Original Article

The Correlation Between Interferon Serum Level and MALAT1 Long Noncoding RNAs Expression in Sample of Systemic Lupus Erythematosus Iraqi Women Patients

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Abstract

Background: Autoimmune disease known as systemic lupus erythematosus (SLE) occurs when the body's immune system assaults its own tissues. **Objectives:** The purpose of the study was to find novel interferon alpha (IFN-α) and MALAT1-based genetic and immunological prognostic indicators of SLE. **Materials and Methods:** Three milliliters of blood were drawn from 60 Iraqi patients with SLE, with a mean age of 31.87 ± 1.08 years, who were matched in age with 60 ostensibly healthy volunteers as a control group. About 300 µL of the blood were placed in TriZol tubes for RNA extraction and subsequently used to estimate MALAT1 gene expression by RT-qPCR, and 2.700 mL of the blood were placed in gel tubes to determine IFN-α serum. **Results:** Patients' Metastasis-Associated Lung Adenocarcinoma Transcript1 (MALAT1) gene expression increased significantly ($P \leq 0.01$) as compared to controls (1.14-fold), increasing by 3.47-fold. ADditionally, the results showed that patients' IFN-α serum levels were significantly higher ($P \leq 0.01$) than controls' were 61.04 ± 0.93 pg/mL versus 125.96 ± 4.45 pg/mL. The results also revealed a substantial positive correlation between MALAT1 lncRNA expression and IFN-α serum level, with significant differences. **Conclusion:** The present study found a direct positive association between MALAT1 gene expression and IFN-α serum level, as well as high levels of MALAT1 gene expression and IFN-α serum level in SLE patients.

Keywords: Autoantibody, INF-α, MALAT1 gene, SLE

Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that affects the skin, joints, kidneys, brain, serosal surfaces, blood vessels, blood cells, lungs, and heart. It is characterized by the production of many autoantibodies.[1] Both genetic and environmental risk factors play a role in the development and pathogenesis of SLE.[2] The availability of full biomarkers for the diagnosis, prognosis, and long-term monitoring of the SLE disease is still restricted.[3] Researchers are working to find a biomarker that will help them choose the best treatment course.[4] The etiology of SLE is heavily influenced by interferon (IFN).[5] Although many different cell types are capable of producing IFN I, neutrophils have drawn increasing attention as one of the main IFN source cells when neutrophils undergo a specific form of cell death called NETosis.[6] Type I IFNs are thought of as a link (bridge) between innate and adaptive immunity and serve a vital role in the immune system that mediates responses against viral invasion.[7] The high level of IFN effects patients' resistance to viral infections in the same way as SLE patients. Throughout the past 50 years, various types of study have been conducted, increasing the understanding of the crucial role that IFN play in the immune pathogenesis of SLE.[8] Long noncoding RNA MALAT1 gene was located within chromosome 11q13.1 and was known to regulate alternative splicing by

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interacting with multiple splicing factors and was found significantly upregulated, so, it plays a significant role in the etiology and progression of SLE.[9,10]

Materials and Methods

The study included 60 SLE patients with ages ranging from 19 to 57 with a mean age of 31.87 ± 1.08 years from the medical lab, rheumatology units at the Baghdad teaching hospital, and 60 age-matched healthy controls. Patients with overlapping autoimmune disorders, other immunological diseases, patients younger than 19 years old, and patients with lupus nephritis (LN) were not included in the study. Additionally, SLE patients who had recently been treated with INF-α, infected, or had cancer were all eliminated.

Molecular study of SLE patients and healthy control

Three main steps were used as clarified: The first step was the extraction of RNA from blood samples which were previously collected from all individuals; 0.30 mL was put into 0.50 mL TriZol (Thermo Scientific, USA) preservation tube and then the RNA was extracted manually by TriQuick Reagent (Solarbio, China), chloroform, isopropanol, and diethylpyrocarbonate (DEPC) water used to resuspend the RNA pellet; followed by the detection of RNA concentration by Quantus Fluorometer (Promega). Secondly, the method of reverse transcription by using AddScript Reverse Transcriptase kit (Addbio, Korea) to convert the RNA to cDNA, and the RNA species were converted into cDNA. The procedure was carried out in a reaction volume of 20 µL. According to the manufacturer's instructions, the total RNA volume to be reversely transcribed was 20 µL. Quantus Fluorometer was used to detect the concentration of converted cDNA according to Mohammed[11] to detect the goodness of samples for downstream applications. And third step was quantitative real-time PCR (RT-qPCR) for MALAT1 expression that was done using Luna Universal qPCR Master Mix (NEB, USA) and RT-qPCR (Bioer LineGene, China) according to Mohammed.[12] The specific primer of the current study is supplied by (Macrogen, Korea), as illustrated in Table 1.

The program of the RT-qPCR included the following steps: RT Enzyme Activation under 37°C, Initial Denaturation and Denaturation under 95°C, Annealing at a temperature between 60°C and 65°C and then the extension step the last at 72°C, only the first step required 1 cycle, but the next steps take place over 40 cycles. The gene expression ratio in all participants was determined using the delta–delta Ct computational approach.[15]

Table 1: Primers of *MALAT1* and their sequences for real-time PCR, with housekeeping gene *GAPDH*

Primer name	Sequence 5′–3′`	Annealing temp. (ºC)	Ref.
GAPDH -F*	TGCACCACCAACTGCTTAGC	55	[13]
GAPDH -R*	GGCATGGACTGTGGTCATGAG		
MALAT1-F*	CAGTGGGGAACTCTGACTCG	61	[14]
MALAT1-R*	GTGCCTGGTGCTCTCTTACC		

*F: forward primer, R: reverse primer

Detection of INF-alpha by ELISA

In short, sandwich ELISA kits from BT Lab (China) were used to detect and quantify IFN-α. The approach was based on the premise that specific antibodies bind to the target antigen to detect the presence and amount of antigen binding. The plate has been pre-coated with human IFN-α antibody. The sample's IFN-α is introduced, and it engages the well-coated antibodies in interaction. IFN-α in the sample is where the antibody attaches when it is introduced. Streptavidin-HRP is subsequently added, which binds the Biotinylated IFN-α antibody. After incubation, unbound Streptavidin-HRP is eliminated by a washing process. The development of color in the presence of the substrate solution is then associated with the level of human IFN-α. The process is stopped by adding an acidic stop solution, and the HumaReader HS (Germany) microplate reader is then used to measure absorbance at 450 nm.

Statistical analysis

The influence of different groups (patients and controls) on study parameters was examined using the Statistical Analysis System—SAS (2018) application.[16] To significantly compare between means, the *t* test was utilized. In this study, a meaningful comparison between percentage 0.05 and 0.01 probability was made using the Chi-square test and also ANOVA one-way.

Ethical approval

The research related to human use has complied with all the relevant national regulations and institutional policies and in accordance with the tenets of the Helsinki Declaration and, both the Iraqi Ministry of Health Research Committee (no. 9198, March 2, 2023) and the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies Committee authorized this study (No. H. T3016, November 28, 2022). Prior to enrolling participants in the study, participant permission was sought.

Results

The distribution of SLE patients by age revealed that the age group 30–40 had the highest frequency in the research sample, with (n = 27, 45%), followed by (n = 26, 43.33%) for those under the age of 30, and a lower percentage of (n = 7, 11.67%) with a highly significant difference for those over 40 ($P \leq 0.01$) as illustrated in Table 2.

The comparison of *MALAT1* gene expression in patients and control groups revealed a substantial increase in *MALAT1* average folding in patients (3.48 ± 0.65-fold) in opposition to control group (1.14 ± 0.18-fold) with significant differences ($P \leq 0.01$) as explained in Table 3 and Figures 1 and 2.

Table 2: Distribution of SLE patients according to age

Age group (years)	*N*	Percentage (%)
30–40	27	45
<30	26	43.33
>40	7	11.67
P value	0.0001**	

**($P \leq 0.01$); **highly significant

The outcome of age effect on *MALAT1* gene expression in SLE patients revealed no significant differences between age groups related to *MALAT1* gene expression in spite of the (<30 years) age group had the highest level among other age groups as clarified in Table 4.

The results of comparison between serum IFN-α levels in SLE patients and control groups revealed a significant elevated in the patients' serum level (125.96 ± 4.45 pg/mL) as compared to the control group (61.04 ± 0.93 pg/mL) with highly significant differences ($P \leq 0.01$) as shown in Table 5.

Table 3: Comparison of *GAPDH* and *MALAT1* genes expression in SLE patients and control groups

Groups	Mean ± SE				
	CT (*GAPDH*)	CT (*MALAT1*)	Delta CT(ΔCT)	Delta–delta CT (ΔΔCT)	*MALAT1* folding
Patients	20.15	33.39	13.53	-0.002	3.48 ± 0.65
Control	18.77	33.47	14.70	1.167	1.14 ± 0.18
P value	–	–	–	–	0.0008**

** ($P \leq 0.01$); **highly significant

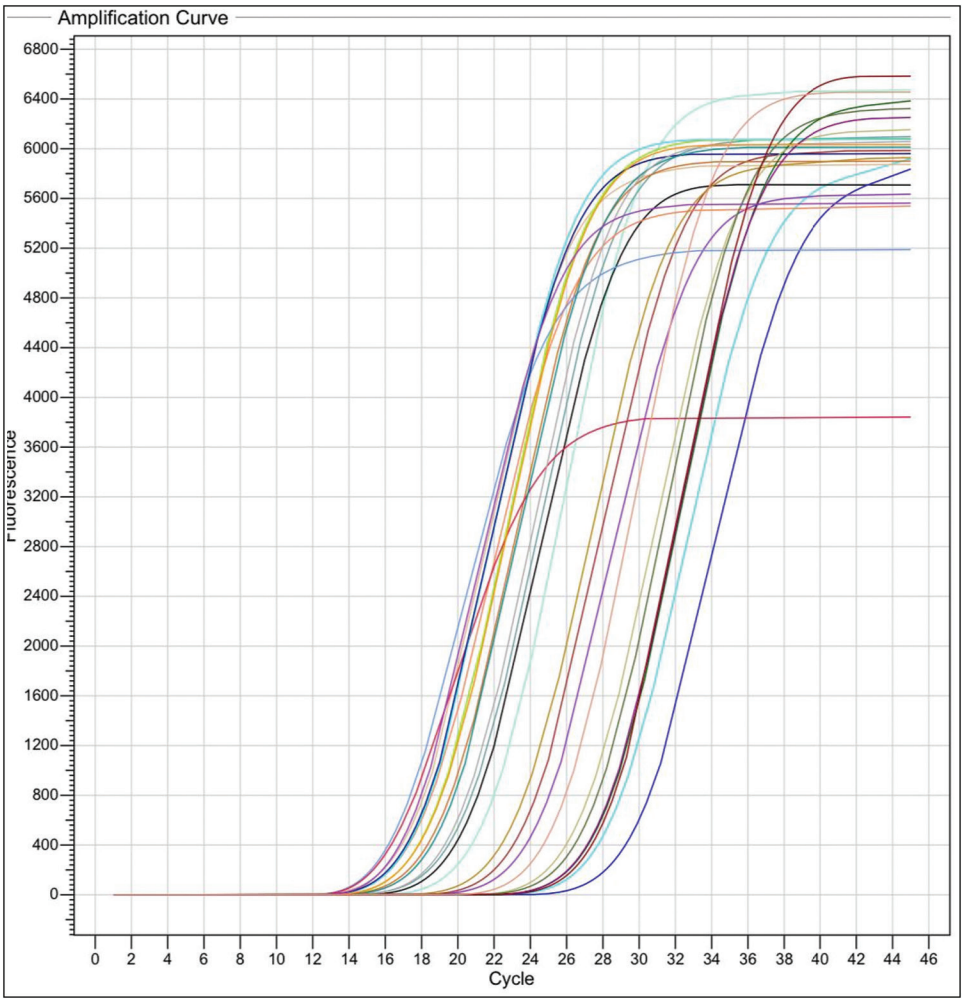

Figure 1: The *MALAT1* expression cycling curve

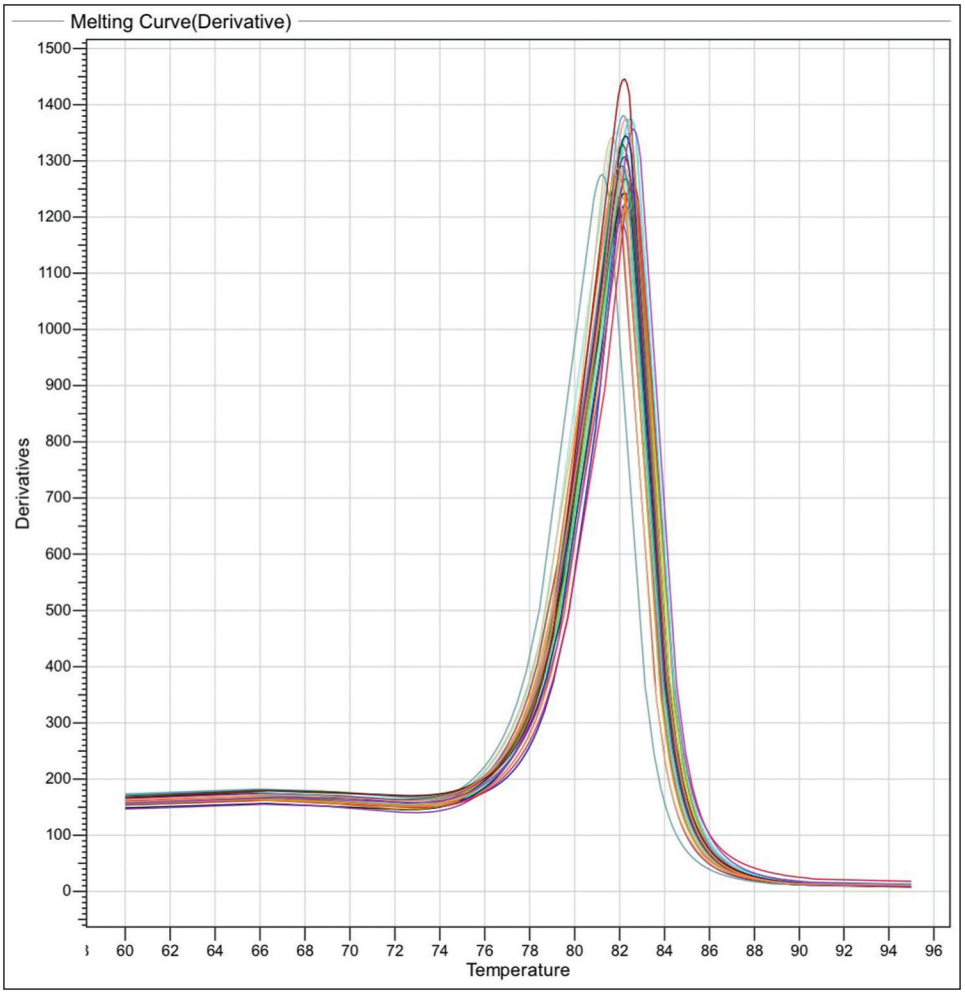

Figure 2: Melting curve of the *MALAT1* expression

Table 4: The relationship between age and *MALAT1* gene expression

Age groups	Mean	%	*P* value
30–40 years	0.98	38.33	0.447 NS
<30 years	1.42	43.33	0.455 NS
>40 years	0.45	18.33	0.171 NS

NS: non-significant

Table 5: Comparison between serum IFN-α levels in SLE patients and control groups

Group	Mean ± SE of INF-α
Patients	125.96 ± 4.45
Control	61.04 ± 0.93
t test	8.99**
P value	0.0001

** ($P \leq 0.01$); **highly significant

The results of age effect on serum IFN-α level in SLE patients revealed no significant differences between age groups related to serum IFN-α level in spite of the 30–40 year age group had the highest level among the other age groups as clarified in Table 6.

Table 6: The relationship between age and IFN-α level

Age group	*N*	Mean	CI 95%	*P* value
<30 years	27	119.24	106.11; 132.37	0.299 NS
30–40 years	26	133.62	120.97; 146.27	
>40 years	7	121.39	93.16; 149.62	

NS: non-significant

Current findings of the association of serum INF-α and MALAT1 gene expression demonstrated that acquire a strong positive association between INF-α serum level and *MALAT1* lncRNA expression with highly significant differences at $P \leq 0.01$.

Discussion

The study's data indicated that SLE is thought to affect younger people more frequently than older persons. The results of the study recorded that the largest percentage (n = 53, 88.33%) of the patient sample was in the age groups at diagnosis of less than 40 years, thus, it is believed that being younger is a key risk factor for SLE.[17,18] This result came in agreement with the study of Medhat,[17] who found that ages (≤45) represented the majority of

patients when compared with >45 age group.[16] Also, this study compatible with[19] which was found the majority of patients were adult-onset. Lifestyle variables such as insufficient physical exercise, poor diet, smoking, alcoholism, drug abuse, instability of the sympathetic nervous system, and poor mental health may have the most impact on disease susceptibility. This may be the case as epigenetics have lately shown a link to the development of numerous illnesses, including autoimmune diseases. According to the information that is, currently available, *MALAT1* had a high expression level in SLE patients. Elevated *MALAT1* gene expression can be brought on by a number of factors, including treatment, patient age, the severity of their SLE, and their immune status. The same gene has been linked to a number of diseases, via influencing numerous transcription factors, which raises the possibility that it also contributes to autoimmune disease. In 2017, Yang[20] mentioned that *MALAT1* was immunologically discovered in monocytes. According to Gao *et al.*[14] in 2020, appeared *MALAT1* suppression was affected $CD19^+$ B, $CD4^+$ T and INF-α levels, where the study also showed that the knockdown of the *MALAT1* could cause a decreasing level of those immune cells and reduce the secretion of INF-α. Interferon alpha genes code for IFN-α proteins, which are classified as type I IFNs.[21] Type I IFN proteins, which are found on the effectors of the immune cells (such as natural killer cells), were bound to the type I IFN-α receptors (IFNAR).[14] The complex formation of the IFN-α receptor subunit 1 (IFNAR1) results in the activation of a series of intracellular signaling pathways, which are mediated by different components like signal transducers, Janus kinases (JAK), and activator of transcription1 (STAT1) phosphorylation cascades.[22] All of these mediators play a vital role in the beginning of the expression of interferon-stimulating genes (ISGs) and are caused dimerization, nuclear translocation, and binding to IRF9 to form IFN-stimulated gene factor 3 (ISGF3).[21] The ISGF3 complex then binds to IFN-stimulated response elements (ISREs) of ISGs, leading to the activation of their transcription, in this manner, IFN-α induces the expression of several hundred ISGs, a large number of which act to induce an antiviral state within the cell. Other studies in 2022 by Amezcua-Guerra *et al*.[23] and Maki *et al.*,[24] mentioned that IFN-α strongly regulates the expression lncRNA *MALAT1*, ISGs expression levels, and interferon-regulated chemokines secretion levels. The amplification of inflammatory response could worsen the condition of autoimmune diseases such as SLE.[25] It is still necessary to thoroughly evaluate many of these new, promising biomarkers in large-scale longitudinal investigations and to find better biomarkers not only for the diagnosis of lupus but also for monitoring, predicting future flares, and evaluating therapeutic response.[26] In this study, IFN-α serum levels were not significantly correspond with age, in spite of the 30–40 age group showing the highest INF-α level than the other age groups, on the contrary, Niewold *et al.*[26] found that the IFN-α serum a statistically significantly correlated with age, especially in people whose ages ranged from 16 to 50, which represented adult-onset, and they made the hypothesis that this pattern of age-related IFN-α may be to blame for the higher incidence rate of SLE disease in younger age groups.[21]

Conclusion

Interferon alpha probably plays a significant impact in the pathophysiology, etiology, and/or persistence of SLE. Although there is a lot of data linking IFN-α to lupus, the link between IFN-α and SLE is primarily conjectural. The precise cellular and immunological pathways by which IFN-α influences lupus were still largely unknown. Future research into these mechanisms and pathways has the potential to be useful. New treatment targets and a better understanding of lupus as a disease are both likely outcomes of these investigations. Also, the study revealed that MALAT1 gene plays an important role in susceptibility to SLE even though its role is understood this gene appears its effect in many diseases including autoimmune diseases like SLE.

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Conflicts of interest

There are no conflicts of interest.

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