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Serum Level Of IL-1β , IL-35 And IL-17A In Systemic Lupus Erythematosus

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Abstract

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease characterized by the presence of autoantibodies directed against nuclear antigens.Profound functional alterations of lymphocytes have been demonstrated including hyperactivity of T and B cells, abnormal T cell-B cell interaction,dysregulated cytokine, and autoantibody production .This study was carried out in laboratories of the College of Science at Wasit University in cooperation with Alyarmook Teaching Hospital and outpatients in Wasit province for the period extended from July 2020 to August 2021.Using ELISA technique,in SLE patients, serum levels of IL-1β and IL-17A were increased significantly compared with control subjects.Whereas, serum levels of IL-35 significantly decreased in SLE patients compared with control subjects.

Introduction

Systemic lupus erythematosus (SLE) is a worldwide chronic autoimmune disease which may affect every organ and tissue such as the skin, joints, kidneys, nervous system, heart and lungs by causing inflammation and organ damage .A complex interaction of impaired apoptotic clearance, upregulation of innate and adaptive immune system, complement activation, immune complexes, and tissue inflammation culminates in a self-sustained autoimmune process **(1)**.Cytokines are a diverse group of soluble proteins and peptides, produced and released by immune system cells , that act as humoral regulators and modulate the functional activities of individual cells and tissues, playing a pivotal role in the differentiation, maturation, and activation of various immune and no immune cells. Cytokine dysregulation is likely to play a role in the loss of immune tolerance that leads to SLE, and in the damage resulting from the disease **(2) .**The immunopathology of systemic lupus erythematosus (SLE) has traditionally been attributed to the deposition in tissues and organs of immune complexes or autoantibodies with specificity for or crossreactivity with locally expressed antigens. These mechanisms are likely to account for an important component of the inflammation that generates tissue damage in this disease, but accumulating data suggest that additional mechanisms should be considered.

Soluble mediators, particularly cytokines and chemokines, are induced by the endogenous and exogenous triggers of innate and adaptive immune system activation and ultimately result in autoimmunity in patients with lupus. These molecules shape the

character of the immune system dysfunction and organ systems involved in SLE. The cytokines are actively involved in both favoring the production of auto-antibodies as generating inflammation in affected tissues. (**3) .**In view of insufficient data about role of inflammatory and anti-inflammatory cytokines in the Iraqi SLE patients, this study was conducted to assess the role of IL-1 , IL-17A IL-35 in clinical disease activity in SLE patients.

Materials and methods

The study included 80 individuals , which were divided into two parts " patients groups " which includes (active and inactive groups) and the second part of the study were 30 of apparently healthy subjects serve as control group . The samples were obtained from both In-patients and out-patients who attended to Rheumatology Unit, Alyarmook Teaching Hospital and out-patients in wasit province after taking their patients consent.Patients groupsthey are included two groups according to SLEDAI .

Detection of HumanInterleukin 1 Beta IL-1β , IL-35 and IL-17A

Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50µl standard to standard well. **Note**: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µl sample to sample wells and then add 10µl anti-IL-1B antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

Statistical analysis

The results of the current study were done using a statistical software package (SPSS 24) and analyzed using an independent t-test. The data were presented as mean standard deviation of the mean (M±SD). The statistical significance, strength, and direction of linear correlation between two normally distributed quantitative variables were calculated by the Pearson Correlation Coefficient. The P-value for all tests was considered significant if it was< 0.05.

Results and Discussion

The results (table 1) showed that the concentration of serum levels of IL-1β had significantly ($p\leq0.05$) increased in all SLE patient groups (41.21±16.62) compared to the healthy control

group (25.11±4.70). Furthermore, elevated serum levels of IL-1β in the group of SLE patients with active disease were 43.65±17.63 in comparison with the inactive group of 33.47±9.99. SLE is a complex disease, and it's becoming evident that individual cytokine patterns play a role in disease stratification (**4).** The findings of current study were consistent with the study conducted (**5)** who reported that the mean level of IL-1β wassignificantly higher in SLE patients than in healthy controls (P< 0.001) as well as that the mean level of IL-1β for patients with active disease was significantly higher than those of inactive disease patients.As well as, similar finding were shown (**6)** who refer to increase serum level of IL-1β in SLE patients compared control group . On the other hand ,(**7)** reported that IL-1β elevated in SLE patients comparison with control subject . IL-1β is a pro-inflammatory cytokine that has a variety of roles in both innate and adaptive immunity. One of its main actions is to promote neutrophil recruitment, which increases inflammation (**8) .**

Table (1) serum level of IL-1β with SLE patients and control .

Cytokine	**IL-1β (pg/l)**	
Groups	**Control (n =30)**	**Total SLE (n=50)**
Mean ± SD	**25.11±4.70 B**	**41.21±16.62 A**
P value	**0.000**	
IL-1β (pg/l)		
Groups	**Active SLE (n=38)**	**Inactive SLE (n=12)**
Mean ± SD	**43.65±17.63 A**	**33.47±9.99 B**
P value	**0.01**	

Data = Mean ± stander deviation

The different capital letter refers to significant differences at level (P ≤ 0. 05) between groups. The similar capital letter refers to Non- significant differences at level (P ≤ 0. 05)between groups.

It seems from table (2) that serum concentration of IL-35 among SLE patients significantly ($p \leq 0.05$) decreased compared to healthy subject , with mean values of (16.53**±**8.83) and (29.54**±**11.62), respectively. However, the serum level of IL-35 was significantly lower in active disease patients than in inactive patients , the mean was (14.94**±**7.82) and (21.59**±**10.23)respectively. According to the data given in this study, IL-35 production was considerably lower in patients with active SLE compared to heathy control and patients with inactive SLE, suggesting that IL-35 levels may play an essential role in SLE regulation. In several autoimmune models, IL-35, a new anti-inflammatory cytokine, can inhibit inflammation. IL-35 has been confirmed to suppress T effector cells (Teff)cell activity and inhibit the differentiation of Th17 cells, and it is critical for regulating the activity of Treg cells. Imbalance between Th17 cells and Treg cells is an important cause of SLE (**9) .** As a result, may be suggest that decreased levels

of IL-35 change the balance of Th17 cells and Treg cells in SLE patients, facilitating the disease's progression.

Table (2) serum level of IL-35 with SLE patients and control .

Cytokine	IL-35(ng/ml)	
Groups	**Control (n =30)**	**Total SLE (n=50)**
Mean ± SD	**29.54±11.62 A**	**16.53±8.83 B**
P value	**0.000**	
IL-35(ng/ml)		
Groups	**Active SLE (n=38)**	**Inactive SLE (n=12)**
Mean ± SD	**14.94±7.82 B**	**21.59±10.23 A**
P value	**0.02**	

Data = Mean ± stander deviation
The different capital letter refers to significant differences at level (P ≤ 0. 05) between groups.
The similar capital letter refers to Non- significant differences at level (P ≤ 0. 05)between groups.

The results (table 3) revealed that all SLE patient groups had significantly ($p \leq 0.05$) higher serum levels of IL-17A (220.13±144.56) than the healthy control group (43.16±22.97). Additionally, IL-17A serum levels were (238.19±150.71) in active SLE patients compared to (162.94±109.53) in inactive SLE patients. Biologic management of the disease, as in autoimmune and auto inflammatory disorders, requires laboratory indicators that are associated to the disease's clinical activity (**10)** .

IL-17A appears to have an important role in the pathophysiology of SLE, according to growing evidence (**11)** .This has encouraged researchers to investigate IL-17A, the major cytokine in the IL17 family, as a potential SLE disease activity indicator and treatment target, using the SLEDAI indexe (**12)** .This study intended to further confirm the function of IL-17A in the serum as a biomarker of disease activity in SLE.

In the current study, the serum level of IL-17A was shown to be higher in SLE patients. These findings are consistent with previous research in which IL-17A levels were found to be higher in SLE patients. **(13)**.

Moreover ,**(14)** showed that serum concentration of IL-17A more significant in SLE patients than in control and serum IL-17A levels in SLE patients were associated with disease Activity Index (SLEDAI) score, These results have implications for both diagnosis and therapy .

In lupus, circulating IL-17A levels are elevated, and tissue staining reveals the presence of IL-17-producing cells in organ lesions. The IL-17A pathway increases autoantibody synthesis, immune complex deposition, complement activation, and tissue injury through a variety of mechanisms **(15).**

Table (3-17) serum level of IL-17Awith SLE patients and control .

Cytokine	**IL-17A(ng/L)**	
Groups	**Control (n =30)**	**Total SLE (n=50)**
Mean ± SD	**43.16±22.97 B**	**220.13±144.56 A**
P value	**0.000**	
IL-17A(ng/L)		
Groups	**Active SLE (n=38)**	**Inactive SLE (n=12)**
Mean ± SD	**238.19±150.71 A**	**162.94±109.53 B**
P value	**0.03**	

Data = Mean ± stander deviation
The different capital letter refers to significant differences at level (P ≤ 0. 05) between groups.
The similar capital letter refers to Non- significant differences at level (P ≤ 0. 05)between groups.

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