in DLE and disease activity, but there was a significant relationship between both CXCL9 and CXCL10 in SLE and disease activity.

This was in accordance with Bauer et al. (2009) who stated that CXCL10 and CXCL9 are elevated in SLE patients' sera, which could be linked to disease activity and contribute to the inflammatory condition.

### Table 5: Relation between serum CXCL9 and CXCL10 levels with disease activity and in DLE group.

<table>
<thead>
<tr>
<th>Disease activity (CLASI score)</th>
<th>DLE group</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive CXCL9</td>
<td></td>
<td>24.87 ± 6.1</td>
<td>18.4 – 36.7</td>
<td>1.688</td>
<td>0.115 NS</td>
</tr>
<tr>
<td>Active CXCL10</td>
<td></td>
<td>30.16 ± 4.76</td>
<td>21.8 – 33.7</td>
<td>0.012</td>
<td>0.757 NS</td>
</tr>
</tbody>
</table>

### Table 6: Relation of serum level of CXCL9 and CXCL10 with disease activity in SLE group.

<table>
<thead>
<tr>
<th>Disease activity (SLEDAI score)</th>
<th>SLE group</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive CXCL9</td>
<td></td>
<td>39.87 ± 5.69</td>
<td>22.2 – 47.1</td>
<td>3.467</td>
<td>0.002 HS</td>
</tr>
<tr>
<td>Active CXCL10</td>
<td></td>
<td>47.62 ± 5.12</td>
<td>36.4 – 58.8</td>
<td>0.012</td>
<td>0.757 NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease activity (SLEDAI score)</th>
<th>SLE group</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive CXCL10</td>
<td></td>
<td>18.84 ± 3</td>
<td>15.5 – 24.3</td>
<td>2.489</td>
<td>0.019 S</td>
</tr>
<tr>
<td>Active CXCL10</td>
<td></td>
<td>22.06 ± 3.48</td>
<td>18 – 27.5</td>
<td>0.012</td>
<td>0.757 NS</td>
</tr>
</tbody>
</table>
Furthermore, according to Bauer et al. (2009) and Kong et al. (2009) data, marked elevations of CXCL10 in the sera of SLE patients, the highest relationships with disease activity were found. So, CXCL10 was consistently the chemokine most significantly linked to present and future disease activity.22,30

CONCLUSION

Based on our results, the chemokines CXCL9 and CXCL10 may have a role in the etiopathogenesis of SLE and DLE as they are increased in both DLE and SLE patients. In addition, serum CXCL10 levels could be used as a standalone biomarker for SLE activity.

REFERENCES


