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Does Novel IL-33 Correlates with TNF-α in RA and SLE?

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ABSTRACT

Key words: SLE, RA, TNF-α, IL-33

Background: Production of high amounts of inflammatory cytokines plays direct role in disease pathogenesis, including that of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Objectives: To investigate whether serum levels of novel IL-33 and TNF-α are higher in Egyptian RA and SLE patients than controls and to compare serum levels of the novel IL-33 with TNF-α in RA and SLE with assessing their correlation to clinical disease activity and laboratory findings.

Methodology: 24 SLE, 25 RA and 25 controls were enrolled in this study. Disease activity was evaluated using SLEDAI for SLE patients and DAS 28 for RA patients. IL-33 and TNF-α serum levels were determined using sandwich enzyme immunoassay. Results: A significant statistical difference of IL-33 level between SLE & RA groups (P=0.002) and between SLE & control groups existed (P<0.001). However, a non-significant difference existed between RA & control groups (P=0.340). There was a significant statistical difference of TNF-α level between SLE & control groups (P<0.001) and between RA & control groups (P<0.001), which didn’t exist between RA & SLE groups (P=0.070). A significant positive correlation of serum IL-33 level with serum TNF-α level in SLE patients occurred (r=0.505, P=0.012), which was not found in RA (r=-0.023, P=0.912) or in the controls (r=-0.168, P=0.601). A significant positive correlation was found between TNF-α level and SLE patients with high disease activity (r=0.446, P=0.029), which didn’t exist with IL-33 (r=-0.168, P=0.601). There was no correlation between serum IL-33 and DAS 28 (r=0.205, P=0.326), however a negative correlation occurred with TNF-α (r=-0.404, P=0.045). Conclusion: Serum levels of TNF-α and novel IL-33 were higher in RA and SLE Egyptian patients than controls. A significant positive correlation occurred between serum IL-33 and serum TNF-α in SLE but not in RA patients. Most clinical and laboratory variables in RA and SLE patients did not correlate with either serum IL-33 or TNF-α levels.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown etiology characterized by persistent symmetric polyarthritis. Its clinical and laboratory features are suggestive of an autoimmune disease with production of high amounts of inflammatory cytokines. It is well established that TNFα and IL-1 are key cytokines that plays a critical role in the pathogenesis of RA. IL-33 is a novel cytokine that belongs to the IL-1 family and a ligand for the IL-1 family receptor ST2L (IL1RL1). The majority of IL-1 family members currently known are pro-inflammatory cytokines in RA. It has been shown that IL-33 was highly expressed in the synovium of RA patients by in situ hybridization experiments and in inflamed parts of collagen-induced arthritis model in mice. In addition, many studies showed that IL-33 levels were significantly increased in synovial fluid and serum of RA patients compared with healthy controls, suggesting that high levels of IL-33 may be associated with the pathogenesis of RA.

Systemic Lupus Erythematosus (SLE) is a rheumatic autoimmune disease characterized by multisystem organ involvement. Abnormal cytokine levels particularly interleukins, interferons, and tumor necrosis factors (TNFs) are important hallmarks of SLE. Elevated serum TNF-α has been correlated with clinical disease activity, lupus nephritis and anti-dsDNA antibodies. Studies have pointed out that abnormal level of IL-33 may be involved in the pathogenesis of

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SLE. On the contrary, other studies showed that elevated IL-33 was infrequently observed in SLE patients and was comparable in frequency and serum level to healthy controls. Therefore, elucidation of the roles of IL-33 in rheumatic diseases would be beneficial to understand the pathogenesis and therapy of these diseases.

The aim of this work was to investigate whether serum levels of the novel IL-33 and TNF-α are higher in Egyptian patients with RA and SLE than healthy control volunteers and to compare serum levels of the novel IL-33 with TNF-α the hallmark cytokine in RA and SLE with assessing their correlation to clinical disease activity and laboratory findings.

**METHODOLOGY**

**Patients:**

Forty nine randomly selected patients from the department of Rheumatology and Rehabilitation, Cairo University were included in the study. They were classified into 2 groups: Group I included 24 SLE patients (22 females and 2 males) diagnosed according to the revised American College of Rheumatology (ACR) criteria with mean age 26.6 ± 8 years. Group II included 25 RA patients (19 females and 6 males) diagnosed according to the American Rheumatism Association (ARA) 1987 revised criteria with mean age 43.1± 12 years. A third group of 25 healthy controls (19 females and 6 males) with mean age 31.1±11.5 years were included. Full history taking, thorough clinical examination, laboratory and plain X-ray investigations of the affected joints were performed for all the patients.

Disease activity for RA patients was assessed using the Disease Activity Score in 28 joints (DAS 28) [19]. According to DAS 28 scoring, there were 9 cases in remission, 3 with low activity, 13 having moderate activity and no high activity was recorded among the studied RA patients. While Disease activity for SLE patients was assessed using Systemic lupus erythematosus disease Activity Index (SLEDAI) score [20]. According to the SLEDAI, 20 cases had high disease activity (SLEDAI > 10) and 4 cases had mild to moderate disease activity (SLEDAI ≤ 10). The study was approved by the local university ethics committee. All patients gave their informed consent prior to their inclusion in the study.

**Laboratory methods:**

Blood samples were collected from each subject under strict sterile conditions. Serum was separated and stored in sterile Eppendorf tubes. Each sample was divided into two aliquots; stored at -20°C until being tested for IL-33 and TNF-α levels using sandwich enzyme-linked immunosorbent assay (sELISA).

a) **Detection of IL-33 (Human IL-33 Platinum ELISA kit, eBioscience)**

The level of IL-33 in serum was determined in all groups using a commercial enzyme immunoassay kit. Anti-human IL-33 antibody bound to the wells of an immunoassay plate were used to bind to IL-33 present in human serum. The results were read with a spectrophotometer at 450/630 nm. A standard curve was generated by plotting the IL-33 standard concentrations (pg/mL) on the X-axis and the corresponding absorbance measurements on the Y-axis. The sample concentrations were then interpolated from the standard curve.

b) **Detection of TNF-α (Human TNF alpha ELISA kit, Boster Immunoleader)**

Quantitative Detection of human TNF-α in serum was determined in all groups using a sandwich ELISA kit. A 96-well plate precoated with anti- human TNF-α antibody were used to bind to TNF-α present in human serum. The optical density was measured at 450 nm using a microplate reader, and the values were obtained from a standard curve using lyophilized recombinant human TNF-α standards (ng/ml) in the ELISA kit.

**Statistical analyses:**

These were performed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Mean and standard deviation were used to describe IL-33 and TNF-α concentrations and other numerical data like laboratory values. Qualitative data were expressed as frequency and percentage. Mann–Whitney test was used for the comparative analysis of 2 quantitative data. Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA) then post-Hoc "Schefe test" was used for pair-wise comparison based on Kruskal-wallis distribution. Spearman-rho method was used to test correlation between numerical variables. A P-value<0.05 was considered significant.

**RESULTS**

Seventy-four subjects were enrolled in this study; 24 SLE, 25 RA and 25 age − sex matched control. The clinical features and manifestations of SLE and RA patients at the time of the study is shown in table (1).

The levels of IL-33 in the three groups are shown in table 2.

There was a highly significant statistical difference of IL-33 levels between SLE group and RA group (P=0.002) and between SLE group and control group (P<0.001). However, a non-significant difference of IL-33 levels between RA group and control group existed (P=0.340). On comparing IL-33 levels between the 3 groups, a significant P-value (<0.001) existed.

The levels of TNF-α in the three groups are shown in table 3.
There was a highly significant statistical difference of TNF-α levels between SLE group & control group ($P=0.001$) and between RA group & control group ($P=0.001$). While there was a non-significant difference of TNF-α levels between RA group & SLE group ($P=0.070$). On comparing TNF levels between the 3 groups, a significant $P$-value ($<0.001$) existed.

The RA group showed a mean value of IL-33 of 24.57±8.93 pg/ml and mean value of TNF-α of 200.84±51.42 ng/ml, while in SLE group the mean values of IL-33 and TNF-α were 29.08±8.42 pg/ml and 172.11±41.04 ng/ml, respectively. The control group showed a mean value of IL-33 of 21.7±3.64 pg/ml and a mean value of TNF-α of 47.69±24.79 ng/ml.

There was a significant positive correlation of serum IL-33 level with serum TNF-α level in SLE patients ($r=0.505$, $P=0.012$). However, no association was found between serum IL-33 levels and serum TNF-α levels in RA patients ($r=-0.023$, $P=0.912$) or in the control group ($r=-0.168$, $P=0.601$).

**Correlation between IL-33 and TNF-α levels with age and gender**

Serum IL-33 and TNF-α levels did not significantly correlate with age in patients with RA ($r=0.013$, $P=0.103$, $P=0.949$, 0.623), SLE ($r=0.190$, 0.141, $P=0.375$, 0.511) or healthy controls ($r=0.135$, -0.105, $P=0.521$, 0.744), respectively. Moreover, IL-33 and TNF-α levels did not differ significantly between women and men in both RA and SLE patients.

**Correlation between serum IL-33 and TNF-α levels with DAS 28 in RA patients**

There was no correlation between serum IL-33 and DAS 28 ($r=0.205$, $P=0.326$), however a significant negative correlation between serum TNF-α and DAS 28 ($r=-0.404$, $P=0.045$) existed.

**Association of serum IL-33 and TNF-α levels with various clinical and laboratory variables in RA patients:**

Most clinical and laboratory variables in patients with RA did not correlate with either serum IL-33 or TNF-α levels. However, there was a statistically significant negative correlation between IL-33 level and the disease duration in years; there was higher IL-33 level in patients with RA with shorter disease duration compared with those with longer disease duration, ($r=-0.537$, $P=0.006$), while no correlation between TNF-α level and the disease duration in years occurred ($r=0.250$, $P=0.228$). Also, there was a statistically significant positive correlation between IL-33 level and duration of morning stiffness in minutes; there was higher IL-33 level in patients with RA with longer duration of morning stiffness compared with those with shorter duration, ($r=0.519$, $P=0.009$) however, such correlation was not noticed with TNF-α level ($r=0.011$, $P=0.959$).

Regarding laboratory variables, there was higher TNF-α level in RA patients with positive RF compared with those with negative RF ($P=0.034$) while IL-33 level was not significantly elevated ($P=0.224$). Also there was a significant negative correlation between TNF-α level and the platelets count ($r=-0.397$, $P=0.050$) which wasn’t noticed with IL-33 ($r=-0.098$, $P=0.640$).

**Correlation between IL-33 and TNF-α levels with SLEDAI in SLE patients**

There was no correlation between serum IL-33 level and SLEDAI ($r=0.227$, $P=0.286$). However, a significant positive correlation was found between TNF-α levels and patients with high disease activity (SLEDAI>10) ($r=0.446$, $P=0.029$).

**Correlation between serum IL-33 and TNF-α levels with renal involvement in SLE group:**

Renal biopsies were done for SLE patients, 16/24 (66.7%) patients showed renal affection and it was found that the mean IL-33 level in these patients was 28.06 pg/ml which was not significant ($P=0.490$), while the mean TNF-α level was 233.64 ng/ml, which was statistically significant ($P=0.046$).

**Association of serum IL-33 and TNF-α levels with various clinical and laboratory variables in SLE patients:**

Most clinical and laboratory variables in patients with SLE did not correlate with serum IL-33 or TNF-α levels. However, there was higher IL-33 level in SLE patients with alopecia compared with those without ($P=0.022$), this difference was not noticed with TNF-α ($P=0.494$). Also IL-33 level significantly correlated with blood urea level ($r=0.445$, $P=0.029$), however this was not noticed with TNF-α level ($r=-0.169$, $P=0.430$).

It was found that TNF-α level correlated much more than IL-33 with some clinical and laboratory variables in patients with SLE. There was higher TNF-α level in SLE patients with Raynaud's phenomenon which was statistically significant ($P=0.042$) while IL-33 level was not significantly elevated ($P=0.537$). Also there was higher TNF-α level in SLE patients with autoimmune haemolytic anaemia compared with those without ($P=0.039$), however IL-33 level was not significantly elevated ($P=0.494$). Spearman’s correlation coefficient demonstrated that TNF-α levels were significantly correlated with low serum level of C4 ($r=0.271$, $P=0.033$), CRP ($r=0.288$, $P=0.022$) and current immunosuppressive dosage ($r=0.315$, $P=0.012$) such correlation was not noticed with IL-33 level ($r=0.061$, $P=0.776$), ($r=0.013$, $P=0.952$) and ($r=0.112$, $P=0.601$), respectively.
Table 1: Demographic and Clinical characteristics of SLE and RA patients:

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>SLE (n = 24)</th>
<th>RA (n= 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female : male</td>
<td>22 : 2</td>
<td>19 : 6</td>
</tr>
<tr>
<td>Age at study, mean (SD), years</td>
<td>26.6 (8.0)</td>
<td>43.1(12.0)</td>
</tr>
<tr>
<td>Age of onset, mean(SD), years</td>
<td>22.1(7.9)</td>
<td>36.6 (12.8)</td>
</tr>
<tr>
<td>Duration of disease, mean (SD), years</td>
<td>4.5 (3.1)</td>
<td>6.5(5.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical manifestations, n (%)</th>
<th>SLE</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis/arthralgia</td>
<td>16 (66.7)</td>
<td>18(72)</td>
</tr>
<tr>
<td>Raynaud's phenomenon</td>
<td>6 (25)</td>
<td>5(20)</td>
</tr>
<tr>
<td>Cutaneous vasculitis</td>
<td>4 (16.7)</td>
<td>4(16)</td>
</tr>
<tr>
<td>Autoimmune haemolytic anaemia</td>
<td>16(66.7)</td>
<td>6(24)</td>
</tr>
<tr>
<td>Oral ulcer</td>
<td>12 (50)</td>
<td>3(12)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>11(45.8)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>8 (33.3)</td>
<td>2(8)</td>
</tr>
<tr>
<td>Lung involvement</td>
<td>0</td>
<td>6 (24)</td>
</tr>
<tr>
<td>S.C. nodules</td>
<td>0</td>
<td>4(16)</td>
</tr>
<tr>
<td>Renal involvement</td>
<td>16 (66.7)</td>
<td>0</td>
</tr>
<tr>
<td>Malar rash</td>
<td>15 (62.5)</td>
<td>0</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>9 (37.5)</td>
<td>0</td>
</tr>
<tr>
<td>Serositis</td>
<td>9 (37.5)</td>
<td>0</td>
</tr>
<tr>
<td>Nervous system involvement</td>
<td>3 (12.5)</td>
<td>0</td>
</tr>
<tr>
<td>Immune thrombocytopenia</td>
<td>1(4.1)</td>
<td>0</td>
</tr>
</tbody>
</table>

Serological features:

<table>
<thead>
<tr>
<th>Feature</th>
<th>SLE</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF (positive)</td>
<td>2 (25)</td>
<td>16 (64)</td>
</tr>
<tr>
<td>ANA (positive)</td>
<td>22 (91.7)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Elevated anti-dsDNA antibody (&gt;100 IU/ml)</td>
<td>14 (66.7)</td>
<td>1 (33.3)</td>
</tr>
<tr>
<td>Low serum C3 (&lt; 76 mg/dl)</td>
<td>19 (79.2)</td>
<td>ND</td>
</tr>
<tr>
<td>Low serum C4 (&lt;9 mg/dl)</td>
<td>16 (66.7)</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 2: Serum IL-33 level in the three studied groups

<table>
<thead>
<tr>
<th>IL -33</th>
<th>N</th>
<th>Mean</th>
<th>Std Deviation</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>N=25</td>
<td>24.57</td>
<td>8.93</td>
<td>22.33</td>
<td>16.54</td>
<td>62.32</td>
</tr>
<tr>
<td>SLE</td>
<td>N=24</td>
<td>29.08</td>
<td>8.42</td>
<td>26.75</td>
<td>22.06</td>
<td>60.11</td>
</tr>
<tr>
<td>Control</td>
<td>N=25</td>
<td>21.7</td>
<td>3.64</td>
<td>20.95</td>
<td>15.99</td>
<td>30.88</td>
</tr>
</tbody>
</table>

Table 3: Serum TNF-α level in the three studied groups

<table>
<thead>
<tr>
<th></th>
<th>Valid N</th>
<th>Mean</th>
<th>Std Deviation</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>N=25</td>
<td>200.84</td>
<td>51.42</td>
<td>210.90</td>
<td>107.30</td>
<td>294.20</td>
</tr>
<tr>
<td>SLE</td>
<td>N=24</td>
<td>172.11</td>
<td>41.04</td>
<td>183.75</td>
<td>87.30</td>
<td>217.60</td>
</tr>
<tr>
<td>Control</td>
<td>N=25</td>
<td>47.69</td>
<td>24.79</td>
<td>38.70</td>
<td>26.80</td>
<td>101.30</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, on comparing IL-33 level between SLE and control group, a highly statistically significant difference occurred (P<0.001). This was in agreement with a study by Yang et al. who found that serum IL-33 level in Chinese SLE patients were significantly up-regulated relative to healthy controls. However, Mok et al. stated that elevated IL-33 was infrequently observed in SLE patients and was comparable to serum levels in controls. They explained that their conclusion may be limited by the sensitivity of the immunoassay.

Many studies reported that IL-33 significantly increased in RA patients, particularly those with active disease compared with healthy controls suggesting that IL-33 may contribute to pathogenesis of RA. In this study, it was found that serum IL-33 level tended to be
higher in RA than in healthy controls, however a non-significant difference existed \( (P=0.340) \) which could be related to the relatively low number of samples analysed.

On comparing the TNF-\( \alpha \) level between the three groups, there was a highly statistically significant difference of TNF-\( \alpha \) level between RA and control group \( (P<0.001) \) and between SLE and control group \( (P=0.001) \). Studies done by Klimiuk et al.\( ^{20} \) and Gheita et al.\( ^{21} \) showed that there was a significant elevation in serum TNF-\( \alpha \)-level in RA in comparison with controls. Also Sabry et al.\( ^{9} \) and Weckerle et al.\( ^{11} \) showed that serum TNF-\( \alpha \) was significantly higher in SLE than in non-autoimmune controls.

Also in this study a highly statistically significant difference of IL-33 level between SLE and RA occurred with SLE showing significantly higher IL-33 levels than RA \( (P<0.001) \). This was in contrast to Yang et al.\( ^{12} \) who found that serum IL-33 level was significantly increased in patients with SLE, compared with healthy controls, but was lower than that with RA. However, there was a non-significant difference of TNF-\( \alpha \)-level between RA & SLE groups \( (P=0.070) \) which could be explained by the fact that TNF-\( \alpha \) is the main starter responsible for most RA symptoms and signs \( ^{22} \) and that elevated serum TNF-\( \alpha \) is a frequent finding in human SLE \( ^{11} \).

This study aimed to compare serum levels of the novel IL-33 with TNF-\( \alpha \) in RA and SLE. A significant positive correlation of both cytokines occurred in SLE patients \( (r=0.505, P=0.012) \). However, no association was found between both in RA patients \( (r=0.023, P=0.912) \) or in the control group \( (r=-0.168, P=0.601) \). However a study by Miller \( ^{23} \) demonstrated that, the level of serum IL-33 decreased after anti-TNF treatment and correlated with production of RA-related autoantibodies including rheumatoid factor and anti-citrullinated protein. Moreover, it showed that serum and synovial fluid levels of IL-33 have decreased in patients who respond to anti-TNF treatment, while they did not change in non-responders. Matsuyama et al.\( ^{19} \) reported that RA patients who responded to TNF inhibitor also showed a reduction in serum IL-33. Also Pei et al.\( ^{24} \) reported that although resting primary synovial fibroblasts from RA patients express little IL-33, the expression level is dramatically up-regulated following stimulation of pro-inflammatory cytokines as TNF-\( \alpha \). While the relation between serum IL-33 level with TNF-\( \alpha \) in SLE patients, to our knowledge hasn’t been studied before. However a study by Xu et al.\( ^{25} \) on murine models showed that treatment with IL-33 exacerbated collagen induced arthritis and elevated production of pro-inflammatory cytokines (IL-17, TNF-\( \alpha \), and IFN-\( \gamma \)).

The present study showed that serum IL-33 didn’t correlate with DAS 28 \( (r=0.205, P=0.326) \), while TNF-\( \alpha \) showed significant negative correlation \( (r=-0.404, P=0.045) \). This was in concordance with Li et al.\( ^{26} \) who suggested a lack of association between IL-33 polymorphism and RA disease activity index (including DAS 28 score). However, Matsuyama et al.\( ^{8} \) stated that serum IL-33 level was significantly higher in high disease activity group than in moderate and low activity groups. While Gheita et al.\( ^{21} \) showed that patients with GA genotype of TNF-\( \alpha \) polymorphism showed a significant negative correlation of the serum TNF-\( \alpha \) level with the DAS28 \( (r=-0.66, P=0.038) \). Most clinical and laboratory variables in patients with RA did not correlate with either serum IL-33 or TNF-\( \alpha \) levels. However, IL-33 level showed a significant negative correlation with disease duration in years \( (r=-0.537, P=0.006) \) where no correlation occurred with TNF-\( \alpha \) \( (r=0.250, P=0.228) \). This was in contrast to Gheita et al.\( ^{21} \) who reported that the disease duration would predict the TNF-\( \alpha \)-level \( (P=0.006) \). Also, there was a significant positive correlation between IL-33 level and duration of morning stiffness \( (r=0.519, P=0.009) \). This was in agreement with Xu et al.\( ^{27} \) who stated that IL-33 may amplify autoantibody induced arthritis by accelerating mast cell maturation and activation, promoting mast cell degranulation in the joints and driving the expression of pro-inflammatory cytokines (IL-1b and TNF-\( \alpha \))\( ^{28} \). However, such correlation was not noticed with TNF-\( \alpha \)-level \( (r=0.011, P=0.959) \) which was in agreement with Gheita et al.\( ^{21} \) who showed that no correlation between TNF-\( \alpha \) polymorphism and morning stiffness duration existed.

Regarding laboratory variables, there was higher TNF-\( \alpha \)-level in RF positive RA patients compared with negative ones \( (P=0.034) \). This was in agreement with Takeuchi et al.\( ^{29} \) who reported that RF significantly increased with high TNF level \( (P=0.007) \). While IL-33 level was not significantly elevated in RA patients with positive RF \( (P=0.224) \). This result did not match with the study by Yang et al.\( ^{12} \) who observed that the higher levels of IL-33 detected in the sera of RA patients are positively correlated with rheumatoid factor.

Also there was a significant negative correlation between TNF-\( \alpha \)-level and platelets count \( (r=-0.397, P=0.050) \) which was not observed with IL-33 \( (r=-0.098, P=0.640) \). Thus it is recommended to further investigate the biologic effects of TNF-\( \alpha \) on the platelets, including effects on their development, maturation and biologic functions.

In this study, a correlation between serum IL-33 level and SLEDAI existed \( (r=0.227, P=0.286) \). This was consistent with Yang et al.\( ^{12} \) who stated that elevated serum IL-33 level correlated with markers of inflammation as ESR and CRP, but not with SLE activity, concluding that it may be part of the acute phase response in SLE, but could not reflect the whole course of SLE. However, Li et al.\( ^{26} \) found that serum IL-33 level correlated with disease activity (SLEDAI) suggesting the value of IL-33 as an indicator for disease activity in SLE and added that anti-IL-33 Ab. treatment provided therapeutic and survival benefit for lupus-prone mice.
While a significant positive correlation was found between TNF-α level and patients with high disease activity (SLEDAI>10) (r=0.446, P=0.029). This agreed with other authors’ observations Aringer et al.36 and Sabry et al.9 who reported that TNF-α level was significantly higher in SLE group with active lupus when compared with SLE patients with inactive disease. While this was in contrast to Gómez et al.31 who reported that TNF-α level was higher in patients with inactive disease compared with patients with very active disease suggesting that TNF-α could be a protective factor in SLE patient.

In the present study, 66.7% of SLE patients showed renal affection and it was found that the mean IL-33 level in these patients was 28.06 pg/ml which was not significant (P=0.490), while the mean TNF-α level was 233.64 ng/ml, which was statistically significant (P=0.046). However, Li et al.26 evaluated the potential of IL-33 inhibition as a treatment option for lupus nephritis using lupus-prone model mice, which revealed that IL-33 represents a potential therapeutic target in SLE disease and that anti-IL-33Ab treatment delayed lupus nephritis as demonstrated by reduced renal immune complex deposition, lessened proteinuria and reduced score of glomerulonephritis. While Sabry et al.9 showed that TNF-α was significantly increased in Egyptian patients with Lupus nephritis and that this data was paralleled by histological observations where TNF-α was found to be highly expressed in glomeruli in all forms of lupus nephritis and the degree of expression correlates with renal inflammatory activity. This correlation again suggests involvement of TNF-α in the inflammatory and destructive process of the disease. However, this was in contrast to Al-Janadi et al.32 who found that serum level of TNF-α was within normal range in SLE patients with lupus nephritis.

Most clinical and laboratory variables in patients with SLE did not correlate with serum IL-33 or TNF-α levels. However, there was higher IL-33 level in SLE patients with alopecia compared with those without (P=0.022), this difference was not noticed with TNF-α (P=0.494). This was in contrast to a study by Emina Kasumagic et al.33 who found that T-cell clones from involved lesions inhibited the proliferation of neonatal keratinocytes released high amounts of TNF-α. In fact, TNF-α is considered by several leading researchers to be the most significant factor in hair cell death, even possibly more significant than dihydrotestosterone 34. It was found that TNF-α level correlated more than IL-33 with some clinical and laboratory variables in SLE patients. There was higher TNF-α level in SLE patients with Raynaud's phenomenon which was statistically significant (P=0.042) while IL-33 level was not significantly elevated (P=0.537). This was in agreement with Farid et al.35 who reported that high TNF-α level was associated with Raynaud's phenomenon and nephritis in patients with TNF-α gene polymorphism-863AA variant. The pathogenesis of Raynaud’s phenomenon is believed to be due to the disturbance of endothelial function. It was proposed that high TNF-α level stimulated the release of endothelin-1 by vascular endothelium resulting in vasoconstriction and shifted the balance that controlled basic functions of microcirculation. Therefore, it is possible that SLE patients with a genetic background of high TNF-α production should have a greater risk of Raynaud’s phenomenon 35.

Our data showed that hematologic disorders were one of the most frequent disorders of patients with SLE including autoimmune haemolytic anaemia in 16 (66.7%), leucopenia 8 (33.3%) and immune thrombocytopenia 1 (4.1%) which was consistent with the previous study by Chung et al.36.

Also there was higher TNF-α level in SLE patients with autoimmune haemolytic anaemia compared with those without (P=0.039), however IL-33 level was not significantly elevated (P=0.494). TNF-α levels were significantly correlated with low serum level of C4 (r=0.271, P=0.033), CRP (r=0.288, P=0.022) and current immunosuppressive dosage (r=0.315, P=0.012) such correlation was not noticed with IL-33 level. The lack of correlation between these data and our results can be explained by the hypothesis that SLE is a genetic disease and we can assume that the difference in the genetics of different populations may be responsible for the difference in clinical presentation.

In Conclusion, serum levels of TNF-α and the novel IL-33 were higher in Egyptian patients with RA and SLE than healthy control volunteers. There was a significant positive correlation of serum IL-33 level with serum TNF-α level in SLE but not in RA patients. Most clinical and laboratory variables in patients with RA and SLE did not correlate with either serum IL-33 or TNF-α levels. To confirm our results we propose that larger scale, multicentre studies with longer evaluation periods are needed in RA and SLE patients.

REFERENCES

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