Correlation of serum interleukin-10 level with disease activity and severity in systemic lupus erythematosus
Mervat I. Abd Elazeem, Rabab A. Mohammed, Nilly H. Abdallah

Departments of *Rheumatology and Rehabilitation, **Clinical Pathology, Faculty of Medicine, *Internal Medicine Department, Beni Suef University, Beni Suef, Egypt

Correspondence to Mervat Ismail Abd Elazeem, MD, 1 Elgeesh Street, Elsaa Square, Elminia, 71111, Egypt
Tel: +20 100 714 7573; fax: +20 822 323 105;
E-mail: miaa_s11@yahoo.com

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Background
Systemic lupus erythematosus (SLE, lupus) is a syndrome of multifactorial etiology, characterized by widespread inflammation, most commonly affecting women during the childbearing years. Virtually, every organ and/or system of the body may be involved. Interleukin-10 (IL-10) production is increased in SLE.

Objective
The aim of the study was to assess serum levels of IL-10 in SLE patients and their relationship with disease activity and severity parameters.

Patients and methods
Totally, 50 patients with SLE and 20 healthy controls were investigated in this study diagnosed according to Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE. Clinical assessment of the disease activity was performed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score. Assessment of SLE disease severity was carried out using the SLICC/American College of Rheumatology Damage Index and laboratory parameters, including erythrocyte sedimentation rate, C-reactive protein (CRP), 24 h urinary proteins, anti–dsDNA antibodies, complement 3, and complement 4 levels. The serum IL-10 levels were determined using enzyme-linked immune sorbent assay technique.

Results
The serum IL-10 levels were significantly higher in SLE patients (mean: 23.07±33.19 pg/ml) compared with the controls (0.52±0.86 pg/ml, P=0.000*). The increase in serum levels IL-10 significantly correlated with the SLEDAI scores (P=0.016*) and CRP (P=0.042*) in the studied patients. There were no significant correlations between IL-10 and SLICC, age, disease duration, erythrocyte sedimentation rate, 24 h urinary protein, anti-DNA, and complement 3–complement 4 (P=0.735; r=0.05, P=0.890, P=0.521, P=0.529; r=0.09, P=0.430; r=0.11, P=0.263; r=0.16, P=0.195; r=0.19, respectively). There was no significant difference between mean IL-10 levels in different classes of lupus nephritis (P=0.702).

Conclusion
The circulating IL-10 concentrations were significantly elevated in SLE patients and correlated with the SLEDAI score and CRP.

Keywords:
enzyme-linked immune sorbent assay, interleukin-10, systemic lupus erythematosus, systemic lupus erythematosus disease activity index

Introduction
Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by B lymphocyte hyperactivity, production of autoantibodies directed against double-stranded DNA, and dysfunction of antigen presenting cells and T lymphocytes. Increased production and decreased clearance of immune complexes lead to immune complex deposition in tissue and damage to multiple organ systems. Interleukin-10 (IL-10) has the ability to induce autoantibody production by B lymphocytes, suggesting that IL-10 plays an important role in the pathogenesis of SLE [1]. The great sources of IL-10 in patients with SLE are B-cells and monocytes. IL-10 overproduction by B lymphocytes and monocytes was described for the first time by Lorente et al. [2].

The IL-10 cytokine is required for regulating immune functions by motivating the widespread suppression of immune responses through its pleiotropic effects. The Interleukin 10 (IL-10) cytokine is required for regulating immune functions by promoting the widespread suppression of immune responses.

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through its pleiotropic effects and the autocrine/paracrine capabilities of IL-10 by direct binding to leukocytes and function of this cytokine. IL-10 secretion from CD4+CD25+FoxP3+regulatory cells (Tregs), macrophages, and other leukocytes, followed by subsequent binding to IL-10 receptors on macrophages and dendritic cells (DCs), has been linked to decreased antigen presentation and increased T-cell anergy [3].

Immune cells express IL-10 with T-cells and B-cells, natural killer cells, mast cells, eosinophils, DCs, and monocytes/macrophages as major sources. IL-10 modulates T-cell responses through the inhibition of major histocompatibility complex class II expression, limited costimulation, and decreased proinflammatory cytokine expression from antigen presenting cells. Conversely, IL-10 promotes B-cell differentiation, proliferation, survival, and antibody production. Thus, IL-10 has been implicated in the pathophysiology of autoimmune disorders [1]. In early lupus, IL-10 was observed 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 it has been illustrated in in-vitro studies that anti-IL-10 antibodies could markedly inhibit immunoglobulin production by SLE peripheral blood mononuclear cells (PBMC), which confirmed the role of IL-10 in mediating autoantibody production [4]. Yin et al. [5] found that the serum IL-10 was significantly higher in SLE patients compared with controls. Furthermore, such an increase in the level of IL-10 showed a highly significant positive correlation with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores in the studied patients. Abnormal production of autoantibodies by B lymphocytes in patients with SLE is IL-10 dependent, and all related studies found that there is a positive correlation of serum IL-10 levels with disease activity [6]. This study aimed to assess serum levels of IL-10 in SLE and their relationship with disease activity and severity, as it might supply future views for targeted therapeutic strategies in SLE.

**Patients and methods**

This study was conducted on 50 clinically diagnosed SLE patients (44 female and six male) who were selected from the Rheumatology and Rehabilitation Department of Beni Suef University hospital from June 2015 to April 2016 and diagnosed according to Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE [7]. Twenty healthy adults served as a control group; consent was taken from all individuals in this study. The study population was subjected to the following clinical and investigational workup: full history taking including age, sex, disease duration, and swollen and tender joint counts of the patients; chest radiography; and echocardiography. Clinical assessment of the disease activity was performed using the SLEDAI score [8]. An SLEDAI score of more than 6 was considered active disease. Laboratory workup included routine laboratory survey with complete blood picture [9] and erythrocyte sedimentation rate (ESR), which was expressed in mm/h [10]. The C-reactive protein (CRP) concentration was determined using immune nephelometry methods; concentrations of 6 mg/l were considered positive for CRP [11]. 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 according to the manufacturer's instructions and expressed in terms of mg/dl [12]. Antinuclear antibody (ANA) [13] and antibodies to double-stranded DNA (anti-DNA) were measured using indirect immunofluorescence [14]. Anticardiolipin and lupus anticoagulant antibodies were measured. Blood samples from SLE patients and controls were centrifuged and sera were obtained for immediate routine laboratory workup. The remaining sera were stored frozen in aliquots at −20°C for the subsequent assays; urine analysis and 24 h urinary protein estimation were performed [15]. Renal biopsy was performed if indicated and classified according to the classification of lupus nephritis by the International Society of Nephrology/Renal Pathology Society [16]. 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 the 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, and then absorbance was read at 450 nm. The normal values for serum IL-10 ranged between 7.8 and 500 pg/ml.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.
Statistical methods
For statistical analysis, statistical package for the social sciences software version 20 (SPSS Inc., Chicago, Illinois, USA) was used.

The following tests were used:

(1) Descriptive analysis of the results in the form of percentage distribution for qualitative data and minimum, maximum, mean and SD calculation for quantitative data.
(2) Cross tabulation test for comparison between percentage values.
(3) Student’s t-test for comparison between mean of two groups with a normal distribution.
(4) The Mann–Whitney or Kruskall–Wallis test was used to compare the mean of variables that did not have a normal distribution.
(5) The Correlation coefficient ($r$): Pearson’s correlation test was used to detect whether change in one variable is accompanied by a corresponding change in the other variable. A significant correlation may be positive, indicating that the change in the two variables is in the same direction or negative, indicating that the change in the two variables is in the opposite direction; the sign of the correlation coefficient (+, −) defines the direction of the relationship, either positive or negative and ‘r’ represents the correlation coefficient.

$P$ value of greater than 0.05 was considered nonsignificant; $P$ value of less than 0.05 was considered significant; $P$ value of less than 0.01 was considered highly significant.

Results
The present study was conducted on 50 SLE patients and 20 age-matched and sex-matched healthy controls.

Among the 50 SLE cases, there were 44 (88%) female and six (12%) male cases. As regards the age, it ranged from 21 to 52 years with a mean value of 27.48±9.58. As for the control group, there were 15 (77.3%) female and five (22.7%) male participants with a mean age of 25.90±4.57 years. As regards the disease duration, it ranged from 1 to 72 months with a mean value of 25.76±17.98 months (Table 1).

The clinical and descriptive data of SLE patients are shown in Table 2.

The number of patients with renal disorders was 33 (66%) patients [mesangial, 12 (36.4%) patients; diffuse proliferative, six (18.2%) patients; membranoproliferative, six (18.2%) patients; mesangiproliferative, four (12.1%) patients; and membranous, one (3.0%) patient] (Fig. 1).

As regards the SLEDAI score in all SLE cases, it ranged from 0 to 48 with a mean of 13.50±10.43, whereas the SLICC score ranged from 0 to 10 with a mean of 1.68±1.73 (Table 3).

The first hour ESR ranged from 8 to 180 mm/h with a mean value of 67.98±41.66 mm/h.

As regards the CRP, the number of patients with negative CRP was 35 (70%), whereas the number of
patients with positive CRP was 15 (30%), which ranged from 3 to 96 mg/l with a mean of 25.48 ±28.119 mg/l.

As regards the complete blood picture, the hemoglobin level ranged from 6 to 15 g/dl with a mean of 10.30 ±2.06 g/dl. The white blood cell count ranged from 2 to 20 (10³/μl) with a mean of 6.32±3.24 (10³/μl). The platelet count ranged from 60 to 477 (10³/μl) with a mean of 259.18±98.31 (10³/μl). The 24 h urinary protein level ranged from 85 to 3500 mg/dl with a mean of 1097.40±1038.17 mg/dl.

All patients had a positive ANA (100%), 31 (62%) patients had positive anti-dsDNA, and 19 (38%) patients had negative anti-dsDNA.

As regards the C3 and C4 levels, the number of patients with consumed C3 and C4 was 23 (46%), whereas the number of patients with normal C3 and C4 was 27 (54%).

The number of patients with positive anticardiolipin was four (8%), and the number of patients with positive lupus anticoagulant was three (6%).

There was a highly statistically significant difference ($P=0.000$) on comparing results of serum IL-10 in cases and controls. The mean serum IL-10 levels was 23.07±33.19 pg/ml in the patient group and it was 0.52±0.86 in the healthy control group.

There was no significant difference between mean IL-10 levels in male patients compared with female patients ($P=0.788$) (Fig. 2).

There was no statistically significant relation found on correlating serum IL-10 with age of patients ($P=0.890$). Correlation coefficient was 0.02, which revealed a weak positive correlation. There was no statistically significant relation found on correlating serum IL-10 with disease duration ($P=0.521$). Correlation coefficient was 0.09.

There was no statistically significant difference on comparing IL-10 levels in SLE patients. According to the disease activity status based on the SLEDAI scoring system, IL-10 levels were higher in the active group (mean: 24.22±32.79 pg/ml) compared with the nonactive group (17.06±36.97 pg/ml) (Table 4).

There was a significant correlation between serum IL-10 and the SLEDAI score in all SLE patients ($P=0.016$; $r=0.34$) (Table 5).

There was no statistically significant correlation between serum IL-10 and the SLICC/ACR score for cumulative organ damage in all SLE patients ($P=0.735$; $r=0.05$) (Table 5).

On comparing results of serum IL-10 level in lupus nephritis and nonlupus nephritis patients, the mean

*Table 3 The systemic lupus erythematosus disease activity index, systemic lupus international collaborating clinics scores in all systemic lupus erythematosus cases*

| Variables      | Range | Mean±SD       |
|----------------|-------|---------------|
| SLEDAI         | 0–48  | 13.50±10.43   |
| SLICC          | 0–10  | 1.68±1.73     |

SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLICC, Systemic Lupus International Collaborating Clinics.

*Table 4 Comparing results of serum interleukin-10 level and Systemic Lupus Erythematosus Disease Activity Index score in systemic lupus erythematosus patients*

| Serum IL-10 (pg/ml) | Nonactive SLE patients | Active SLE patients | Mann–Whitney $U$ test ($Z$) | $P$ value |
|---------------------|------------------------|---------------------|-----------------------------|-----------|
| Mean                | 17.06±36.97            | 24.22±32.79         | 1.80                        | 0.072     |
| Median              | 3.90                   | 12.20               | 7.52–30.92                  |           |

IL-10, interleukin-10; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index. $P>0.05$, nonsignificant.
serum level of IL-10 was 20.90±22.48 in lupus nephritis, whereas it was 27.28±48.32 in nonlupus nephritis. There was no significant difference between mean IL-10 levels in lupus nephritis and nonlupus nephritis ($P=0.743$).

There was no significant difference between mean IL-10 levels in different classes of lupus nephritis ($P=0.702$). The mean serum level of IL-10 was 25.85±12.32 in mesangial, 25.70±26.72 in focal proliferative, 15.23±22.13 in diffuse proliferative, 18.55±26.47 in membranoproliferative, 16.77±19.04 in mesangioproliferative, and 8.30±0 in membranous class.

There was a statistically significant difference on comparing IL-10 levels in the CRP-positive and CRP-negative groups. The mean serum IL-10 level was 38.02±47.36 pg/ml in the CRP-positive group and was 16.66±22.88 in the CRP-negative group ($P=0.043$) (Fig. 3). Moreover, correlation analysis between IL-10 and CRP was significant ($r=0.29; P=0.042^*$) (Fig. 4).

There was no statistically significant relation found on correlating serum IL-10 with ESR ($P=0.529$).

**Discussion**

SLE is an autoimmune disease with variations in incidence, prevalence, disease activity, and prognosis.

![Figure 3](image3.png)

The mean serum interleukin-10 (IL-10) level in the C-reactive protein (CRP)-positive and CRP-negative groups.

![Figure 4](image4.png)

Correlation of serum interleukin-10 (IL-10) in all patients with C-reactive protein (CRP).

| Variables | Correlation coefficient ($r$) | $P$ value |
|-----------|-------------------------------|----------|
| SLEDAI score with serum IL-10 in all patients | 0.34 | 0.016* |
| SLICC score with serum IL-10 in all patients | 0.02 | 0.913 |

| Variables | Correlation coefficient ($r$) | $P$ value |
|-----------|-------------------------------|----------|
| ESR       | 0.09                          | 0.529    |
| CRP       | 0.29                          | 0.042*   |
| ANA       | –                             | –        |
| Anti-DNA  | 0.16                          | 0.263    |
| C3–C4     | 0.19                          | 0.195    |
| 24 h protein | 0.11                        | 0.430    |

ANA, antinuclear antibodies; C3–C4, complement 3 and 4; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; IL-10, interleukin-10.
based on race and ethnicity [17]. SLE is a chronic systemic disease with variable clinical presentation. The exact pathological mechanisms of SLE remains elusive, and the cause of SLE is multifactorial, involving genes, sex hormones, and environmental factors, including sunlight, drugs, and infections [18].

Immune complexes that are produced in SLE acting through Fcγ receptor II stimulate IL-10 production from PBMC, thus perpetuating the pathological cycle. Rönnelid et al. [19] showed a significant increase in IL-10 production in cell cultures incubated with SLE sera, in comparison with cell cultures incubated with control sera. This effect gives a possible explanation for the enhanced production of IL-10 in patients with SLE, which leads to B-cell hyperactivity, autoantibody production, immune complex production, PBMC stimulation, and also production of IL-10. Stimulation of this cycle leads to increased deposition of immune complexes in tissues and SLE-related pathology [19].

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 one of the most pathogenetically significant cytokines that have attracted researchers, 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 this cytokine. The present study included 50 SLE patients who were compared with 20 age-matched and sex-matched healthy unrelated controls. Both the patient and control groups were subjected for the analysis of IL-10 using enzyme-linked immune sorbent assay technique.

This study included 70 participants, and 50 of them were suffering from SLE. An overall 88% of SLE patients were female and 12% were male. This finding indicates that female sex is considered as one of the predisposing factors of the disease and that hormones share through unknown mechanisms to increase the prevalence of SLE among women. The X chromosome may share in increasing the severity of the disease, as the gene known to contribute to the pathogenesis of SLE is CD40, which is placed on chromosome X [20]. In the current case–control study, there was a highly statistically significant difference on comparing IL-10 levels in SLE patients and healthy controls. The mean serum IL-10 level was 23.07±33.19 pg/ml in the patient group and it was 0.52±0.86 in healthy controls (P=0.000).

The overproduced IL-10 in SLE patients may be due to B-cells and monocytes [21]. Moreover, B-cell secretion of IL-10 could regulate DCs and T-cell function to motivate Th2 cell deviation of the immune response [22]. So, the increased excretion of IL-10 may be due to an increasing number of the earlier peripheral B-cell abnormalities including plasma cell spread [23].

In accordance with the results of the current study, Sahar et al. [24] indicated that in SLE patients the mean IL-10 levels was 120±27.2 pg/ml compared with a mean of 76±14.2 pg/ml in healthy controls (P=0.001). In addition, our results on the significant difference in IL-10 level detected between SLE patients and healthy controls were in agreement with the results of the studies conducted by Liviu et al. [25], Zhihua et al. [26], Yang et al. [27], and Ahmad et al. [28].

However, contrary to our results, Dhir et al. [29] and Chen et al. [30] found that there was no difference in serum IL-10 level between patients and controls. These conflicts may have arisen probably from many potential factors such as sample size, patients with different demographics, clinical characteristics, or types of therapy. In addition, in some indices measured for SLE activity, qualitative data with high heterogeneity between studies can also contribute to this discordance.

In the current case–control study, we compared the IL-10 levels in SLE patients according to the disease activity status based on the SLEDAI scoring system. IL-10 levels were higher in the active group (mean: 24.22±32.79 pg/ml) compared with the nonactive group (17.06±36.97 pg/ml); however, the results did not reach statistical significance (P=0.072). However, correlation analysis between IL-10 and SLEDAI was statistically significant (r=0.34, P<0.016*).

In accordance with the results of the current study, Ahmad et al. [28] found that positive correlation between IL-10 levels and SLEDAI was highly significant (r=0.503, P<0.001).

Similarly, Yang et al. [27] indicated that IL-10 level was higher in active patients than in nonactive ones, and serum IL-10 level was correlated with SLEDAI.

In addition, several research groups [31–34,24] had reported that the serum IL-10 level was correlated with SLEDAI. However, contrary to our results, on evaluating the correlation between the two variables,
they detected a significant negative correlation. These results were contradicted by Lorente et al. [35] and Arora et al. [36]; 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 Lorente and colleagues the authors studied its concentration in peripheral mononuclear cells cultures after 24 h.

In our study, there was a statistically significant difference on comparing IL-10 levels in the CRP-positive and CRP-negative groups. The mean serum IL-10 level was 38.02±47.36 pg/ml in the CRP-positive and 16.66±22.88 in CRP-negative groups (P=0.043). Moreover, correlation analysis between IL-10 and CRP was significant (r=0.29, P=0.042\textsuperscript{*}).

In accordance with the results of the current study, Zhihua et al. [26] and Sahar et al. [24] had found that there was a significant positive correlation between IL-10 and CRP (r=0.373, P=0.001). In contrast, Lacki et al. [37] and Capper et al. [38] had found that there was no correlation between IL-10 and serum level of CRP.

Our study had found an increase in IL-10 serum in lupus nephritis patients in comparison with those without renal involvement (without statistical significance). The mean serum IL-10 level was 20.90±22.48 in lupus nephritis and was 27.28±48.32 in nonlupus nephritis (P=0.743).

There were no significant differences in serum level of IL-10 among different pathological classes (P=0.702). The mean serum level of IL-10 was 25.85±12.32 in membranoproliferative, 15.23±22.13 in diffuse proliferative, 18.55±26.47 in membranoproliferative, 16.77±19.04 in mesangioproliferative, and 8.30±0 in membranous.

In accordance with the results of the current study, Sikka et al. [39] and Sameh et al. [40] had found that there was no difference on comparing IL-10 levels in lupus nephritis patients with those without renal involvement.

In contrast with the results of the current study, Ishida et al. [31], Chun et al. [33], and Zhihua et al. [26] found that IL-10 negatively correlated with C3 and C4. Ma et al. [43] found that IL-10 negatively correlated with C3. Moreover, Sahar et al. [24] found no significant correlations between serum IL-10 and C3 levels (r=0.25, P>0.05).

There was no statistically significant difference detected on correlating IL-10 with ESR. In accordance with the results of the current study, Sahar et al. [24] found that there were no significant correlations between serum IL-10 and ESR (r=-0.208, P<0.05). Moreover, Ma et al. [43] in their study found that IL-10 negatively correlated with ESR. In contrast, a significant correlation was found between serum IL-10 and ESR in the studies of Capper et al. [38] and Zhihua et al. [26].

In the current study, SLICC/ACR score for cumulative organ damage was correlated with IL-10 in all SLE patients to determine whether IL-10 has a role in SLE organ damage. There was no statistically significant correlation detected on correlating SLICC/ACR score with IL-10 in SLE patients (P=0.735, r=0.05).

In accordance with the results of the current study, Eoghan et al. [44] in their study found that there was no statistical significance detected on correlating SLICC/ACR score with IL-10 (P=0.072).

In accordance with the therapeutic effect of anti-IL-10 antibodies, the continuous administration of recombinant IL-10 increased disease activity. Similarly, in a small, uncontrolled, open label study including patients with mild disease, anti-IL-10 monoclonal antibody improved skin lesions, joint symptoms, and the SLEDAI [45].

Therefore, the study concluded that the circulating IL-10 concentrations were significantly elevated in SLE patients and correlated with the SLEDAI score and CRP and it might be used as a serum marker to evaluate disease activity. Contribution of our studied cytokine might supply future ideas for targeted therapeutic strategies in SLE.
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