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Association of Interleukin-6 and Interleukin-10 Levels with Severity of Systemic Lupus Erythematosus Patients Attending in a Tertiary Care Hospital in Dhaka, Bangladesh

Nasrin Nahar[1], Raisa Enayet Badhan[*,2], Sharmeen Ahmed[3], Rehana Razzak Khan[3], Shanjida Khondakar Setu[3]

Abstract

Background: Multiple cellular and cytokine abnormalities have been identified to be associated with systemic lupus erythematosus (SLE) during the past decades. Aim of the study was to demonstrate the serum level of Interleukin-6 (IL-6) and Interleukin-10 (IL-10) in Bangladeshi SLE patients and to find out the association of these cytokine levels with disease severity.

Materials and Methods: According to 2019 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) criteria, total 75 patients with SLE and 75 healthy controls fulfilling the inclusion criteria were enrolled in this study. Serum cytokine levels were measured by Chemiluminescence and Enzyme linked immunosorbent assay (ELISA) method. Disease severity was measured by Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) score. All statistical analysis was performed using the statistical pakage for social science (SPSS) program, 26 version. $P<0.05$ was considered to be statistically significant.

Results: Among 75 SLE patients, 94.66% were female and 5.34% were male. According to SELENA-SLEDAI score, 59 patients were in mild to moderate case group and 19 patients were in severe case group. Serum level of IL-6 and IL-10 were significantly higher in SLE patients compared to controls. Mean IL-6 and IL-10 levels in severe group were significantly higher than that of mild to moderate group.

Conclusion: Serum level of IL-6 and IL-10 in patient group were positively correlated with disease severity. From the study, it can be concluded that serum level of IL-6 and IL-10 may be useful for clinical evaluation of severity of SLE in Bangladeshi population.

Keywords: IL-6, IL-10, SLE, SELENA-SLEDAI

Introduction

Multiple organs are affected by SLE, an autoimmune illness [1]. This autoimmune illness is typified by the development of autoantibodies against nuclear antigens, the deposition of immune complex in many organs, and a loss of tolerance to self-antigens, which activates autoreactive B and T cells [2]. In lupus patients, these events have been shown to permanently damage organs like the brain, kidneys, and skin [3]. SLE typically affects women who are of reproductive age and affects females ten times more frequently than males [4]. There are notable differences in the incidence and prevalence of

Affiliation:

[1]Department of Microbiology, Sher-E-Bangla Medical College, Barisal, Bangladesh

[2]Department of Microbiology, National Institute of Burn and Plastic Surgery, Dhaka, Bangladesh.

[3]Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

***Corresponding author:**
Raisa Enayet Badhan, National Institute of burn and plastic surgery, Dhaka, Bangladesh.

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SLE among various racial and ethnic groups. In the USA, the prevalence of lupus is thought to be as high as 51 cases per 100,000 individuals. Data from 24 Asian nations show that the prevalence of SLE ranges between 30 and 50 per 100,000 people [5]. It is worth noting that, while SLE occurs worldwide, it is more prevalent in some nations, and within a country, certain ethnic groups appear to be more prone to developing this condition than others. Different studies indicated the prevalence of SLE to be 30-70/100000 in China, 3.2/100000 in India [5]. The prevalence of SLE in Bangladesh is not known [6].

SLE develops in genetically susceptible people who have encountered particular environmental or stochastic triggers. The complicated combination of genetics, environment, and hormones disrupts immune function, leading in autoantibodies, inflammation, and organ damage [3]. Autoantibodies against a wide range of nuclear and cell surface autoantigens produce immune-mediated tissue damage, which manifests clinically as arthritis, vasculitis, nephritis, mouth ulcers, skin rash, and photosensitivity [7]. Because of the disease's heterogeneous presentation and unpredictable course, there is a strong interest in identifying biomarkers that can predict SLE susceptibility, the risk of future organ involvement, and how they alter with disease activity [8]. Moreover, many cellular and cytokine abnormalities have been found in SLE throughout the past three decades. Cytokines are soluble substances produced primarily by immune cells, and they play important roles in the differentiation, maturation, and activation of numerous immune cells. Depending on the local microenvironment, these cytokines might have pro- or anti-inflammatory effects. The aberrations of various cytokines may suggest an imbalance among distinct immune cell subsets, such as Th1/T h2 and Th17/ Treg, contributing significantly to SLE pathogenesis [9]. Several cytokines, including B-lymphocyte stimulator, Interferon-α, TNF-α, IL-6, Interleukin-2, Interleukin-17, and IL-10, have been linked to SLE [10]. However, most studies concur that SLE patients exhibit higher levels of IL-10 and IL-6, which are recognized to play an essential role in SLE pathogenesis [11].

IL-6 is a versatile pro-inflammatory cytokine that operates on a variety of cell types and is a major inducer of acute phase proteins [12]. IL-6 plays an important role in the pathogenesis of human SLE by hyperactivating B cells, resulting in increased autoantibody synthesis, which is a major immunological abnormality in SLE [13]. Some studies have linked elevated blood IL-6 levels to illness flares in patients with active SLE [14,15]. IL-10 is an essential pleiotropic cytokine with both anti-inflammatory and stimulatory properties [16]. Furthermore, IL-10 overproduction influences autoantibody biosynthesis, which happens in SLE patients [17]. The clinical improvements in SLE patients following the administration of an anti-IL-10 monoclonal antibody suggest the potential role of IL-10 in SLE etiology [18,19]. To the best of our knowledge, no such study has been undertaken on the Bangladeshi SLE population. The purpose of this study was to correlate IL-6 and IL-10 levels with clinical characteristics and severity of SLE in the Bangladeshi population.Thus this study's findings will be useful in understanding the role of IL-6 and IL-10 in SLE patients, allowing for a better knowledge about their role in disease pathophysiology, host immune response and their association with disease severity.

Methodology

This case control study was conducted from March 2021 to February 2022 in the department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka.

Study population

A total of 75 SLE patients attending both outdoor/ SLE clinic and the department of Rheumatology, BSMMU, Shahbag, Dhaka were selected as cases. Healthy control groups were selected from the laboratory and nursing stuff, post graduate students and general people of same geographical area without any diagnosed connective tissue diseases.

Inclusion criteria

Systemic Lupus Erythematosus patients diagnosed by expert Rheumatologists of the department of Rheumatology, BSMMU fulfilling "2019 EULAR/ACR classification criteria for SLE" were enrolled in the study as cases. On the other hand, person without SLE and who were anti neuclear antibody (ANA) test negative were included as controls.

Exclusion criteria

Patients having overlap syndrome in association with SLE, patients with pregnancy, with acute infection, severely ill patients and person unwilling to participate are excluded from case group. On the other hand, person having family history of SLE or other rheumatic diseases, pregnant woman, acute infection and person unwilling to participate were excluded as controls.

Laboratory procedure

Sample collection

With disposable sterile syringe 3 ml of venous blood from all patients and healthy controls were drawn and taken in tube without anticoagulant for collection of serum. Sample were brought immediately to the Department of Microbiology and Immunology, BSMMU for the further procedure. Blood were centrifuged at 4000 rpm for 5 minutes for collection of serum. Separated serum were stored at -80°C till analysis of serum cytokine levels.

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Detection of serum level of IL-6: Stored serum from patients and healthy controls were used for determination of IL-6 level by Chemiluminescence Immunoassay (Bechman Coulter) according to manufacturer's instruction (ADVIA centaur IL-6 assay kit,Siemens).

Detection of serum level of Il-10: Stored serum from patients with SLE and heatlthy controls were used for determining the level of IL-10 using ELISA kit according to manufacturer's instruction (Elabscience, USA; catalog no: E-EL-H6154). According to principle, the test was performed by sandwich ELISA method.

Statistical analysis: Data was collected in a pre-designed data collection sheet. After collection, data was checked for inadequacy, irrelevancy and inconsistency. Irrelevant and inconsistent data was discarded. Collected data was checked, edited and analysis was performed with SPSS software package version-26 (Strata Corporation, College station, Texas). Continuous parameters were expressed as mean±SD and categorical parameters as frequency and percentage. P value calculated by Mann-whitney U test to compare the serum cytokine levels in SLE patients and control. Comparison of serum level of cytokines of SLE patients groups (mild to moderate group and severe group) was calculated by Mann-whitney U test. Association of SLEDAI-score with serum level of cytokines was calculated by Pearson correlation test. To see the difference between two groups (cytokines and genotypes) Man-Whitney U test was done. For all test a P value <0.05 was considered as statistically significant.

Ethical consideration

The study was ethically approved by the Institutional Review Board BSMMU (No. BSMMU/ 2021/ 3557) on 21/ 08/ 2021.

Results

Clinicodemographic data of patient with SLE are shown in table 1. The mean age of patient with SLE was (29.43± 8.08) years. Out of 75 patients, 71 (94.66%) were female and 4 (5.34%) were male with a female to male ratio of 17.75:1. Among the patients with SLE, 56 (74.7%) patients were in mild to moderate group and 19 patients (25.3%) were in severe group according to SELENA-SLEDAI score. Most of the patients (68.0%) had arthritis followed by rash (52.0%), lupus nephritis (33.3%), oral ulcer (30.7%) and hematological disorders (2.7%).

The comparison of IL-6 and IL-10 levels between cases and controls are shown in table 2. The mean serum levels of IL-6 and IL-10 were found to be significantly higher in SLE patients compared to controls (13.83 pg/ml vs 2.81 pg/ml, p=.001 and 12.35 pg/ml vs 2.37 pg/ml, p=.001 respectively).

Association of disease severity with serum IL-6 and serum IL-10 are shown in Table 3. Mean IL-6 level in severe case group was comparatively higher (30.27 pg/ml) than mild to moderate case group (8.25 pg/ ml) (P=<0.001). Mean IL-10 level in severe case group was higher (18.45 pg/ml) than mild to moderate case group (10.28 pg/ml) (p=0.001).

Figure 1 and 2 show that both the serum level of IL-6 and IL-10 in patient group were positively correlated with disease severity (r=0.472, p<0.001; r=0.494, p=<0.001).

Table 1: Clinicodemographic data of patients with SLE

Variables	No of patients	Percentage (%)	Mean±SD
Age (years)			29.43±8.1
Gender			
Female	71	94.7	
Male	4	5.3	
Female: Male ratio	17.75:1		
SELENA-SLEDAI score			
Mild to moderate (<12)	56	74.7	
Severe (>12)	19	25.3	
Clinical manifestations			
Rash	39	52	
Oral Ulcer	23	30.7	
Arthritis	51	68	
Lupus nephritis	25	33.3	
Hematological disorders	2	2.7	

Table 2: Serum levels of IL-6 and IL-10 in SLE patients and controls

Cytokine levels	Case (n=75)	Control (n=75)	p-value
IL-6 (pg/ml)			
Mean±SD	13.83±20.03	2.81±1.41	**<0.001***
Median	6.85	2.43	
Range (min-max)	1.11 - 101.36	1.08 - 8.18	
IL-10 (pg/ml)			
Mean±SD	12.35±8.21	2.37±1.29	**<0.001***
Median	11.77	1.9	
Range (min-max)	1.44 -39.20	1.15 -8.10	

p-value measured by Mann-Whitney U test, *significant

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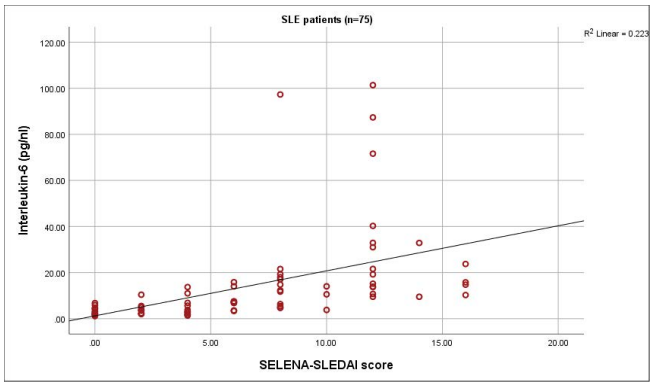
Table 3: Association of disease severity with IL-6 and IL-10 levels

Cytokine level	Disease severity		P-value
	Mild to moderate (n=56)	Severe (n=19)	
IL-6 (pg/ml)			
Mean±SD	8.25±13.18	30.27±27.13	**<0.001***
Median	4.73	19.21	
IL-10 (pg/ml)			
Mean±SD	10.28±6.18	18.45±10.38	**0.001***
Median	11.35	13.5	

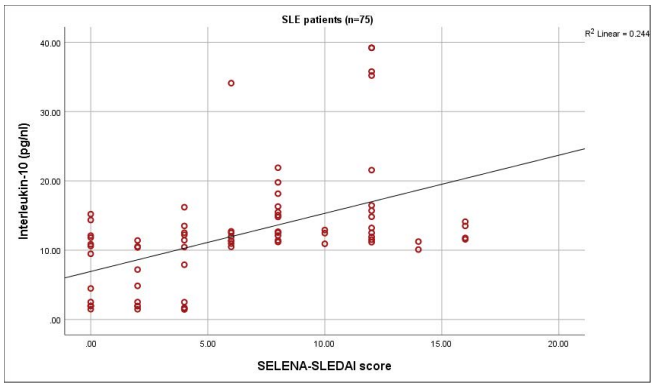
p-value reached from Mann-Whitney U test, *significant

Figure 1: Correlation between serum IL-6 levels and disease severity according to SELENA-SLEDAI score.



Pearson's Correlation test, *significant

Figure 2: Correlation between serum IL-10 levels and disease severity according to SELENA-SLEDAI score.



Pearson's Correlation test, *significant

Discussion

SLE is a chronic systemic disease with varying clinical manifestations. The precise pathogenic mechanisms of SLE remain unknown, however the etiology is believed to be complex, involving genes, sex hormones, and environmental variables such as sunshine, medicines, and infections. Cytokines, which play important roles in immune response control, have been linked to the pathophysiology of SLE [20]. This study's high female prevalence is consistent with a study conducted in Iraq [21], in which females made up 98% of the total number of patients. A larger American study [22] found that 88% of 1103 cases were female. This research suggests that female sex is one of the disease's risk factors, and hormones (oestrogen) lead to an increase in the prevalence of SLE among women. CD40, a gene on chromosome X, has been linked to the etiology of SLE [23].

The study found higher levels of serum IL-6 in SLE patients, indicating its inflammatory function in autoimmune SLE disease. IL-6 plays a key role in SLE pathogenesis by accelerating autoantibody synthesis by low density B cells and promoting the development of naïve T cells into Th17, causing neutrophils to migrate to the site of inflammation and release IL-17. Tissue damage is caused by proteases, tissue damage agents, and reactive oxygen species produced by neutrophils [24]. Katkam et al. (2017) similarly found a substantial increase in serum IL-6 in SLE patients compared to healthy controls [25]. In this investigation, there was an increase in IL-10 concentration in all SLE patients, which was determined to be statistically significant when compared to the control group. The severe case group had a higher concentration of IL-10 than the mild to moderate case group. A similar result was obtained in a study [26,27,28] where there was an increase in IL-10 concentration in SLE patients compared to control; furthermore, there was a higher mean concentration of IL-10 in the severe case group compared to mild to moderate case group. IL-10 is a multifunctional cytokine that is critical in the development of SLE. In addition to its immunoregulatory action, it promotes B-cell proliferation, differentiation, survival, and immunoglobulin class switching while decreasing auto-reactive B-cell death, which results in autoantibody synthesis in SLE [29]. In this study, IL-6 and IL-10 serum concentrations were found to be favorably linked with the SLEDAI score in SLE patients. In other research, IL-6 and IL-10 concentrations were found to be positively correlated with SLEDAI in SLE patients [15,30]. The study's goal was to show that serum cytokine levels correlated with illness severity. This study's findings show that greater levels of IL-6 and IL-10 are relevant for clinical evaluation of the severity of SLE in Bangladeshi population. There are some limitations to this study. First, the sample size was small compared to some other studies, due to financial and time issues. Second, samples were collecteded and cytokine levels were measured in some patients during treatment course, that might have impacted the study results.

Conclusion

Serum levels of IL-6 and IL-10 are significantly elevated in SLE patients and their serum levels in SLE patients are positively correlated with disease activity assessed by

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SELENA-SLEDAI score. IL-6 and IL-10 may be useful biomarkers for clinical evaluation of severity of SLE in Bangladeshi population.

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Authors' contributions

Conception: NN and SA.

Methodology: NN, SA, SKS, RRK and REB.

Sample collection and laboratory analysis: NN.

Statistical analysis: NN and SA.

Writing—first draft: NN.

Writing—revision and editing: NN and REB.

Supervision: SA, SKS and RRK.

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Declarations Competing interests

The authors declare that they have no competing interests.

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