**Introduction**

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the production of a large quantity of autoantibodies and the formation of immune complexes causing tissue and organ damage [1]. The disease affects predominantly women in their reproductive years, probably due to an estrogen hormonal effect [2,3]. Aberrant production and imbalance of Th1/Th2 lymphocyte function resulting in an enhanced production of cytokines that upregulate autoantibody production by B cells. Accordingly, in murine models of SLE, an altered production of both Th1 (such as IFN-γ, IL-18, and IL-2) and Th2 (such as IL-4 and IL-10) cytokines has been reported [6].

From Th1 cells, we have selected IL-18 as a proinflammatory cytokine, predominantly released by antigen-presenting cells such as macrophages and dendritic cells. IL-18 acts as a Th1 cytokine, as it promotes both proliferation of Th1 lymphocytes and IFN-production by these cells [7]. IL-18 shares functional similarities with IL-12, which induces the production of IL-18 by activation of natural killer (NK) cells and cytotoxic T lymphocytes [8].

Evidence is now accumulating to suggest the potential role of IL-18 in SLE; IL-18 may prove to be an important target for developing new drugs and treatment in SLE [9], as the primary function of IL-18 involves...
the induction of IFN-γ and tumor necrosis factor α (TNF-α) in T cells and NK cells, the upregulation of Th1 cytokines, including IL-2, granulocytes-macrophage colony stimulating factor, and IFN-γ, and stimulation of proliferation of activated T cells [10]. IL-18 has been found to be elevated in serum of lupus patients and to correlate with disease activity. It has been postulated that IL-18 may act together with TNF-α and IL-1 to mediate the inflammatory mechanism in SLE patients [11]. It accelerates spontaneous lupus disease with characteristic glomerulonephritis and vasculitis. This is further exacerbated by the synergistic action of IL-12 and IL-18, a combination known to promote Th1 cell development including reciprocal enhancement of their receptor expression. In contrast, several studies have reached the opposite conclusions that the levels of serum IL-18 are irrelevant to disease activity and kidney involvement [7,12]. Thus, the functions of IL-18 in the pathogenesis of Lupus Nephritis (LN) are still debated.

However, from the Th2 cells, we have selected IL-10 prototyp to study, as IL-10 is a key immune-regulatory cytokine produced by a multiplicity of immune cells including monocytes, macrophages, mast cells, NK cells, eosinophils, and neutrophils and by adaptive immune cells such as Th1, Th2, and CD8+ T and B cells [13,14]. IL-10 has been shown to exert a potent suppressor effect on macrophage activity, in addition to its recognized direct inhibitory effects on the proliferation of CD4+ T cells. It was also found to suppress the production of proinflammatory cytokines such as IL-2, IFN-γ, IL-4, IL-5, and TNF-α. In contrast, IL-10 has been shown to stimulate the growth of mast cells, B and Th2 lymphocytes and promote adaptive immunity [15] and type 2 cytokine pattern by inhibiting the IFN-γ production of T lymphocytes [16–18]. In early lupus, IL-10 was found to play a downregulatory role, whereas at later phases of disease excessive production of IL-10 might result in enhanced autoantibody production and subsequent formation of pathogenic autoantibody–antigen complexes as has been illustrated in in-vitro studies that anti-IL-10 antibodies could markedly inhibit immunoglobulin production by SLE peripheral blood mononuclear cells, which corroborated the role of IL-10 in mediating autoantibody production [19,20].

This study aimed to assess serum levels of IL-18 and IL-10 in SLE and their relationship with disease activity, as it might provide future insights for targeted therapeutic strategies in SLE.

Patients and methods

Patients were selected from outpatient clinics of the Rheumatology Department of Faculty of Medicine, Sohag University, between December 2011 and December 2012. The study included 30 patients (female/male = 27/3) with the established diagnosis of SLE classification performed according to the American College of Rheumatology criteria [21]. Their ages ranged from 25 to 44 years. Twenty age-matched and sex-matched normal healthy individuals (female/male = 17/3) were included as the control group. Their ages ranged from 23 to 42 years with a mean of 32.67 ± 2.1 years. Informed consents were taken from the participants and the study was approved by the ethics committee of the research organization.

The study population was subjected to the following clinical and investigational work-up:

Full history taking including age, sex, disease duration as well as swollen and tender joint counts of the patients was performed; chest radiography and echocardiography were performed. Clinical assessment of the disease activity was performed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score. A SLEDAI score of more than 6 was considered active disease [22].

Laboratory work-up included routine laboratory survey with complete blood picture (Coulter STKS Hematology Flow Cytometer; Block Scientific Inc., Bohemia, New York, USA); erythrocyte sedimentation rate (ESR) was expressed in mm/h. The C-reactive protein (CRP) concentration was determined by immunonephelometry methods (Orion Diagnostica, Finland); concentrations of 6 mg/l were considered positive for CRP [23,24]. The serum levels of complement 3 (C3) and complement 4 (C4) were quantitatively measured in all samples by means of immune-turbidimetry with the Turbid Time System (Dade Behring, USA) according to the manufacturer instructions and expressed in terms of mg/dl. Antinuclear antibody (ANA) and antibodies to double-stranded DNA (anti-DNA) were measured using indirect immunofluorescence. Blood samples from SLE patients and controls were centrifuged and sera were obtained for immediate carry on of routine laboratory work-up. The remaining sera were stored frozen in aliquots at –20°C for the subsequent assays; urine analysis was performed. Patients with lupus nephritis were defined by persistent proteinuria greater than 0.5 g/24 h or by the presence of cellular casts or persistent hematuria; renal biopsy was not performed.

Determination of serum IL-18 was performed using an enzyme-linked immunosorbent assay (ELISA) kit (Biosource International Inc., Camarillo, California, USA). Samples and standards of human
IL-18 (Hu IL-18) and controls were pipetted into these wells and incubated for 1 h. Thereafter, a monoclonal antibody specific for Hu IL-18 was added and incubated for 1 h. Hu IL-18 was captured and immobilized onto the plate. After washing, a horseradish peroxidase (HRP)-conjugated anti-Hu IL-18 antibody was pipetted into the wells and incubated for 1 h. The HRP activity was detected by addition of trimethyline benzidine chromogenic reagent. Substrate and the color produced was proportional to the quantity of Hu IL-18 contained in the sample. Finally, the reaction was stopped with the addition of stop solution, which terminates the HRP-catalyzed reaction. The absorbance of each well was measured at 450 nm using a microtiter plate reader. A calibration curve was plotted and IL-18 concentration in samples was determined by interpolation from the calibration curve. The normal values were set according to the calibration curve based on manufacturer’s instructions and they ranged from 36.05 to 257.75 pg/ml with a cutoff level of 259.41 pg/ml. Determination of serum IL-10 was performed using ELISA kits (Biosource International Inc.), following the manufacturers’ instructions; the samples, standard, and controls were added to wells followed by incubation buffer. After incubation, biotin-conjugated anti-IL-10 antibody was added to each well. After 2 h of incubation, streptavidin–HRP working conjugate was added; finally, chromogen was added followed by the stop solution then absorbance was read at 450 nm. The normal values for serum IL-10 ranged between 7.8 and 500 pg/ml.

Statistical analysis
Statistical analysis was performed using SPSS program (version 15; SPSS Inc., Chicago, Illinois, USA). Data were presented as number (%), with mean ± SD. The Mann–Whitney U-test was used for the analysis of two quantitative data. Spearman’s correlation was used for detection of the relationship between two variables. The differences between categorical variables were investigated using the χ²-test. P value was considered significant if less than 0.05.

Results
The study included 30 patients with the diagnosis of SLE and 20 healthy control individuals with the mean age 34.62 ± 4.3 and 32.67 ± 2.1 years, respectively. The mean duration of the disease in the studied patients was 5.6 ± 3.4 years. Patients were on the following treatments: prednisolone 10–50 mg/day (mean 25.20 ± 5.75 mg/day), hydroxychloroquine 200–400 mg/day (mean 280 ± 172.76 mg/day), and azathioprine 50–150 mg (mean 115.45 ± 25.59 mg/day) daily in combination. The clinical and descriptive data of SLE patients and statistical comparison of laboratory work-up between the studied SLE patients and healthy controls are shown in Tables 1–3. The study found that ANA was positive in 40% of the patients, whereas anti-ds DNA was positive in 30%. The study reported a SLEDAI score that ranged from 1 to 44 with a mean ± SD 23.69 ± 18 in the studied patients with SLE; 53.3% of the studied SLE patients had a SLEDAI score of at least 6 who were considered as the active disease group (Table 4). The number of patients with lupus nephritis was four (13%) and vasculitis was five (17%), which was too small number to give statistical significance, if wanted to differentiate the patients according to each of them. Surveying serological biomarkers of inflammation in the examined SLE patients results illustrated a highly significant decrease in the mean value of serum C3 and C4 level in the studied SLE patient compared with controls (P = 0.000), and such decline in complement levels significantly correlated with an observable increase in the SLEDAI score values (r = −0.431, P = 0.003, and r = −0.68, P = 0.001, respectively) (Table 5).

### Table 1 Descriptive data of the studied patients with systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Patients (n = 30)</th>
<th>Range</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25–44</td>
<td>34.26 ± 4.3</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>2–9</td>
<td>5.6 ± 3.4</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>1–44</td>
<td>23.69 ± 18</td>
</tr>
</tbody>
</table>

SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

### Table 2 Cumulative clinical manifestations of the patients studied (n = 30)

<table>
<thead>
<tr>
<th>Clinical manifestations</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis</td>
<td>21 (70)</td>
</tr>
<tr>
<td>Myositis</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Facial rash</td>
<td>24 (80)</td>
</tr>
<tr>
<td>Raynaud’s phenomena</td>
<td>5 (17)</td>
</tr>
<tr>
<td>Discoid lupus</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mucosal ulceration</td>
<td>5 (17)</td>
</tr>
<tr>
<td>photosensitivity</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>19 (63)</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Leukopenia (&lt;4 × 10^9/l)</td>
<td>6 (20)</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;100 × 10^9/l)</td>
<td>13 (43)</td>
</tr>
<tr>
<td>Lymphopenia (&lt;1.5 × 10^9/l)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Seizure</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Psychosis</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Myelopathy</td>
<td>13 (43)</td>
</tr>
<tr>
<td>Neuropathy (peripheral or cranial)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Optic neuritis</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Renal</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Serositis</td>
<td>5 (16)</td>
</tr>
</tbody>
</table>
The study found significantly higher levels of serum IL-18 (mean ± SD 3131.2 ± 1211.41 pg/ml) in SLE patients compared with the reported values in the matching control group (mean ± SD 110.65 ± 30.37 pg/ml, P < 0.001) (Table 3). A statistically significant correlation was reported between serum IL-18 levels and the total SLEDAI score in all SLE patients (r = 0.556, P = 0.003). Moreover, a significant correlation was found between serum IL-18 levels and seropositivity to ANA as well as anti-ds DNA in the studied patients (for IL-18 and ANA r = 0.412, and for IL-18 and anti-ds DNA r = 0.584, P = 0.001). The serum levels of IL-18 did not show significant variations with respect to sex (female 2113.00 ± 53.31 pg/ml and male 2002.56 ± 48.19 pg/ml, P = 0.08), age, or disease duration (r = 0.24, 0.123, respectively, P > 0.05) in the studied SLE patients. Negative correlations were found between serum IL-18 and C3 and C4 (r = −0.253 and −0.241, respectively, P > 0.05), although they did not reach statistical significance. The study also could not detect any significant correlation between the increase in serum levels of IL-18 and ESR in the studied patients (Table 5).

Levels of serum interleukin-10 in the studied population

Results of this study showed that the serum levels were significantly higher in patients (120 ± 27.2 pg/ml) compared with their matching healthy controls (76 ± 14.2 pg/ml, P < 0.001) (Table 3). Similarly, serum levels of IL-10 similar to those of IL-18 did not show significant variations with respect to sex (male 96 ± 23.31 pg/ml and female 72.56 ± 18.19 pg/ml, P = 0.21), patient’s age, or disease duration (r = 0.25, 0.123, respectively, P > 0.05). In contrast, a significant correlation was found between serum IL-10 and the SLEDAI score in all SLE patients (r = 0.551, P < 0.01). Moreover, results illustrated a significant positive correlation between serum levels of IL-10 and C4 (r = 0.741, P < 0.05) as well as with seropositivity to ANA and anti-ds DNA in the studied SLE patients (r = 0.72, r = 0.584, respectively, P = 0.001). Similarly, the current study found a significant positive correlation between serum IL-18 concentration and serum levels of IL-10 (r = 0.404, P = 0.037). The increase in serum levels of IL-18 and IL-10 positively correlated with the values of CRP in patients with active disease (r = 0.48 and 0.44, respectively, P < 0.05).

In contrast, the study did not find any significant correlations between serum IL-10 and each of the following: C3 levels or ESR (r = 0.25 and −0.208, respectively, P > 0.05) (Table 5).
Discussion

SLE is classified as an autoantibody–mediated immune complex disease. The role of Th2 cytokines in disease pathogenesis has always been dominant and attracted attention as a potential target for therapeutic interventions [25–33]. The current research aimed to assess the relationship between SLE disease activity in a population of Egyptian patients in terms of the SLEDAI score and two of the most pathogenetically significant cytokines that have attracted researchers, IL-18 and IL-10; in addition, the study tried to explore the possible influence of the serologic profile and the results of the inflammatory biomarkers on the level of these two cytokines. The study included 30 patients with SLE; over 50% of the patients had a clinically significant SLEDAI score greater than 6 manifesting significantly active SLE, despite being on multiple Disease Modifying Anti Rheumatic Drugs (DMARDs). The study found that ANA was positive in 40% of the patients, whereas anti-ds DNA was positive in 30%. This might be attributed to the influence of the immune-modulatory effects of drug combinations used in the studied patients. Regarding the surveyed cytokines, for the first cytokine, which is IL-18, the study found significant elevation of serum IL-18 in all SLE patients included compared with the control group, and this elevation correlated significantly with clinically active disease illustrated in terms of SLEDAI score. Elevated levels of IL-18 in SLE patients have been reported in several late studies particularly in patients with clinically active systemic illness [34–37]. Results of the current study are in agreement with those obtained by Esfandiari et al. [33], Wong et al. [37], as well as Amerio et al. [38], where the researchers illustrated a significant elevation in IL-18 concentration in patients with SLE compared with controls (\( P < 0.001 \)), which correlated with the SLEDAI activity score in their patients population [39]. In contrast to these studies, Robak et al. [7] did not find significant differences in the levels of IL-18 between active and inactive SLE in their work, which might have been explained by the fact that SLE is a heterogeneous disease in which variability in the mean levels of certain cytokines is expected with different phases of disease activity as well as with therapeutic interventions. Furthermore, cytokine levels in the plasma might not always reflect the locally high concentration produced in lymphoid tissues, as cytokines in the plasma might be bound to proteins or form complexes with soluble receptors or autoantibodies, which might affect their detection [7,40]. Age and sex were shown to have no significant influence on the serum levels of IL-18 in SLE patients [34,41], which is consistent with results of this study.

The second cytokine assessed in the current research was IL-10. The immune-regulatory effects of IL-10 in SLE have been successively reported in a variety of human as well as experimental studies, some of which illustrated that continuous administration of anti-IL-10 has been reported to delay the onset of lupus reflecting a relatively protective immune-regulatory effect of this cytokine against the induction and development of lupus-like disease in mice. Other studies showed that peripheral blood mononuclear cells from active SLE patients were found to spontaneously produce high amounts of IL-10 in vitro; furthermore, antagonism to the biologic functions of IL-10 was found to decrease immunoglobulin and anti-DNA production by SLE lymphocytes both in vivo and in vitro [42–45]. In contrast, Chun et al. [46] found a positive correlation between circulating levels of IL-10 and the titer of anti-ds DNA antibodies as a marker of disease activity in humans. Ishida et al. [43] and Chun et al. [46] demonstrated that serum IL-10 levels are higher in SLE patients than in controls and showed a positive correlation with SLEDAI and anti-ds DNA and negative correlation with C3, C4, and peripheral lymphocyte counts. These findings in combination with current data where serum IL-10 levels were significantly elevated in the studied population of SLE patients indicated that IL-10 might be part of the regulatory response that was not fully capable of compensating for the prevailing proinflammatory response. This suggests that IL-10 might play an important role in the pathogenesis of SLE. There were significant associations between IL-10 and disease activity score, anti-DNA antibodies and C4 in our population of SLE patients (\( P < 0.05 \)); yet, serum IL-10 levels showed a negative correlation with C3 levels. These findings were similar with the results reported by Houssiau et al. [44], El-Sayed et al. [47], and Park et al. [48], where they showed that IL-10 positively correlated with SLEDAI and anti-DNA antibodies but negatively correlated with C3 levels; however, the authors did not comment on C4 titers in these studies. In contrast, Llorente et al. [45] in their study could not find any significant association between IL-10 and disease activity in SLE; such discrepancy might be attributed to the difference in the assessment technique as the current study measured IL-10 in the sera of patients, whereas in the study by Llorente and colleagues, the authors studied its concentration in peripheral mononuclear cells cultures after 24 h. IL-10 plays a role in inhibition of proinflammatory cytokines; despite this, it appears that, in patients with active disease as reported in the current study, the increase in serum IL-10 failed to inhibit production of the proinflammatory cytokine IL-18. The authors hypothesize that, during an immune response, both IL-10 and IL-18 are cytokines that need to be coexpressed and it is the balance between the inflammatory and anti-inflammatory cytokines that...
there are no conflicts of interest.

---

**References**

1. Manson JJ, Rahman A. Systemic lupus erythematosus. Orphanet J Rare Dis 2006; 1:


---

**Acknowledgements**

**Conflicts of interest**

Sahar Abou El-Fetouh Hanan S Abozaid. Mohammed received financial support from the Department of Medical Research in Sohag University Hospital, Sohag University, for this research. For Reem M Hamdy, there are no conflicts of interest.


Wong CK, Ho CY, Li EK, Lam CW. Elevation of proinflammatory cytokine (IL-18, IL-17, IL-12) and Th2 cytokine (IL-4) concentrations in patients with systemic lupus erythematosus. Lupus 2000; 9:589–593.


