THE CORRELATION BETWEEN INTERFERON-α AND INTERLEUKIN-6 WITH SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY

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ABSTRACT

Background

Systemic lupus erythematosus is a chronic autoimmune disease affecting many organ systems with diverse clinical manifestations in association with autoantibodies to components of the cell nucleus. Cytokines, like interferon-α and interleukin-6, are important components of immune response regulation and their imbalance play an important role in the pathogenesis of the disease.

Objectives

To compare the serum levels of interferon-α and interleukin-6 in systemic lupus erythematosus patients and the apparently healthy subjects, and to find out the correlation between the serum levels of the two cytokines and the disease activity according to systemic lupus erythematosus disease activity index.

Methods

A cross-sectional analytic study conducted on 37 systemic lupus erythematosus patients. The patients were investigated for the serum level of the two cytokines, and the results were compared with those of 31 apparently healthy subjects. Then, the disease activity was measured in the patients according to systemic lupus erythematosus disease activity index and arranged into groups of different disease activity, and their corresponding cytokine levels were compared.

Results

The serum levels of interferon-α and interleukin-6, in systemic lupus erythematosus patients were significantly higher than those of the healthy subjects (37.26 ± 27.58 IU/ml), (18.09 ± 21.02 pg/ml) respectively in the patients, and (13.29 ± 23.63 IU/ml), (7.10 ± 19.80 pg/ml) respectively in the healthy subjects, with *p*-values of (< 0.001) and (0.031) respectively. Eight (21.6%) of systemic lupus erythematosus patients had inactive disease, 9 (24.3%) had mild to moderately active disease, and 20 (54.1%) had highly active disease. There were a significant positive correlation between the serum levels of interleukin-6 and systemic lupus erythematosus disease activity, with *p*-value of (0.016), while there were no significant correlation between the serum levels of interferon-α and disease activity, with *p*-value of (0.734).

Conclusions

The serum levels of both cytokines in systemic lupus erythematosus patients are significantly higher than their levels in the serum of healthy subjects, and interleukin-6 is significantly correlated with the systemic lupus erythematosus disease activity.

Keywords: *Systemic lupus erythematosus (SLE), Interferon-α (IFN-α), Interleukin-6 (IL-6), Systemic lupus erythematosus disease activity index (SLEDAI).*

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INTRODUCTION

SLE is a systemic autoimmune disease with diverse clinical manifestations in association with autoantibodies to components of the cell nucleus [1]. SLE primarily is a disease of young women, with a peak incidence between the ages of 15 and 40 and a female: male ratio of 6 -10: 1. The age at onset, can range from infancy to advanced age [1]. The clinical manifestations of SLE are diverse, ranging from fatigue and oral ulcerations to life-threatening renal and neurologic disease. Disease activity fluctuates with periods of remissions and flares [2]. SLE affects the joints, skin and blood in over 80% of patients and the kidneys, central nervous system and cardiopulmonary system in 30%- 50% of patients [3].

SLE is an autoimmune disease of unknown origin affecting virtually all organ systems. Beyond genetic and environmental factors, cytokine imbalances contribute to immune dysfunction, trigger inflammation, and induce organ damage [4]. Unrestricted hyper-activation of innate and adaptive responses of the immune system may lead to the overproduction of autoantibodies, immune complex deposition, inflammatory cytokine release, enhanced apoptosis and eventually disease onset [5].

Cytokines are important components of immune response and its regulation, and play an active role in activating, differentiating, and maturing immune cells [6]. An imbalance between pro- and anti-inflammatory cytokines is a well-known characteristic of SLE [7]. They are heavily integrated in T-Cell and B-Cell signaling systems and abnormal cytokine levels, particularly interleukins and interferons are important hallmarks of SLE [8].

Interferon-α (IFN-α)

Interferons derive their names from their ability to "interfere" with the host cells of viruses during the infection process and have been heavily associated with the pathogenesis of SLE [9]. The interferon cytokine family is composed of type I IFNs including (IFN-α, IFN-β), type II IFN (IFN-γ), and the recently described type III IFNs (IFN-*λ*). Extensive data have suggested an important pathogenic role for IFN-*α* and IFN-*γ* in SLE [7, 10] .

The major producers of IFN-α are plasmacytoid dendritic cells, although virtually any cell type can elaborate interferon. IFN-α is produced both in response to exogenous stimuli, such as bacterial and viral pathogens, and to endogenous stimuli, such as self-nucleic acids and nucleic acid-containing immune complexes [11].

By binding to its receptor, IFN-*α* create broad effects of activating dendritic cells; promoting proliferation, survival, and differentiation of monocytes into antigen-presenting cells and B cells into plasma cells; stimulating the T-helper 1 pathway and preventing apoptosis of activated cytotoxic T cells; suppressing regulatory T cells, enhancing natural killer cell activity, and modulating each of these cells' respective cytokine production and signaling responses [12].

A number of patients treated with IFN-α, (like hepatitis-B or C infected patients), have developed lupus or lupus-like syndrome and, many specific manifestations of idiopathic lupus such as malar or discoid rash, oral ulcers, photosensitivity, renal involvement, anti-Sm and anti-dsDNA antibodies were represented. Discontinuation of IFN-α; typically resulted in remission of SLE symptoms, supporting a causal relationship with IFN-*α* [13]. Type I interferon levels have correlated with disease activity in a number of cross-sectional studies [14], and the use of elevated levels of type I interferon and interferon gene expression as biomarkers for disease activity was suggested in early studies [15].

Plasmacytoid dendritic cells (the main producers of IFN-α) also accumulate in the glomeruli of SLE nephritis patients and can also be found in cutaneous lesions where they promote continuous IFN-α release [16]. Based on those observations, the efficacy of two humanized anti-IFN-α monoclonal antibodies, (rontalizumab, sifalimumab), in the treatment of SLE is currently under study [2].

Interleukin-6 (IL-6)

IL-6 is a pleiotropic cytokine mainly produced by monocytes, fibroblasts, and endothelial cells, but its secretion may also be found in keratinocytes, mesangial cells, and T and B lymphocytes [17]. IL-6 receptor consists of two subunits, IL-6R and glycoprotein 130 (gp130). Binding of IL-6 to IL-6R leads to dimerization of gp130, subsequent activation of gp130-associated kinase, janus kinase 1 (JAK1), and then tyrosine phosphorylation of gp130, thereof accomplishing the transmission of intracellular signals [18].

In SLE patients, B cells express IL-6R spontaneously and produce great amounts of IL-6 [19, 20]. Moreover, auto-reactive T-cell clones from SLE patients also produce large amounts of IL-6, and thereby promote B-cell activation and autoantibody production [21]. Indeed, the copious spontaneous production of immunoglobulin by SLE B cells can be enhanced by exogenous IL-6 and diminished by neutralizing antibodies to IL-6 [22].

Abnormal IL-6 levels were observed in patients with SLE in serum and local tissues. Serum levels of IL-6 were significantly elevated and correlated with disease activity and anti-dsDNA titers [23]. In patients with active lupus nephritis, the urinary level of IL-6 was higher than that of normal controls [24]. Infiltrating inflammatory cells in the kidney, mainly macrophages and monocytes, are the main source of IL-6. In addition to the high IL-6 serum levels, significant over expression of IL-6 is found in diffuse proliferative lupus nephritis whereas healthy kidneys show little IL-6 expression [25]. The absence of IL-6 resulted in significant reduction of infiltrating macrophages in the kidney, a decrease in renal immunoglobulin G (IgG) and complement 3 (C3) depositions, and a reduction of CD4+ and CD8+ lymphocytes. These results indicate that IL-6 is a strong promoter for diffuse proliferative lupus nephritis [26].

In SLE patients with neuropsychiatric involvement, the levels of IL-6 in the cerebrospinal fluid were elevated [27]. The raised serum IL-6 presented an inverse correlation with hemoglobin level, indicating that IL-6 may involve in anemia [28]. Latest studies also revealed that SLE patients with ongoing synovitis and joint deformities had increased IL-6 which correlated with ESR and anti-dsDNA levels [29]. Moreover, Lupus patients with pulmonary involvement had higher serum IL-6 levels compared with those without pulmonary involvement [30]. Tocilizumab is a humanized monoclonal antibody against IL-6 receptor. Tocilizumab was discovered to be well tolerated in SLE treatment. In addition, tocilizumab treatment resulted in significant clinical improvement among SLE patients, especially in patients with arthritis and lupus nephritis [2, 26].

Disease activity assessment

Accurate clinical assessment of SLE is desirable because this disease has a complex process, a variable disease course, and cumulative morbidity over time, as new organ system involvement may be seen over time in many patients even 5 to 10 years after diagnosis [31]. Three patterns of disease activity have emerged: the flare (or "remitting relapsing pattern"), chronically active disease, or long inactive quiescence. These patterns can be discerned using systematic clinical assessments, routine laboratory tests, and standardized measures of disease activity [32].

Because no single measure can describe status in all SLE patients, standardized indices for assessing SLE disease activity have been created. The most common measure used now is the SLE disease activity index (SLEDAI), designated by the American College of Rheumatology [33]. The SLEDAI is perhaps the easiest assessment tool to use. Twenty-four features that are attributed to lupus are listed, with a weighted score given to any one that is present. The more serious manifestations (such as renal, neurologic, and vasculitis) are weighted more than others (such as cutaneous manifestations) [34].

PATIENTS AND METHODS

Thirty seven new and longstanding SLE patients enrolled in this cross-sectional analytic study, which had been diagnosed according to the American College of Rheumatology (ACR) criteria of 1997 revision. The patients were considered to have SLE if any 4 or more of the 11 criteria were present, during any interval of observation.

The SLE patients were recruited from the department of rheumatology in general teaching hospital and the center of rheumatology and rehabilitation in Sulaimani city. Thirty one of apparently healthy control subjects were selected among paramedical personnel and normal healthy relatives of the patients visiting the medical center of Ali Kamal after obtaining their informed consent, with matched age and sex variables to those of SLE patients.

Both SLE patients and normal controls were involved in the period from October 2012 to April 2013. The normal controls were interviewed, general information recorded, past medical and drug history taken, physical examination done, and several hematological and serological tests, including hemoglobin level, erythrocytes sedimentation

rate, C-reactive protein level, leukocytes and thrombocytes counts, in addition to anti-nuclear antibodies, were done to ensure that they have no any inflammatory or connective tissue disease; and the normal limits of tests were derived from the lab reference values.

A protocol was designed for all cases to record age, sex, address, occupation, duration of illness, drug history. Verbal informed consent was taken from all cases. A proper history and physical examination were undertaken and all clinical and laboratory parameters involved in SLE disease activity index (SLEDAI), were measured to classify SLE cases into: inactive, mild to moderately active and highly active patients.

The blood samples were taken from both SLE patients and healthy controls for the estimation of the serum levels of INF-α and IL-6.

The IFN-α ELISA kit was from (TSZ ELISA). The low serum level of circulating IFN-α was considered to be less than 5 IU/ml, while the elevated serum level of circulating IFN-α considered to be equal or more than 5 IU/ml, as assessed by the test performed by: Focus Diagnostics, Inc. and reported by Mayo Medical Laboratories.

The IL-6 ELISA kit was from (Immunotech). The low serum level of circulating IL-6 was considered to be less than 17.4 pg/ml, while the elevated serum level of circulating IL-6 considered being equal or more than 17.4 pg/ml, as assessed by the test performed by (Viracor-IBT Laboratories) and reported by Mayo Medical Laboratories.

Statistical package for social sciences (SPSS) version 17.0 was used for statistical analysis. Student's *t*-test and analysis of variance (ANOVA) test were used and *p*-values of less than 0.05 were considered to be statistically significant.

RESULTS

A total number of 68 subjects participated in the study, 37 (54.0%) of them were SLE cases and 31 (46.0%) were healthy controls. The 37 SLE patients were composed of 4 (10.8%) males and 33 (89.2%) females. The 31 apparently healthy controls were composed of 3 (9.7%) males and 28 (90.3%) females. The ages of patients ranged from 13 to 56 years (31.1±12.8 years); while controls' ages ranged from 15 to 45 years (31.1±8.1 years) (Table 1).

Twenty six (70.27%) of SLE patients, had symmetric polyarticular arthritis and/ or arthralgia; while mucocutaneous manifestations of malar rash, discoid rash, photosensitivity and mucosal ulcers, were found in 31(83.78%) of patients, 21 (56.75%) of the patients had haematologic manifestations of anemia, leukopenia and thrombocytopenia; renal manifestations indicated by generalized body swelling, periorbital and lower peripheral oedema associated with proteinuria and lupus nephritis were found in 23 (62.16%) of the patients. Neuropsychiatric manifestations of convulsions, lupus headache, confusion and peripheral neuropathy were present in 8 (21.62%) of patients; while pleuropulmonary features of pneumonitis, pleural effusion and/or pleurisy were found in 4 (10.81%) of all SLE patients; and cardiovascular manifestations of pericarditis and pericardial effusion also were found in 4 (10.81%) of all patients (Figure.1).

It was found that 8 (21.62%) of SLE patients were inactive (remitted) patients with SLEDAI group 1, while 9 (24.32%) of cases were considered to have mild to moderate disease activity with SLEDAI group 2, and 20 (54.05%) of SLE cases were considered to have high disease activity with SLEDAI group 3 (Table 2).

The serum levels of the two inflammatory cytokines were significantly higher in SLE patients, compared to the healthy controls. As they were (37.26 ± 27.58 IU/ml) and (13.29 ± 23.63 IU/ml) respectively for INF-α (p-value < 0.001); (18.09 ± 21.02 pg/ml) and (7.10 ± 19.80 pg/ml) respectively for IL-6 (p-value < 0.001)

Twenty five (75.75%) of all the thirty three female patients had elevated serum level of IFN-α, and 19 (57.57%) of them had high serum level of IL-6; while 3 (75%) out of the four male patients had elevated serum level of IFN-α, and 2 (50%) of them had elevated serum levels of IL-6.

The serum levels of the two cytokines were compared among the three SLEDAI groups, and the results showed that IL-6 had a significant positive correlation with disease activity (p-value =0.016), while INF-α was not correlated with the disease activity (p-value=0.734).

Table 1. Sex and Age Distribution in SLE Patients and Healthy Controls.

Demographic Variables	Patients		Controls	
Sex	Number (N)	Percentage (%)	Number (N)	Percentage (%)
Females	33	89.2%	28	90.3%
Males	4	10.8%	3	9.7%
Age	Patients		Controls	
Minimum (years)	13		15	
Maximum (years)	56		45	
Mean & Standard Deviation (years)	31.1 ± 12.8		31.1 ± 8.1	

Table 2. Disease activity According to SLEDAI.

SLEDAI Groups	Cases N (%)	SLEDAI Scores (Mean ± SD)
SLEDAI 1*	8 (21.6%)	2.13 ± 0.64
SLEDAI 2**	9 (24.3%)	8.33 ± 2.00
SLEDAI 3***	20 (54.1%)	20.45 ± 8.30

***SLEDAI 1 (Inactive Disease), *SLEDAI 2 (Mild to Moderate Active Disease), ***SLEDAI 3 (Highly Active Disease)w**

Table 3. Comparison of INF-α and IL-6 Levels in the Two Groups Using Student's *t*-test.

Cytokines	Cases	Controls	*p*- value
IFN-α M±SD (IU/ml)	37.26 ± 27.58	13.29 ± 23.63	**< 0.001**
IL-6 M ± SD (pg/ml)	18.09 ± 21.02	7.10 ± 19.80	**0.031**

Table 4. Comparison of INF-α and IL-6 Levels in the Three SLEDAI Groups Using ANOVA.

SLEDAI groups	INF-α (IU/ml) (Mean ± SD)	p-value	IL-6 (pg/ml) (Mean ± SD)	p- value
SLEDAI 1	40.113 ± 37.787		5.588 ± 5.548	
SLEDAI 2	30.866 ± 25.309	0.734	8.320 ± 5.548	0.016
SLEDAI 3	38.995 ± 24.882		23.410 ± 11.983	

DISCUSSION

Systemic lupus erythematosus is a chronic autoimmune disease that is characterized by a defect in immune tolerance at which both innate and adaptive types of immune response are affected. SLE associated immune hyperactivity can be detected systemically as elevations in levels of cytokines. Importantly, the cytokines that have significant roles in SLE pathogenesis include IFN-α, IL-6, and TNF-α [34, 35].

The female to male ratio in this study is close to the prevalence supported by Gaubitz M. [36] and George Bertsias *et al* [37], who reported female predominance by (90%), and female to male ratio of (9:1) among SLE patients.

In this study there was a mild female predominance in the state of elevated serum levels of IFN-α, as compared to male patients, which is supported by the studies of Weckerle CE *et al* [38] and Hughes GC *et al* [39]. Also there was a female predominance among SLE patients with elevated serum levels of IL-6, which is supported by the studies of Chapman BP *et al* [40], who reported that females appear to have higher serum levels of IL-6 than male SLE patients. This higher serum levels of IFN-α and IL-6 among females might be due to female sex hormonal effects [39 40].

The ages at disease onset of the SLE patients in this study, was close to that of the (Lupus Foundation of America) statistics about Lupus in 2004 [41], which showed that SLE may be diagnosed at any age, but the peak age includes second to fourth decades with the mean age of 30 years. This result was also similar to the findings reported by Ishaq M *et al* [42], who revealed that the ages at disease onset were (31.6 ± 10.5 years).

In this study, the serum level of IFN-α is significantly higher in SLE patients compared with healthy controls, which is close to the results of Kirou KA *et al* [43], Kim T *et al* [44] and Dina Shahin *et al* [45] who referred also to highly significant difference, (p-value < 0.01, < 0.001 and < 0.001) respectively, between the serum levels of IFN-α in SLE patients and healthy subjects.

Similarly, in this study, the serum level of IL-6 is significantly higher in SLE patients compared with the healthy controls, which is comparable to the results of Robak E *et al* [46], Sabry A *et al* [47] and Ghada S. Azkalany *et al* [48], who revealed significant differences between the levels of IL-6 in SLE patients and healthy subjects, with (p-values < 0.02, = 0.002 and = 0.008) respectively.

There were no significant differences in the serum levels of IFN-α in the three SLEDAI groups in this study, which indicates that the serum level of IFN-α is not correlated with SLE disease activity. This result is comparable to the findings reported by Kirou KA *et al* [43], who revealed that the serum level of IFN-α is not correlated with disease activity as measured by SLEDAI. This could be explained by the positive significant association between SLE disease activity and the (IFN gene expression signature), rather than serum IFN-α, that can be identified from the nuclei of peripheral blood mononuclear cells (PBMCs). This

appears to be a more sensitive readout for activation of (INF) pathway than cytokine levels in serum and associate with the severe features of SLE, (neural, hematologic and renal features), as that reported by Baechler EC *et al* study [49] and Feng X et al [50], who referred to a significant correlation between (IFN gene signature) and SLE disease activity index with *p*-values = 0.004, 0.001) respectively.

In the current study, there was a significant positive correlation between the serum level of IL-6 and disease activity measured by SLEDAI, which might indicate that IL-6 could be used as an indicator of disease activity in SLE. This finding is supported by the results reported by Mellor-Pita S *et al* [51] (*p*-value = 0.010), *Spronk PE et al* [52] (*p*-value < 0.05), and Sabry A *et al* [47] (*p*-value = 0.002).

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