# A POSSIBLE ROLE FOR INTERLEUKIN-17 IN SYSTEMIC LUPUS ERYTHEMATOSUS.

***Samia Ali Ibrahim*, Sahar Zaglol and Naglaa Khalifa***.***

*Dermatology*,General Medicine**and Clinical Pathology***Departments, Faculty of Medicine, Zagazig University.*

## ABSTRACT

Systemic lupus erythematosus (SLE) is a chronic inflammatory – autoimmune disease. IL-17 is a Th-17 cytokine and is implicated in many chronic inflammatory and autoimmune diseases**. Aim of the work:** was to investigate the serum level of IL-17 in patients with SLE and its association with disease manifestations and activity, in an attempt to find a possible role of it in the pathogenesis of SLE**. Patients and Methods**: this study included 30 patients with SLE, diagnosed according to American College of Rheumatology (ACR) diagnostic criteria and 30 healthy control subjects. Serum IL-17 levels were measured using ELISA method for patients and controls. **Results:** Serum Levels of IL-17 were found to be significantly increased in patients when compared to control group. Non significant associations were detected between serum IL-17 level and both clinical manifestations and laboratory parameters in SLE patients. In SLE patients, non significant differences were found in IL-17 levels in patients with or without nephritis and those with less and more active disease. No association was found between serum IL-17 levels and SLE disease activity index (SLEDAI) score. **Conclusion**: Serum IL-17 is increased in SLE patients, suggesting that this cytokine may trigger the inflammatory response in SLE .

## INTRODUCTION

Systemic lupus erythematosus (SLE) is sometimes labelled the "great imitator" because of the wide variety of symptoms, it often confused with other disorders. It is a chronic inflammatory autoimmune disease in genetically prone individuals that affects the skin, joints, kidneys, lungs, nervous system and other organs. Usually patients have skin rashes and arthritis, as well as fatigue and fever[(1)].

The immune system normally protects the body by producing antibodies that attack foreign germs and cancers. With lupus, the immune system misfires. Instead of producing protective antibodies, the immune system begins manufacturing auto-antibodies which attack the patients' own tissues. As attacks continue, other immune system cells join the fight. This leads to inflammation and blood vessel abnormalities (i.e vasculitis)[(1)].

More than one mechanism could contribute to the disease and different mechanisms may be responsible for the different disease manifestations[(2)]. Cytokines produced by abnormal T-helper cells have been implicated in the pathogenesis of SLE[(3)].

Involvement of T-helper (TH) cells in the pathogenesis of SLE is controversy. It has been suggested that SLE is a Th2 – polarised disease because of its production of auto-antibodies specific for self antigens[(4)]. However, other studies have demonstrated that Th1 response including IFN-γ and IL-12 were also significantly increased in patients with SLE[(5,6)]. In fact, Th1 dominant immune responses have been commonly considered to be pathological in autoimmune disease via the induction of inflammatory reaction[(3)].

Interleukin-17 is a Th17 cytokine associated with inflammation, autoimmunity and defence against some bacteria, it has been implicated in many autoimmune diseases including psoriasis, multiple sclerosis and systemic sclerosis[(7)]. However, whether IL-17 plays a role in the pathogenesis of SLE remains unclear. So the aim of this work was to investigate the serum level of IL-17 in patients with SLE and its association with disease manifestations and disease activity.

## PATIENTS AND METHODS

Thirty patients with SLE (28 females and 2 males), their ages ranged from 16-60 years, were recruited for this study from outpatient clinics of Dermatology, Rheumatology and General Medicine Departments in Zagazig University Hospitals. Diagnosis of SLE was established by the presence of four or more of American Collage of Rheumatology (ACR) diagnostic criteria [8]. All the patients are newly diagnosed cases, not starting treatments. In addition 30 age and sex matched healthy subjects (29 females and 1 male) were also chosen as a control group, their ages ranged from 16-66 years old. We excluded from our study patients with any inflammatory or autoimmune condition that is known to increase IL-17 levels other than SLE as psoriasis, inflammatory bowl diseases, systemic sclerosis, rheumatoid arthritis and other autoimmune diseases.

Sera were obtained from 5 ml whole blood and stored at -80 °C until tested. All subjects gave their written consent.

**Collection of data:**

1-Demographic data, clinical data and laboratory data were collected from hospital records or by questionnaire and reviewed by experienced physician.

2- Clinical features of SLE such as butterfly erythema, oral ulcer, arthritis, nervous system disorders, myocarditis, renal involvement, serositis, alopecia, anaemia and photosensitivity were recorded.

3- Renal involvement of SLE was defined according to ACR criteria, i.e. any one of the following: A- Persistent proteinuria ≥ 0.5 g/day. B- Presence of active cellular casts[8].

4- Disease activity was quantified using SLE disease activity index (SLEDAI) score[9], more active SLE was defined as SLEDAI score ≥ 10. Those patients with SLEDAI <10 were classified as less active.

5- Laboratory abnormalities were also recorded, including leukopenia (wBCS count<4.000/$mm^3$ ), thrombocytopenia (platelet count<100.000/$mm^3$ ).The occurrence of blood in urine(hematuria) or proteinuria, elevated erythrocyte sedimentation rate (male: >15mm/hour, female: >20mm/hour),the presence of anti-dsDNA, anti-nuclear, anti- sm, anti-SSA, anti-SSB, anti-3NP(by indirect immunofluorescence); IgG, IgA, IgM and serum levels of C3/C4 were also recorded.

**Measurement of serum IL-17 levels:**

Serum IL-17 levels were measured by specific ELISA kits according to the manufacture's recommendation (R&D systems, Minneapolis, MN, USA), reproducibility of IL-17 measurement was: Intra-Assay: CV>10%. Inter Assay: CV<15%.These results were expressed as pg /ml[10].

**Statistical Analysis:**

All quantitative data were expressed as mean±SD. Statistic analyzes of were performed by SPSS 10.01 software. Analysis of covariance was used to compare serum IL-17 levels among different groups. Association of serum IL-17 levels with clinical and laboratory parameters of SLE patients were analyzed by independent samples t-test. For the correlation analysis between serum IL-17 levels and SLEDA, Pearson correlation coefficient was used. Difference was considered statistically significant if P<0.05 in two tailed test.

## RESULTS

1-Demographic characteristics of patients and controls: there were non significant differences between SLE patients and control subjects regarding the age and sex (p values were 0.081 and o.31 respectively). The mean age and sex distribution among patients and controls were shown in tables (1,2).

2- Serum IL-17 levels of SLE patients and controls: In this study, 14 patients had lupus nephritis, 21 SLE patients were classified as having more active disease. Serum IL-17 levels were found to be significantly elevated in patients with or

without nephritis than controls. Also, there was a non significant difference between patients with and without nephritis regarding serum IL-17 level. No significant difference was found between less and more active SLE in the mean IL-17 levels as shown in table (3) .

3- Association of serum IL-17 levels and clinical & laboratory parameters of SLE patients: No correlation was found between serum IL-17 levels and major clinical & laboratory parameters in SLE patients as shown in tables (4,5).

4- Relation between serum IL-17 levels and disease activity: Correlation analysis between serum IL-17 levels and SLEDAI found no association (r=0.175, P=0.195), using Pearson correlation coefficient as shown in figure (1).

**Table (1): The ages of SLE patients and controls**

| | Age in years | | |
|---|---|---|---|
| | **Range** | **Mean±SD** | P value |
| **Patients** | 16-60 | 37.63±12.55 | 0.081 |
| **Controls** | 15-66 | 39.52±11.20 | |

**Table (2): Gender distribution in SLE patients and controls.**

| | **Patients NO** | | **Controls No** | | **P value** |
|---|---|---|---|---|---|
| **Females** | 28 | 93.3% | 29 | 96.6% | 0.31 |
| **Males** | 2 | 0.06% | 1 | 0.03% | |

**Table (3): Comparison of serum levels of IL-17 in different groups.**

| | **NO.** | **Serum IL- 17 in pg/ml** | **P value** | |
|---|---|---|---|---|
| **Normal controls** | 30 | 16.89±6.00 | 0.000 | a |
| **Patients without nephritis** | 16 | 25.79±8.39 | 0.357 | b |
| **Patients é nephritis** | 14 | 23.64±9.70 | 0.003 | c |
| **Patients é less active SLE** | 9 | 23.19±9.89 | 0.566 | d |
| **Patients é more active SLE** | 21 | 23.90±9.00 | - | |

Analysis of covariance: Covariates appearing in the model are evaluated at the following values: AGE.

a- Versus SLE without nephritis.

b- Versus SLE with nephritis

c- Versus normal controls.

d- Versus more active.

**Table (4): Association of IL-17 and clinical parameters in SLE patients**

| Group | ± | Number | Serum IL- 17 level (pg/ml) | *P* value | |
|---|---|---|---|---|---|
| **Butterfly Erythema** | + | 15 | 24.05 (20.50-28.72) | 0.073 | N.S |
| | | | 20.73 (16.21-25.26) | | |
| | - | 15 | | | |
| **Oral ulcer** | + | 5 | 25.90±7.08 | 0.433 | N.S |
| | - | 25 | 23.00±9.24 | | |
| **Arthritis** | + | 13 | 23.80±9.90 | 0.758 | N.S |
| | - | 17 | 23.47±8.37 | | |
| **Nervous system** | + | 2 | 24.26±8.89 | 0.787 | N.S |
| | - | 28 | 23.00±9.24 | | |
| **Renal involvement** | + | 14 | 24.62±4.39 | 0.635 | N.S |
| | - | 16 | 23.52±9.36 | | |
| **Serositis** | + | 2 | 29.22±14.58 | 0.263 | N.S |
| | - | 28 | 23.31±8.72 | | |
| **Alopecia** | + | 7 | 23.52±7.13 | 0.877 | N.S |
| | - | 23 | 23.64±9.58 | | |
| **Anemia** | + | 18 | 24.12±10.18 | 0.168 | N.S |
| | - | 12 | 22.87±7.11 | | |
| **Photosensitivity** | + | 6 | 24.55±6.30 | 0.803 | N.S |
| | - | 24 | 23.39±9.60 | | |

**Table 5: Association of IL-17 levels with laboratory parameters in SLE patient**

| | ± | Number | Serum IL-17 level (pg/ml) | *P* value | |
|---|---|---|---|---|---|
| **Antinuclear antibody** | + | 28 | 23.93±8.82 | N.S | 0.712 |
| | - | 2 | 21.24±12.64 | | |
| **Anti-dsDNA** | + | 11 | 24.73±9.99 | N.S | 0.837 |
| | - | 19 | 23.35±8.72 | | |
| **Anti-Sm** | + | 4 | 24.60±5.55 | N.S | 0.718 |
| | - | 26 | 23.57±9.40 | | |
| **Anti-SSA** | + | 19 | 23.97±7.43 | N.S | 0.392 |
| | - | 11 | 25.72±11.43 | | |
| **Anti-SSB** | + | 7 | 23.25±6.80 | N.S | 0.756 |
| | - | 23 | 23.98±9.45 | | |
| **Anti-RNP** | + | 12 | 24.75±8.45 | N.S | 0.568 |
| | - | 18 | 23.38±9.43 | | |
| **C3 ↓** | + | 24 | 24.44±9.56 | N.S | 0.463 |
| | - | 6 | 21.61±7.21 | | |
| **C4 ↓** | + | 18 | 24.90±9.20 | N.S | 0.183 |
| | - | 12 | 21.53±8.80 | | |
| **Blood urine** | + | 8 | 26.12±11.24 | N.S | 0.258 |
| | - | 22 | 22.83±8.14 | | |
| **Thrombocytopenia** | + | 7 | 22.59±7.65 | N.S | 0.634 |
| | - | 23 | 23.91±9.54 | | |
| **Leukopenia** | + | 14 | 23.82±6.59 | N.S | 0.979 |
| | - | 16 | 23.76±10.92 | | |
| **Ig G ↑** | + | 8 | 25.39±6.52 | N.S | 0.398 |
| | - | 22 | 23.11±10.00 | | |
| **Ig A ↑** | + | 5 | 24.28±8.24 | N.S | 0.864 |
| | - | 25 | 23.50±9.48 | | |
| **Ig M ↑** | + | 2 | 26.52±11.47 | N.S | 0.527 |
| | - | 28 | 23.34±9.00 | | |

± With/Without

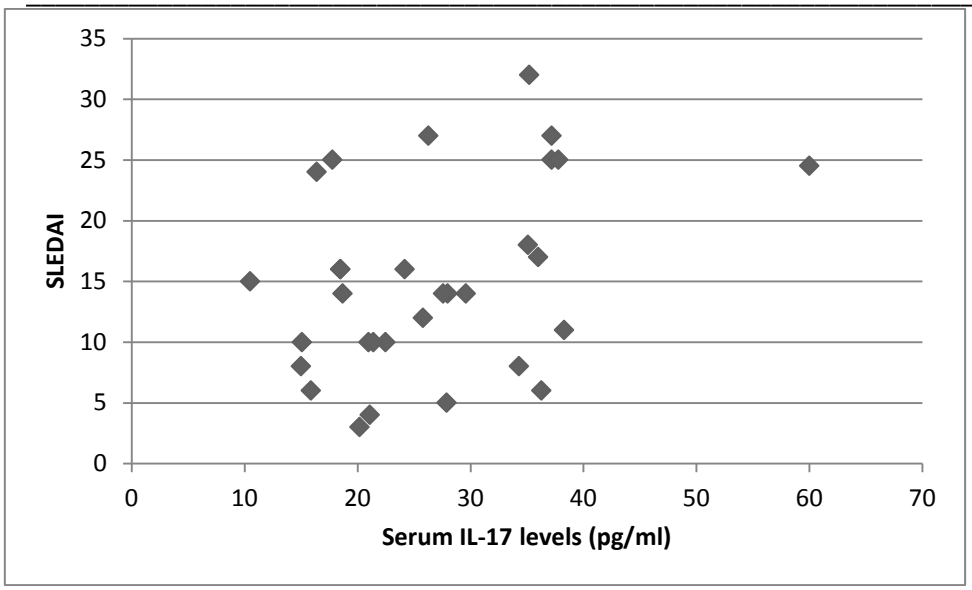


**Figure 1: Correlation between Serum IL-17 levels and SLEDAI**

## DISCUSSION

Systemic lupus erythematosus is a systemic autoimmune disease characterized by production of auto-antibodies, complement activation and immune complex deposition causing tissue and organ damage, leading finally to a fatal outcome[7].

There is an increasing evidence that Th-17 cells are crucial in host defence against extracellular bacteria, fungi and parasites[11]. Th-17 cells also drive inflammatory response[12]. Importantly, manipulations of Th-17 subset holds a great clinical promise as Th-17 cells have been implicated in the initiation and progression of many inflammatory and autoimmune pathologies, including mouse models of MS[13], colitis[14] and human inflammatory bowel disease[15] and RA[16] via production of IL-17 and other pro inflammatory cytokines as IFN-γ, IL-1 and IL-6[17].

IL-17 is a 32KD cytokine produced by Th-17 and its level was found to be increased in the serum of patients with SLE and correlated with disease activity [7]. In addition, the frequency of IL-17 producing cells in the peripheral blood of patients with SLE is increased[18]. In the present study IL-17 levels were significantly increased in the serum of SLE patients compared to normal controls, going in line with the previous results obtained by Zhang et.al.[7] This suggested that IL-17 may have a role in the inflammatory response in SLE. However in the current study, no correlation was found between serum IL-17 levels in patients with SLE and any of the clinical and laboratory parameters. There was a non significant difference between serum IL-17 levels in SLE patients with and without nephritis and those with more and less active disease, in contrary to the results obtained by Wong et al[10], who found a correlation with the disease activity. This preliminary data can be explained by heterogenic nature of SLE or by small size of the sample in this study. This suggested that IL-17 may trigger the inflammatory response in SLE either directly or through production of other pro-inflammatory cytokines, that will need further investigations.

In consistency with the role of IL-17 in autoimmune inflammations, IL-17 deficient mice either were resistant to experimental autoimmune encephalitis (EAE) and collagen induced arthritis (CIA) induction or showed reduced disease severity[19]. Administration of IL-17

monoclonal antibodies in CIA and EAE mouse models also significantly reduced disease severity.[20]

Mice immunization with IL-17 VLD induced high levels of anti-IL-17 antibodies and have lower incidence of disease severity in both CIA and EAE. Neutralisation of IL-17 by passive or active immunisation may be an novel approach in the therapeutic aspect of SLE[21].

Apart from its direct pro-inflammatory capacities and production of other pro-inflammatory cytokines as IF-γ, IL-12, IL-23, IL-21.....etc, the effect of IL-17 in other cell types may contribute to the pathogenesis of SLE[22]. In addition, the immune involvement in patients with SLE, suited for generation of IL-17, probably has broad effects on the immune system that induce B- cell stimulation[23]. Increased production of IgG, anti-ds-DNA IgG and IL-6 by peripheral blood mononuclear cells in patients with lupus nephritis was observed when they cultured in the presence of IL-17[24]. Also, patients with SLE have a higher frequency of IL-17-producing T cells[25].

## CONCLUSION

Serum IL-17 is increased in patients with SLE, its level probably contributes to recruitment and activations of immune cells( neutrophiles and T-cells) to target organs and thus amplifying an ensuing immune response. The precise pathway through which IL-17 contributes to SLE. pathology will need to be identified in the future work as IL-17 may prove to be a promising treatment target in SLE.

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## دور انترلوكين -17 فى مرض الذئبة الحمراء

يعد مرض الذئبة الحمراء مرض ذاتى المناعة ويتميز با لتهاب المزمن

يعتبر انترلوكين-17 أحد السيتوكينا ت التى تلعب دورا فى ألآمرا ض الأ لتهابية المزمنة والأمرا ض ذاتية المناعة.

هد ف البحث : هو قياس مستوى أنترلوكين-17 فى مصل مرضى الذئبة الحمراء وعلاقتة بمظاهر المرض الأكلينيكية والمعملية ومدى علاقتة بنشاط المرض لأ كتشا ف ما اذا كان له دور فى أمكانية حدوث ذلك المرض.

اشتمل هذا البحث على ثلاثون من مرضى الذئبة الحمراء وثلاثون من الأشخاص الطبيعين كمجموعة ضابطة.

الطريقة: تم قياس مستوى أنترلوكين-17 فى أ مصال المرضى والمجموعة الضا بطة باستعما ل طريقة الأليزا.

النتائج: وجد أ ن مستوى أنترلوكين-17 عالى فى أمصا ل المرضى عنه فى المجموعة الضا بطة . ووجد أنه لاتوجد علاقه بين مستوى أنترلوكين-17 وأى من مظا هر المرض الأكلينيكية و النتائج المعملية. كما وجد أ نه لم يوجد اختلا ف فى مستوى أنترلوكين-17 بين مرضى الذئبة الحمراء الذين يعانون من التهاب فى الكلى والذين لا يعانوا وبين مرضى الذئبة الحمراء الذين يعانون من مرض فى حالة نشطة والذين مرضهم أقل نشاطا. كما لم توجد علاقة بين مستوى أنترلوكين-17 ونشاط المرض.

الخلاصة: أنترلوكين- 17 ربما يلعب دورا فى تسبب مرض الذئبةالحمراء.