Evaluation of Interferon Alpha (IFN-α) in Women with Systemic Lupus Erythematosus in Iraq

Ruaa Hameed Abdulridha[1], Asmaa Mohammed Saud[2], Mohammed Hadi Alosami[3]

[1]*Medical laboratory techniques Department, Al-Hikma University College, Baghdad, Iraq.*
[2]*Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq.*
[3]*Department of Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq.*

Abstract:

Systemic Lupus Erythematosus (SLE) is a multifactorial chronic systemic autoimmune disease. It is characterized by a lack of immune tolerance to autoantigens such as nuclear antigens. The aim of the study is to assess the interferon-alpha (IFN-α) serum level in Iraqi patients with SLE and determine its potential relation to different clinical and laboratory parameters and disease activity. 100 SLE patients were all females and with a mean of age 31.3 ± 10 years (16-63years) and disease duration of 5.8 ± 3.7years (1 month to 15 years). The average of SLEDAI score ranged from 2 to 22 with a mean of (8.53 ± 3.42). Proteinuria, ESR, creatinine and AST were significantly higher (65% vs. 10% and 0.62 ± 0.11 vs. 0.70 ± 0.14 mg/dl respectively) while the PLT was significantly lower (231.9 ± 88.8 vs. 282.3 ± 67.3 10^3/mL) ($p< 0.001$) among SLE patients as compared to control. Serum levels of IFN-α were increased in the SLE patients compared to control, and no significant difference has been observed (208.7 ± 530.0 vs. 63.7 ± 34.8 pg/ml) respectively ($P=0.245$). Interferon-alpha showed a significant negative correlation with the SLE Disease Activity Index (SLEDAI) in the active and inactive groups. There were no significant variations in all study parameters across IFN-α serum levels (p greater than 0.05). In conclusion, the results suggest a risk effect for female gender and age in etiology of SLE. IFN-α could not be considered as biomarker or to have a risk effect in SLE patients or perpetuate the disease activity. No evidence for any correlation between the IFN-α serum level and any clinical manifestations or laboratory investigation of the disease in current study except for age and disease duration, which suggests them as a risk factor for increasing the IFN-α serum level.

Keywords: Autoimmune disease, Anti-nuclear autoantibody, Interferon-Alpha, Systemic lupus erythematosus.

تقييم انترفيرون ألفا (IFN−α) في النساء المصابات بالذئبة الحمامية الجهازية في العراق

رؤى حميد [1] ، اسماء محمد [2] ، محمد هادي [3]

[1]قسم تقنيات المختبرات الطبية، كلية الحكمة الجامعية ، بغداد، العراق.

[2]قسم التكنولوجيا الحيوية، كلية العلوم ، جامعة بغداد ، بغداد العراق.

[3]. قسم الطب ، كلية الطب ، جامعة بغداد ، بغداد ، العراق.

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*Email: roaaaltaee@gmail.com

الخلاصة

الذئبة الحمامية الجهازية هي مرض مناعي ذاتي جهازي متعدد العوامل. يتميز بنقص التحمل المناعي للمضادات الذاتية مثل المستضدات النووية ، والهدف من الدراسة هو تقييم مستوى مصل مضاد للفيروسات انترفيرون ألفا (α–IFN) في المرضى العراقيين المصابين بمرض الذئبة الحمراء وتحديد علاقته المحتملة بمختلف العوامل السريرية والمختبرية ونشاط المرض. 100 مريض بمرض الذئبة الحمامية المجموعية كانوا جميعهم من الإناث ومتوسط أعمارهم 31.3 ± 10 سنوات (16–63 سنة) ومدة المرض 5.8 ± 3.7 سنوات (من شهر واحد إلى 15 سنة). تراوح متوسط درجات SLEDAI من 2 إلى 22 بمتوسط (8.53 ± 3.42). كانت البيلة البروتينية والكرياتينين أعلى بشكل ملحوظ (65٪ مقابل 10٪) و 0.62 ± 0.11 مقابل 0.70 ± 0.14 مجم / ديسيلتر على التوالي) بينما كان PLT أقل بشكل ملحوظ (231.9 ± 88.8 مقابل 282.3 ± 67.3 103 / مل) (0.001> p) بين مرضى الذئبة الحمراء مقارنة بمجموعة التحكم. تمت زيادة مستويات α–IFN في المصل في مرضى الذئبة الحمراء مقارنة بمجموعة التحكم ، ولم يلاحظ أي فرق كبير (208.9 ± 530.0 مقابل 46.2 ± 34.8 بيكوغرام / مل) على التوالي (P = 0.98). أظهر Interferon–alpha ارتباطًا سلبيًا كبيرًا مع مؤشر نشاط مرض SLE (SLEDAI) في المجموعة النشطة وغير النشطة. لم تكن هناك اختلافات كبيرة في جميع معلمات الدراسة عبر مستوى مصل α–IFN (أكبر من 0.05). في الختام ، تشير النتائج إلى وجود تأثير خطر على الجنس والعمر للإناث في مسببات مرض الذئبة الحمراء. لا يمكن اعتبار α–IFN علامة بيولوجية أو أن لها تأثير خطر على مرضى الذئبة الحمراء أو استمرار نشاط المرض. لا يوجد دليل على أي علاقة بين مستوى مصل α–IFN وأي مظاهر سريرية أو تحقيق معمل للمرض في الدراسة الحالية باستثناء العمر ومدة المرض ، مما يشير إلى أنها عامل خطر لزيادة مستوى مصل α–IFN.

Introduction

Systemic Lupus Erythematosus (SLE) is a multifactorial chronic systemic autoimmune disease[1]. It is characterized by a lack of immune tolerance to autoantigens such as nuclear antigens [2]. The production of multiple autoantibodies against host DNA and other cellular elements and antigen-antibody complexes would lead to damage to various organs and tissues inflammation [3]. SLE is a multifactorial disease caused by the interaction of multiple genetic and environmental factors [4, 5]. In addition, this disease is a heterogeneous disease with various clinical and laboratory features, that make a diagnosis, assessment of disease activity, and treatment difficult and challenging for physicians [6].

The list of biomarkers that aid the physician in decision making regarding diagnosis and prognosis of the disease activity has remained very limited [7, 8], and includes mostly antinuclear antibody (ANA), anti-dsDNA antibodies, complement 3 protein(C3), complement 4 protein (C4) and leukopenia [9]. Those are now insufficient to use in the management of disease and it is, therefore necessary to identify reliable and advanced new biomarkers [10]. Interferon-alpha (IFN-α) plays a vital role in immune system regulation, it is mainly synthesized by plasmacytoid dendritic cells (pDCs), where it stands as a link bridge between innate and adaptive immunity [11].

IFNA genes code for IFN-α proteins, which are classified as Type I interferons (IFNs) [12]. In humans, the IFN-β, IFN-ε, IFN-κ, and IFN-ω classes are represented by a single functional gene whereas IFN-α consists of a 13 member multigene family [13]. IFN-α has 80% of amino acid sequence similarities which are consisted of 165 to 166 amino acids [14, 15]. Several prior studies in animal models and humans have described a disordered expression of type I IFN family-regulated genes in peripheral blood and damaged tissues in SLE patients, which is commonly known as the IFN signature [16]. The inciting events that

induce a persistent type I IFN proteins production are not completely understood but some evidence shows that a combination of genetic and environmental factors is implicated [17]. In addition, hormonal roles have been identified too such as the estrogen that was founded to enhance the activation of IFN-α signaling [17]. The present study aimed to assess the interferon-alpha serum levels in Iraqi patients with SLE and determine its potential relation with different clinical and laboratory parameters and disease activity.

Methods

Subject

A case-control study was conducted on one hundred SLE female patients. Cases were referred to the inpatient-outpatient clinic at Baghdad Teaching Hospital (the Rheumatology Unit) from October 2020 – February 2021. The diagnosis of the disease was handled by the rheumatologists at the unit clinic by following the European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) diagnostic criteria for SLE [18]. The clinical and laboratory assessments were all performed. Furthermore, the score of the Systemic Lupus Erythematosus disease activity index (SLEDAI) for every individual patient was determined directly by the physician during blood draw [19]. Depending on the disease activity score results, SLE patients were classified into two groups: active disease (scores≥4) and inactive disease (scores<4) [20]. Fifteen healthy controls were enrolled in this study; the controls were recruited from the Units of Healthcare in Baghdad. The physicians at the Units ascertained their health status, which did not have any autoimmune diseases or treatment by immunosuppressive agents.

All the participants have been provided with written informed consent to be included in the study. The protocol of the study was approved by the Ethics Committee at the Iraqi Ministry of Higher Education and Scientific Research on (No. CSEC/0121/001 on January 29, 2021).

Inclusion and Exclusion criteria

We applied available data to common inclusion criteria including age ≥16 years, positive for EULAR/ACR) diagnostic criteria for SLE [18].

The study excluded patients with other immune diseases, or suffering from overlapped autoimmune diseases, patients with juvenile SLE (early-onset) that age less than 16 years old, or with lupus nephritis (LN).

Laboratory Investigations

The demographic data information and clinical manifestation were collected from the medical record for each patient. The laboratory investigations were performed to assess the immunological and routine tests. All the hematological parameters that assess by complete blood count (CBC), Erythrocyte sedimentation rate (ESR), and the amount of proteinuria were determined at the time of sampling. The anti-nuclear autoantibody (ANA) was determined by the enzyme-linked immunosorbent assay (ELISA) technique (Human Company, Germany) [21]. The Blood urea and creatinine were determined by automated Fujifilm according to manufacturer instructions.

Interferon Alpha Measurement

Serum level of IFN-α titer was measured by using ELISA technique kit for researcher purposes, commitment to the instructional manual that provided by manufacturer Cell Biolabs Inc, United States (Cat. No RDEEH3252). The technique was very sensitive and detected a very low titer of IFN-α in serum with high precision reached to <9.375 pg/ml.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 22 has been used in the current study for dealing with obtained data. Mean (median) and interquartile range has been used to calculate parametric results(quantitative) while non-parametric data(qualitative) was calculated by means and standard deviation. Additionally, Pearson Chi-square test was used also for comparisons, and Spearman correlation was used to test the correlation between different study parameters. P-value is significant if < 0.05 at confidence interval 95%.

Results and Discussion

The studied population consisted of one-hundred female SLE patients; fifty healthy control with a mean age of 31.3±10 (years) and 34.0±12 (years), respectively. Meanwhile, active SLE represented 60% and inactive 40% of total SLE patients, adult-onset (16-50 years) consisted of 94% of SLE patients and late-onset 6% (>50 years) the variation was significant (P<0.001). The majority of patients (82%) had no history of SLE in their families. The mean disease duration for patients was 5.8±3.7. Most clinical manifestations presented in our study patients were (52%) malar rash, (45%) oral ulcers, (54%) arthritis, and (45%) neurological disorder. Demographic and laboratory parameters of the female SLE patients and healthy control enrolled in this study were described in Table1.

**Table 1:** Demographic, clinical, and laboratory parameters in all studied groups.

Parameters Mean ±SD or N (%)	Patients (N=100)	Controls (N=50)	*P*
Age	31.3±10.2	34.0±12.4	0.230
Age groups			
Adult-onset	29.8 ±8.5		
Late-onset	54.3±4.8		
P	**<0.001***		
Disease duration(years)	5.8±3.7		
Family History	18(18)		
SLEDAI-2K	7.7±5.3		
Active SLE	60(60%)		
Inactive SLE	40(40%)		
Proteinuria	65(65%)	5(10%)	**<0.001***
WBC (10^3/mL)	6.66 ± 2.63	6.38 ± 1.52	0.514
HB (g/dl)	10.91 ± 1.77	10.59 ± 1.86	0.301
PLT (10^3/mL)	231.9 ± 88.8	282.3 ± 67.3	**<0.001***
Urea (mg/dl)	25.57 ± 7.47	28.80 ± 6.32	0.10
Creatinine (mg/dl)	0.62 ± 0.11	0.70 ± 0.14	**<0.001***
ANA positivity	90(90%)		

Values are expressed as N: number in each parameter, %: percentage in each group and mean ± SD. The abbreviations are; WBC: white blood cell, Hb: hemoglobin, S: serum, ANA: antinuclear antibody. Normal ranges: WBCs (10^9/L), 4.00-10.00; HB (g\dl), 12-17; Platelets (10^3/μl), 150-450; urea (mg\dl), 15-45; Creatinine (mg\dl), 0.3-0.7. *Correlation is significant at the 0.05 level (2-tailed).

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease, characterized by the increased production of autoantibodies directed against self-antigens and variable clinical manifestations with totally unpredictable flares course [22]. Despite great and advances in the SLE pathophysiology understanding, patients with SLE still have a high risk of organ damage and mortality. Predicting new tools allows for earlier diagnosis of SLE, which lead to earlier monitories for disease activity and choosing a proper treatment [23, 24].

The results presented suggest a risk effect for female gender and age in etiology of SLE. With respect to age, SLE is regarded as a disease that mostly afflicts younger-aged more than middle-aged or elderly people. The recorded age range at diagnosis is 16-63 years with mean (of 28.9 years). So the younger age for adults is considered as a crucial risk factor for SLE [25]. The current study described that 100 SLE cases were female despite the collection time is about 5 months; most studies agree that SLE occurs more frequently in women than in men [26, 27]. The demonstrated gender difference may result from the interaction between the environment, genotype, and sex hormones during individual development; also all cases were adults and this is similar to previous research that clarified, that females with SLE have a higher prevalence than males after puberty due to high levels of circulating estrogen [28].

The IFN-α serum levels were higher in SLE patients (208.7±530.0 pg/ml) than in healthy controls (63.7±34.8 pg/ml) with an insignificant association has been described (P=0.245) as shown in (Figure1a). Furthermore, analysis confirmed the low specificity and sensitivity of IFN-α as a potential biomarker of SLE disease. IFN-α serum levels at a cutoff point of 20.1 had a specificity and sensitivity of 100% in SLE patients and controls. The mean of IFN-α serum levels shows no significant difference (P=0.637) between active (182.2±391.1 pg/ml) and inactive (248.9±696.4 pg/ml) SLE disease groups as shown in (Figure2b).
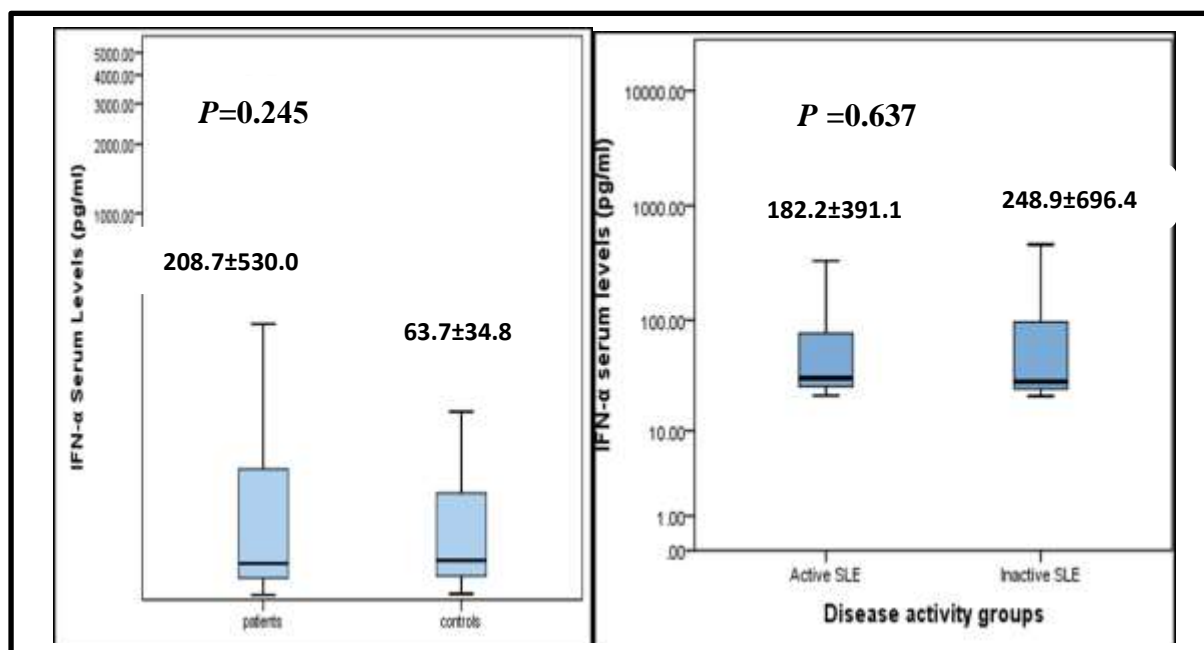
Figure 1: (a) IFN-α serum levels in SLE patients and healthy controls **(b)** IFN-α serum levels in active and inactive SLE patients.

The current work did not reveal any significant difference in mean of IFN-α serum levels between SLE patients and healthy control. On the contrary in the majority of previous studies, that done in USA [29, 30] or Egypt [31, 32]. Also, Shahin et al. have suggested the IFN-α as a useful biomarker for identifying SLE patients [33]. The contrast in our observation could be explained by the ethnic difference of Iraqi population with a genetic basis or by the effective treatment course that described by the physicians especially that 88% of our subjects undergo treatment. This is in agreement with Sinicato *et al.*, who reported an increase in IFN-α serum levels in patients who are not taking medication [34].

Regarding the correlation between the IFN-α serum levels and demographic, clinical manifestations, laboratory investigations, as well as disease activity, are explained in Table 2.

A significant relation was detected between IFN-α serum levels and age as well as disease duration (P=0.019; P=0.035) respectively. All the clinical manifestations, laboratory investigations showed an insignificant correlation with IFN-α serum levels so do the SLE disease activity (SLEDAI).

Table 2: IFN-α serum levels correlated with demographic, clinical manifestations, laboratory investigations as well as disease activity.

Parameter	IFN-α in SLE patients (N=100)	
	r	p
Malar rash	0.187	0.152
Oral ulcers	0.090	0.494
Arthritis	-0.003	0.980
Neurologic disorder	-0.820	0.534
Age	-0.302	**0.019***
Age groups	0.143	0.277
Disease duration	-0.273	**0.035***
Family History	-0.198	0.130
SLEDAI-2K	0.062	0.637
Proteinuria	0.192	0.142
WBC	-0.019	0.884
HB	0.070	0.593
PLT	-0.070	0.593
ESR	-0.093	0.478
Urea	0.070	0.593
Creatinine	-0.102	0.438
ANA	0.020	0.882

r: Pearson correlation, *Correlation is significant at the 0.05 level (2-tailed).

The patients in the current study, those with malar rash, oral ulcers, arthritis and neurologic disorder had an insignificant correlation with IFN-α serum levels that agree with the findings of Oke and his colleagues [35]. Several previous investigators demonstrate that mucocutaneous (malar rash) associates with high type I IFN activity in vitro and with high levels of circulating IFN-α [36], suggesting that this subgroup might benefit from IFN-α blocking agents [37]. Besides, they reported that high IFN activity was observed among patients with the following clinical manifestations such as arthritis, low complement, carrying anti-dsDNA, and anti-Sm or antiRo60 antibodies [35]. The IFN-α Serum levels significantly correlated with age. The study by Niewold et al. found a significant correlation especially in those with age ranged between 16-50 years which represented the adult-onset, who suggested that this pattern of age-related to IFN-α may contribute to the increased incidence rate of SLE disease in early adulthood [38]. The IFN-α serum levels were not associated with SLEDAI. This result goes with what was reported formerly, that IFN-α had no role with the disease activity [31]. Contrast results were found in various studies that reported the significant association between IFN-α serum levels and SLEDAI [39-41]. The laboratory investigations in total showed an insignificant correlation with IFN-α, similar conflicting results were described [31, 33]. The negative correlation observed in the current study between hematological parameters that include WBCs count, Hb level and IFN-α, may provide further evidence for the suggestion said leucopenia and anemia could be connected to chemokines [42] and adhesion particles that drive lymphocytes out of the vascular space and increase leukocytes adhesion to vessel wall [43]. Other studies suggest a possible suggestion is that IFN-α could act as an inhibitory molecule that inhibits B-cell lymphopoiesis that occurs in the bone marrow [33]. The present study finds that ANA positivity reaches 90% in SLR patients;

an observation that also has been made in many studies, few studies reported a 100% frequency while the more common demonstrate a lower number from 95% to 99% although lower frequency of ANA positivity is usual in cross sectional studies [44, 45]. Previous Iraqi studies showed a significant variation in ANA with a wide range of frequency variations of 98% [46] and 93% [47].

Conclusions

The results suggest a risk effect for female gender and age in etiology of SLE. IFN-α could not be considered as biomarker or to has a risk effect in SLE patients or perpetuate the disease activity. No evidence for any correlation between the IFN-α serum level and any clinical manifestations or laboratory investigation of the disease in current study except for age and disease duration, which suggests them as a risk factor for increasing the IFN-α serum level.

Ethical Clearance

This research was ethically approved by the Research Ethical Committees of the Ministry of Environmental and Health and the Ministry of Higher Education and Scientific Research, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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