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Evaluation of *IL10* Gene Expression in Samples of Systemic Lupus Erythematous Iraqi Patients

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Abstract: Systemic Lupus Erythematous (SLE) is a type of chronic multisystemic autoimmune disease which impacts women more frequently than males. The antibodies produced by the immune system attack healthy cells The goals of the current research were to evaluate *IL10* gene expression in a sample of Iraqi women patients with Systemic Lupus Erythematous and determine the influence of IL10 gene expression level on SLE disease. A total of 54 women blood samples were obtained from individuals diagnosed with Systemic Lupus Erythematous (SLE) in Iraq. Additionally, 46 seemingly healthy women were included as a control group. The age range of all participants was between 15 and 51 years. The assessment of *IL10* gene expression was conducted using Real-time Polymerase Chain Reaction (RT-PCR). The results of *IL10* gene expression detection showed that no differences between the control group (1 fold change) and the patient group(1.043 fold change). It was concluded no effect of IL10 gene expression in development SLE Iraqi patients.

Keyword: IL10, SLE, gene expression.

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Introduction

The SLE is a type of chronic multisystemic autoimmune disease which impacts women more frequently than males. The antibodies produced by the immune system attack healthy cells and can interact with various cell organelles, These immune complexes are deposited and attack the various internal organs of the body such as the lung, heart, joints, blood, kidneys, brain, skin, and blood vessels , In the most advanced cases of the disease, the kidneys are severely affected, causing inflammation, which leads to an increase in mortality rates (1). Majority of those infected with the autoimmune disease are women, and many research in Iraq refer to this (2,3) which showed that women are more susceptible to autoimmune diseases compared to men.

The study of SLE indicated genetic susceptibility and various environmental factors that can cause the development of the disease in genetically susceptible individuals (4).

The basic mechanisms that underlie the onset and progression of SLE are still unclear. However, immunological tolerance issues, increased antigenic load, T cell hyperactivity, and improper B cell control all play a role in the production of pathogenic autoantibodies. Additionally, Environmental factors and sex hormones that affect the immune system appear to either induce or prevent the development of SLE (5).

The diagnosis is based on international standards. The 2019 classification criteria for SLE EULAR/ACR (The European Alliance of Associations for Rheumatology /American College of Rheumatology), one of this criteria is Double-stranded DNA antibody (dsDNA) used to detect SLE(6). The *IL-10* gene is located on chromosome 1 q21-q32 , split into four introns and five exons codes for a polypeptide with 178 amino acids, including a signal peptide with 18 amino acids and a single chain glycoprotein of 160 amino acids,. The mRNA for the gene contains 1.8 kb(7). IL-10 is a major B cell stimulating cytokine, recently reported to be a crucial driver of the extrafollicular B cell response, a pleiotropic cytokine with potent immune tolerogenic effects, recent research suggests that it has multiple effects in SLE, where it may suppress pro-inflammatory effector activities but also appears to be a key driver of the extra follicular antibody response (8, 9).The main aim of the current study was to investigate the correlation between the expression of the IL10 gene and the pathogenesis of SLE in patients from Iraq.

Material and methods

The study was conducted in accordance with the guidelines and regulations set out by an ethical committee responsible for overseeing human research. A total of 54 individuals diagnosed with SLE were included in this study (Patients with other autoimmune disorders were not included in the study, as well as those with(early-onset) or lupus nephritis). On the other hand, controls 46 people had no family history of SLE previously or any other autoimmune disease came from the same area and were of the same age and sex, with ages ranging from 15 to 51 years.. Venous blood samples of three milliliters were collected from all participants. The blood samples in gel tubes were centrifuged at 3000 rpm for 15 minutes, within an hour of their collection, for sera separation. Enzyme-linked immunosorbent assay (ELISA) kits were employed to assess levels of total dsDNA antibody (ds-DNA Screen ELISA, Demeditec/Germany). A blood sample of 250 µl was obtained from each subject and afterwards mixed with 750 µl of Trizol preservation solution for RNA extraction, used TransZol Up Plus RNA Kit (TransGen biotech /China) and The estimation of RNA concentration and purity was conducted using the Nanodropspectrophotometer instrument manufactured by ThermoFisher cientific/USA. Then used *EasyScript*® One-Step gDNA Removal and cDNA Synthesis Super Mix from (TransGen biotech /China). Total RNA was reverse-transcribed to cDNA,the operation was performed in afinal reaction volume of 20 µl (Table 1). Total RNA (4µl) had to be reversely transcribed. The primers were designed using the Primer 3plus, V4 tool, and their reference sequences were checked using the NCBI database and the University Code of Student Conduct (UCSC) programs as shown in (Table 2). The information received from NCBI was relied upon for this purpose. The expression levels of the *IL-10* gene were estimated by the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) method, The Quantitative Real Time PCR (qRT–PCR) using the QIAGEN Rotor gene Q Real-time PCR System.

Table (1): Strand cDNA synthesis reaction component

Component	volume reaction
Total RNA	4 µl
Anchored Oligo(dT)18 Primer (0.5 µg / µl)	1 µl
Random Primer (0.1 µg / µl)	1 µl
2xES Reaction Mix	10 µl
EasyScript ® RT/RI Enzyme Mix	1µl
gDNA Remover	1µl
RNase-free Water	1µl

Table (2): Primers of IL10 and GADPH genes with their sequences

Primer Name	Sequence 5`- 3`
IL-10-F	AGGTTAGAGAAGGAGGAGCT
IL-10-R	GAGCTGTGCATGCCTTCTTT
GADPH-F	GAAATCCCATCACCATCTTCCAGG
GADPH-R	GAGCCCCAGCCTTCTCCATG

Results and discussion

The Statistical Analysis System (SAS) was used to identify the impact of various variables on research parameters (10). Furthermore, the T-test was implemented. Similarly, to determine significant differences between percentages at the 0.05 and 0.01 probability levels, the Chi-square test was employed.

Distribution of SLE patients among Family history

The findings indicated that 24% of patient with family history and 76% without any family history of SLE,as shown in figure (1)many previous studies suggested that family history of the disease is a possible indicator of the possibility of inheriting this disease genetically, in addition to other causes(11,12).

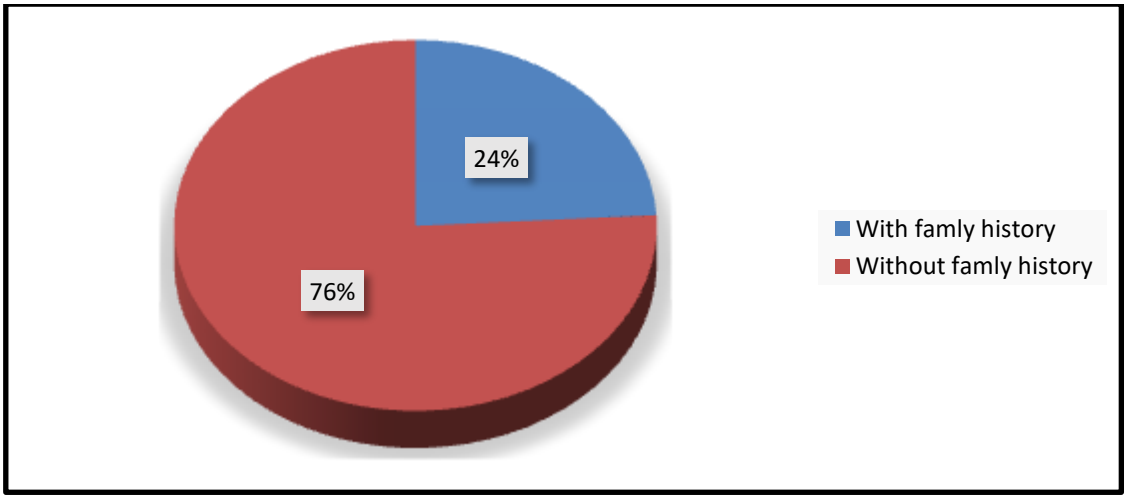

Figure (1): Distribution of Sample Study According to Family History

The levels of double-stranded DNA antibodies

The results of the statistical analysis in Table (3), which represents the levels of double-stranded DNA antibodies in the groups of patients and control, showed that there were significant differences in the levels of the group of patients, which amounted to (58.79) ml/u compared to the groups

of control, which amounted to (21.62) ml/u, where they were Its P value=0.0009. Studies have proven its high specificity for this disease, as it can be used as a diagnostic marker for the disease and the severity of disease activity Its high levels in the blood leads to an increase in the damage caused by disease to tissues and cells within the body(6,13).

The effect of IL10 on SLE diseases

According to the the delta delta cycle threshold value relative fold change ($2^{-\Delta\Delta Ct}$) method, the result of *IL10* gene expression show a slight increase in IL10 fold change in patient (1.043 fold) when compared with that for control group (1 fold)) as elucidated in (Table 4) and (Figure 2). This result don't meet with the results of other researches which pointing to increase or decrease of the IL10 fold change when compared between patient and control group (14,15,16,17).The change in *IL10* gene expression which appear in these studies attributed to the function that IL10 plays in the body, as it has been observed to be pleiotropic cytokine that is particularly well-known for its suppressive effects on different immune cells (6). The difference between the present study and other studies according to the *IL10* gene expression

levels between studies can be attributed to several reasons, the most important of which is the heterogeneous nature of the disease. Also, the different patterns of disease onset, severity, and course can often be linked to differences in racial or ethnic origin, level of education, income, and health insurance status,the degree of social support, adherence to medication, as well as environmental and occupational factors,are affected factors on different studies.

Conclusions

Considering the results of the current study and noting that there are no statistically significant differences between the patient group and the control group in the gene expression of interleukin 10, we can conclude that this disease may be less affected by this interleukin in Iraq, or that the drugs used in its treatment directly affect its concentration and because small size of the samples studied, the result requires further studies to address it and study its relationship with the medications used in Iraq.

Table (3): Comparison between patients and control groups in dsDNA

C	Mean Ct *GAPDH*	Mean Ct *IL 10*	Δ Ct	Δ Ct Calibrator	ΔΔCt	2^-$^{\Delta\Delta Ct}$	Experimental / Control group	Fold change
Controls	17.474	32.64528	15.17128	21.3353	-6.16402	71.7059	71.7059/ 71.7059	1
SLE Patients	17.437	32.74981	15.31281	21.3353	-6.2249	74.7965	74.7965/ 71.7059	1.043

Table 4): Comparison between GADPH and *IL10* genes expression in SLE patients and control groups

Group	Patients	Control	t-test	P-value
dsDNA Mean ± SE	58.79 ±8.48	21.62 ±1.30	21.391**	0.0009
	** (P≤0.001),			

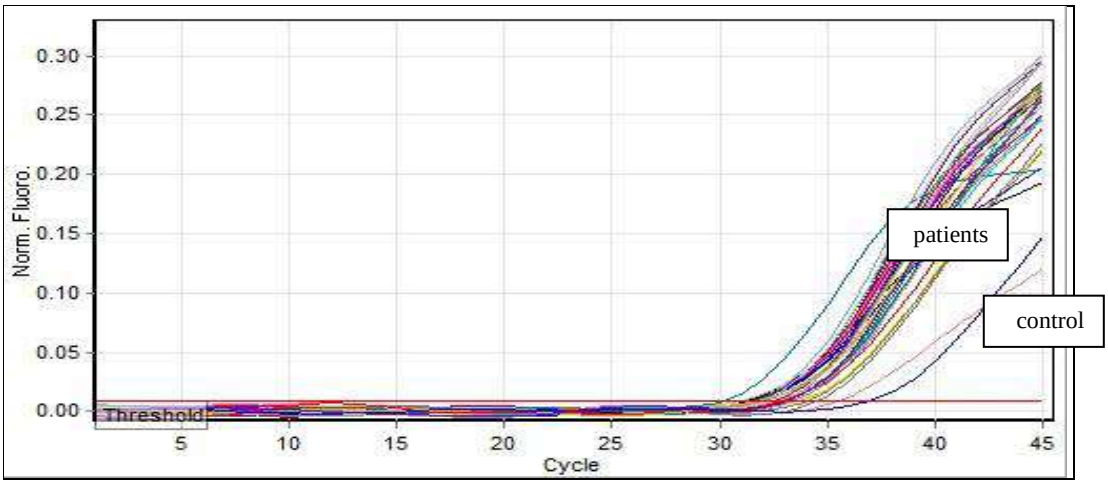

Figure (2): *IL10* gene expression in sample SLE patients and control groups

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