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Serum level of Interleukin -17 in Systemic lupus erythematosus: clinical associations with disease activity and lupus nephritis.

KEYWORDS: Interleukin-17, Systemic lupus erythematosus, lupus nephritis.

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ABSTRACT Background: Serum IL-17 concentration correlates poorly with Systemic lupus erythematosus (SLE) disease activity and lupus nephritis. The aim of the present study was to further investigate the possible role of IL-17 in the pathogenesis of SLE and development of lupus nephritis (LN). Patients and Methods: This study included 46 adult patients with SLE (21 with LN and 25 without LN) and 15 apparently healthy volunteers. SLE Disease Activity Index(SLEDAI) and renal disease activity were calculated for all patients. Laboratory investigations included: full blood picture, ESR, CRP, serum creatinine, simple urine analysis & estimation of 24 hours urinary protein, C3, C4, ALT, AST, C3, C4, ANA, anti-dsDNA and assay for Interleukin-17 were done for patients and controls. Renal biopsy was done for patients with LN. Results: SLE patients had higher IL-17 than control group (P <0.001). In SLE group; there was significant higher serum levels of IL-17, antidsDNA titer and degree of proteinuria in LN subgroup than non LN subgroup (p<0.001). In LN subgroup, there was statistically significant positive correlation was found between serum level of IL-17 and SLEDAI (r= 0.643, P= 0.001) & renal disease activity (r= 0.798, P< 0.001). In renal biopsy; there was significant positive correlation between IL-17& activity index (r= 0.624, p=0.002) but no correlation with chronicity index (r= 0.214, P= 0.146). On the other side, in non LN subgroup; IL-17 was poorly correlated with SLEDAI (r=0.108& P= 0.649). Conclusion: Serum level of IL-17 was positively correlated with the severity of lupus nephritis and could be a promising strategy for treatment of lupus nephritis in near future

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoantibody mediated chronic autoimmune disease characterized by the deposition of immune complexes that contribute to severe organ damage. Lupus nephritis (LN), which occurs most often within five years of lupus onset, is one of the most serious manifestations and one of the strongest predictors of a poor outcome [1].

Cytokines are intimately involved in SLE pathogenesis. They contribute to the underlying immune dysfunction and to immune-mediated events that damage target organs[2]. Multiple cytokines have been implicated in the disease activity or organ involvement in SLE. Among these, Interleukin 17 (IL-17) which is thought to play an important role in the creation of the characteristic milieu in SLE and promotes B-cell survival and autoantibody production. IL-17 is a proinflammatory cytokine that is involved in defending the host against extracellular, some intracellular pathogens and fungi[3]. IL-17 promotes inflammation on several levels, as its receptors are expressed on both hematopoietic cells and non hematopoietic cells. In addition to its potent proinflammatory capacity, IL-17 exerts its effects through the recruitment of monocytes and neutrophils by increasing the local production of chemokines (IL-8, monocyte chemoattractant protein-1, growth-related oncogene protein-α)[4,5], the facilitation of T cell infiltration and activation by stimulating the expression of intercellular adhesion molecule-1[6]. IL-17 can also stimulate B-cell antibody production[7]. Recent studies have reported that production of IL-17 is abnormally high in patients with SLE. Its levels are increased in SLE sera and correlate with SLE disease activity. Moreover, the frequency of IL-17-producing T cells is increased in the peripheral blood of patients with SLE[8,9].

LN is a major complication of SLE that affects 50% of patients. It is mediated by glomerular deposition of immune complexes that trigger a number of inflammatory events leading to tissue damage[10]. The presentation can range from asymptomatic urinary abnormalities to rapidly progressive renal failure leading to end-stage renal disease. Renal failure remains an independent risk factor for death in patients with LN[11]. Some studies have highlighted the potential importance of the Th17 immune response in renal inflammatory disease. These include the identification and characterization of IL-17-producing T cells in nephritic kidneys of mice and humans, as well as evidence for the contribution of IL-17 and the IL-23/Th17 axis to renal tissue injury in LN[12]. On the other hands, there are studies have reported that serum IL-17 concentration correlate poorly with SLE disease activity and LN[13]. Thus, the role of IL-17 in the pathogenesis in SLE & LN is still debated. The aim of the present study was to further investigate the possible role of IL-17 in the pathogenesis of SLE and development of LN, and to explore its relationship with renal pathology according to WHO pathological classes of LN.

PATIENTS AND METHODS

Patients group:

This study was carried out on 46 SLE patients recruited from the Nephrology and Rheumatology departments in Riyadh national hospital in Riyadh, KSA over 15 months (from May, 2012 to July, 2013) and 15 apparently healthy subjects (12 females & 3 males, with mean age 31.22±

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7.36 years) were served as a control group. They were age and sex matched with the patient group. Our exclusion criteria were any patients had diabetes mellitus, malignancy, and chronic infections.

Diagnosis of SLE was established according to the American College of Rheumatology revised classification criteria for SLE[14]. SLE patients were subjected to thorough history taking, general and local examination. SLE patients were divided into two groups according to the presence of renal involvement: 21 patients (19 females &2 males) non LN subgroup (11 patients with arthritis, 4 patients with serositis, 3 patients with CNS manifestations and 3 patients with hematological manifestations) and 25 patients (22 females & 3 males) LN subgroup.

Patients with LN were defined by persistent proteinuria > 0.5 g/24 h, or the presence of cellular casts, persistent hematuria or renal biopsy results consistent with LN[15]. Renal ultrasound was done for all patients at the start of the study to exclude possible renal causes of kidney impairment.

Disease activity was assessed using the SLE Disease Activity Index (SLEDAI) [16]. Renal disease activity was measured by the Systemic Lupus International Collaborating Clinics (SLICC) Renal Activity Score. It was calculated as follows; proteinuria 0.5–1 g/day (3 points), proteinuria 1–3 g/day (5 points), proteinuria > 3 g/day (11 points), urine RBC's > 5/hpf (3 points), and urine WBC's > 5/hpf (1 point) [17]. Blood sample (5 ml) was extracted from each subject. Serum was isolated and stored frozen at - 80 C for later IL-17 assay. Laboratory investigations included: full blood picture, ESR, CRP, serum creatinine, simple urine analysis & estimation of 24 hours urinary protein, C3, C4, ALT, AST, C3, C4, ANA, and anti-dsDNA. Blood and urine samples were collected on the same day. A written consent from all participants in the study and an approval from the local ethics and scientific committees were obtained.

Renal biopsy:
It was obtained from all patients with LN during the study period for pathological classification of LN according to the criteria defined by the WHO[18]. The renal histopathological examination included light microscopic examination and immunofluorescence. The activity index (AI) and chronicity index (CI) of each biopsy specimen were also determined according to the previously accepted indices[19]. Evaluation of the biopsy specimens was performed by a single pathologist.

Measurement of serum IL-17:
Bender Med Systems GmbH, The human IL-17A platinum ELISA (BMS2017 / BMS2017TEN) was used for the measurement of serum IL-17(Campus Vienna Biocenter 1030 Vienna, Austria, www.eBioscience.com) [20]. This assay employs an anti-human IL-17A coating antibody which is adsorbed into micro wells. Human IL-17A present in the sample or standard binds to antibodies adsorbed to micro wells. A 100 µl biotin- Conjugated antibody is added and binds to human IL-17A captured by the first antibody. Following incubation unbound biotin-conjugated anti-human IL-17A antibody is removed during a wash step. A 100µl horseradish peroxidase (HRP)-conjugated streptavidin (Streptavidin-HRP) is added and binds to the biotin-conjugated anti-human IL-17A antibody. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of human IL-17A present in the sample or standard. The reaction is terminated by addition of acid (100 µl tetra-methylbenzidine substrate solution) and color develops in proportion to the amount of IL-17 bound. The Stop Solution (100 µl) changes the color from blue to yellow, and absorbance is measured at 450 nm. A standard curve is prepared from 7 human IL-17A standard dilutions and human IL-17A sample concentration determined. The Sensitivity of the test was 0.50 pg/ml and the range of positive results was 3.5 - 100 pg/ml.

Statistics:
The Data were analyzed by computer using the statistical package SPSS for windows version 16 (software). Quantitative variables were reported as mean +/- SD, and qualitative variables as number and/or percentages. Comparing means was performed by independent samples T test. The relationship between variables was assessed by bivariate Pearson correlation test to determine Correlations between IL-17 and other variables (SLEDAI, renal activity score, activity & chronicity indices of renal biopsy &others). For all tests P values <0.05 were considered statistically significant.

RESULTS:
Compared to controls, SLE patients presented with significantly higher level of ESR, serum creatinine, 24 h urinary protein, , ANA, and anti-dsDNA(P< 0.001) while C3& C4 levels were significantly lower in SLE group (P<0.001) and also IL-17 was higher in SLE group than control group (61.96 ±15.11 Vs 2.15± 0.6 pg/ml, P <0.001, respectively) . There were no significant differences between both groups as regards age & gender distributions (table 1).

In LN subgroup; there were significant higher serum levels of IL-17(70.59 ±14.73 Vs 43.48 ± 9.45 pg/ml), serum creatinine(183.14 ±43.92 Vs 93.09± 14.05 µmol/L) , 24 hours urinary protein(1234.13± 644.96 Vs 96.70± 23.83mg/day), and anti-dsDNA (178.59 ± 56.85 Vs 33.72± 6.52IU/ml) than among non LN subgroup(p<0.001) while the levels of C3& C4 were significantly lower in LN subgroup than among non LN subgroup(1.56±0.34 Vs 2.18± 0.49 & 0.74±0.69 Vs 2.77± 0.39 mg/dl, P=0.003& P <0.001)respectively (table 2).

Among SLE patients; a statistically significant positive correlation was found between serum level of IL-17 and SLEDAI (r= 0.618, P< 0.001, fig.1). In LN subgroup, a statistically significant positive correlation was found between serum level of IL- 17 and SLEDAI (r= 0.643, P= 0.001) (fig. 2) & renal activity score (r= 0.798, P< 0.001) (fig. 3). IL- 17 also showed significant positive correlation with 24 hours urinary protein (r= 0.891, P<0.001) and anti-dsDNA (r= 0.940, P <0.001) (fig. 4) while no correlations between IL-17 and C3, C4 and serum creatinine levels (r =-0.152, r=-0.210& r=0.182, P>0.05 respectively) (table 3). As regards renal biopsy; according to WHO classifications for LN, it was found that class IV had the highest levels of IL-17 compared to other classes with the lowest levels in class V with no statistically significant differences between all 5 classes (p = 0.083) and there was positive & significant correlation between IL-17& activity index (r= 0.624, p=0.002) and poor correlation with chronicity index (r= 0.214, P= 0.146) (table 3). On the other side, in non LN subgroup; IL-17 was poorly correlated with SLEDAI (r=0.108& P= 0.649, fig. 5)

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Table 1: The demographic & laboratory data of patients and control groups.

parameters	Patients group	Control group	P value
Gender (Female/Male)	40/6	12/3	NS
Age, years (mean± SD)	31.46±8.19	29.97±9.08	NS
Duration of the disease (yrs)	4.29 ± 1.78		
IL-17(pg/ml))	61.96 ±15.11	2.15± 0.6	<0.001
Anti-dsDNA titer (IU/ml	130.75 ± 50.38	13.72± 3.52	<0.001
Proteinuria (mg/day)	854.23 ± 734.72	82.45 ± 42.95	<0.001
Serum creatinine (umol/l) (mean± SD)	147.84 ± 32.63	93.09 ± 14.05	<0.001
ESR 1st hour (mm/h)	71.32± 14.95	14.73± 4.23	<0.001
C3 (mg/dl)	1.83 ±0.46	3.44 ± 1.01	<0.001
C4(mg/dl)	1.71 ± 1.1	3.21 ± 1.06	<0.001
SLEDAI score (mean± SD)	20.28±5.27		

Anti-dsDNA= anti-double stranded desoxy ribonucleic acid, ESR=erythrocyte sedimentation rate SLEDAI=systemic lupus erythematosus disease activity index, C3 = complement 3, C4=complement 4 & SD= standard deviation

Table 2: The demographic & laboratory data of patients with LN and non LN patients.

Parameters	Patients with LN	Patients with no LN	P value
Gender (Female/Male)	21/4	19/2	NS
age, years (mean ± SD)	30.89±8.06	31.70±7.08	NS
Duration of the disease (yrs)	3.91 ± 1.47	4.39 ± 1.54	NS
IL-17(pg/ml))	70.59 ±14.73	43.48 ± 9.45	<0.001
Anti-dsDNA titer (IU/ml	178.59 ± 56.85	53.72± 6.52	<0.001
Proteinuria(mg/day)	1234.13 ± 644.96	96.70 ± 23.83	<0.001
Serum creatinine (umol/l) (mean± SD)	183.14 ± 43.92	93.09 ± 14.05	<0.001
ESR 1st hour (mm/h)	75.65± 7.31	66.34± 11.17	NS
C3 (mg/dl)	1.51 ±0.34	2.18 ± 0.49	0.003
C4(mg/dl)	0.74 ± 0.54	2.77 ± 0.43	<0.001
SLEDAI score (mean± SD)	21.08±4.67	19.95±4.80	NS
Renal activity score (mean ±SD)	7.12 ±1.69		
WHO Class of LN (n %) II III IV V	 4 (16%) 6 (24%) 11 (44%) 4 (16%)		
Activity index (/24) (mean ±SD)	9.89±3.61		
Chronicity index (/12) (mean ±SD)	3.17 ±1.06		

Anti-dsDNA= anti- double stranded desoxy ribonucleic acid, ESR=erythrocyte sedimentation rate SLEDAI=systemic lupus erythematosus disease activity index, C3 = complement 3, C4=complement 4 & SD= standard deviation

Table 3: Correlation between serum level of IL-17 and laboratory tests for assessment of renal function and renal activity score in LN subgroup.

parameters	Interleukin-17	
	r	P
Serum creatinine	0.182	NS
24 hours Urinary Proteinuria	0.891	<0.001
Anti-dsDNA	0.653	0.001
Renal activity score	0.798	<0.001
C4	-0.210	NS
C3	-0.152	NS
Activity index (/24) (mean±SD)	0.624	0.002
Chronicity index (/12) (mean ±SD)	0.214	NS
SLEDAI	0.643	0.001

Pearson correlation co-efficiency test–P is significhant if <0.05.

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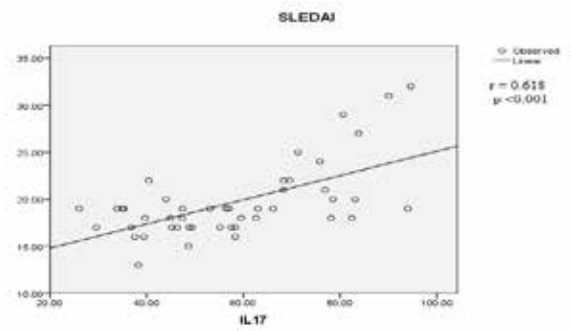

Fig. 1: Correlation between IL-17 and SLEDAI score in patients with SLE

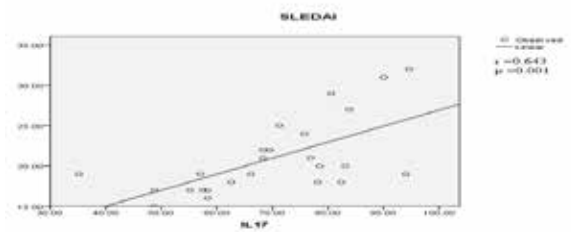

Fig. 2: Correlation between IL-17 and SLEDAI score in patients with LN

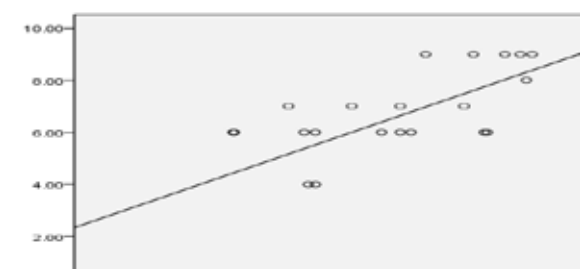

Fig. 3: Correlation between IL-17 and Renal Disease Activity in patients with LN

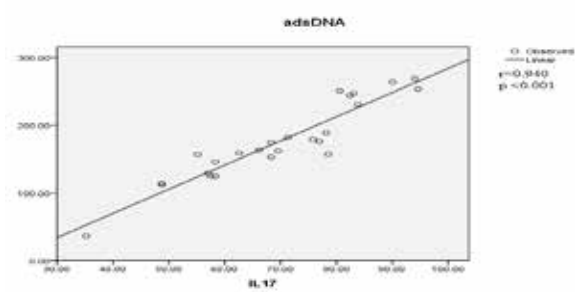

Fig. 4: Correlation between IL-17 and anti-dsDNA in patients with LN

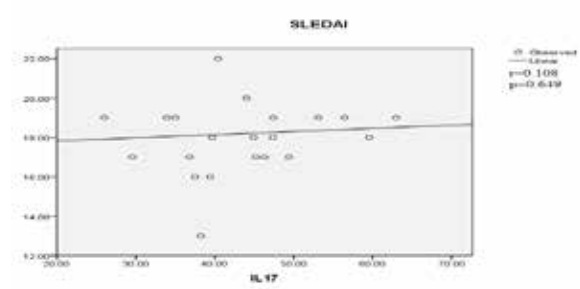

Fig. 5: Correlation between IL-17 and SLEDAI score in patients without LN

DISCUSSION:

The SLE is a complex autoimmune disease in which a T cell-driven autoimmune response against universally expressed autoantigens results in clinically and pathologically diverse manifestations[21]. Renal involvement in SLE is present in over 50% of patient with active SLE and remains a major cause of end-stage renal disease and it is associated with a greater than four-fold increase in mortality in recent series [22]. The picture of cytokines present in LN is already complex [23]. Recent evidence indicates that IL-17 plays a role in the pathogenesis of SLE [24]. IL-17 is a pleiotropic cytokine that participates in tissue inflammation by inducing expression of proinflammatory cytokines, chemokines and matrix metalloproteases [25]. Elevated numbers of IL-17-producing T cells were also infiltrated in the kidneys of patients with LN [26,27]. Moreover, the frequency of IL-17-producing T cells is increased in peripheral blood of SLE patients [26.28] . IL-17 production is increased in vitro stimulated lymphocytes from SLE patients when compared with normal lymphocytes [27]. IL-13, IFN-γ and IL-17 were the main cytokines produced by infiltrating T cells in nephritic kidneys of MRL/lpr mice [29]. Apart from the obvious proinflammatory activities attributed to IL-17, its effects in other cell types may contribute to SLE pathogenesis. Accordingly, increased production of total IgG, anti-dsDNA IgG and IL-6 by peripheral blood mononuclear cells of patients with lupus nephritis was observed when they were cultured with IL-17 [30].

The present study demonstrated that SLE patients had higher serum level of IL-17 than in healthy control subjects and among SLE patients with renal involvement compared to those without .These results were in line with recent studies which had reported that production of IL-17 is abnormally high in patients with SLE and higher than healthy controls [28,31]. In our study, we found that IL-17 correlate poorly with disease activity in patients without LN which is supported a recent study which had reported that serum IL-17 concentration correlated poorly with SLE disease activity [13] but other studies reported that plasma IL-17 levels showed a positive correlation with SLE disease activity [28]. Moreover, the frequency of IL-17-producing T cells is increased in the peripheral blood of patients with SLE [8,9]. IL-17 plays a role in the pathogenesis of SLE which is supported by many studies that indicate that IL-17 production is increased in patients with SLE as well as in animals with lupus-like diseases. This could be a consequence of systemic inflammation and augmented T cell activation [32], or could indicate that the pathways that guide T cell differentiation into IL-17- producing cells (either Th17 or double-negative (DN) T cells) are facilitated in SLE patients or could also be a consequence of skewed T cell differentiation and as well as could also be an amplification of the immune response by inducing the production of IL-6, pros-

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taglandin E2, granulocyte–macrophage colony stimulating factor and granulocyte colony-stimulating factor [33,34]. Additionally, IL-17 synergizes with other cytokines, in particular with IL-1β, tumour necrosis factor (TNF)-α, and interferon (IFN)-γ [35,36]. The CD4+ T cell effector subset termed 'Th17' has been considered a remarkable discovery which was named after its signature cytokine, IL-17 [38]. Th17 cells are considered as a distinct T helper cell subset because: (i) they arise from naïve T cells when primed in the presence of specific factors; (ii) their differentiation is controlled by exclusive transcription factors; (iii) they exhibit a particular cytokine production profile; and (iv) their differentiation into Th17 cells excludes the acquisition of other effector phenotypes (i.e. Th1 and Th2) [39].

In LN subgroup; we found a statistically significant higher serum level of IL-17 than in patients with non LN and was supported by the statistically significant positive correlation between IL-17 & laboratory markers (anti – dsDNA, proteinuria, RDA score) & histopathological findings of lupus nephritis and it is well acknowledged that anti-dsDNA antibody, which is closely correlated with the clinical syndrome and hence of diagnostic and even prognostic value, is an important pathogenic autoantibody involved in immune complex deposition that resulted in development of lupus nephritis [40,41].

A recent study explored the potential role of IL-17 in anti-dsDNA antibody production and reported that the serum IL-17 expression level was closely correlated with the serological level of anti-dsDNA antibody in activated lymphocyte derived DNA induced lupus mice. Of important, it revealed that treatment with exogenous IL-17 increased anti-dsDNA antibody production, while in vivo blockade of IL-17 decreased anti-dsDNA antibody production and also the study showed that up-regulation of IL-17 enhanced the immune complex deposition and complement activation in kidney, While blockade of IL-17 alleviated the immune complex deposition and complement activation in kidney. These findings strongly demonstrated that IL-17 was crucial for increasing anti-dsDNA antibody production in lupus [42]. Consistently, recent study showed that down regulation of IL-17 production by T cells was correlated with the amelioration of murine lupus after treatment with either low-dose peptide tolerance therapy or nasal anti-CD3 antibody [43,44]. However, it should be noted that a murine lupus model cannot fully reproduce the complexity of clinical SLE in human patients.

Further studies to reproduce our current findings in clinical SLE patients were still needed. On the other hand; our finding are matched with a study which reported that by using laser-manipulated micro dissection (LMD) andreal-time quantitative PCR analysis of renal biopsy samples from LN patients had showed a negative correlation between the level of IL-2 and renal damage while a positive correlation between IL-17 and renal damage was evidenced [45]. Moreover, recent studies have highlighted the potential importance of the Th17 immune response in renal inflammatory disease. These include the identification and characterization of IL-17-producing T cells in nephritic kidneys of mice and humans, as well as evidence for the contribution of IL-17 and the IL-23/Th17 axis to renal tissue injury in LN [12,46] and also acceleration of nephritis in SLE may indeed be associated with the IL17/Th17 pathway [47]. Similarly, the demonstration of IL-17+ T cells in kidneys affected by lupus nephritis indicates that it may play a role in the amplification and perpetuation of the inflammatory response in organs targeted by SLE [30].

In summary, IL-17 production is increased in patients with SLE. Elevated IL-17 levels probably contribute to the recruitment and activation of immune cells (e.g., neutrophils and T cells) to renal affection and amplify immune response. The exact mechanism through which IL-17 contributes to LN pathology will need to be identified in future work.

Conclusion:

Serum level of IL-17 was positively correlated with the severity of LN and may have a role in anti-dsDNA antibody production. IL-17 could be a promising strategy for treatment of lupus nephritis

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