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Role of Interleukin-12 in Pathogenesis of Systemic Lupus Erythematosus

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Abstract: Systemic Lupus Erythematosus (SLE) is an autoimmune disorder noticed by the decreasing of immunological tolerance to the auto antigens such as the nuclear antigen and the production of autoantibodies and others immune complexes, leading to tissue inflammation and organ deterioration. Many genetic and environmental variables combine to create SLE, more than 90% of SLE affects females. This study aimed to evaluate the serum Interleukin-12 (IL-12) levels in SLE patients as well as their possible association with the Pathogenesis of disease. This study applied to a sample of Iraqi females, the sample was collected from Baghdad Teaching Hospital, including 100 patients and 50 healthy controls with an age range of (16-65) years old, and mean ages of (35.72 ±1.65 and 34.05 ±1.74) respectively. The current study used enzyme-linked immunosorbent assay (ELISA) and the automated Fujifilm to estimate the serum levels of (Anti-Nuclear Antibody, Anti-dsDNA, IL-12, vitamin D3, Urea, and creatinine) as well as a laboratory examination for ESR, hemoglobin, and white blood cells. ANA, Anti-dsDNA, urea, creatinine, and Erythrocyte Sedimentation Rate were significantly higher, at the same time the Vitamin D3, hemoglobin, and special component of blood cells (white blood cells) were remarked lower ($p< 0.01$) patients that are suffered from SLE as compared to control. Also, levels of IL-12 serum were increased unhealthy people compared to control, and a significant difference has been observed ($p< 0.01$) between patients and control. Additionally, significant relation observed between IL-12 serum levels with ANA and hemoglobin, and a positive non-significant correlation was noticed between IL12 and V.D3, while other laboratory parameters show a negative non-significant correlation with IL-12.

Keywords: Anti-dsDNA, Antinuclear autoantibody, Haemoglobin, Interleukin-12, SLE, WBC.

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Introduction

Systemic lupus erythematosus (SLE) can be defined as a chronic autoimmune disease having a wide range of clinical symptoms it is defined by autoantibodies and systemic inflammation that affects different organ systems, its typical connective tissue disease that can produce neurological and psychiatric syndromes (1,2). Its etiology is influenced by a variety of genetic, hormonal, immunologic, and environmental variables, and its varied biological basis and phenotypic manifestations make therapy development complicated (3,4)

IL-12 known as cytokine secreted before the inflammation, innate immune cells responsible for its secretion upon microbial stimulation it triggers Th cells differentiation into Th1 cells, it represents a “bridge” between the two main immune system (innate and adaptive). For more than a decade following its identification, the cytokine like interleukin-12 (IL-12) was assumed to have a key role in T cell-mediated inflammatory responses. IL-12 is made

up of a 70-kDa heterodimeric structure with p40 (IL-12B) and p35 (IL-12A) which are two covalently linked subunits (5). IL-12 plays a critical function in the defense against microbial pathogens. Dendritic cells (DCs) release IL-12 quickly after being triggered by toll like receptor (TLR) ligands such as lipopolysaccharides (LPS), oligodeoxynucleotides (CpG), and polyinosinic-polycytidylic acid. Once produced, IL-12 promotes Th cell development into Th1 cells, which produce IFN-γ and are responsible for antimicrobial responses (6). According to studies, the activation and role of cytotoxic T cells relies on IL-12 which is vital for these processes. IFN-γ induced by IL-12 causes the development of different autoimmune diseases. The concept that cytokine dysregulation has a role in kidney disorders, including LN, is supported by evidence from both human and experimental models (7, 8). SLE can be developed by IL-12 which is considering a key immune mediator. In comparison to healthy people, people that are suffering from SLE have a higher concentration of IL-12 (9). B cells stimulated by human T follicular helper (Tfh) cells to create immunoglobulin, including pathogenic autoantibodies, are also influenced by IL-12 (10). IL-12, such as the genetic deletion of p35, have been connected to the development of SLE. Despite the fact that multiple cytokines may play a role in SLE pathogenesis, The relative importance of IL-12 in SLE pathogenesis is unknown until recent days (11). This study was focused on the association between IL-12 serum levels with clinical and laboratory findings in a sample of Iraqi female SLE patients.

Methods

Subject

The study was began after Ethics Committee of the Biotechnology Department, College of Science, University of Baghdad approved and written agreement from all its members.then, One hundred and fifty participants were enrolled in this study divided into two groups, 100 as an SLE patient and 50 as a control. Participants were 16 to 65 years old and attended Baghdad Teaching Hospital (the Rheumatology Unit) from January to April 2022. Patients were diagnosed based on a pre-diagnosis done by a physician and a diagnosis of SLE disease based on the guidelines set by the European League against Rheumatism/American College of Rheumatology (EULAR/ACR) as diagnostic criteria for SLE (12). Controls were diagnosed by physicians at the Units to ensure that they were free from any other autoimmune disorders and immunosuppressive medications.

Inclusion and exclusion criteria

Available data was used to apply typical inclusion criteria for SLE, including the age of (16-65) years and a positive diagnosis of SLE EULAR/ACR (12). Patients with overlapped autoimmune disorders or other inflammatory diseases were not included in the study, as well as those with juvenile SLE (early-onset) or lupus nephritis (LN)and patients who are pregnant. On the other hand, controls 50 people who appeared healthy and had no family history of SLE previously or any other autoimmune disease came from the same area and were of the same age and gender. All participants in the study patients and healthy controls were unrelated, and their ethnic background was determined by self-description

based on ancestry questions.

Laboratory investigations

The Laboratory investigations, including total serum levels of IL-12, routine blood testing, complete blood count (CBC), ANA, anti-dsDNA, Erythrocyte sedimentation rate (ESR), Vitamin D3 (25(OH)D), blood urea and creatinine levels. The blood samples from each subject (patients and controls) that participated in this study were placed into sterile gel tubes and EDTA tubes. Under an aseptic technique, two milliliters of venous blood were withdrawn from each subject by vein-puncture by multi-sample syringe. The blood samples in gel tubes were centrifuged at 3000 rpm for 15 minutes, within an hour of their collection, for sera separation. Then, the sera were directly stored at -80˚C until the time for cytokine analysis. ELISA kits were employed to assess levels of total IL-12(BioSource Inc, British), Anti-ds-DNA and ANA (13). The blood urea and creatinine levels were measured using an automated Fujifilm. Hematology analyzer was used to estimate complete blood count after whole blood was placed in EDTA tubes.

Interleukin-12 measurement

Enzyme-linked immunosorbent assay (ELISA) technique was used to estimate cytokine IL-12 level for researcher purposes, according to the instructions of manufacturer MyBiosource Inc, British (Cat. No RDEEH0176).

Statistical analysis

Cytokines serum level was analyzed by the computer programmer SPSS (Statistical Package for Social Sciences) version 22. All data were showed in terms of means ± standard errors (S.E.), ANOVA, and Duncan's tests were used to study differences between means. When the probability (P) value was ≤ 0.05 significant difference was considered. The probability for non-parametric data was calculated using Pearson's chi-square test. The correlation between the analyzed factors was determined using Pearson's correlation.

Results

One hundred (100) SLE patients and fifty (50) participants in the control group, were matched for age and sex. All subjects were Iraqi females. The clinical presentations of SLE patients and controls are shown in Table 1. Patients with SLE ranged in age from 16 to 65 years old, with a mean and standard error value equal to (35.72 ±1.65years). The age range of the controls was identical to the age range of patients (34.05 ±1.74years). The participants were divided into three age groups; 16-29, 30-49 and > 50 years. The percentage of systemic lupus patients in the age group of 30-49 years (54.0%), which is higher than that in the age groups of 16-29 years age group was (36.0) % and > 50 years (10.0 %).

Table (1): Systemic lupus erythematosus patients and control distributed according to age groups.

Groups	Patients (No.=150)	Controls (No.=50)	p-value
Age (mean±SE.) year	35.72 ±1.65	34.05 ±1.74	0.491 N.S
Age Group (N,%): 16-29years	36 (36.00%)	21 (42.00%)	0.382
30-49 years	54 (54.00%)	24 (48.00%)	0.379
<50years	10 (10.00%)	5 (10.00%)	1.00

p-value: probability; No.; Number, S.E.; standard Error.

Significantly higher levels of IL-12 were found in the SLE group (p < 0.001) compared to normal control. The overall serum concentration of this

cytokine is depicted in Table-2:

Table (2): Median serum levels of IL-12 of systemic lupus patients among healthy controls.

Groups/(No.) of subject	Median IL-12 (pg/ml)	P Value
Patients/ (100)	309.92	0.0001
Control/ (50)	165.75	

p-value: probability; No.; Number of subjects.

Evaluation of the blood of SLE patients for severity markers. All the laboratory tests of the female, SLE patients and healthy control enrolled in this study were described in Table -3. ANA, Anti-dsDNA, urea, creatinine, and ESR were significantly higher, at the same time the Vitamin D3, hemoglobin, and white blood cells were significantly lower ($p< 0.01$) among unhealthy peoples as compared to control(healthy).

Table (3): Laboratory investigations in all studied groups.

Parameters Mean ± SE or NO%	Patients (N=100)	Controls (N=50)	P
ESR (mm/hr.)	27.12 ±2.16	15.40 ±0.89	0.0001
WBC (10^3/mL)	4.21 ±0.28	7.61 ±0.27	0.0001
HB (g/dl)	10.48 ±0.33	13.01 ±0.26	0.0001
V.D 3	24.78 ±1.30	37.53 ±0.94	0.0001
Urea (mg/dl)	51.58 ±3.65	29.12 ±1.23	0.0001
Creatinine (mg/dl)	1.906 ± 0.24	0.720 ±0.03	0.0001
ANA positivity	98%	0%	0.001
Anti-ds-DNA	32%	0%	0.001

Values are expressed as N: number in each parameter, Median. The abbreviations are; WBC: white blood cell, Hb: hemoglobin, S: serum, V.D3: vitamin D3, ANA: antinuclear antibody. Normal ranges: WBCs (10^9/L), 4.00-10.00; HB (g\dl), 150-450; urea (mg\dl), 15-45; Creatinine (mg\dl), 0.3-0.7, V.D3: (ng/ml), 30-50. *Correlation is significant at the 0.05 level (2-tailed)

Correlation of serum cytokine level IL-12 with laboratory parameters in all SLE patients

The correlation between serum cytokine IL-12 in SLE patients and Laboratory investigations is shown in Table 4. Correlations were high Significantly positive between IL-12 with ANA, and HB levels in SLE patients. A negative correlation was observed significantly for other laboratory parameters.

Table (4): Correlation analysis between serum cytokine IL-12 with laboratory investigations in all SLE patients.

Parameters	IL-12 in SLE patients (N=100)	
	r	P-value
Anti-ds-DNA	-0.15 NS	0.289
ANA	0.48**	0.0004
WBC	-0.03 NS	0.812
Hb	0.28*	0.043
ESR	-0.09 NS	0.522
B. Urea	-0.06 NS	0.662
Creatinine	-0.12 NS	0.409
Vit. D3	0.12 NS	0.391

***Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). R: Pearson's correlation.**

Discussion

SLE (systemic lupus erythematosus) is a multi-system inflammatory autoimmune disease marked by the deterioration of immunological tolerance, resulting in excessive inflammation and tissue damage. This study was conducted to observe the relationship between IL-12 and laboratory parameters in SLE patients among Iraqi females. The probability of female's infection are more than males, according to previous researches [23, 24, 25] as a result of hormonal and sex chromosomal differences (14). Furthermore, the severity was generally higher among Iraqi females. Previous studies suggested that female gender and age affect the onset of SLE (15). This study was applied to females of different ages in SLE cases. When it comes to age SLE is thought to be a disease that affects middle-aged individuals more than younger or older persons. The recorded age of this study ranged from 16 to 65 years with a mean age of (35.72years). As a result, the current study revealed that the thirtieth decade is regarded as a critical risk factor. This concurs with (16) who found that the most susceptible age for SLE is 20–39 years for females. One of the multifactorial causes of SLE pathogenesis is an imbalance in cytokine levels, which contributes to disease severity, so interleukins (cytokines) have a key role in SLE pathogenesis (17, 18).

Cytokine imbalance has been established in systemic lupus erythematosus patients (19). On the other hand, various cytokines may play various roles in SLE patients. But, the role of each singular cytokine and their relation with disease severity in SLE remains elusive (20). IL-12 is a pro-inflammatory cytokine, mainly formed by antigen-presenting cells (21).

SLE has been associated with IL-12 overexpression (22). In the current study there was a significant difference in the median of IL-12 serum levels between SLE patients (309.92) and healthy controls (165.75), (P= 0.0001). This is because IL-12 stimulates the JAK/STAT pathway, promoting the development of naive CD4+ T cells into IFN-producing Th1 cells as well as the differentiation of T follicular helper cells (Tfh). In SLE, both Th1 and Tfh cells are overexpressed (23). Current study is supported by (24) who showed that total IL-12 serum levels were found to be greater in SLE patients compared to controls.

SLE is indicated when the autoantibodies are present. For this reason, laboratory assessment of autoantibodies, including anti-nuclear antibody (ANA), and anti-dsDNA were used markedly for the detection of SLE (25).

Although anti-dsDNA antibodies are highly specific for SLE, the greater majority of patients might test negative for anti-dsDNA for these antibodies during the disease course (26). These are founded in 70% to 100% of SLE patients and have a very high specificity (around 97%) for the disease (27). Anti-dsDNA antibodies interact with renal antigens directly or indirectly, resulting in immunological complexes (28). In the current study, Anti-dsDNA compares the patient group to the control group and shows a significant difference. (p=0.0001), these findings corresponded with results revealed by (28) who reported that Anti-dsDNA antibodies are found in the serum of SLE patients, especially in 80% of SLE

patients. While it does not show any significant correlation with IL-12 serum levels, this result is supported by (29) who reported that no significant correlation was found between IL-12 and anti-dsDNA antibody (29). Also, a study by (30) has recorded substantially high levels of anti-dsDNA in SLE cases, as compared to control.

Antinuclear antibodies (ANA) is a more specific autoantibody testing that could be useful in the diagnosis of suspected SLE or ANA-associated disease (31). The current study finds that ANA positivity of 98% in SLE patients, as a result, shows a significant difference in the mean of ANA serum levels between SLE patients and healthy controls(P=0.0001), however, these results correspond with (32) study on Iraqi SLE patients who recorded that ANA positivity test was 98% in SLE patient. Also, the current study shows a significant correlation with IL-12 (P =0.0004) This is in agreement with evidence that suggests ANAs, either individually or in immune complexes, cause cytokine induction (33).

In this study, Haemoglobin (HB) shows a significant part in the pathogenesis of SLE. This is endorsed by (*S. A. Ahmed et al*)(34). who reported that anemia can be present in about 50% of lupus patients (34). Also, the current study result shows a significant correlation between HB with IL-12 (P=0.043). This result is in agreement with (35) who suggested that haemoglobin has well-documented inflammatory effects in a lupus milieu that drives inflammatory responses and the production of lupus-associated cytokines, as well as increases the production of potentially pathogenic lupus-associated autoantibodies (35).

Evidence suggested that 1,25-dihydroxy vitamin D3 (1,25(OH)2 D3) impedes IFN-secretion and inversely controls IL-12 synthesis by down-regulating NF-B (36). In the current study, V.D3 show no significant correlation with IL-12.

Other laboratory parameters in this study (Blood Urea, Serum Creatinine, WBC, ESR) show no significant correlation with IL-12. This is supported by (37) who reported that no significant correlation was found with IL-12 cytokine (37,38).

Conclusions

In conclusion, it's clear that there is a noticed relation between IL-12 and SLE disease. Its association with laboratory parameters would be a possible biomarker in observing and Predicting disease severity, which may be helpful for SLE patients for increasing diagnostic and treatment options.

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