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Association between Interleukin-6 and Hyperferritinemia in Systemic Lupus Erythematosus Patients

Radhi F. Shlash*
Faculty of Medicine/ University of Al-Qadisiyah/Iraq, 58001

Abstract

Background: Systematic lupus erythematosus (SLE) is a long-lasting autoimmune ailment that disturbs nearly several system structures, involving the skin, joints, kidneys, and heart.

Objectives: this study was achieved to define the levels of serum interleukin-6 (IL-6) and its association with serum iron and serum ferritin levels in patients with systemic lupus erythematosus (SLE).

Materials and Methods: 50 serum samples from SLE patients were collected besides 20 (compatible with gender and age) served as control subjects. Clinical considerations of ailment were measured, involving seropositivity test for systematic lupus erythematosus (SLE), erythroid sedimentation rate (ESR). Serum level of iron was measured spectrophotometrically. Serum levels of interleukin-6 (IL-6) and ferritin were assessed by an enzyme-labeled immunosorbent assay (ELISA).

Results: the concentration of serum interleukin-6 (IL-6) and serum level of ferritin was considerably rising ($P<0.0001$) in persons suffering from SLE paralleled to whom of healthy individuals, while serum level of iron was significantly decreased ($P< 0.0001$) paralleled to whom of healthy individuals.

Conclusions: this study concluded that the higher levels of ferritin and IL-6 in SLE patients and these alterations strongly associated with the inflammatory status of the patients (significant elevation and ESR, C-reactive protein). In overall, changed iron behavior, inflammation and anemia of prolonged ailment collectively create a life-threatening triad in SLE.

Keywords: Systemic lupus erythematosus (SLE); interleukin-6 (IL-6); hyperferritnemia; ELIZA

1. INTRODUCTION

Systematic lupus erythematosus (SLE) is a long-lasting autoimmune ailment can disturb nearly any system structures, involving the integument system, skeletal system, renal system, serosal membranes, and cardiovascular system. SLE considers a main rheumatic ailment, and above of 95% of patients suffering from SLE has polyarthralgia. The SLE considered commonest ailment by an occurrence of nearly one case every 1500 individuals in some inhabitants [1]. Highest frequency of SLE happens amongst individuals with 15 to 40 years. There is a women/men ratio about 9/1, and this ratio approaches nearer to 30/1 throughout the childbirth periods. The high frequent distribution of the disease in Negro of United Sates and Asians, and some relatives are more than in others [2].

The etiology of this autoimmune disease is mysterious. High serum availability of auto-antibodies proposes impairment of surveillance role of the immune system. These circulating auto-antibodies have been recognized in contradiction of self-components particularly nuclear and systolic components of the cell that have specificity to tissue or organ system. The antibodies that are directed in contradiction of numerous nuclear antigens is called antinuclear antibodies (ANAs), which involving genetic elements, basic proteins (histones), non-histone proteins, and protein components of nucleolus [3]. An additional types of auto-antibodies are in contradiction of antigenic proteins exist in cell surface of erythrocytes.

Once a cytokine like interleukin-6 is stimulated, severe inflammatory reactions like pyrexia or anaemia are promoted. This cytokine activates B lymphocytes propagation, therefore is elaborate in the autoantibodies generation [4]. Ferrous has significant role in oxidative disturbance-facilitated cell injuries and immupathogenesis of SLE. Several years ago it had been proposed that ferrous might have a major role in the inflammation advancement of the SLE [5]. Ferrous ions exist in minute quantities are vigorous to make depolymerization of refined hyaluronic acid and this is accompanied by the little viscidness of synovial fluid (SF) of persons suffering from SLE. Spectrographic investigations revealed that synovial fluid from persons suffering from SLE checked high iron concentration [6]. Spectrophotometric tests revealed that the iron concentration was higher in the synovial fluid of SLE persons compared with healthy ones. [7]. In SLE, it is assessed that 33-65% of persons are suffering from anaemia. One of the utmost common reasons of anaemia in SLE persons is iron deficiency anaemia (IDA). Anaemia of chronic disease (ACD) which does not normally respond to iron administrations is the additional main reason of anaemia in persons suffering from SLE [8]. However, Bloxham and his coworkers found that the majority of anemic patients were ACD, with rather fewer patients demonstrating iron deficient [9]. Therefore, the study was designed to assess the concentration of important inflammatory cytokines (interleukin-6) in the serum of persons suffering from SLE beside to explore the correlation between this cytokine and serum iron and ferritin.

2. MATERIALS AND METHODS

2.1 Patients and clinical evaluation

Fifty patients attended to Al-Diwanyiah Teaching Hospital/ Iraq in the period between February and May 2016, the standardizations of the American college of rheumatology (ACR) 2010 for the identification of SLE were applied [10]. 20 persons (compatible with age and sex) served as

healthy subjects deprived of any evidence of long-lasting inflammatory conditions. At standard, this study calculated the twenty eight-joint disease activity score (DAS28) using the number of joints with sensitivity or enlargement. Subjects experienced full clinical and laboratory assessments, involving whole medical history, serology test for c-reactive protein (CRP), SLE, and assessment of erythroid sedimentation rate (ESR).

6 ml of blood samples were obtained by intravenous route from each person, one ml for assessment of ESR, though the serum were put in cut-glass tubes devoid of anti-coagulant, kept about an half hour at room temperature, and underwent centrifugation (3000 rpm about ten minutes at 4°C) and collected with malleable tubes prior to kept at minus twenty centigrade till examination.

2.2 Classification of patients

Patients with SLE were grouped along with the period of the disease as follows (Fig. 1)

2.3 Methods

2.3.1 Systemic lupus erythematosus (SLE) and C-reactive protein (CRP) serology test

SLE latex serology test and C - reactive protein was measured by agglutination test

2.3.2 Erythroid Sedimentation Rate (ESR)

ESR was measured according to the Westergren method procedure which recommended by International Committee for Standardization in Haematology (ICSH) [11, 12].

2.3.3 Serum iron measurement

Serum levels of iron and was measured spectrophotometrically in Biochemistry Laboratory Al-Diwanyiah Teaching Hospital the Colorimetric test method was used via RANDOX reagents (RANDOX kit, U.K) according to Ceriotti and Ceriotti (1980) [13].

2.3.4 Measurement of IL-6and serum ferritin

The serum level of interleukin-6 and ferritin was assessed by an AssayMax enzyme-labeled immunosorbent assay (ELISA) kit (Assaypro, USA).

2.3.5 Data analysis

Parametric statistical analyses were accomplished by ANOVA followed postthoc Tukey's test using GraphPad prism software version 6. Pearson correlation analysis was also performed to evaluate the correlation between studied parameters. The limit of significance was set at 5%t [13].

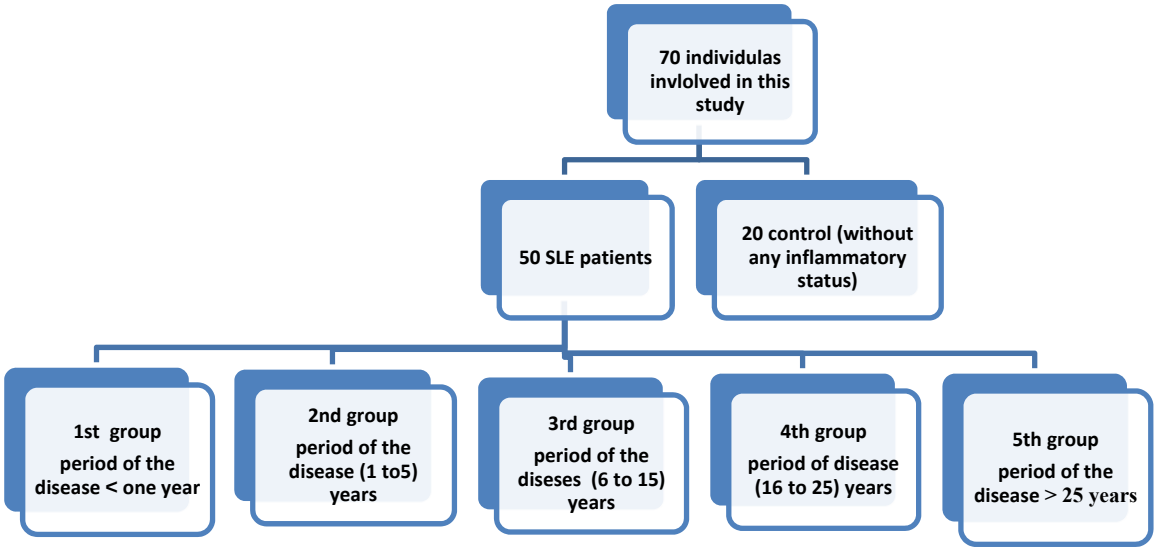

Fig. 1: schematic representation showing the study design

3. RESULTS

3.1 Characteristics of the SLE patients

Table1: Clinical with Demographic Characteristics of SLE patients

Characteristics		SLE patient (n=50)				
	Healthy Control	GI	GII	GIII	GIV	GIV
Number of subjects	20	8	12	9	14	7
Age	41±1.33	42±4.12	45±4.11	48±1.12	46±4.9	56±5.13
Women to men fraction	16/4	6/2	9/3	8/1	10/4	5/2
Period of the disease per years		0.44±0.05	2.17±0.33	7.44±2.54	18.4±0.66	25.5±0.8
%SLE seropositive		92.5%	96.8%	91.2%	91%	94%
ESRmm/hour	20.9±0.4	56.1±4.2	55.6±2.6	58.4±5.16	68.19±6.6	49.3±3
%CRP seropositive		90%	89%	90%	90.9%	80%

Table 2: Iron status in SLE patients and control subjects

Iron status	Control Subjects (n=20)	SLE patients (n= 50)				
		GI	GII	GIII	GIV	GV
S. Iron (µmol/L)	21.71±1.00	$8.44±2.07_a^{***}$	$7.81±1.49_a^{***}$	$7.88±1.49_a^{***}$	$10.12±1.79_a^{***}$	$10.66±1.07_a^{***}$
Ferritin (ng/mL)	131.2±5.96	$220.4±23.73_a^{ns}$	$233.2±28.37_a^{**}$	$218.2±28.47_a^{*}$	$198.5±27.38_a^{ns}$	$217.5±21.26_a^{ns}$

Values are stated by means of means ± standard error (SE).
Stars refer significant changes according to Tukey multiple comparable analysis.
Similar small letters refer insignificant changes amongst groups according to Tukey multiple comparable analysis.
*** indicate extremely significant (P value <0.001).
* indicate significant (P value 0.01 to 0.05).
ns: not significant.

3.1 Serum iron and ferritin

Serum level of iron and ferritin showed significant elevation (P value less than 0.0001) in patients with SLE paralleled in comparison with control subjects (Table 2).

3.2 Serum concentration of IL-6

Serum values of interleukin-6 of SLE patients showed a significant increase (P value less than 0.0001 in comparison with the healthy control, particularly very early duration of the disease was much higher (Fig. 2)

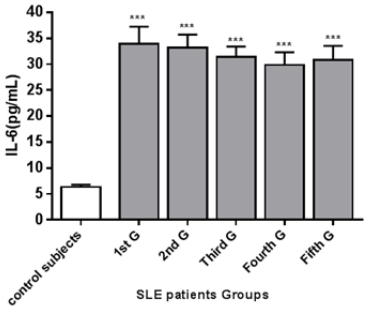

Fig. 2: Interleukin-6 levels in SLE patients and control subjects

Values are stated by means of means ± standard error (SE).
Stars refer significant changes according to Tukey multiple comparable analysis.
P amount less than 0.0001
*** indicate extremely significant (P value less than 0.001).

3.3 Erythrocyte Sedimentation Rate (ESR) (mm/hour)

ESR of SLE patients showed significant increase (P value less than 0.0001) in the in cmparison with healthy control, particularly with long duration of the disease was much higher (Fig. 3)

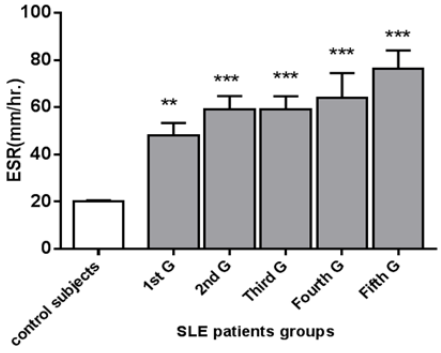

Fig 3: ESR in SLE patients and control subjects

Values are stated by means of means ± standard error (SE).
Stars refer significant changes according to Tukey multiple comparable analysis.
P amount less than 0.0001
*** indicate extremely significant (P value <0.001).
** indicate very significant (P value 0.001 to 0.05).

3.4. Association of interleukin-6 values to serum iron

Interleukin -6 values showed inverse correlations with serum iron (Fig. 4)

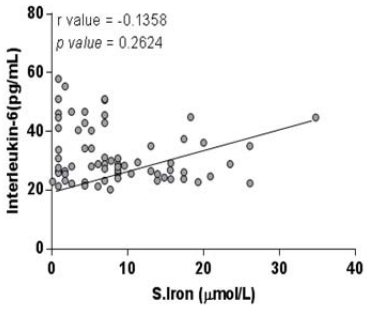

Fig.4: Correlation between interleukin-6 and serum iron in SLE partients

3.5 Association of interleukin-6 values to serum ferritin and ESR

The current study revealed significant and positive relation between Interleukin-6 values and serum ferritin values (r value = 0.9480, p value less than 0.0001) the highest coefficient association with erythroid sedimentation rate (r value= 0.9776, p value <0.0001), respectively (Fig.5 - 6).

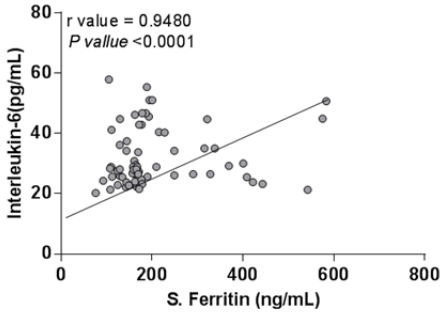

Fig.5: Correlation between interleukin-6 and serum Ferritin in SLE partients

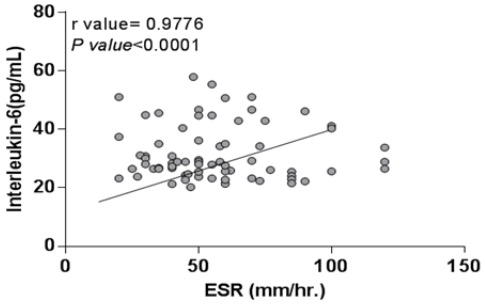

Fig.6: Correlation between interleukin-6 and ESR in SLE partients

4. DISCUSSION

The present study revealed a significant rising in the values of interleukin-6 in all persons with SLE in comparison with those in healthy subjects. As realized in formerly investigations, this outcome agreed with the postulation in that this inflammatory cytokine plays a major role in the pathogenesis of SLE [15-18]. In addition, it is well known that this cytokine causes an acute inflammatory response, and has an important role in the pathogenesis of several inflammatory diseases comprising SLE [19, 20]. Also, this obvious significant elevation noted in the present study refer that this inflammatory cytokine play significant role in eliciting inflammatory reactions or intervening anti-inflammatory responses in the pathogenesis of SLE [20].

On the other hand, this study also revealed significant elevation in iron levels, in persons with SLE. As understood in former investigations, this result agreed with the suggestion that the anaemia is the main reason of extra-articular symptoms of the SLE [21, 22]. Additionally, it is reported that 35-65% of SLE patients are anaemic. The iron deficiency anemia (IDA) considered the major reason of anaemia in patients with SLE along with anaemia of chronic disease (ACD) which not respond to iron administration, is also considering another major reason of anaemia in SLE patients [23].

The present study also indicates significantly increased in serum ferritin, in all groups of patients with SLE. However, serum ferritin was considered as surrogate indicator of iron storage and the precise alone marker of entire iron of the body [24]. Serum ferritin remains an acute phase reactant and elevated serum level of ferritin in RA patients is mainly due to the inflammation [25].

Patel et al., (2011) was concomitant with this study when they describe the coexistence of iron insufficiency with the severe ferritin elevation as distinguishing of SLE. The combined effect has clinical significance and permits assumptions around the occurrence of hyperferritinaemia despite obvious iron depletion. The co-occurrence of iron insufficiency must be deliberated at what time estimating the patient with anaemia of chronic diseases despite the ferritin concentrations are increased numerous hundredfold. Additional perceptions on the metabolism of ferritin are proposed by the probability that the severe hyperferritinaemia in inflammatory status of SLE disease was approximately whol of the iron-free apoferritin form [26].

Data from Masson, (2011) provide a reasonable linkage amongst interleukin-6 generation and the progression of anaemia in patients with the long-lasting disease. This cytokine inhibits the quantity of reticulocytes by interfering with the erythropoiesis inside haemopoiteic tissues and depresses the iron amounts, and those combined effect defects could be ameliorated by administration of interleukin-6 blockers [27]. Dakhil and Mezher (2014) reported that inflammatory hypoferraemia is facilitated by interleukin-6 which stimulates production of hepcidin, a hormone which regulate the iron metabolism [8].

5. CONCLUSION

This study has been concluded that the inflammatory cytokine levels were significantly related with inflammatory state represented by serum ferritin and ESR. Taking together with the handling of serum iron, the interleukin-6 plays a major role in the pathogenesis of SLE including anemia of chronic disease.

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