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# Decreased expression of IL-4 Gene and Exploring of mutable lymphotoxin alpha (TNF-β) gene in Patients with Systemic Lupus Erythematosus

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**Abstract** Rapid genetic system identification characterizes the complex illness known as systemic lupus erythematosus (SLE). The classic cytokine, IL-4, is known to stimulate the Th2 route of differentiation and to effectively inhibit the Th1 response. The pathogenesis of systemic lupus erythematosus (SLE) has been linked to immunological and genetic variables. Therefore, this study aimed to characterize the gene expression of IL-4 in peripheral blood mononuclear cells (PBMC) and explore potential links between the functional Interleukin-4 gene and SLE. Additionally, lymphotoxin alpha (LTA) is a key cytokine in the pathogenesis of SLE. In SLE, cytokines have a significant role in controlling lymphocyte function.

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**Keywords:** *TNF-β gene, peripheral blood mononuclear cells (PBMC), lymphotoxin alpha (LTA), TNF-cellular pathways, and systemic lupus erythematosus (SLE).*

## 1. INTRODUCTION

An inflammatory, multisystemic autoimmune disease is called systemic lupus erythematosus, or SLE. The development of the illness is significantly influenced by both genetic and environmental factors. The hallmarks of systemic lupus erythematosus include aberrant complement system function, autoantibodies, and inflammatory cytokine modulation. (1). About 8–10 times as many women as men have systemic lupus erythematosus, and African Americans are roughly 3–4 times as likely to have it than Caucasian Americans. [/2] An important role in genetic vulnerability to SLE has been identified by studying the family and genetics, especially in relation to monozygotic and dizygotic twin research. [/3] The human leukocyte antigen (HLA) region is thought to be a possible SLE vulnerable area. However, there are other regions that are sensitive in relation to SLE disease besides HLA. *[4] Also, the pathophysiology of SLE is impacted by cytokine-mediated immunity. [5, 6]

Produced by CD4+ Th2 cells, basophils, and mast cells, interleukin-4 is an anti-inflammatory cytokine that helps control the humoral immune response [7]. Interleukin-4 has several anti-inflammatory properties, is cytotoxic, and prevents the activation of nitric oxide synthase and the production of superoxide anions by macrophages. The genes for IL-1β and IL-4 are found on chromosomes 2q14–21 and 5q31–33, in that order [8].

An intricate autoimmune disease called SLE results in inflammation. Genetics and environment worked together to cause the illness. Autoantibodies, proinflammatory cytokines, and abnormalities in the complement system are the defining features of this illness. In [16] SLE is more common in African Americans than in Caucasians, and it affects women around eight times more frequently than it does men. (17) Studies in families and genetics, including those involving monozygotic and dizygotic twins, demonstrate the critical role that hereditary predisposition to SLE plays. 18] The human leukocyte antigen's (HLA) HLA region may be related to SLE. However, this is not the only site where SLE pathogenesis can occur. 19] It has been discovered that the pathophysiology of SLE is significantly influenced by cytokine-mediated immunity. (20, 21) SLE and tumor necrosis factor (TNF) are related. It is uncertain, therefore, how TNF polymorphisms relate to the pathophysiology of SLE.

Two distinct cytokines were identified in 1984 with the aid of lymphocyte and macrophage cells. These are TNF-β and



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tumor necrosis factor α (TNF-α), in that order. 21] Numerous cell types, including T cells, B cells, and NK cells, control TNF-β. 23]

## 2. MATERIALS AND METHODS

Three consecutive SLE patients were recruited for the current experiment at the Baghdad Medical City Rheumatology Clinic. Those with malignant tumors, infections, or other rheumatic disorders were not allowed. The University of Baghdad's College of Nursing's ethical committee gave the researcher its blessing. The study participants were informed of the procedures before any data were collected. The American College of Rheumatology (ACR) 1997 criteria stated that all patients met at least four SLE elements [9]. Two milliliters of blood from each participant were drawn and stored at -20°C in sterile EDTA tubes. Three Iraqi SLE patients had their blood serum DNA extracted using the Quick-DNATM Blood MiniPrep Catalog at a concentration of between 1 and 1.2 ng/µl. To corroborate the genotypic diagnosis, healthy serum was utilized as a comparison.

| | | | |
|---|---|---|---|
| Forward | 5'- AGGCTGAAAGGGGGAAAGC - 3' | 57.9 | 57.9 |
| Reverse | 5'- CTGTTCACCTCAACTGCTCC - 3’ | 55.7 | 55 |

***Table 1.*** *RT-PCRCycling Program for IL-4 gene*

| Step | Temp. (°C) | Time | Cycle | Scanning |
|---|---|---|---|---|
| Enzyme activation | 95 °C | 05:00 min | Hold | |
| Denaturation | 95.0 °C | :00:20 | 40 | |
| Annealing | 60.0 °C | :00:20 sec | | |
| /Extension | 72.0 °C | :00:20 sec | | |

Patients involved in this study met a minimum of four ACR, American College of Rheumatology, 1997 criteria for SLE. [24] two mL of Blood samples were collected using sterile EDTA tubes and froze under -20°C. Using the Quick-DNATM Blood MiniPrep Catalog, DNA was extracted from the blood serum of three Iraqi SLE patients between (1-1.2 ng/l) and healthy serum (control) was used to corroborate the genotypic diagnosis of IL-4 primer.

| | | | | |
|---|---|---|---|---|
| Forward | 5'-CCG TGC TTC GTG CTT TGG ACT A- 3' | 59.8 | 54.5 | |
| Reverse | 5'-AGA GCT GGT GGG GAC ATG TCT G - 3’ | 61.1 | 59.1 | 740bp |

PCR reaction consisted of 25 µl PCR reaction mixture using INtRON's Maxime PCR PreMix Kit, the reaction was carried out in accordance with an established protocol.

| | | | |
|---|---|---|---|
| **Initial Denaturation** | 95°C | 3 min. | 1 cycle |
| **Denaturation -2** | 95°C | 45sec | |
| **Annealing** | 60°C | 45sec | 35 cycle |
| **Extension-1** | 72°C | 45sec | |
| **Extension -2** | 72°C | 7min. | 1 cycle |
| **For TNF-β gene** | | | |

Using an ABI 3730 sequencer, fluorescent dye terminator chemistry was used to sequence and examine the DNA of purified PCR amplicons. Also, Using the NCBI nucleotide database (www.ncbi.nlm.gov/nucleotide), the prime repair exonuclease TNF- was matched with the database and added to the multiple alignment (clone man)



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## 3. RESULTS

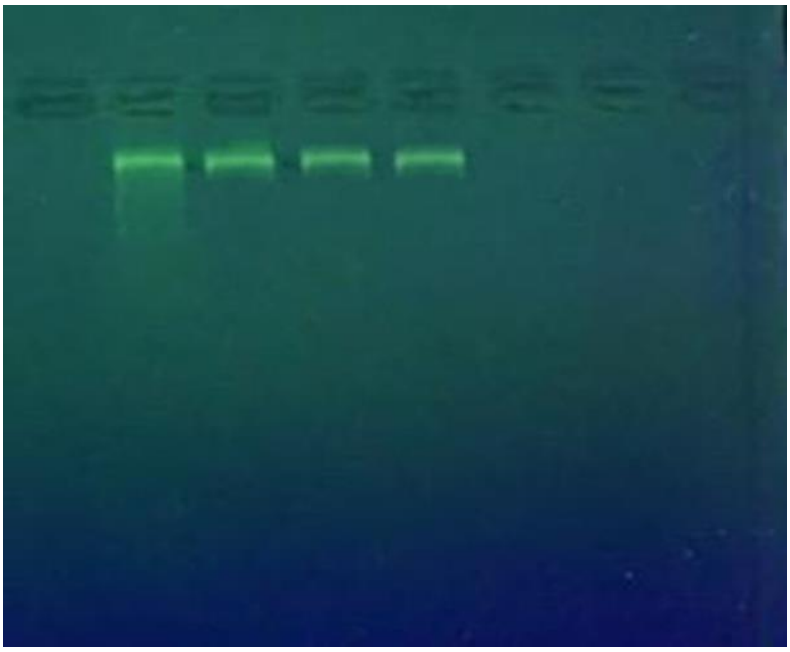

***Figure 1.*** *Gel electrophoresis of genomic DNA extraction from blood 1% agarose gel at 30 min*

***Table 2.*** *IL-4 gene expression by real-time PCR*

| Number of the hole | Identificator of the tube | Cp, Fam |
|---|---|---|
| A1 | Sample_1 (CONTROL) | 25.4 |
| A2 | Sample_2 A | 24.2 |
| A3 | Sample_3 A | 24.3 |
| A4 | Sample_4 A | 23.8 |
| A5 | Sample_5 B | 22.9 |
| A6 | Sample_6 B | 22.3 |
| A7 | Sample_7 B | 22 |

IL-4 decreased expression in SLE patients compared with a healthy control group

**The mutable TNF-β gene identification**

The TNF- gene mutation was amplified using whole DNA that was taken out of the blood serum of Iraqi patients. DNA was amplified using primers from the conserved region of the TNF gene, and the PCR products were then separated on a 2% agarose gel. The results of the gel electrophoresis were published in (Fig. 1,2), which demonstrated the presence of the mutant TNF- gene.



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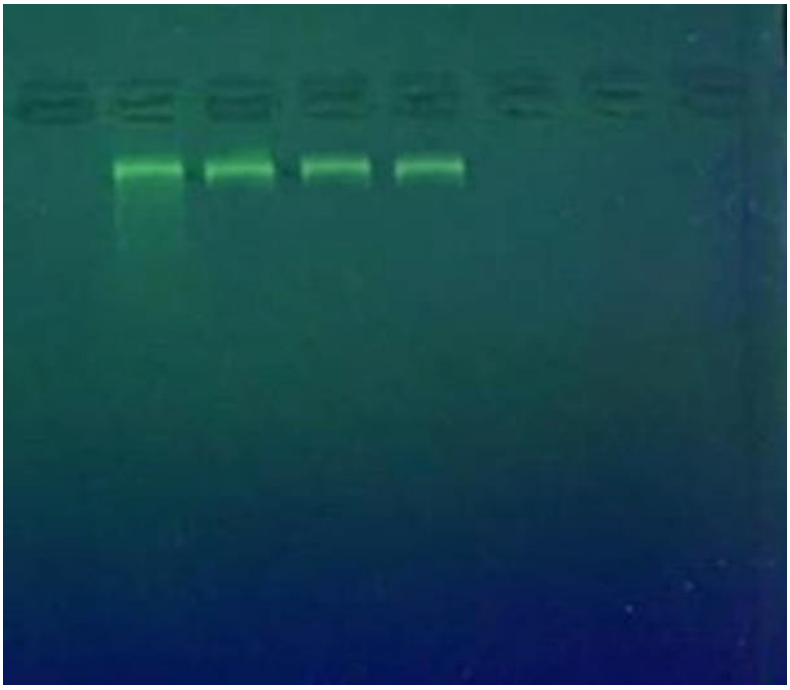

***Figure 3:*** *Gel electrophoresis of genomic DNA extraction from blood 1% on agarose gel for 30 min.*

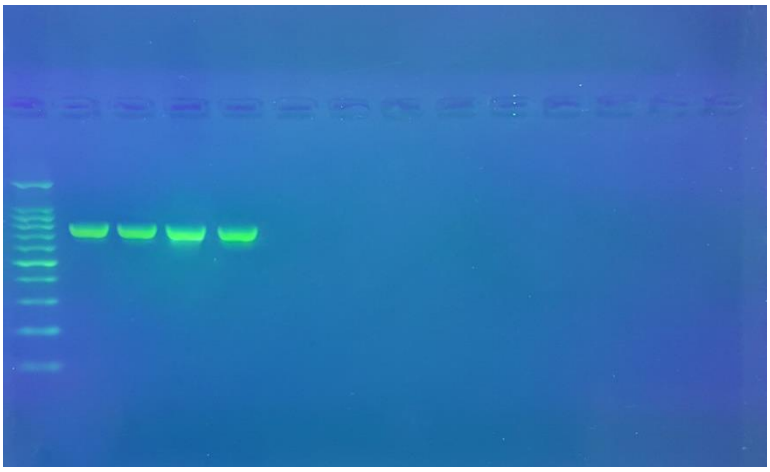

***Figure 4****:PCR product the band 740 bp for TNF-β. The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100)*

**Sequences analysis of Mutable TNF-βgene**

This study suggests that TNF may be connected to autoimmune diseases in Iraqi patients. The present study then examined the DNA of individuals suffering from autoimmune illnesses such as SLE. The importance of genetic risk factors for SLE has recently improved due in part to a greater understanding of the genetic components that influence SLE risk that has emerged over the last ten years. Every susceptibility gene that a person carries increases their relative risk of developing SLE and may have an effect on the disease's clinical manifestations and onset age (13). Therefore, it is expected that a significant portion of the "missing heritability" in SLE will be explained by the use of sequencing methods and gene-gene interaction analysis (14).

Three patients from Iraq (16–20 years old) and three controls underwent full sequencing of their TNF-coding region in order to search for missense mutations. Using the clone manager demo 9.2, we were able to determine the information displayed in the red point with cycles of Figure 4.

DNA was isolated using standard methods from blood serum. TNF-'s coding sequence is contained fully within one exon. The gene was amplified into three overlapping segments, which macrogen Korea then examined and sequenced. BLAST, a free application available at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov), was used to do homology searches. The application Bio Edit was used for additional editing. Analytical parameters and primer sequences are made available on demand. The position of the detected



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variations is referred to the sequence (G>C AY070, C>G 490.1, A>G AY070, C>A 940.1, A>G G>A G>T G>A AY070/490.1 G>C AY070/940.1) (fig 3) To ascertain the genetic composition of the detected missense variations, controls underwent genotyping. We used NCBI analysis data (https://blast.ncbi.nlm.nih.gov/Blast.cgi), which integrates genetic data through (clone manager demo 9.2) software, to evaluate the influence of synonymous variations on genes in order to ascertain the possible functional significance of the discovered missense variants (13, 14).

***Table (3)*** *Homo sapiens lymphotoxin alpha (TNF superfamily, member 1) (LTA) gene*

| No. of sample | Type of substitution | Location | Nucleotide | Sequence ID | Score | Identities | Source |
|---|---|---|---|---|---|---|---|
| 1 | Transvertion | 2490 | G>C | ID: AY070490.1 | 610 | 99% | Homo sapiens (TNF) |
| | Transvertion | 2568 | C>G | | | | |
| 2 | Transition | 2374 | A>G | ID: AY070490.1 | 1218 | 99% | Homo sapiens (TNF) |
| | Transvertion | 2847 | C>A | | | | |
| 3 | Transition | 2374 | A>G | ID: AY070490.1 | 866 | 99% | Homo sapiens (TNF) |
| | Transition | 2518 | G>A | | | | |
| | Transvertion | 2681 | G>T | | | | |
| | Transition | 2693 | G>A | | | | |
| 4 | Transvertion | 2490 | G>C | ID: AY070490.1 | 1219 | 99% | Homo sapiens (TNF) |

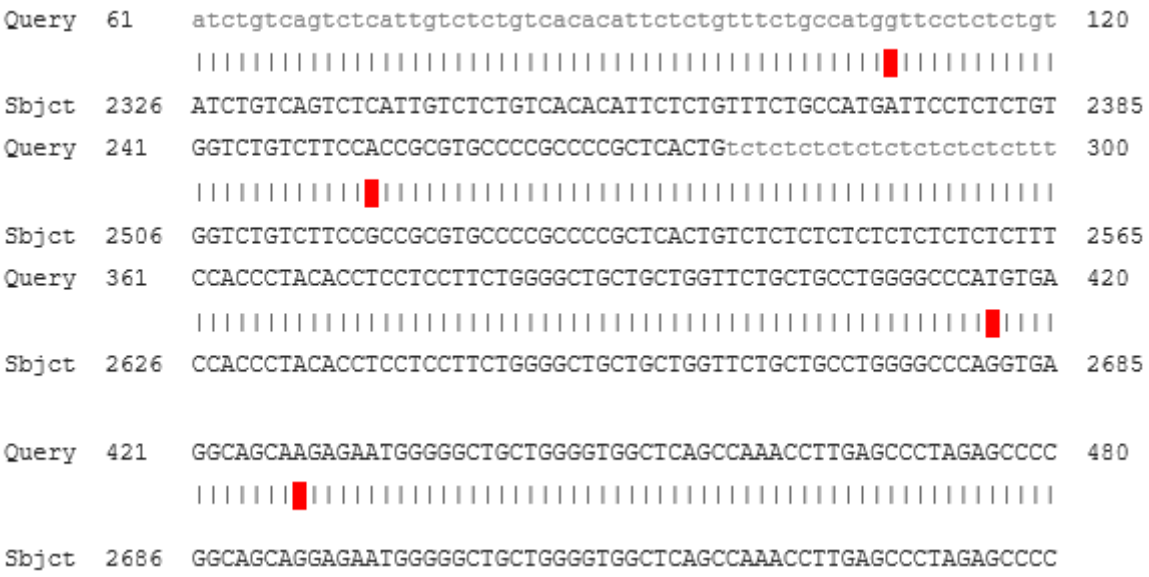


***Figure 5 1-*** *Homo sapiens lymphotoxin alpha (TNF superfamily, member 1) (LTA) gene, complete cds*

Sequence ID: AY070490.1Length: 5033Number of Matches: 1
Related Information
Gene-associated gene details
Range 1: 2229 to 2571GenBankGraphicsNext MatchPrevious Match
Alignment statistics for match #



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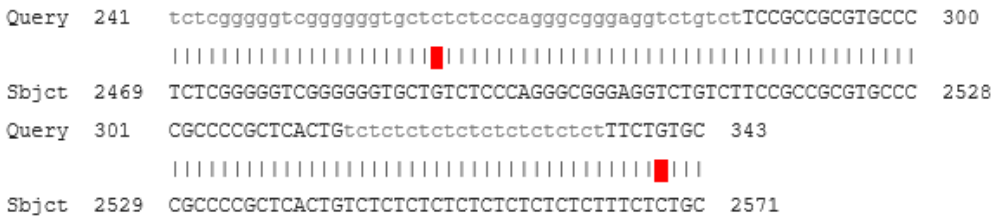



***Figure 6****-Homo sapiens lymphotoxin alpha (TNF superfamily, member 1) (LTA) gene, complete cds*

Sequence ID: AY070490.1Length: 5033Number of Matches: 1
Related Information
Gene-associated gene details
Range 1: 2223 to 2902GenBankGraphicsNext MatchPrevious Match
Alignment statistics for match #1

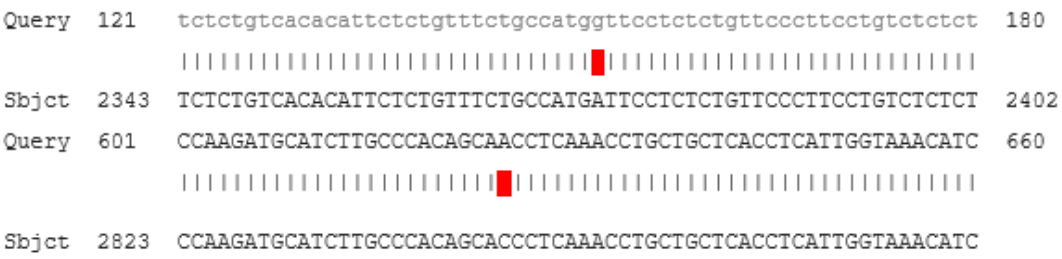
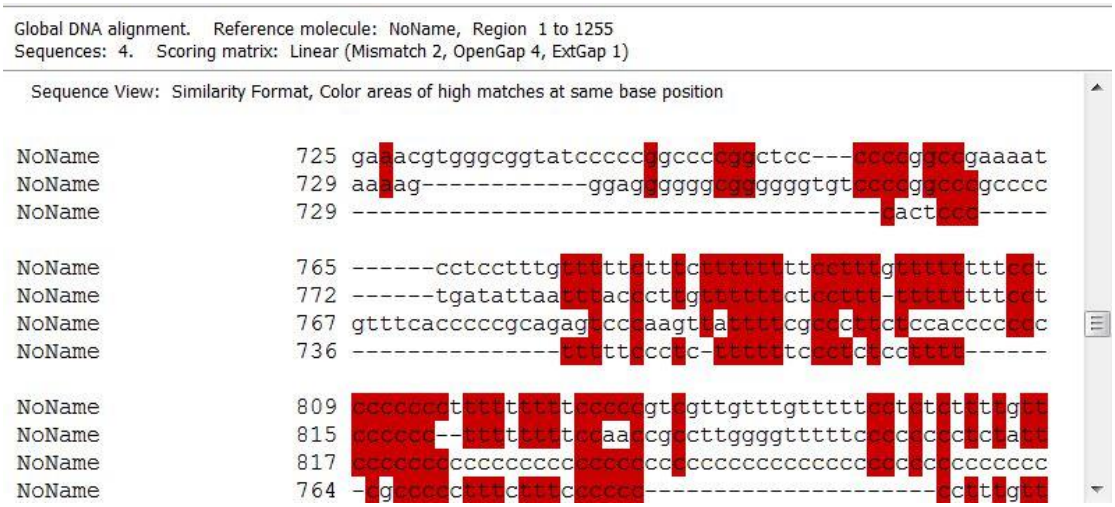



***Figure 7*** *Homo sapiens lymphotoxin alpha (TNF superfamily, member 1) (LTA) gene, complete cds*

Sequence ID: AY070490.1Length: 5033Number of Matches: 1
Related Information
Gene-associated gene details
Range 1: 2266 to 2755GenBankGraphicsNext MatchPrevious Match
Alignment statistics for match #1



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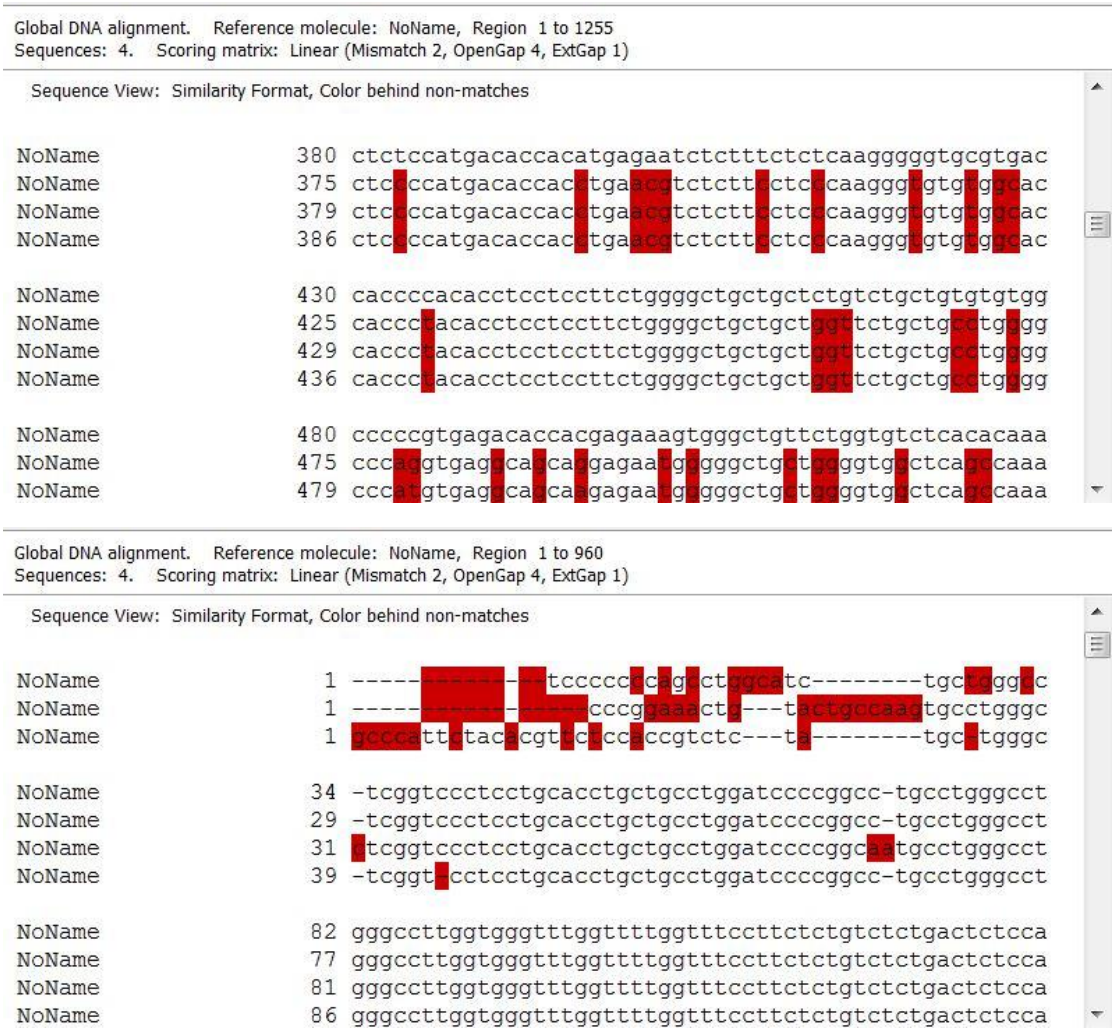


***Figure 8**: Scheme of disease-associated TNF-β mutations so far reported in literature to compare alignment of DNA lengths of health TNF-β gene (control) of DNAes III in 3 patients, The mutation regions (red point) like missense mutations found in the present study are in cycles.*

## 4. DISCUSSION

IL-4 is an anti-inflammatory cytokine that mediates the humoral immune response and is necessary for the helper-2 cell fraction to be properly controlled by the immune system. One important cytokine that aids in T lymphocyte formation and B cell activation and differentiation is IL-4 .[10]

According to the current, IL-4 expression was lower in SLE patients than in healthy controls. There are currently no effective treatments for SLE; instead, current approaches focus on postponing the onset of the illness. An earlier in vivo investigation showed that animals given vitamin D3 together with other medications had higher levels of IL-4 in SLE. 11] Cytokines are important modulators of lymphocyte activation in SLE. However, it is still uncertain what Th1 and Th2 cytokine profiles circulating lymphocytes in human SLE create. In [12] Initially, it was stated that IL-4 promoted class-switch and early activation signals to lymphocytes [13]. Furthermore, it has been shown that IL-4 expression is overexpressed in the murine model of SLE, making it a crucial B cell stimulant [14]. Here, we postulated that elevated IL-4 levels are associated with SLE in humans. But the current study found that unstimulated PBMC from SLE patients had significantly less IL-4 mRNA expression. This result validates the earlier research conducted by Horwitz et al. [15], who demonstrated a decrease in Th2 mRNA in SLE patients' peripheral blood cells. Similarly, PBMC from SLE patients had lower levels of IL-4 expression.In[12]

Tumor necrosis factor (TNF) and lymphotoxin alpha (LTA) are two examples of cytokines that are important in the development of systemic lupus erythematosus (SLE). The lymphotoxin-alpha (LTA) (TNF-) gene has been connected to rheumatoid arthritis, psoriasis, and systemic lupus erythematosus (SLE). In [27] The detection of a heterozygous missense variant in alternative splicing results in a variety of transcript variants; patients with SLE have been reported to have mutations in lymphotoxin alpha, and connections have been observed between lymphotoxin dysfunction and disease(28) .

The amount of IL_4 expression was found to be lower in SLE patients as compared to healthy controls, and the researchers



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deduced that malfunctioning TNF-cellular pathways may contribute to the pathophysiology of common, complex forms of systemic lupus erythematosus. The sequence (G>C AY070, C>G 490.1, A>G AY070, C>A 940.1, A>G G>A G>T G>A AY070/490.1 G>C AY070/940.1) is the order in which the variations were discovered.

**Declaration of interest:** None

**Author contributions**: Noor Alhuda Khaleel Ibrahim: Data collection, writing the introduction, and resources.

Shahad Fawzi Obeid: Data analyses and results communication

Wasnaa Jomaa Mohammed: Conceptualization, methodology, and resources

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