Sohag University Sohag Medical Journal Faculty of Medicine

Interleukin 17 role as a biomarker in Systemic Lupus Erythematosus patients.

Asmaa Tarazan Mostafa*, Amany Abbass Abd Allah*, Sahar Abo alfotoh Abd Alwahed*, Rania Hafez, Safenaz Hussien**, Rania Mohamed Mohamed Bakry ***.**

* Department of Clinical and Chemical Pathology - Faculty of Medicine - Sohag University.
** Department of Hematology and Bone Marrow Transplantation - Department of Internal Medicine - Faculty of Medicine - Assiut University.
*** Department of Clinical Pathology - South Egypt Cancer Institute - Assiut University.

Abstract:

Objective: The purpose of this study is to determine the interleukin 17 (IL-17) level in cases with Systemic lupus erythematosus (SLE), in addition to assessing the correlation of IL-17 with the activity of the disease. **Patients and Methods:** The present research recruited 60 adult SLE cases versus 60 healthy subjects serving as controls. Subjects had a full clinical evaluation, history taking as well as evaluation of disease activity in cases with SLE via the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). The serum level of IL-17 was measured in controls as well as SLE patients. **Results:** The IL-17 levels were substantially elevated in the SLE cohort than in controls. In addition, its level was positively related to SLEDAI. **Conclusion:** The present study findings revealed maximized IL-17 levels in SLE cases, denoting their contribution to the activity as well as the pathogenesis of the disease. Serum IL-17 levels were significantly positively related to the activity of the disease, 24 h protein in the urine, and anti-dsDNA.

Keywords: Systemic lupus erythematosus, Interleukin-17, Disease Activity.

Introduction:

SLE is known as a chronic autoimmune illness manifested by infiltration of immune complex deposits along with inflammatory cell infiltration in different organs in the body, such as the nervous system, skin, joints, kidneys, and mucosa[1]. The etiology

y of SLE is unclear, although a genetic vulnerability with immune system abnormalities contributes to SLE progression. Antigen-presenting cells absorb undigested dead cell components that are not eliminated, resulting in elevated secretion in antinuclear antibody production[2]. Cytokines are volatile substances that contribute to the activation, maturation, as well as distinction of numerous immune cells implicated in the SLE's immunological dysregulation of different immune cells as well as local inflammatory responses that lead to tissue damage[3]. The IL-17 cytokine family has five receptors (IL-17RA to IL-17RE), besides six proteins (IL-17A to IL-17F) that are functionally irrelevant to any other defined cytokine receptors. Th17 cells generate IL-17F and IL-17A, while other cell types generate the other family members[4]. IL-17 is involved in malignan-

cies, inflammation, and autoimmuneity, as well as host defenses against fungal and bacterial infections [5]. Serum IL-17 levels were shown to be greater in cases with active SLE than in healthy subjects and associated with the activity of the disease[6-7]. IL-17 contributes to the pathophysiology of SLE; it causes damaged tissues, and inflammation and leads to the disintegration of tolerance in SLE cases[8]. Increased IL-17 levels in SLE are likely to aid in activating and recruiting immune cells like T cells and neutrophils to target organs and increase the response of the immune system[9].

Patients and Methods:

This research was done on 60 subjects visiting the outpatient clinic in Sohag University Hospital and 60 healthy controls of both sexes from February 2019 to February 2020. They were assigned to two primary cohorts; Group I (SLE cohort) consisted of 60 SLE subjects diagnosed by the SLE classification criteria of the American College of Rheumatology (ACR)[10]. Group II (Controls) consisted of 60 healthy female and male subjects.

1. Ethical considerations:

This research was authorized by the Faculty of Medicine's Scientific and Ethical Committee, Sohag University. Patients and controls provided written consent after discussing the aim of the study and methods.

2. Inclusion criteria:

New-onset SLE patients of both sexes are included and those more than 18 years old.

3. Exclusion criteria:

Patients receiving biologic blocking antibodies, patients treated with any immunosuppressive drugs, and patients who had malignancy and chronic infections were excluded from this study.

4. Clinical examination:

Patients had full history taking as well as complete clinical examination, such as the chest, general, skin, locomotor system, cardiovascular, vascular, abdominal, and neurological examinations.

5. Disease activity:

Disease activity was evaluated in cases of SLE based on SLEDAI[11]. SLEDAI scores were used to determine the categories of activity: very high activity (SLEDAI: 20), high activity (SLEDAI: 11-19), moderate activity (SLEDAI: 6-10), mild activity (SLEDAI: 1-5), and no activity (SLEDAI: 0).

6. Sample collection:

Under aseptic conditions, 5 ml of venous blood were taken from each patient as well as controls. They were divided into two portions. Two ml of blood was added to EDTA vacutainer to be used for complete blood count and ESR. Three ml of blood in a sterile plain vacutainer for other investigations and IL-17 detection through serum separation. Twenty-four-hour urine samples were collected from both groups.

7. Laboratory investigations:

Protein in 24 hours collected urine sample, complete blood picture, liver function tests, serum creatinine, the rate of erythrocyte sedimentation, complement 3, complement 4, Anti-dsDNA, and ANA are among the tests performed.

8. IL-17 assay:

IL-17 was detected utilizing (Lum-inex® 100/200™ System, Austin, Tex-as, USA, serial number LXSD 1310-7003) and Luminex x ponent® (one lambda) software version 4.2 for an-alysis of the results. Luminex® assays utilize a series of fluorescent beads, each of which falls in a distinct region of the luminous spectrum. The beads are cov-

ered with capture antibodies specific to the analyte of interest [12], with a normal range of 7- 13.5 pg/ml.

9. Statistical analysis:

The coding, tabulation, and analysis of data were performed utilizing the 26th version of [SPSS]. The Chi-square test was utilized to evaluate the relationship between categorical variables, while qualitative data were reported as percentages and numbers. Quantitative data were represented as mean as well as standard deviation (Mean SD), as well as means for groups, were compared utilizing a -test. In order to assess the link between quantitative data, the Pearson correlation test was used. A p-value of 0.05 was deemed statistically significant.

Results:

1.The patients' and controls' demographic data:

In this research, the 60 SLE cases were 58 females as well as two males, with an age range of (19-48 years), with an average age of 31.52 ± 8.30 years. While 60 controls were 56 females and four males, with an age range of (20-50 years) with an average age of 32.47 ± 9.19 years **(Table 1).**

2. Patients' and controls' laboratory investigations:

There was a substantial elevation in the levels of ESR, 24-hour urinary protein, anti-dsDNA, ANA, and IL-17 (P<0.0-01), as well as an elevation in serum creatinine level(P<0.05) in SLE subjects than controls. While there were a highly significant decrease in the levels of C3, C4, PLT (P<0.001) and a considerable decline in WBCs, RBCs, and HGB levels (P<0.05) in cases with SLE than controls **(Table 2).**

3. SLE patients' disease activity :

As regards SLEDAI activity, 5% of subjects had no activity, 15% had mild activity, 21% had moderate activity, 28% had high activity, and 30 % had very high activity **(Fig 1).**

4.Correlation of IL-17 with laboratory investigations of SLE patients:

There were susbstantial positive correlation between IL-17 with SLEDAI (r= 0.809, P< 0.001) **(Fig 2)**, with anti-ds-DNA (r= 0.785, P< 0.001) **(Fig 3)** and with 24 hour protein in urine (r= 0.653, P< 0.001) **(Fig 4)**. In contrast, a substantial negative realtioship was detected between IL-17 with C3 (r= -0.573, P< 0.001) **(Fig 5),** and with C4 (r= -0.321, P= 0.012) **(Fig 6).**

Table (1): Patients' and controls' demographic data.

Parameters	patient group	Control group	P. value
Gender (female/male)	58/ 2	56/4	0.402 (NS)
Age,years(mean± S.D)	31.52 ± 8.30	32.47 ± 9.19	
range	19-48	20-50	0.554 (NS)

-NS: No significant. -S.D: standard deviation.

Table (2): Laboratory investigations of patients and controls.

Parameters (mean±S.D)	patient group	Control group	P. value
ESR	41.47±34.94	11.78±3.36	<0.001
WBCs	6.13±2.85	7.02±2.01	0.01
RBCs	4.43±0.49	4.94±0.28	0.02
HGB	12.03±1.38	13.51±1.05	0.01
PLT	218.77±93.94	241.65±72.29	<0.001
serum creatinine	1.83±1.13	0.89±0.22	0.03
24 h urinary protein	316.17±229.99	92.33±32.49	<0.001
C3	59.17±46.54	111.85±26.56	<0.001
C4	10.20±9.33	20.95±8.96	<0.001
ANA	4.85±3.11	0.72±0.34	<0.001
anti-dsDNA	189.57±109.03	9.78±5.07	<0.001
IL-17	35.41±16.98	9.83±2.19	<0.001

-S.D: standard deviation. -ESR=erythrocyte sedimentation rate.
-ANA: antinuclear antibodies. -Anti-dsDNA: anti-double-stranded DNA**.**
-C3: complement 3. -C4: complement 4.
-WBCs: white blood cells. -RBCs: red blood cells**.**
-HGB: heamoglobin. -PLT: platelet.
-IL-17: interleukin 17.

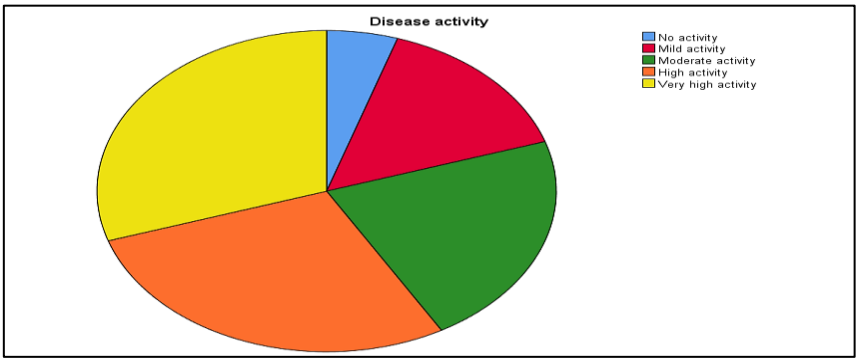

Fig (1): SLE Disease Activity Index (SLEDAI) of SLE patients.

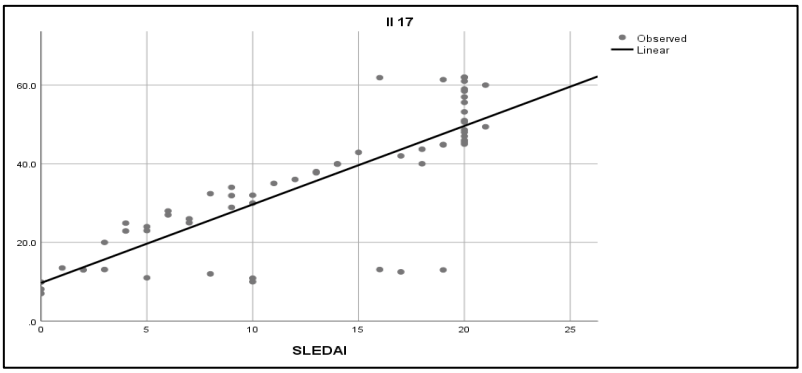

Fig (2): The association between IL-17 and SLEDAI.

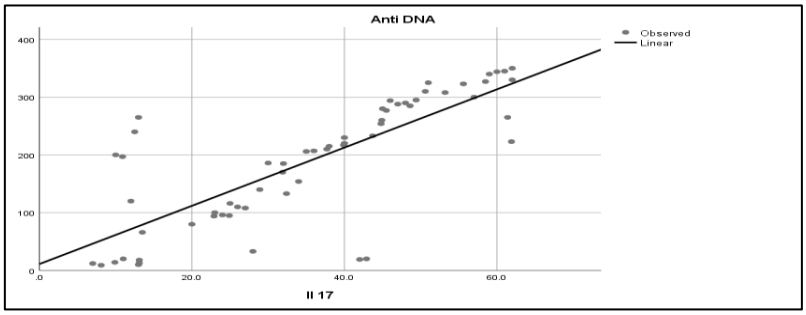

Fig (3): Correlation between anti-dsDNA and IL-17.

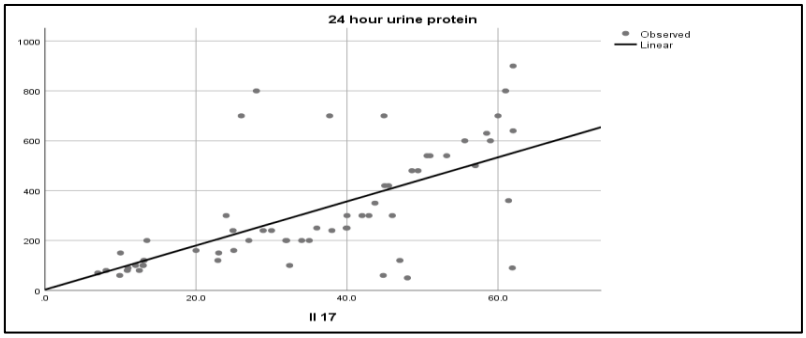

Fig (4): Correlation between 24 h protein in urine and IL-17.

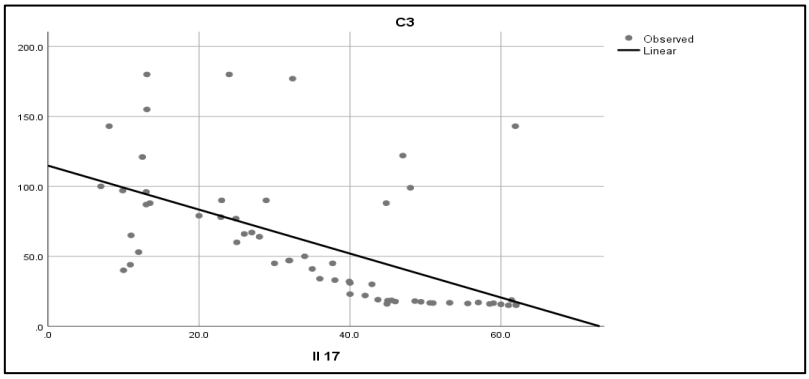

Fig (5): association between C3 and IL-17.

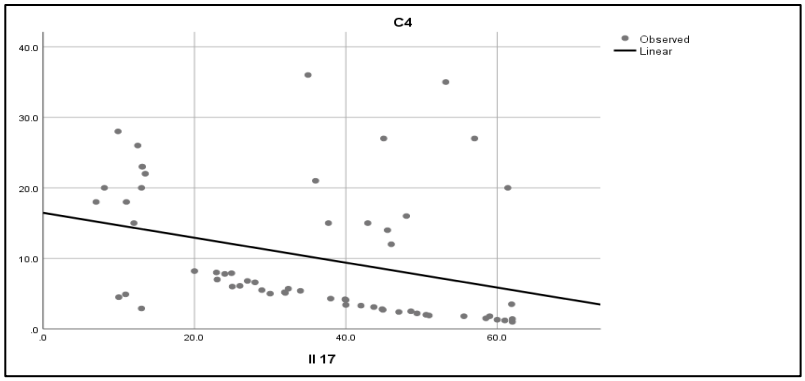

Fig (6): Association between C4 and IL-17.

Discussion:

SLE is a multisystem autoimmune illness with numerous clinical manifestations[13]. Multiple organ impairment in SLE is caused by the formation of autoantibodies against many organs, such as the vascular, renal, and brain tissues, in addition to phospholipids, nuclear antigens, and ribosomes[14-15]. SLE is usually thought to be a Th2-mediated illness in its early stages [16]. Nevertheless, Th1 bypasses the Th2 pathway, taking over SLE development to active nephritis[17]. IL-17 is synthesized by the Th17 fraction of CD4+ T cells in humans[18]. In addition, it is generated by CD8+Tcells, natural killer cells, as well as neutrophils[19]. This research attempted was to identify the function of Il-17 as a biomarker in cases with SLE.

In this research, a substantial elevation in the levels of IL-17 was revealed in the SLE cohort than in controls, which is in agreement with many studies[7-20-21-22]. Zickert et al. [23] found that the SLE cohort experienced elevated Th17 cell numbers in the tissues as well as sera of people with lupus nephritis (LN) and kidney damage. Another study found that increased serum IL-23 in cases with SLE contributes to pathogenic Th17 cell development, which synthesizes IL-17, resulting in attracting neutrophils to tissues involved in SLE pathogenesis.[24]

IL 17 may promote SLE by inhibiting Treg cell formation and function. Treg cells appear to have qualitative or quantitative abnormalities in their ability to control the secretion and proliferation of proinflammatory cytokines in ESL's effector immune cells[25]. Th17 cell growth is caused by a decrease in IL-2 and elevated IL-6 levels[26]. Environmental variables such as smoking, infection, and UV radiation induce oxidative stress and elevated IL-6 production, which may be the major reason for Treg loss in SLE[27]. IL 17 contributes to the advancement of clinical SLE symptoms in LN, vasculitis, and the CNS [28].

Raymond et al. [29] found no elevation in IL-17 level in SLE; the previously mentioned level could be explained that they detected serum IL-17 by different technology. They found that blood IL-17 levels may not be a precise representation of the overall production of IL-17, that IL-17 may be confined to inflammatory tissue such as the brain in certain situations, and that serum IL-17 levels may not be a precise representation of its endogenous synthesis[30].

In this study, a substantial positive association was detected between IL-17 and SLEDAI, which other studies recorded[31-32]. Wong et al. [33] found an association in only cases with no kidney disease. In contrast, other researchers reported no relationship between the activity of the disease as well as the levels of IL-17 in any case subset[29-34]. These diverse findings may be explained by disease heterogeneity. The expression of the disease may vary between patients in terms of the involvement of significant organ involvement that probably influenced these results, as well as small sample sizes utilized in some studies and variations in the principle, the sensitivity of the ELISA test, and uncontrolled other factors as immunosuppressive drugs could be strong effectors in the levels of serum IL-17 [30].

In this study, there was a substantial positive association between anti-dsDNA as well as IL-17 in this research, which is compatible with previously reported findings[35]. It was demonstrated that elevated anti-dsDNA generated by IL-17 is dose-dependent and can be totally prevented by the monoclonal antibodies of IL-17; nonetheless, it

does not have the potential to stimulate normal controls' peripheral blood mononuclear cell (PBMC) to enhance the secretion of anti-dsDNA. They concluded that the impact of IL-17 on the elevated levels of anti-dsDNA by PBMC probably depends on the features of genetic as well as immunological anomalies detected in SLE patients' PBMC[7]. A substantial positive relation was revealed between 24-hour protein as well as IL-17 in urine, which is consistent with various studies.[32] In another study that randomized 60 SLE subjects into cases with nephritis (LN cohort) and cases without nephritis (SLE cohort), the frequency of Th17 cells was more elevated in the LN cohort than in both controls and the SLE cohort. Furthermore, both the LN as well as SLE groups experienced elevated IL-17 levels than normal subjects[36]. In this study, a substantial negative association was detected between C3 and C4 with IL-17, and these findings are compatible with various studies[32-37].

Conclusion:

Serum IL-17 levels were more elevated in SLE cases than in controls, contributing to SLE pathogenesis. Serum IL-17 levels were significantly positively related to the activity of the disease, 24 h protein in the urine, and anti-dsDNA. In addition, IL-17 serum levels were substantially negatively related to C3 and C4.

References:

1-Enrique, M., Maria, G., & Hernando, T. (2021). Update on Lupus Nephritis: Looking for a New Vision. Nephron, 145: 1-13.

2-Lisnevskaia, L., Murphy, G., & Isenberg, D. (2014). Systemic lupus erythematosus. Lancet, 384: 1878-1888.

3-Jianwen, D., Shujun, S., Tao, Y, et al., (2020). Serum interleukin-6 level is correlated with the disease activity of systemic lupus erythematosus: a meta-analysis. Clinics, 75.

4-Korn, T., Bettelli, E., Oukka, M., & Kuchroo, V.K. (2009). IL-17 and TH17 cells. Annual Review of Immunology, 27: 485-517.

5-Iwakura, Y., Ishigame, H., Saijo, S., & Nakae, S. (2011). Functional specialization of interleukin-17 family members. Immunity, 34: 149-162.

6-Alisa, N., Angel, J.V., Patrick, E.A., &Peter, P.K. (2016). Levels of Interleukin-17 and Interleukin-23 in Patients with Systemic Lupus Erythematosus (SLE) in Trinidad and TobagoNobee. Immunochem Immunopathol, 2:1.

7-Robert, M., & Miossec, P. (2020). Interleukin-17 and lupus: enough to be a target? For which patients?. Lupus, 29: 6-14.

8-Weaver, C.T., Hatton, R.D. (2009). The interplay between the TH17 and TReg cell lineages: a (co-)evolutionary perspective. Nat Rev Immunol, 9: 883-9.

9-Doreau, A., Belot, A., Bastid, J, et al., (2009). Interleukin 17 acts in synergy with B cell-activating factors to influence B cell biology and the pathophysiology of systemic lupus erythematosus. Nat. Immunol,10: 778-785.

10-Tan, E.M., Cohen, A.S., Fries, J.F, et al., (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum, 25: 1271.

11-Bombardier, C., Gladman, D.D., Urowitz, M.B, et al., (1992). Derivation of the SLEDAI. A disease activity index for lupus patients. The committee on prognosis studies in SLE. Arthritis Rheum, 35: 630-40.

12- Kupcova, S.H., Katerina, V.K., Shum, K., & Petr, V. (2020). Luminex Xmap Assay To Quantify Cytokines In Cancer Patient Serum. Methods Mol Biol, 2108: 65-88.

13-Askanase, A., Shum, K., & Mitnick, H. (2012). Systemic lupus erythematosus: an overview. Soc Work Health Care, 51(7): 576-86.

14-Podolska, M.J., Biermann, M.H., Maueroder, C, et al., (2015). Inflammatory etiopathogenesis of systemic lupus erythematosus: an update. J Inflamm Res, 8: 161-71.

15-Pego-Reigosa, J.M., Lois-Iglesias, A., Rua-Figueroa, I, et al., (2016). Relationship between damage clustering and mortality in systemic lupus erythematosus in early and late stages of the disease: cluster analyses in a large cohort from the Spanish Society of Rheumatology Lupus Registry. Rheumatology (Oxford), 55(7): 1243-50.

16-Nayera, Z.S., Sherif, H.M., Dina, A.S., & Marwa, S.F. (2017). Expression of T helper 17 cells and interleukin 17 in lupus nephritis patients. The Egyptian Rheumatologist, 39: 151-157.

17-Miyake, K., Akahoshi, M., & Nakashima, H. (2011). The subset balance in lupus nephritis. J Biomed Biotechnol, 980286.

18-Murugaiyan, G., & Saha, B. (2009). Protumor vs antitumor functions of IL-17. J Immunol, 183: 4169-75.

19-Ohl, K., & Tenbrock, K. (2011). Inflammatory cytokines in systemic lupus erythematosus. J Biomed Biotechnol, 432595.

20-Chen, X.Q., Yu, Y.C., Deng, H.H, et al., (2010). Plasma IL-17A is increased in new-onset SLE patients and associated with disease activity. J Clin Immunol, 30: 221-5.

21-Ashraf, H.M., Abdelnaser, B., Emad, E, et al., (2014). Serum level of Interleukin 17 in Systemic lupus erythematosus: clinical associations with disease activity and lupus nephritis. Medical Science, 4(10): 2249-555X.

22-Keshav, R.S., Lihua, D., Yin, W, et al., (2016). Serum Cytokines Th1, Th2, and Th17 Expression Profiling in Active Lupus Nephritis-IV: From a Southern
Chinese Han Population. Mediators of Inflammation, 10.

23-Zickert, A., Amoudruz, P., Sundstrom, Y, et al., (2015). IL-17 and IL-23 in lupus nephritis association to histopathology and response to treatment. BMC Immunol, 16: 1-10.

24-Cornelissen, F., Van-Hamburg, J.P., Lubberts, E. (2009). The IL-12/IL-23 axis and its role in Th17 cell development, pathology, and plasticity in arthritis. Curr Opin Investig Drugs, 10: 452-462.

25-An, N., Chen, Y., Wang, C, et al., (2017). Chloroquine Autophagic Inhibition Rebalance Th17/Treg-Mediated Immunity and Ameliorates systemic lupus erythematous. Cellular physiology and biochemistry, 44: 412-422.

26-Koga, T., Ichinose, K., & Tsokos, G.C. (2017). T cells and IL-17 in lupus nephritis. Clin Immunol, 185: 95-99.

27-Shah, K., Lee, W.W., Lee, S.H, et al., (2010). Dysregulated balance of Th17 and Th1 cells in systemic lupus erythematosus. Arthritis Res Ther, 12: R53.

28-Wu, Q., & Antonio, L. (2012). Cava IL-17 in systemic lupus erythematosus Clin. Invest, 2(4): 417-421.

29-Raymond, W., Ostli-Eilertsen, G., Griffiths, S., & Nossent, J. (2017). IL-17A
levels in systemic lupus erythematosus associated with inflammatory markers and lower rates of malignancy and heart damage: evidence for a dual role. Eur J Rheumatol, 4: 29-35.

30-Vincent, F.B., Northcott, M., Hoi, A, et al., (2013). Clinical associations of serum interleukin-17 in systemic lupus erythematosus. Arthritis Res Ther, 15(4): 97.

31-Lu, X.Y, Zhu, C.Q., Qian, J, et al., (2010). Intrathecal cytokine and chemokine profiling in neuropsychiatric lupus or lupus complicated with central nervous system infection. Lupus, 19: 689-695.

32-Enass, A.E., Omyma, Z., Enas, I.M., & Ghada, B. (2014). The role of interleukins 4, 17 and interferon-gamma as biomarkers in patients with Systemic Lupus Erythematosus and their correlation with disease activity. Egyptian Society for Joint Diseases and Arthritis. The Egyptian Rheumatologist, 36: 21- 27.

33-Wong, C.K, Lit, L.C, Tam, L.S, et al., (2008). Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implications for Th17-mediated inflammation in auto-immunity. Clin Immunol, 127: 385-393.

34-Zhao, X.F, Pan, H.F., Yuan, H, et al., (2010). Increased serum interleukin 17 in patients with systemic lupus erythematosus. Mol Biol Rep, 37: 81-85.

35-Ashraf, H.M., Abdelnaser, B., Emad, E, et al., (2014). Serum level of Interleukin 17 in Systemic lupus erythematosus: clinical associations with disease activity and lupus nephritis. Medical Science, 4(10): 2249-555X.

36-Xing, Q., Wang, B., Su, H, et al., (2012). Elevated Th17 cells are accompanied by FoxP3+ Treg cells decrease in patients with lupus nephritis. Rheumatol. Int, 32(4): 949-58.

37-Sheren, E.M., Hanan, A.T., Walaa, G.H, et al., (2019). Study of balance between T-helper 17 /T-regulatory cells in systemic lupus erythematosus and its relation to disease activity. Curr Pediatr Res, 23(2): 49-55.