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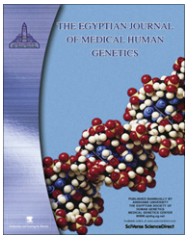

ORIGINAL ARTICLE

Serum interferon-alpha level in first degree relatives of systemic lupus erythematosus patients: Correlation with autoantibodies titers

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KEYWORDS

Interferon-α;
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Abstract *Background and objectives:* Interferon-α (IFN-α), a cytokine with both antiviral and immune-regulatory functions, was suggested as a useful tool which can evaluate current systemic lupus erythematosus (SLE) disease activity and identify patients who are at risk of future disease flares. In the current study, serum IFN-α levels and associated demographic, and serological features in Egyptian SLE patients and their first degree relatives (FDRs) in comparison to unrelated healthy controls (UHCs) were examined, in order to identify individuals at the greatest risk for clinical illness.

Abbreviations: IFN-α, interferon-α; SLE, systemic lupus erythematosus; FDRs, first degree relatives; UHCs, unrelated healthy controls; DCs, dendtritic cells; ANA, antinuclear antibodies; ds DNA, anti double stranded DNA; ACR, American College of Rheumatology; ILE, incomplete lupus erythematosus; ELISA, enzyme linked immune sorbent assay; Hb, hemoglobin; WBCs, white blood cell count; ESR, erythrocyte sedimentation rate; Ig, immunoglobulin.

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Methods: In a cross-sectional study, blood samples were drawn from 54 SLE patients, 93 of their FDRs who consented to enroll into the study and 76 UHCs. Measurement of serum IFN-α by a modified ELISA was carried out. Data were analyzed for associations of serum IFN-α levels with autoantibodies titer.

Results: Mean serum IFN-α in FDRs was statistically higher than the UHCs and lower than in SLE patients ($P < 0.0001$) and it was correlated with ANA titer ($r = 0.6$, $P < 0.0001$) and anti ds DNA titer ($r = 0.62$, $P < 0.0001$).

Conclusion: IFN-α is a crucial player in the complicated autoimmune changes that occur in SLE and serum IFN-α can be a useful marker identifying persons who are at risk of future disease development.

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by wide spread immunologic abnormalities and multiple organ involvement [1]. The precise pathogenesis of SLE remains unknown, however, genetic factors in the presence of a permissive environment are involved [2]. One of the factors suggested to play a role in the pathogenesis of SLE is interferon-α (IFN-α), a cytokine with both antiviral and immune-regulatory functions [3], which has been found in increased levels in SLE patients [4–9]. The potential role of this cytokine in SLE development is further suggested by the finding that SLE patients produce IFN-α in an abated fashion and this cytokine induces monocytes to mature into dendritic cells (DCs) [10]; a key regulator of antigen presentation.

A causative role for IFN-α in SLE is suggested more directly by the observation that IFN-α therapy in patients with nonautoimmune disorders, induced autoantibody production, including antinuclear antibodies (ANA) and anti double stranded DNA (ds DNA), and occasionally also a lupus-like syndrome [11–13] which typically resolved after IFN-α therapy discontinuation [13,14]. Furthermore, IFN-α administration frequently lead to other autoimmune disorders such as immune mediated thyroid diseases [15], type I diabetes mellitus [16], multiple sclerosis and inflammatory arthritis [17].

SLE is a familial disease and SLE family members have higher susceptibility to develop SLE and non-SLE autoimmune disorders [18,19], and showed more prevalent autoantibodies than the unrelated healthy population [20–22]. Abnormally high levels of serum IFN-α were frequently found in healthy first degree relatives (FDRs) of SLE patients [23]. Advances in genetics, immunology and environmental epidemiology suggest that early or even preclinical identification of individuals at high risk to evolve into lupus may be feasible [20,24,25].

In the current study, we examined serum IFN-α levels and associated demographic, immunologic and laboratorial features in Egyptian SLE patients and their FDRs in comparison to unrelated healthy controls (UHCs) in order to identify individuals at the greatest risk for clinical illness.

2. Patients and methods

2.1. Study design

In a cross-sectional analysis, SLE patients, who were consecutively seen in, or referred to the Rheumatology and Immunology clinics – Mansoura University Hospital and Mansoura University Children's Hospital between September 2009–2010 were invited to participate if they had at least one sibling who or their guardians consented to enroll in the study.

The study was approved by the Ethical Committee of Mansoura School of Medicine and informed consent was obtained from every study participant or their guardians. All the clinical data were assessed at the time of blood donation and all the demographic, clinical, and serological characteristics of the studied subjects were evaluated and recorded by a rheumatologist.

2.2. Study population

The studied subjects were divided into three categories:

(1) SLE patients: All included lupus patients fulfilled the 1997 American College of Rheumatology (ACR) revised criteria for SLE Classification [26]. No more criteria were required for patients' enrollment.
(2) First-degree relatives (FDRs): Defined as brothers and sisters of included SLE patients who or their guardians provided informed consent. They underwent thorough clinical examination to screen for autoimmune diseases. FDRs having at least one but fewer than four of the criteria for SLE were defined as incomplete lupus erythematosus (ILE) [27].
(3) Unrelated healthy controls (UHCs): They were recruited from medical students, nursing staff, healthy relatives of non autoimmune disease patients. All the controls were subjected to a thorough history taking and clinical examination to ascertain the absence of any personal or family history of autoimmune disease.

2.3. Exclusion criteria

Individuals with a personal history of malignancy, recent infection, viral hepatitis or treatment by IFN-α were not eligible for the present study.

2.4. Laboratorial analysis

From each patient 5 ml venous blood was collected, one milliliter was withdrawn into plastic tube containing the dipotassium salt of EDTA at a final concentration of 1.2 mg EDTA/ml venous blood which was used for performing CBC using a cell counter (Sysmex K× 20), 2 ml was added into a tube containing trisodium citrate for perform-

Table 1 General features of studied population.

	SLE patients (n = 54)	FDRs (n = 93)	UHCs (n = 76)	P for ANOVA*	Scheffe post hoc test	
					P value patients vs. FDRs	P value FDRs vs. UHCs
Age/in years (mean ± SD)						
Total participants	21.2 ± 11.1	19.5 ± 9.8	20.9 ± 11.8	ns		
Adults	29.5 ± 9.4	28.3 ± 6.1	31.6 ± 8	ns		
Pediatrics	12.2 ± 2.9	11.3 ± 3.5		ns		
Gender n (%)						
Female	44 (81.5%)	63 (67.7%)	48 (63.2%)			
Male	10 (18.5%)	30 (32.3%)	28 (36.8%)			
Age category n (%)						
Adults	28 (51.9%)	45 (48.4%)	36 (47.4%)			
Pediatrics	26 (48.1%)	48 (51.6%)	40 (52.6%)			
ILE	–	18	–			
Hb	11.3 ± 1.9	13.3 ± 1.6	13.5 ± 1.6	<0.0001	<0.0001	ns
WBCs	4.6 ± 1.6	4.9 ± 1.1	6 ± .8	<0.0001	<0.0001	ns
Platelet	262.3 ± 107.3				342.6 ± 96.2	374.3 ± 119.9
<0.0001	ns	0.001				
ESR	71.6 ± 35.2	14.5 ± 3.6	12.9 ± 2.6	<0.0001	<0.0001	ns

P significant <0.05.
ns = non significant.
FDRs = first degree relatives, ILE = incomplete lupus erythematosus, UHCs = unrelated healthy controls, Hb = hemoglobin, WBCs = white blood cell count, ESR = erythrocyte sedimentation rate, ANA = antinuclear antibody, dsDNA = double stranded DNA.
* One way ANOVA.

ing ESR in a ratio of one volume citrate to four volume blood.

Two ml was collected into clean dry plastic tube and allowed to clot, the yielded serum was used for performing ANA assay using ANA serum enzyme immune assay kit (BinDazyme, Birmingham, UK), double stranded DNA antibody assay using enzyme linked immune sorbent assay (ELISA) (Immulisa, Immco Diagnostic, NY, USA) and serum level of IFN-α which was measured using an enzyme linked immuno sorbent assay (ELISA) kit (Bender, Medsystems, Vienna, Austria). To neutralize heterotrophile antibodies and avoid false-positive levels of IFN-α, a modified protocol described by Aly et al. [28] by adding 5% mouse serum to the assay buffer was used in the current assay.

2.5. Statistical analysis

Data were analyzed using SPSS (version 17). Data were expressed as mean ± SD and frequencies. Differences between continuous variables were analyzed by t-test and one way ANOVA followed by Tukey's post hoc test to determine significance between groups. Dichotomous variables were analyzed by ^{2} and Fisher's exact test whenever applicable. P values <0.05 were considered significant.

3. Results

3.1. General characteristics of studied population

Demographic features and laboratorial characteristics of the included participants were given in Tables 1 and 2. The study population consisted of fifty-four SLE patients, ninety-three FDRs and seventy-six UHCs with mean age of (by years) 21.2 ± 11.1, 19.5 ± 9.8and 20.9 ± 11.8, respectively. Meanwhile, females represented 81.5% of SLE patients, 67.7% of FDRs and 63.2% of UHCs, adult population consisted of 51.9% of SLE patients, 48.4% of FDRs and 47.4% of UHCs. Table 1. However, 33.3% of the ILE (n = 18) subset of FDRs were adults and 66.7% were pediatrics and 52% of the asymptomatic FDRs (n = 75) were adults and 48% were pediatrics the difference was not statistically significant.

The mean hemoglobin levels were comparable in the studied groups. SLE patients and FDRs had significantly lower WBCs than did the UHCs and only SLE patients showed significantly higher ESR and lower platelet count as compared with FDRs and UHCs. (Table 1).

3.2. Serum INF-α

The mean levels of IFN-α were 65.3 ± 53.4 pg/ml for SLE patients, 19.5 ± 23 pg/ml for FDRs and 5.2 ± 5.2 pg/ml for UHCs as shown in Fig. 1. The difference was statistically significant by ANOVA (P < 0.0001). Tukey's post hoc test revealed that the difference between patients and FDRs was statistically significant (P < 0.0001 with a 95% CI 33.6–58) and the difference between FDRs and UHCs was found to be statistically significant (P = 0.007 with a CI of 3.3–25.4) (Fig. 1).

Within the subset of FDRs, the mean serum IFN-α in FDRs with ILE (53.8 ± 31.4 pg/ml) was significantly higher than in asymptomatic FDRs (11.3 ± 8.4 pg/ml) (P < 0.0001) and the asymptomatic FDRs had a significant higher mean serum IFN-α than UHCs (P < 0.0001). (Table 2).

Table 2 Characteristics of FDRs subsets in comparison to unrelated healthy controls.

	UHCs (n = 76)	FDRs (n = 93)		p for ANOVA	Scheffe post hoc test	
		Asymptomatic FDRs (n = 75)	FDRs with ILE (n = 18)		p^1	p^2
Age (in years)		20.9 ± 11.8	20.3 ± 10	16.2 ± 8.6	ns	–
–						
Hb	13.5 ± 1.6	13.6 ± 1.6	12 ± 1.4	<0.0001	ns	
<0.0001	WBCs	5.6 ± .8	5.1 ± 1.1	4.3 ± .7	<0.0001	
<0.0001	<0.0001					
Platelet		374.3 ± 119.9	343.2 ± 104.2		339.8 ± 52.1	0.04
ns	ns					
ESR	12.9 ± 2.6	13.5 ± 3	18.5 ± 2.8	<0.0001	ns	
<0.0001	ANA	(mean ± SD) (IU/ml)	6 ± 4.9	10 ± 6.7	24.2 ± 2.8	
<0.0001	<0.0001	<0.0001				
Anti ds DNA (mean ± SD) (IU/ml)	11.2 ± 9.9	17.4 ± 10.3	37.7 ± 6.4	<0.0001	<0.0001	
<0.0001	IFN-α	(mean ± SD) (pg/ml)	5.2 ± 5.2	11.3 ± 8.4	53.8 ± 31.4	
<0.0001	<0.0001	<0.0001				

p Significant <0.05.
p^1 = p Value for asymptomatic first degree relatives vs. unrelated healthy controls.
p^2 = p Value for first degree relatives with incomplete lupus vs. asymptomatic first degree relatives.
ns = non significant.
IFN-α = interferon-α, FDRs = first degree relatives, ILE = incomplete lupus erythematosus, UHCs = unrelated healthy controls, Hb = hemoglobin, WBCs = white blood cell count, ESR = erythrocyte sedimentation rate, ANA = antinuclear antibody, ds DNA = double stranded DNA.

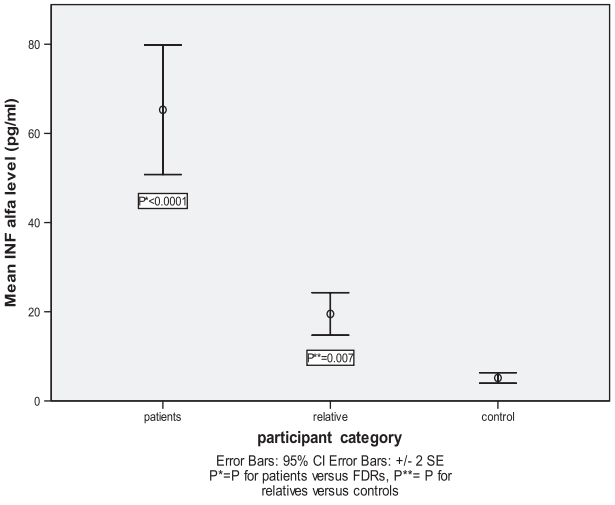

Figure 1 Mean serum INF-alpha levels in SLE patients, their first degree relatives and healthy unrelated controls (ANOVA P < 0.0001).

3.3. Autoantibodies

1) *Antinuclear antibody (ANA)*: The overall mean ANA titer in SLE patients (66.2 ± 26.5 IU/ml) was higher than in FDRs (12.7 ± 8.3 IU/ml) or the UHCs (6 ± 4.9 IU/ml), the difference was significant between the three groups (P < 0.0001) (Fig. 2). Further analysis was carried out and the difference between patients and FDRs (P < 0.0001, 95% CI 47.7–59.3), on one side, and FDRs and UHCs, on the other side, (P = 0.008, 95% CI 1.4–11.9) were statistically significant. (Fig. 2). Similarly FDRs with ILE showed higher mean ANA titre (24.2 ± 2.8 IU/ml) than in asymptomatic FDRs (10 ± 6.7 IU/ml) or the UHCs (6 ± 4.9 IU/ml) (P < 0.0001) (Table 2).
2) *Anti ds DNA antibody (ds DNA)*: Although FDRs in the current study showed significantly lower anti ds DNA (21.3 ± 12.6 IU/ml) as compared to SLE patients (107.2 ± 34.2 IU/ml) (P < 0.0001, 95% CI 78–93.8), they had higher overall mean levels as compared with UHCs (11.2 ± 9.9) (P = 0.003, 95% CI 3–17.3) (Fig. 3). Further analysis of FDRs subgroups and UHCs, a rising anti ds DNA titer was observed; starting by 11.2 ± 9.9 in UHCs to 17.4 ± 10.3 in asymptomatic FDRs to 37.7 ± 6.4 in FDRs with ILE. The difference was statistically significant, P < 0.0001 (Table 2).

3.4. Pearson's correlations between serum IFN-α levels and participants characteristics

FDRs revealed, On one hand, a significant negative correlations between serum IFN-α levels and FDRS age (r = −0.34, P = 0.001), mean hemoglobin (r = −0.5, P < 0.0001), and

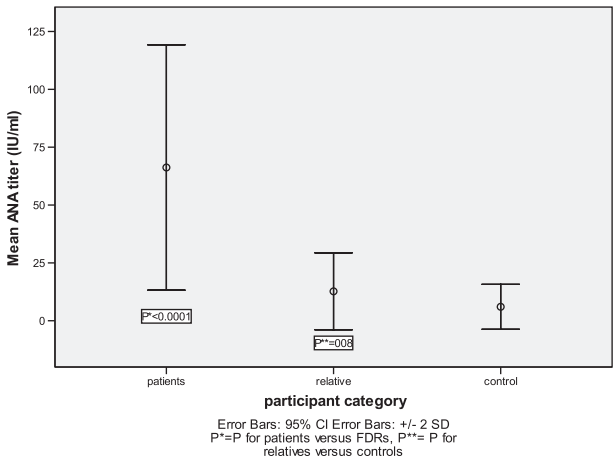

Figure 2 Mean serum ANA titer in SLE patients, their first degree healthy unrelated controls (ANOVA $P < 0.0001$).

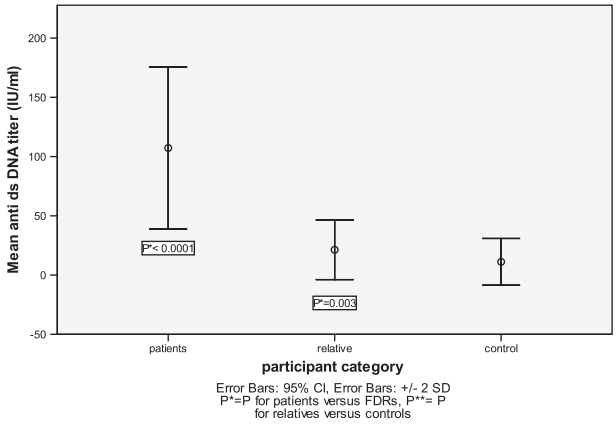

Figure 3 Mean serum anti ds DNA titer in SLE patients, their first degree relatives and healthy unrelated controls (ANOVA $P < 0.0001$).

WBCs ($r = -0.3$, $P = 0.006$), and on the other hand, it was positively correlated with ESR ($r = 0.5$, $P < 0.0001$), ANA titer ($r = 0.6$, $P < 0.0001$), and anti ds DNA ($r = 0.62$, $P < 0.0001$) (Table 3). Furthermore, a nearly similar pattern of correlations was observed in the total participants (Table 3).

4. Discussion

Detecting autoimmune disease in early or preclinical stages is clinically important because the institution of treatment prior to the onset of organ damage has a greater chance to ameliorate or even cure the disease [29]. Lately, much insight has been gained regarding IFN-α as a causal factor for SLE, and was suggested as a useful tool which can evaluate current disease activity and identify patients who are at risk of future disease flares [30]. Even though preclinical identification of individuals at risk of SLE might be feasible [11,20,24,31], the development of quantitative SLE risk profile is still deficient [24].

Many previous investigators [4–9] observed increased levels and activity of IFN-α in SLE patients. This was consistent with the findings in the present study. More interestingly, FDRs in the current study had significant higher IFN-α than UHCs. Given that SLE family members share the same genetic background with their SLE relative patients [19,32] and that serum IFN-α activity was estimated to have a broad-sense heritability of 48% in SLE families [23]; FDRs of SLE patients may probably produce IFN-α in an abated fashion as in SLE patients [3]. Indeed, SLE blood represented a dendritic cell (a key controller of immunity, tolerance, and IFN-α production) inducing environment [10,33]. It was found that the serum of SLE patients contains an IFN-α inducing factors that induce the production of large quantities of IFN-α in normal blood leukocytes in vitro [34,35].

Further investigations of this IFN-α inducing factor in SLE revealed that immune complexes containing nucleic autoantibodies were essential for the induction of expression of IFN-α [14]. In agreement with these observations, autoantibodies titers and serum IFN-α, in the current study, clearly showed stepwise increase in the studied groups going from UHCs to asymptomatic FDRs to FDRs with ILE to SLE patients. Similar findings were recently reported by Li et al. [24]. They showed that IFN-α genes expression were the specificities that most clearly showed stepwise increase in their studied groups starting by low ANA healthy controls, through high ANA healthy controls and ending by SLE patients. Data from earlier studies on auto-antibodies profile of FDRs of SLE patients [20,36] were in accordance to the data from the present study, indicating a familial basis to mount an immune response.

Previous investigators suggested at least three phases in the development of SLE autoimmunity [37,38]; normal immunity progresses to benign autoimmunity through the influence of genetic composition and environment. Later, benign autoimmunity progresses to pathogenic autoimmunity. Symptoms of clinical illness appear soon after pathogenic autoimmunity develops. Our serologic and clinical findings along with their strong association with serum IFN-α levels, in the current study, further supported a crescendo of autoimmunity culminating in clinical illness. First, the UHCs in the present study represented the phase of normal immunity, and the asymptomatic FDRs represented the phase of benign autoimmunity. Finally, the FDRs with ILE, in the present study, symbolized the third pathogenic autoimmunity phase, that is marked by the presence of more ominous autoantibodies and inflammatory markers.

In-depth reading of the strong association between IFN-α levels and autoantibodies titer in the current study further indicated an essential role for IFN-α in the development of SLE. Our results could be explained by the hypothesis formulated by RÖnnblom and Alm [3] in which type I IFN system is crucial to the development of SLE in two distinct phases. Initially, autoantibodies against nucleic acids and associated proteins are generated during infections (exogenous IFN-α inducers). In the second phase, immune complexes form and act as endogenous IFN-α inducers, which sustain the autoimmune process by prolonging the production of IFN-α. Furthermore, persistently elevated IFN-α might promote autoantibody class switch from immunoglobulin (Ig) M to the more pathogenic IgG class [39]. This proposed mechanism will operate in the genetically predisposed individuals, such as SLE family members, to develop SLE. In accordance to the results of the present work, data from previous studies showed a good

Table 3 Pearson's correlations between serum IFN-α levels and characteristics of study participants, FDRs and asymptomatic FDRs subset.

	INF-α in total participants (n = 223)		INF-α in total FDRs (n = 93)		INF-α in asymptomatic FDRs (n = 75)	
	r	p	r	p	r	p
Age	−0.05	ns	−0.34	0.001	−0.1	ns
Hb	−0.5	< 0.0001	−0.5	< 0.0001	−0.3	0.02
WBCs	−0.36	< 0.0001	−0.3	0.006	−0.05	ns
Platelet	0.3	< 0.0001	−0.1	ns	−0.2	ns
ESR	0.77	< 0.0001	0.5	< 0.0001	0.6	< 0.0001
ANA	0.75	< 0.0001	0.6	< 0.0001	0.2	0.05
Anti dsDNA	0.77	< 0.0001	0.62	< 0.0001	0.4	0.001

p significant < 0.05.
ns = non significant.
IFN-α = interferon-α, FDRs = first degree relatives, Hb = hemoglobin, WBCs = white blood cell count, ESR = erythrocyte sedimentation rate, ANA = antinuclear antibody, ds DNA = double stranded DNA.

correlation between IFN-α level and/or activity and autoantibody titer and/or profiles in SLE patients [6,8,9,40] and individuals with ILE [39,41]. On the other hand, Niewold et al. [23] reported no relation between IFN-α activity and ANA in their studied healthy family members, and hypothesized that IFN-α is an independent primary risk factor that might be familial. This contrast between our results and Niewold's may be related to different laboratory methods used for assessment of IFN-α. Niewold et al. [23] developed a functional bioassay to measure the ability of patients' sera to cause IFN induced gene expression. However, in the current work, a modified ELISA, in which 5% mouse serum was added to the assay buffer to adsorb the heterophile antibodies and improve the specificity as recommended by Aly et al. [28], was used for direct estimation of serum IFN-α levels.

The clinical observation that patients with active SLE are often leucopenic may be related to chemokines [42] and adhesion molecules [43] driving lymphocytes out of the vascular space and increasing leukocytes adhesion to vessel wall. Bauer et al. [40,44] demonstrated that up to 12 chemokines were upregulated in the serum of SLE patients, and most of these chemokines were inducible by IFN-α. The negative correlation between WBCs and IFN-α levels observed in the current work lends further support for this idea. Another possible explanation is that IFN-α, itself, has an inhibitory effect on B cell lymphopoiesis in bone marrow [45,46]. The current observed correlation between IFN-α and clinical laboratory tests such as elevated ESR, lower leucocytic count, and lower platelet count was supported by a number of reports in the literature [6,8,9,41,47].

The finding that the expression of type I IFN signature was almost universal in pediatric SLE patients [48] had shed light on the inverse correlation, found in the current study, between serum IFN-α and the age of the participants, and explained, even though not statistically significant, the relatively higher number of the pediatric ILE as compared to the adult ILE. A similar inverse correlation in female SLE patients and their healthy female FDRs was also reported [49].

The relatively high percentage of ILE cases among FDRs in the present study could be explained on the basis that the relatives of patients with lupus became more aware by the disease than other population and seek medical consultation on suspicion, thus facilitating early diagnosis. Another possible explanation is that siblings who share a common genetic predisposition to SLE tend to reach the threshold for manifestation of SLE within similar time period during which accumulated environmental factors played a role [50].

This study was limited by the cross-sectional design which does not enable follow up of longitudinal changes that evolve over time for further interpretation of the significance of IFN-α in predicting those individuals who evolve into the full clinical lupus.

In conclusion, our results supported that IFN-α is a crucial player in the complicated autoimmune changes that occur in SLE, and it can be a useful marker identifying persons who are at risk of future disease development. Furthermore, down-regulation of IFN-α could be a therapeutic or possibly even preventive approach, reducing the overall SLE disease burden and preventing end organ damage.

Authors contributions

All the authors shared the study design, data collection and literature research. DS assembled the patients' cohorts, wrote the initial version of the manuscript and critically revised the final version of the manuscript and she was responsible for the statistical analysis of the clinical results. AMR, AKH, AAS, SM, coordinated patient recruitment and clinical data collection and revised the manuscript. NAS and RMF were responsible for performing the biochemical analyses and helped in writing the initial version of the manuscript.

Disclosure

All the authors of this paper report no conflicts of interest.

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