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Research Article

The Role of IL-12 in the Aetiology of SLE and its Connection to HBV Infection in Iraqi Patients

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I N F O

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A B S T R A C T

Introduction: Systemic lupus erythematosus (SLE) is a significant social and public health issue because the medications used to treat it can only manage the symptoms and halt the progression of the disease, not totally cure it. The purpose of this study is to determine the association between the blood level of IL-12 and SLE, as well as its consequences, such as lupus nephritis (LN), and its active or dormant condition, in Iraqi patients.

Method: The study enrolled 90 SLE patients from AL-Sadar teaching hospital, with 66(73.3%) developing lupus nephritis (LN) and 24(26.7%) without LN. Additionally, there were 60 (90.9%) and 6 (9.1) active patients. Only 48 of 90 individuals tested positive for HBsAg, whereas 42(46.6%) tested positive for HBV. Each participant donated 5 ml of blood for anti-dsDNA and IL-12 testing.

Results: In SLE patients with LN, IL-12 serum levels were considerably higher than in those without LN($p = 0.05$). Different levels of IL-12 were found in the LN groups. Serum IL-12 concentrations were considerably lower in patients with HBV-associated SLE (3.41±0.85 pg/ml), non-HBV-associated SLE (2.05±0.66 pg/ml), and healthy controls (0.53±0.28 pg/ml). WBC count, ESR, anti-ds antibody, and ANA levels were all elevated in lupus patients and in the active groups.

Conclusion: Our research established that SLE patients have elevated IL-12 levels. IL-12 levels were significantly greater in SLE with LN as compared to SLE without LN, and they differed significantly between lupus nephritis classes II, IV, and V. Finally, significant differences in IL-12 levels were seen between HBV and non-HBV SLE patients.

Keywords: IL-12, HBV, SLE, Lupus Nephritis

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with protean manifestations, that predominantly affects young women and isassociated with high morbidity and mortality.[1] SLE is a chronic multi-systemic autoimmune disorder with diverse immune-pathological abnormalities and varying degrees of organ damage.[2] Disease activity is classified asmildform manifested as arthritis or mucocutaneous lesion, moderateform such as cutaneous vasculitis or pericarditis, and severe life-threatening disease.[3] SLE affects 20-50 of every 100,000 individuals and its morbidity and mortality are usually

associated with cardiovascular events or damage to major organs, particularly the kidneys, nervous system, and haematopoietic organs.[4]

Among SLEs, according to complications, the most frequent and dangerous is lupus nephritis (LN) which occurs in about 50-60% of all patients, a major source of morbidity and mortality in patients with systemic lupus erythematosus (SLE).[5] SLEis characterised by interleukin instability (SLE). Itacts as a mediator in pathogenesis pathways and local inflammation which leads to tissue injury and organ damage with disease severity.[6]

Antigen-presenting cells (APCs) primarily generate interleukin-12 in reaction to infections or foreign materials, making it a key immune-regulatory cytokine.[7]

Over 100 million people worldwide have been infected with hepatitis B virus (HBV), which causes liver damage and death. 3.6 percent of the world's population is infected with the hepatitis B surface antigen (HBsAg).[8] The risk of hepatitis B virus reactivation is high in lupus patients receiving immuno suppressive therapy. Clinical and immunological evidence suggests a causal association between HBV carriage and the development of nephropathy.

Methodology

The present case-control study was conducted for 90 SLE Iraqi patients who were attending AL-Sadar Teaching Hospital between February and July 2021. Patients classified as SLE with LN and SLE without LN were included in the study. 66 (73.3%) patients among them were classified as having lupus nephritis, Additionally, 60 (90.9%) patients were in an active state, whereas 6 (9.1) patients were in an inactive state.

Lupus nephritis was further classified into three groups (II, IV, and V), with the remainder being non-lupus nephritis. 24 (26.7%) patients were based on a physician's physical and clinical assessment. All patients were divided according to infectionwith HBV. 48 (53.3%) patients were HbsAg positive and were further divided depending on the viral load into low viral load patients (22,45.8%) and high viral load patients (26, 54.2%), while 42(46.7%) SLE patients showed a negative result for HBsAg and were regarded as SLE non-HBV infection. 30 apparently healthy subjects were free from clinical evidence or family history of any auto immune disease based on their clinical history and physical examinations.

5 ml of blood was collected from all participants. 2 ml was put into EDTA tubes for total WBC count evaluated by hemoanalyzer system(Abbott, United States) and ESR level, and 3 ml was put in gel tubes to separate the serum. It was then stored in Eppendorf tubes at -20°C until it was used for measuring IL-12 (Elabscience, USA), Screatinine, auto antibody by ELISA method for detecting antinuclear antibody and anti-dsDNA antibody (Paramedical/Itali). Finally, HbsAg was done by rapid test (Biopanda Reagents, The United Kingdom).

Exclusion Criteria

The following patients were excluded:

- Patients suffering from kidney disease or any other type of autoimmune disease
- SLE patients infected with any other type of virus
- Pregnant women
- Patients suffering from any chronic disease
- Patients who did not fill completeinformation

Ethical Approval

This study was ethically approved by the medical ethics committee in Al-Sadder Medical City, Najaf and the Faculty of Science, Kufa University (No 168871). Informed consent was obtained from all participants before their samples were collected.

Statistical Analysis

The results were presented as means and standard error (SE) and were analysed using one-way analysis of variance (ANOVA) test via Graphpad prism 5.04. $P< 0.05$ was considered significant.

Results

The male: female ratio for SLE disease patients in the current study was 1:9. The mean age of patients was 42 years with the age range between 7 and 55 years. The same frequency was observed for gender and age in the control group with no significant difference.

The patients were categorised as SLE without lupus nephritis (24, 26.7%), and patients with lupus nephritis (66, 73.3%), out of which, 22 (33.3%) patients were classified as Class II, 24(36.3%) patients were classified as Class IV, and 20 (30.4%) patients were classified asClass V as shown in Table 1.

Active SLE patients in this study were 60 (90.9%) and inactive patients were 6 (9.1%). WBCs, S creatinine, ESR, Antinuclear antibody, and anti-ds DNA antibody increased was significantly in active patients, as shown in Table 2.

The present study illustrated a highly significant elevation in serum level of IL-12 in SLE patients (2.56± 0.8pg/ ml) as compared to healthy persons (0.53±0.28 pg/ml) as shown in Figure 1.

This study revealed an increased serum level of IL-12 in SLE with LN patients (4.26±0.9) as compared to SLE without LN(2.5±0.6) with significant difference at $p \leq 0.05$ (Figure 2).

The results showed that the level of IL-12 varies according to classes of lupus nephritis (3.4±0.67, 4± 0.74, and 5.6 ± 0.43 in classes II, IV, and V respectively) as shown in Figure 3.

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Table 1.Basic Subgroups of Patients with SLE and their Features

Characteristics	SLE (n=90)		Control (n=30)
	LN (66)	Without LN(24)	
Age range (mean)	9-48 years (40)	7-55 years (44)	6-50 years (43)
Gender (male: female)	1:9	100% female	1:9
LN classes			
Class II, No. (%)	22 (33.3)		
Class IV, No. (%)	24 (36.3)		
Class V, No.(%)	20 (30.4)		
Activity index			
Active, No. (%)	60 (90.9)	10 (41.6)	
Inactive, No. (%)	6 (9.1)	14 (58.4)	
SLE DAI			
Mild, No. (%)	0 (0)	2 (20)	
Moderate, No. (%)	24 (40)	8 (80)	
Severe, No. (%)	36 (60)	0 (0)	

No: Number of patients; SLE: Systemic lupus erythematosus; LN: Lupus nephritis

Table 2.Haematological and Auto-antibody Parameters in Active SLE, Inactive SLE, and Healthy Control Group

Category	Active SLE(n=60) (Mean ± SE)	Inactive SLE(n=6) (Mean ± SE)	Control (n=30)
WBC (10^9/L)	6.39 ± 1.56	5.62 ± 1.87	4.22
S creatinine (µmol/L)	3.33 ± 14.66	2.87 ± 11.78	0.56
ESR (mm/hr)	70.33 ± 23.89	53.65 ± 22.65	20.95 ± 9.04
ANA	38.22 ± 14.75	19.4± 3.63	
Anti-dsDNA Ab (pg/ml)	135.65± 24.98	66.78 ± 17.83	

No: Number of patients, ANA: antinuclear antibody. Data are presented as mean ± SE.

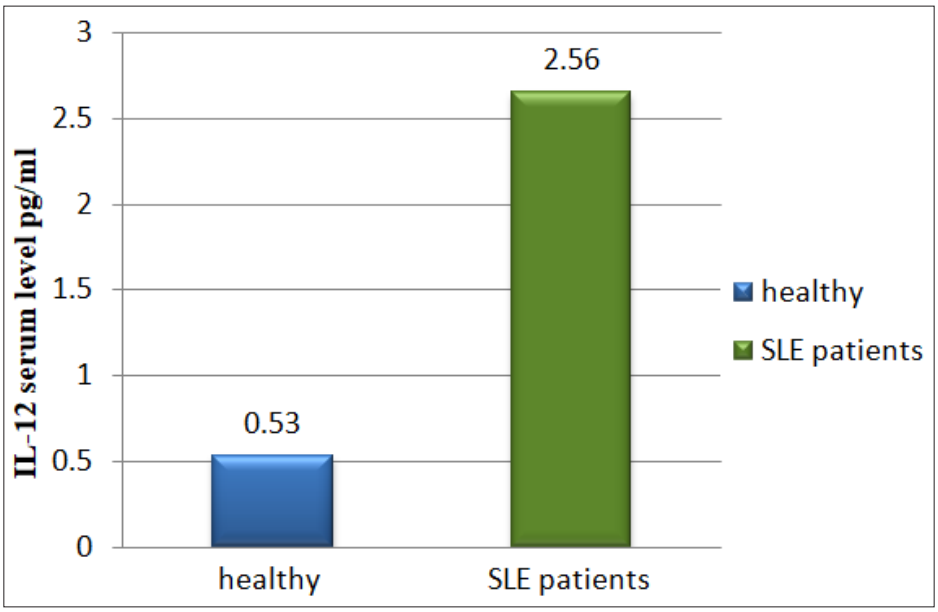

Figure 1.IL-12 Serum Level in SLE Patients and Healthy Subjects

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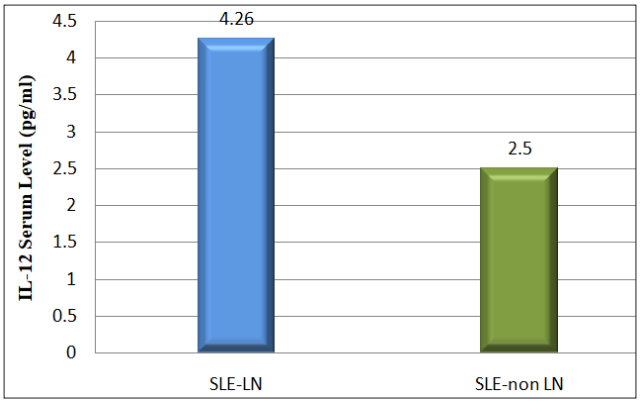

Figure 2.Mean Serum Level of IL-12 in SLE-LN and Without LN Patients

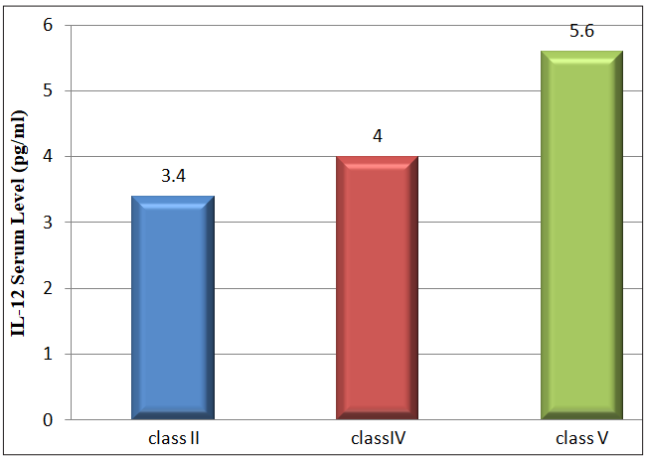

Figure 3.Levels of IL-12 according to Classes of Lupus Nephritis

Figure 4 shows a significant difference ($p < 0.05$) in the concentration of serum level of IL-12 among hepatitis B patients with SLE (3.41± 0.85 pg/ml), patients with SLE but non-HBV (2.49 ±0.66pg/ml), and healthy control (0.53±0.28pg/ml).

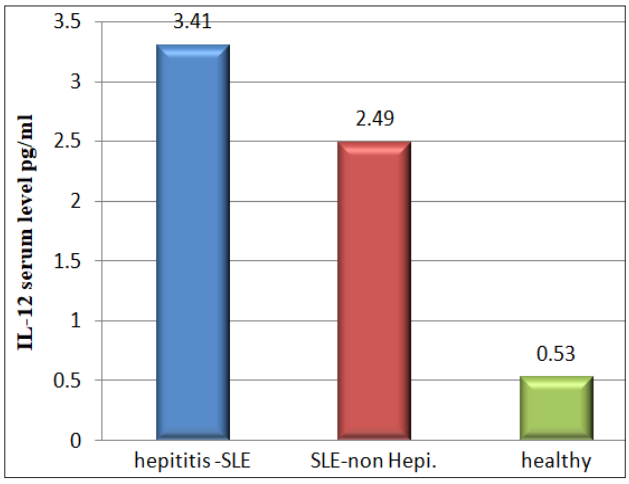

Figure 4.Serum Levels of IL-12 among Patients

Discussion

SLE is one of the most prevalent immunological systemic disorders impacting more women than males due to hormonal and chromosomal variations between men and women.[9] The results agree with that of Nusbaum et al.[10] who reported that 90% of patients were women with an average illness duration of 15 (4-52) years. This investigation demonstrated that SLE was more common in women than males. Stojan and Petri observed a higher prevalence and incidence rate of SLE in women compared with men.[11] Izmirly et al.[12] found in a study conducted in the United States that the prevalence estimate was 9 times higher among females than among males (128.7 versus 14.6 per 100,000).

Selvaraja et al.[13] discovered that 100% of SLE patients with LN were in a severe active condition, whereas 53.2% of non-LN patients were in a moderate active state, and 46.8% were in a severe active state. ESR, protein, and creatinine levels were higher in the SLE-LN group than in the SLE without LN group. Also, Ruchakorn et al.[2] observed that active SLE patients showed a significantly high increase in WBC count, ESR, anti-ds antibody, and ANA, especially in the lupus nephritis group in comparison with non-lupus and inactive groups. In the same line, Lourenço and La Cava explained that all serum biomarkers - WBC, ESR and Screatinine were increased in lupus patients as compared with healthy subjects. They were also highly increased in active as compared toinactive patients.

Patients suffering from active lupus nephritis showed increased serum creatinine levels and high ESR in comparison to inactive lupus patients.[14] Mohammed et al.[15] revealed significant statistical differences regarding anti-dsDNA in active lupus patients. ESR and serum creatinine were also higher in patients with active state than inactive state.

Selvarajaet al.[13] verified that autoantibodies such as anti-nuclear antibody (ANA), anti-dsDNA, and anti-nucleosome are SLE markers that are widely used to assess disease activity and severity.

Screening mediators implicated in lupus development might be useful in determining clinical SLE activity.[16] Several mediators are altered in SLE patients depending on the seriousness of the disease or the kind of clinical manifestations. According to Tokanoet al.[17], the blood levels of IL-12 in SLE patients were greater than in normal participants. Different cytokine profiles are associated with disease activity and specific clinical symptoms in a subset of SLE patients.[18]

The results indicated a substantial correlation between serum IL-12 and HBV DNA levels and non-detected viral load (ND), low viral load (119.53 ± 61.60), and high viral load

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(247.74 ± 50.37). This result closely agrees with that of Zare et al.[8] who found that IL-12 may have an essential role inviral clearance in HBV infection. Therefore, the host immunity through the interaction of different cytokine expression profiles with HBV may affect the disease progression of hepatitis.

On the other hand, our findings are consistent with those of Larosa et al.,[19] who suggested that the IL-23/ Th17 axis has a role in SLE and that the interaction between this axis and IL-12 is substantial in SLE patients. Also, Liu et al.[20] confirmed that SLE is highly associated with IL-12/IL-12R pathway and found elevated levels of IL12A, IL12B, and IL12RB2. Dysregulation of IL-12 expression was reported in SLE patients as compared to healthy people. This demonstrated that IL-12 is considered a biomarker for the progress ofthe auto immune disease.[21]

The disparities in IL-12 concentrations between SLE-HBV and SLE without HBV patients are due to the fact that cytokine balance has been proven to be a key immunological feature in the initiation and progression of hepatitis B, and also in the progression of the disease.[22] HBVr was shown to be frequent in SLE patients who tested positive for HBsAg and was linked to poor survival according to Chen et al.[23] IL-12 has effective roles in the responses against HBV infection.[24]

Conclusion

Our research established that SLE patients have elevated IL-12 levels. IL-12 levels were significantly greater in SLE with LN compared to SLE without LN, and they differed significantly between lupus nephritis classes II, IV, and V. Finally, significant differences in IL-12 levels were seen between HBV and non-HBV SLE patients.

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Conflict of Interest: None

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