VER
Vascular & Endovascular Review

Elevated IFN-λ and IL-28R Serum Levels and Upregulated IFNL3/IFNLR1 Gene Expression in Iraqi Women with Systemic Lupus Erythematosus

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a chronic inflammatory disease characterized by complex interactions of cytokines that are important mediators in the pathogenesis of SLE. However, the important roles of IFNL3 and IFNLR1 in immune responses and their correlation with gene expression levels and serum levels of SLE patients have been identified.
Aim of the study: investigated serum IFN-λ and IL-28 levels along with IFNL3/IFNLR1 gene expression in Iraqi women with SLE to determine their association with disease activity .
Materials and Methods: The study comprised of 90 participants (60 SLE female patients aged 20-40 years, in addition to 30 healthy females (control group)) , all patients were seen at Medical City (Consultants of Arthritis, Consultant of Dermatology, Lobby of Hematology and Arthritis)/Baghdad Teaching Hospital, with data collected between 1 December 2024- 1 March 2025. The samples were diagnosed by the consultant and the immunology unit in the previously described hospitals according to their protocols . The 60 patients were divided into two groups. Group 2 (G2) consisted of 30 newly diagnosed patients, while Group 3 (G3) included 30 patients who were being treated with hydroxychloroquine 200 mg and prednisolone 5 mg twice daily. The control group consisted of 30 healthy females (G1). 5ml peripheral blood samples were withdrawn from all participants divided into two parts .4.5ml for serological study which performed by elisa assay. Where as , 0.5 ml mixed with TRIzol™ Reagent to extract mRNA and to determine the level of gene expression
Result: Newly diagnosed SLE patients (G2) showed a significantly elevated serum IFN-λ and IL-28R levels versus healthy controls (G1) and treated patients (G3) ($p<0.001$), with both markers exhibiting high diagnostic accuracy (AUCs 0.99 & 0.96). IFNL3 and IFNLR1 gene expression was also significantly upregulated in G2 compared to G1 and G3; IFNL3 expression itself had high diagnostic value (AUC 0.96). While IFNL3 and IFNLR1 expression correlated strongly, serum cytokine levels did not correlate with their corresponding gene expression.
Conclusion: In newly diagnosed SLE patients (G2), serum IFN-λ and IL-28R levels were much higher than those present in treated patients (G3) and controls (G1), with these serum biomarkers normalizing after appropriate treatment. Both of these showed very high diagnostic accuracy (AUC 0.99 and 0.96, respectively), and gene expression of IFNL3 and IFNLR1 was upregulated in newly diagnosed SLE patients (G2) vs both age matched treated SLE patients (G3) and controls (G1). Finally, to note, BMI had no significant effect on serum IFN-λ or IL-28R levels in any patient group (G1, G2, G3) respectively. However, significant differences ($p<0.001$) were found across these three patient groups for both biomarkers across each age bracket.

KEYWORDS: IFNL3 , IFNLR1 , gene expression, Iraqi women with SLE.

How to Cite: Mohammed Amer Kamil1, Hazima Mossa AlAbassi2, (2025) Elevated IFN-λ and IL-28R Serum Levels and Upregulated IFNL3/IFNLR1 Gene Expression in Iraqi Women with Systemic Lupus Erythematosus, Vascular and Endovascular Review, Vol.8, No.3, 9-17.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune condition marked by the body's production of autoantibodies and systemic inflammation that can impact various organ systems, (1). As a multisystem immune-mediated disease, SLE presents a wide range of clinical phenomena at its onset (2). Recent data suggest that the development of SLE is attributable to the interplay of genetic background and environmental exposure, which alter immune regulation and self-tolerance to produce autoantibodies, leading to widespread inflammatory injury (3). SLE refers to a relatively wide spectrum of autoimmune disease with heterogeneous clinical features inclusive of serositis, which denotes inflammation of the serous membrane and may be localized to pericarditis, pleuritis, or peritonitis (4, 5).

Typically, the disease has a relapsing-remitting pattern ranging from minimal symptoms to life-threatening effects. According to (6), SLE is most common in women, demonstrate the highest occurrence rates in females aged 15 to 44. This disease has

multiorgan involvement, and kidney impairment and central nervous system dysfunction are especially troublesome (7). Additionally, treatment-related complications and active disease may lead to a progressive injury to important organs for patients (8).

Interferons (IFNs) are key cytokines in the immune response and are used to set up a robust antiviral response. They are divided into three main classes based on their structural characteristics, receptor complexes and biological function, type I, type II, and type III (9). Early in 2003, a large advance in interferon research occurred with the identification and characterization of the interferon lambda (IFN-λ) family. The IFN-λ family has three genes that encode closely related proteins: IFN-λ1, which is interleukin-29 (IL-29), IFN-λ2 which is IL-28A, and IFN-λ3 which is IL-28B (10). Collectively this group of proteins makes up the type III interferons. It is interesting to note that type III interferons (IFN-λ) activate similar intracellular signaling pathways and exhibit many biological activities, including anti-autoimmune properties in many different target cells (11).

The synthesis of IFN-λ is provoked in the milieu of viral infections, and in the context of autoimmune diseases, thus being part of the overall immune process. As a result, these expression patterns and biological effects of type III IFNs (IFN-λs) mimic that of type I IFNs (12). In addition, IFN-λ receptors are primarily located on epithelial cells, which further suggests the clinical promise of IFN-λ as a potential therapy for both antiviral diseases and autoimmune diseases (13, 14).

Interferons are critical to inducing immune aberrancy, facilitating autoantibody and immune complex production and tissue damage. Recent studies have illustrated that systemic lupus erythematosus (SLE) is characterized by variation of type I and type III interferons. Multiple studies(15, 16) found higher IFNλ1 and IFNλ3 levels in serum from SLE patients than healthy controls. Reduced IFNL1 transcripts were also observed from SLE peripheral blood mononuclear cells (PBMCs) and increased IFNL2 and IFNL3 transcripts in activated CD4+ T cells (17, 18). Higher serum concentrations of IFNλ were found correlating with more severe clinical manifestations and laboratory findings, such as increased anti-double-stranded DNA (dsDNA) autoantibodies and SLE Disease Activity Index scores(19, 20). Higher levels of IFNλ have also been associated with varying manifestations of the disease, from arthritis to nephritis, serositis to skin symptoms (15, 21).

Interestingly, one case study showed that during clinical remission, serum levels of IFNλ1 significantly decreased after treatment with glucocorticoids and hydroxychloroquine (22, 23). IFNλ signaling inhibition through IFNLR1 blockade is expected to reduce autoimmune pathology in SLE by decreasing Th1/Th17 polarization, autoantibody production, and end-organ damage.

The objective of this study was to assess serum concentrations of interferon lambda (IFN-λ) and interleukin-28 (IL-28), while also examining the expression patterns of the IFNL3 and IFNLR1 genes, in Iraqi women with systemic lupus erythematosus (SLE).

MATERIAL & METHODS

The present research study involves a sample of 90 female participants who were aged between 20 and 40 years. The sample included 60 female patients diagnosed with systemic lupus erythematosus (SLE) and 30 female healthy individuals. The participants were recruited from Medical City, namely from the departments of Arthritis, Dermatology, and Hematology and Arthritis lobby of Baghdad Teaching Hospital, from December 1, 2024 to March 1, 2024.

Consultants and the immunology unit made the diagnoses at the hospitals stated and followed the established protocols and guidelines from their documentation, (2019 EULAR/ACR SLE classification criteria). Of the SLE patient subgroup, 30 was de novo diagnosed (untreated) - Group 2 (G2) and 30 was treated {hydroxychloroquin 200mg , predneslone 5mg (twice daily)} - Group 3 (G3). The control group was comprised of 30 healthy females - Group 1 (G1).

Participants who had existing diseases, specifically hypertension, cardiovascular disease, Type I or II diabetes, or other chronic autoimmune diseases, were excluded from this study. Data collection included the participation of names, age, residency, occupation, and the systematic administration via questionnaire. The study protocol received approval from the Scientific Research Committee of the Iraqi Ministry of Health.

Venous blood sample collection of 5 ml was performed for the control and patient groups using disposable medical syringes and sterilizing the area of withdrawal with 70% ethanol. Blood samples were divided into test tubes as follows: 4.5 ml of blood was placed for serum isolation to enable serological analysis. The serum was then centrifuged at 4000 revolutions per minute for 10 minutes and then stored at -20 °C for use in the ELISA serum for IFN-λ and IL-28R levels tested by commercial ELISA kits (MyBioSource) in accordance with relevant commercial protocol. Furthermore, 0.5 ml of whole blood was placed in separate tubes with equal volume of TRIzol™ Reagent. Gene expression of IFNL3 and IFNLR1 was assessed with SYBR Green-based qPCR using designed primers.

Table (1): The study's primers (gene expression)

Primer Name	Seq.	Annealing Temp. (ºC)
β-Globin-F	ACACAACTGTGTTCACTAGC	65
β-Globin-R	CAACTTCATCCACGTTCACC	
IFNLR1_exp-F2	TGGCAGTCAGCCCTAATA	
IFNLR1_exp-R2	GAACGTGTAGATGGTTCTGG	

IFNL3_exp-F	TCGCTTCTGCTGAAGGACTGCA	60
IFNL3_exp-R	CCTCCAGAACCTTCAGCGTCAG	

Statistical Analysis

Data analysis was performed with SPSS V.28 software; independent t-test and Anova One Way ANOVA (LSD) were utilized to calculate p values and significant differences between the highlighted groups as well as analyzing the correlations for the studied data via Pearson correlation coefficient. All data were presented as mean±S.D or standard deviation. A significance difference was considered if $p<0.05$.

RESULTS

Serological study

IFN-λ & IL-28R concentration

As indicated in Table (2), serum values of IFN-λ were statistically significantly increased in newly diagnosed SLE patients (G2: 112.37±38.10 pg/mL) compared to control (G1: 40.85±9.86 pg/mL) and treated patients (G3: 45.14±11.48 pg/mL) ($p < 0.001$). This is consistent with (24) reported earlier. On the contrary, IL-28R serum level observed a statically significant increase in newly diagnosed patients (G2) versus control (G1) and under treatment patients (G3), the result for 3 studied groups [G1, G2, G3] [0.52±0.25)(1.74±0.88)(0.62±0.22) ng/ml respectively (p-value < 0.001).We found this result agreed with (25) which referred to IL-28R level in SLE patients is a sensitive indicator to assess SLE disease activity and correspondingly, IL-28R might give B cell help and autoantibodies in patients. The result also shows that treatment group and control groups have very close IL-28R ratio, which is evidence that treatment group diminish IL-28R levels. This finding is in agreement with what recorded (26) who referred to the enhancing in the expression of IL-28R level was found in lupus peripheral blood.

Table (2): distribution of IFN-λ& *IL-28R* serum level among studied groups

Parameter	Groups	Concentration (Mean±S.D.)	LSD	P value
IFN-λ (pg/mL)	Control	40.85±9.86 a	12.15	<0.001**
	Newly Diagnosed patients	112.37±38.10 b		
	Under treatment patients	45.14±11.48 a		
Parameter	**Groups**	**Concentration (Mean±S.D.)**	**LSD**	**P value**
IL-28R (ng/mL)	Control	0.52±0.25 a	0.28	<0.001**
	Newly Diagnosed patients	1.74±0.88 b		
	Under treatment patients	0.62±0.22 a		

Different letters indicate a significant differences between the groups. Similar letters indicate non- significant differences

Receiver Operating Characteristics test for serology

The ROC analysis highlights the potential of IFN-λ and IL-28R as diagnostic biomarkers for SLE,. ROC and AUC (Area under the Curve is a measurement point used to determine how test can distinguish between patients and healthy individuals. According to the Table (3) and Figure (1), IFN- λ has a high sensitivity and high specificity the result was (97%,100%) P- value (≤0.001), AUC (0.99), cutoff (69.22). IL-28R has a high sensitivity and high specificity the result was (83%,96%) P value (<0.001), AUC (0.96), cutoff (1.06).

Table 3. ROC curve results for studied parameters in patients with SLE comparing with controls

Parameter	AUC	Cutoff	Sensitivity	Specificity	Asymptotic 95% Confidence Interval		P Value
					Lower Bound	Upper Bound	
IFN- λ	0.99	69.22	97%	100%	0.989	1.005	<0.001**
IL-28R	0.96	1.06	83%	96%	0.917	1.001	<0.001**

ROC curves for these biomarkers provide valuable insights into their diagnostic and prognostic roles in SLE. Higher specificity and sensitivity values indicate their potential utility in clinical practice. Continued research into their combined effects may improve the stratification of SLE patients and therapeutic strategies.

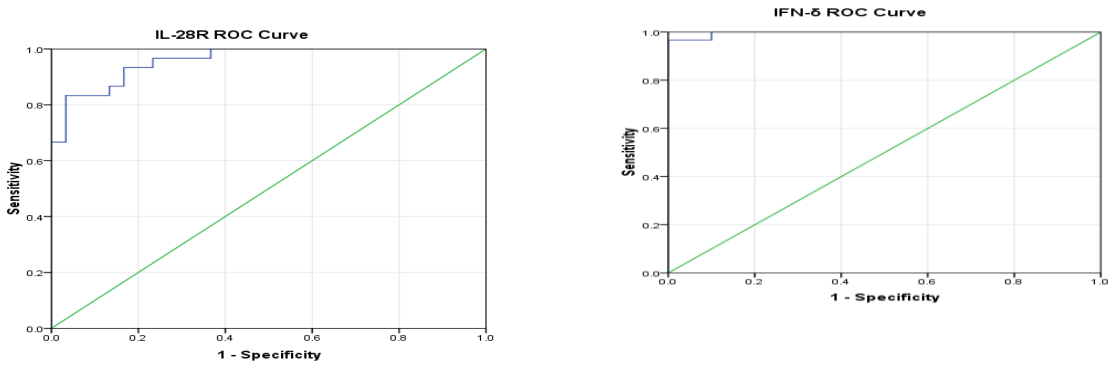

Figure 1. Model discrimination of the ROC curve and AUC of the predicting model

The Body Mass Index (BMI) and age

IFN-λ & IL-28R serum level distribution in studied groups based on BMI

As shown in Table (4) there was no significant difference on IFN-λ serum level between two BMI category (20-25 , 25.1-30) kg/m² for each studied groups (G1, G2, G3) .

The result fot two BMI category in G1was (39.94±9.08 , 41.54±10.64) kg/m² p-value <0.6 , as for G2 (105.11±36.86, 123.25±38.88) kg/m² p-value<0.2 and for G3 (45.75±13.77 , 44.54±9.08) kg/m² p-value<0.77 . where as an impact was seen for each BMI category among three studied groups with p-value<0.001.

As for IL-28R an identical manner was seen, BMI has no impact between two BMI groups in each studied groups (G1, G2, G3) , the result for two BMI groups in G1 was (0.53±0.26, 0.50±0.25) kg/m² p-value <0.7, for G2 was (1.84±0.84, 1.58±0.95) kg/m² p-value <0.4, and for G3 was (0.58±0.21, 0.67±0.23) kg/m² p-value<0.27 , while a significant difference was seen among studied groups for each BMI groups p-value(<0.001).

Table 4. IFN- λ and IL-28R serum level distribution in studied groups based on BMI

IFN- λ / BMI	Control (Mean±SD)	Newly diagnosed patients (Mean±SD)	Under Treatment patients (Mean±SD)	LSD	P value
20-25	39.94±9.08 a	105.11±36.86 b	45.75±13.77 a	19.06	<0.001**
25.1-30	41.54±10.64 a	123.25±38.88 b	44.54±9.08 a	17.10	<0.001**
LSD	7.39	28.75	8.72	-	
P value	0.6 NS	0.2 NS	0.77 NS		
IL-28R / BMI	Control (Mean±SD)	Newly diagnosed patients (Mean±SD)	Under Treatment patients (Mean±SD)	LSD	P value
20-25	0.53±0.26 a	1.84±0.84 b	0.58±0.21 a	0.43	<0.001**
25.1-30	0.50±0.25 a	1.58±0.95 b	0.67±0.23 a	0.41	<0.001**
LSD	0.19	0.7	0.17	-	
P value	0.7 NS	0.4 NS	0.27 NS		

Duncan's test: Different letters indicate a significant differences between the groups. Similar letters indicate non- significant differences

IFN-λ & IL-28R serum level distribution in studied groups based on age

As shown in table (5) Age has no impact on IFN-λ serum level between two age groups in each studied groups (G1, G2, G3) , the result for the age groups (20-30)and (31-40) years for control was (37.78±8.37 , 43.91±10.54) years with p-value<0.09 , for Newly diagnosed were (102.19±32.93 , 124.00±41.39) years with p-value<0.12 . and for under treatment were (46.09±7.98 , 44.51±13.50) years with p-value<0.72 . where as a significant was recorded among three studied groups with two age groups p-value(<0.001).

As for IL-28R an identical manner was seen, age has no impact between two age groups in each studied groups (G1, G2, G3) , the result for two age groups in G1 was (0.57±0.30 , 0.46±0.19) years p-value<0.5 , for G2 was (1.48±0.58 , 2.03±1.09) years p-value<0.1 , and for G3 was (0.66±0.23, 0.60±0.22) years p-value<0.5 , while a significant difference was seen among studied groups for each age groups p-value(<0.001).

Table 5. IFN-λ & IL-28R serum level distribution in studied groups based on age

IFN- λ / Age (Yrs.)	Control (Mean±SD)	Newly diagnosed patients (Mean±SD)	Under Treatment patients (Mean±SD)	LSD	P value
20-30	37.78±8.37 a	102.19±32.93 b	46.09±7.98 a	16.35	<0.001**
31-40	43.91±10.54 a	124.00±41.39 b	44.51±13.50 a	17.43	<0.001**
LSD	7.12	27.81	8.05	-	
P value	0.09 NS	0.12 NS	0.72 NS		
IL-28R / Age (Yrs.)	Control (Mean±SD)	Newly diagnosed patients (Mean±SD)	Under Treatment patients (Mean±SD)	LSD	P value
20-30	0.57±0.30 a	1.48±0.58 b	0.66±0.23 a	0.32	<0.001**
31-40	0.46±0.19 a	2.03±1.09 b	0.60±0.22 a	0.46	<0.001**
LSD	0.19	0.64	0.17	-	
P value	0.5 NS	0.1 NS	0.5 NS		

Different letters indicate a significant differences between the groups. Similar letters indicate non- significant differences, NS= Non-Significant

Molecular study

Gene Expression

Gene Expression for IFNL3

As shown in Table (6), the mean Ct of the IFNL3 gene for the control ,newly diagnosis ,under treatment respectively (30.66, 28.77,28.78), while the mean of Ct for β-Globin gene expression was (15.71, 15.83,14.85)respectively.

. In the present study, the β-Globin gene has been used as a standard gene for comparison in the IFNL3 . The present study found a significant upregulation of IFNL3 gene expression (5.93±3.01) in (G2) patients compared to (G1) and (G3).

The present study supports a previous study by (25) which found that SLE patients had beneficial changes in their IFNL3. Comparison may imply a link between increased IFNL3 and SLE patients (26). The study concluded that IFNL3 is linked to SLE, the lambda IFNs are cytokines that play a central role in immune host defense at endothelial and epithelial barriers.

IFNLs engage with their heterodimeric receptor, which has two subunits: IFNLR1 and interleukin (IL)10, and this receptor determines the cellular specificity of the cytokines effects. Recent data show that IFNL signaling affects CD4+ T cell differentiation and supports the formation of Th1 cells, and in fact this mechanism as resulted in the identification of IFNL as a potential therapeutic target for autoimmune diseases. In addition, IFNL induced immunomodulation in the innate and adaptive immune cells and in many cases led to expression of autoimmunity especially in the cases of SLE. However(27), the underlying mechanisms for these interactions could be special, especially considering the association between the mechanism of IFNL3 expression in SLE. Based on this findings and comparisons to the previous work, the expression of IFNL3 gene fold should be normal. Thus, the up regulation of IFNL3 in SLE patients, could become one of the sources of the abnormal system in SLE.

Table 6. Fold change of IFNL3 gene expression Depending on 2-ΔΔCt method

Groups	**Mean Ct of *IFNL3***	**Mean Ct of *B-Globin***	**ΔCt (Mean Ct of *IFNL3*- Mean Ct of *β-Globin*)**	**Mean ΔCt (*IFNL3* of control)**	**ΔΔCt**	**$2^{-\Delta\Delta ct}$**	**Fold of gene expression**	**Fold Change (Folding /control)**
Control	30.66	15.71	14.95	15.5	-0.55	1.45	1.45±1.12 a	1
Newly diagnosed patients	28.77	15.83	12.94	15.5	-2.57	5.93	5.93±3.01 b	2.54
Under treatment patients	28.78	14.85	13.93	15.5	-1.57	2.98	2.98±1.66 a	0.6
P value							<0.001	-

Duncan's test: Different letters indicate a significant differences between the groups. Similar letters indicate non- significant differences

Gene Expression for IFNLR1

As shown in Table (7), the mean of Ct for the *IFNLR1* gene for the control ,newly diagnosis ,under treatment respectively (27.76, 28.07, 27.63), while the mean of Ct for GAPDH gene expression for the control was 15.9 and for the G2,G3 was (17.56, 15.4) respectively

the *β-Globin* gene has been used as a standard gene for comparison in *IFNLR1* .The present study found a significant upregulation of *IFNLR1* gene expression (3.08±2.53) in G2 group compared to G1,G3 respectively (1.21±0.64 , 0.75±1.11). Numerous investigations have demonstrated that the upstream of *IFNLR1* may contribute to SLE through increased expression of *IFNLR1*. *IFNLR1* encodes IFNλ receptor 1, which binds IFNλ with high affinity and recruitsinterleukin-10 receptor β, a partner receptor

for IFNλ signaling (28).

Additionally, this finding parallels the report by (29), who stated that type III IFN signaling may have non redundant role in autoimmunity. The production of IFNλ is significantly greater at mucosal sites in epithelial and myeloid cells subsequent to a viral infection. In poly-IC induced lupus with Toll-like receptor 7 signaling, IFNλ cytokines levels were increased, and Ifnlr1 knock out mice exhibited decreased immune cell activation and organ damage without any affects on autoantibody production. The current finding suggests that these findings support IFNLR1 upregulation may be related to SLE and noted that this could be added to the evidence of additional factors in SLE patients.

Table 7. Fold change of *IFNLR1* gene expression Depending on 2-ΔΔCt method

Groups	**Mean Ct of *IFNLR1***	**Mean Ct of *B-Globin***	**ΔCt (Mean Ct of *IFNLR1*- Mean Ct of *β-Globin*)**	**Mean ΔCt (*IFNLR1*of control)**	**ΔΔCt**	**2⁻ΔΔct**	**Fold of gene expression**	**Fold Change (Folding/control)**
Control	27.76	15.9	11.86	12.14	-0.28	1.21	1.21±0.64 a	1
Newly diagnosed patients	28.07	17.56	10.51	12.14	-1.62	3.08	3.08±2.53 b	2.55
Under treatment patients	27.63	15.4	12.23	12.14	0.1	0.75	0.75±1.11 a	0.62
P value	-	-	-	-	-	-	0.001	
Duncan's test: Different letters indicate a significant differences between the groups. Similar letters indicate non- significant differences								

Quantitative Reverse Transcription PCR of β-Globin) (housekeeping Gene):

Because housekeeping genes exhibit stable expression in the cells or tissues being studied, they are utilized in molecular studies. β-Globin is one of the housekeeping genes most frequently used in gene expression data companions. Beta globin is a housekeeping gene that is used in molecular diagnosis to verify that the PCR conditions are optimum , to control amplification .The present study results show that therange of Ct value for β-Globin (reference gene) in the control group(G1) was (15.71,15.9) respectively ,while for the G2 (15.83,17.56) and G3 group, it was (14.85, 15.4) as shown in table (6)(7).

Receiver Operating Characteristics test for QPCR

Table (8) and Figure (2), IFNLR1 has a high sensitivity and high specificity the result was (60%,100%) P value (≤0.01), AUC (0.76), cutoff (2.71). IFNL3has a high sensitivity and high specificity the result was (100%,87%) P value (≤0.001), AUC (0.96), cutoff (2.53).

Table 8. ROC curve results for *IFNL3* & *IFNLR1* in patients with SLE

Parameter	AUC	cutoff	Sensitivity	Specificity	Asymptotic 95% Confidence Interval		P Value
					Lower Bound	Upper Bound	
IFNLR1	0.76	2.71	60%	100%	0.568	0.943	0.01*
IFNL3	0.96	2.53	100%	87%	0.906	1.023	<0.001**

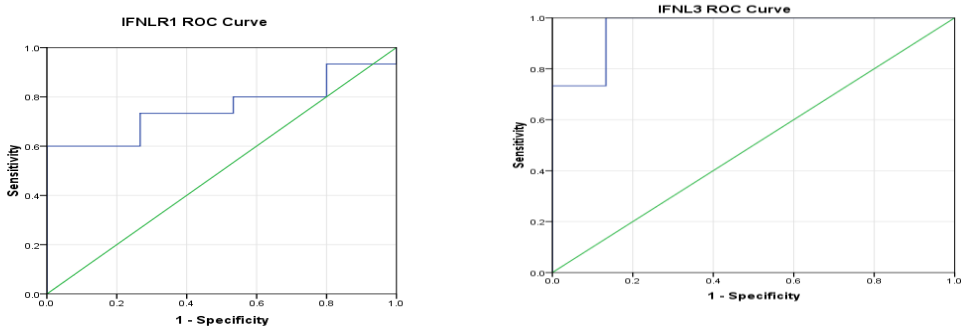

Figure 5. Model discrimination of the ROC curve and AUC of QPCR

Correlation between ELISA and Genes

Table (9) presents the correlation coefficient between the IFNL3 and IFNLR1 gene expressions and serum levels of the patient group. the data presented in table (9) indicated no significant correlation between IFNL3 and IFN-λ or IL-28R. Similarly, no significant correlation was found between IFNLR1 and IFN-λ or IL-28R. However, It should be noted that a significant positive relationship was also found among IFNLR1 and IFNL3.

Table 9. Correlation between ELISA and Genes

Parameters	Pearson Correlation coefficient (r)	P value
IFNLR1 & IFN-λ	0.106	0.57 NS
IFNLR1 & IL-28R	-0.082	0.66 NS
IFNL3 & IFN- λ	-0.172	0.258 NS
IFNL3 & IL-28R	-0.138	0.365 NS
IFNLR1 & *IFNL3*	0.65**	0.001

NS= Non-Significant

DISCUSSION

This study provides compelling evidence for the role of the interferon-lambda (IFN-λ) pathway in the pathophysiology of systemic lupus erythematosus (SLE). Our integrated analysis demonstrates significant elevations in both serum levels (IFN-λ and IL-28R) and gene expression (IFNL3 and IFNLR1) in newly diagnosed SLE patients, supporting their potential as biomarkers and reinforcing the involvement of this signaling axis in disease pathogenesis. The marked increase in serum IFN-λ and IL-28R in newly diagnosed patients, and their decrease in treated patients to levels measured in healthy controls, indicate the potential of these molecules as sensitive, dynamic biomarkers for SLE activity and therapeutic response. The superb diagnostic accuracy of IFN-λ (AUC = 0.99) and IL-28R (AUC = 0.96) aligns with worldwide studies showing elevated IFN-λ in active SLE patients, particularly in those with renal involvement [28]. Our results are consistent with longitudinal studies in which IFN-λ levels decreased after immunosuppressive therapy [15].

At the transcriptional level, a significant upregulation of both the ligand (IFNL3) and its receptor (IFNLR1) within newly diagnosed patients provides a molecular correlate with the previous serological data. The very strong positive association between their expression ($r = 0.65$, $p = 0.001$) suggests that the entire pathway was robustly and enlighteningly upregulated. This work fits in with the emerging evidence that IFN-λ signaling aggravates SLE pathogenesis through increasing Th1-cell differentiation and immune cell activation, as has been proposed with murine models and in studies of human disease [24]. While the accuracy of IFNL3 gene expression (AUC = 0.96) was higher than IFNLR1 (AUC = 0.76), it suggests ligand overexpression may be a driving factor in pathway. A notable and insightful finding was the lack of significant correlation between serum protein levels and their respective mRNA transcripts in peripheral blood. This finding suggest either robust post-transcriptional regulation occurred, or the circulating proteins are from tissue-specific sources such as renal epithelium in lupus nephritis [15], and or not entirely from circulating immune cells. This calls into question the multifaceted nature of IFN-λ biology in SLE. Moreover, the consistency of IFN-λ and IL-28R levels across varying age and BMI groupings increases their validity as generalized biomarkers. This demographic stability contrasts with other inflammatory cytokines and supports potential use in different patient demographics [24]. The heightened activity of the IFN-λ pathway in SLE emphasizes its aspect of pathogenesis beyond viral host defense. Mechanistically IFN-λ has been shown to:

Chemokine secretion (e.g. IP-10, MIG). These are responsible for recruiting inflammatory cells that migrate into the target organs. Localized direct renal inflammation with local expression (either glomerular or tubulo-interstitial) is correlated with the severity of lupus nephritis. Autoantibody production is also associated with infection and depletion of complement observed in our cohort and other similar studies [30]. In summary, IFN-α and IFN-λ engage similar signaling pathways, but our findings support a non-redundant role for both during SLE. IFN-λ also correlates better with the production of Th17 cytokines, and has a unique tropism for epithelial and renal cells. In support of therapeutic approaches, the non-redundant differences shown would suggest that it may be necessary for certain subsets of patients to target type I and type III IFNs concurrently.

CONCLUSION

Our research reveals that untreated SLE patients have significantly elevated serum levels of IFN- λ and IL-28R, as well as increased expression of IFNL3/IFNLR1 gene expression, relative to healthy controls and treated patients. These findings support a link between the IFN-λ pathway and early SLE pathogenesis. The impressive diagnostic ability of serum IFN-λ (AUC=0.99) and IL-28R (AUC=0.96) as candidate biomarkers is noteworthy. The finding of considerable discordance between gene and serum protein levels, is suggestive of post-transcriptional regulation, either by translational control or protein degradation. The normalization of these biomarkers in the treated category suggests that the therapies they received would impact the IFN-λ pathway, identifying opportunities to trial targeted inhibitors of the pathway. However, highly significant differences ($p < 0.001$) demonstrated that serum IFN-λ and IL-28R levels and gene expression within the treated and untreated categories remain significantly different from one another within their respective age brackets. Finally, the lack of impact of BMI indicates that these biomarkers can be regarded as indicative of SLE-associated pathology rather than potential confounding by metabolic effects.

Acknowledgment
Authors are gratful to Department of Biology, of Education for pure Science (Ibn Al-Haitham),University of Baghdad, Iraq for their support to complete the current research.

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