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The Correlation between Single Nucleotide Polymorphisms (SNPs) rs360719 and rs1012356 with *IL-18* and *IL-22* Gene Expression in Iraqi SLE Patients

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Abstract

Systemic Lupus Erythematosus (SLE) is a multisystemic autoimmune illness distinguished by biological and clinical variability. Interleukin-18 (IL-18) is a cytokine linked to some immunological responses, and elevated amounts of IL-18 have been found in autoimmune animal models and the biological fluids and organs of people with various autoimmune diseases, previous studies have indicated the importance of IL-22 (another interleukin) in the development and pathogenesis of several autoimmune diseases, including SLE. This study investigates the molecular correlation between gene expression of both IL-18 and IL-22 and different genotypes of rs360719 and rs1012356 SNPs, exploring their roles in the pathogenesis of SLE in a sample of Iraqi patients. By analyzing samples from 63 SLE patients and 42 health controls, the study investigated the expression levels of IL-18 and IL-22 expression through the qRT-PCR technique. The results of gene expression represented by fold change ($2^{-\Delta\Delta Ct}$) revealed an increased expression of IL-18 mRNA in total SLE patients, but such increase was not significantly different between male and female groups. The results obtained also suggest that there is no association between SLE and IL-22 rs1012356, and the data obtained show that the IL-18 rs360719 polymorphism may be associated with susceptibility to SLE and the expression levels of both genes were higher in female SLE patients than in female controls, indicating that these cytokines might have a role in the disease's preponderance in females. By providing possible biomarkers for early detection, prognosis, and the development of targeted therapeutics, the study paves the way for additional research into the molecular pathways of these molecules with the goal of improving the care and results for SLE patients in Iraq.

Keywords: SLE, SNP, Interleukin-18, Interleukin-22, Gene expression.

العلاقة بين تعدد الأشكال *rs360719* و *rs1012356* والتعبير الجيني لـ *IL-18* و *IL-22* في مرضى الذئبة الحمراء العراقيين

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الخلاصة

داء الذئبة الحمراء الجهازي (SLE) هو مرض مناعي ذاتي يمتد إلى العديد من أجهزة الجسم ويتميز بالتنوع بيولوجياً وسريرياً. يرتبط بين ابيضاض-18 (IL-18)، وهو محرك خلوي تفرزه كريات الدم البيضاء ببعض الاستجابات المناعية، وقد تم العثور على كميات مرتفعة منه في النماذج الحيوانية للإصابة بأمراض المناعية الذاتية وكذلك في السوائل البيولوجية وأعضاء الأشخاص المصابين بأمراض المناعة الذاتية المختلفة. وقد أشارت الدراسات السابقة إلى أهمية *IL-22* (وهو بين ابيضاض آخر) في تطور العديد من أمراض المناعة الذاتية، بما في ذلك الذئبة الحمراء. تبحث هذه الدراسة في الارتباط الجزيئي بين التعبير الجيني لكل من *IL-18* و*IL-22* و وكل من تعدد الأشكال للقاعدة المفردة rs360719 (SNP) وrs1012356، واستكشاف أدوارها في تطور داء الذئبة الحمراء بين المرضى العراقيين. من خلال تحليل عينات من 63 مريضًا بمرض الذئبة الحمراء و42 فرداً كسيطرة، بحثت الدراسة في مستويات التعبير عن IL-18 وIL-22 من خلال تقنيات qRT-PCR. كشفت نتائج طية التعبير الجيني ($2^{-\Delta\Delta Ct}$) عن زيادة في التعبير عن mRNA لـ *IL-18* في إجمالي مرضى الذئبة الحمراء، لكن هذه الزيادة لم تكن معنوية بشكل كبير بين مجموعات الذكور والإناث. وتشير النتائج التي تم الحصول عليها أيضًا إلى عدم وجود ارتباط بين داء الذئبة الحمراء و*IL-22* في rs1012356، وتُظهر البيانات التي تم الحصول عليها أن تعدد أشكال *IL-18* في rs360719 قد يكون مرتبطًا بقابلية الإصابة بالذئبة الحمراء وأن مستويات التعبير عن كلا الجينين كانت أعلى لدى مرضى الذئبة الحمراء الإناث مقارنة بأفراد السيطرة الإناث، مما يشير إلى أن هذه المحركات الخلوية قد يكون لها دور في زيادة نسبة المرض لدى الإناث. ومن خلال توفير المؤشرات الحيوية المحتملة للكشف المبكر والتشخيص وتطوير العلاجات المستهدفة، تفتح الدراسة الباب أمام إجراء أبحاث إضافية في المسارات الجزيئية لهذه الجزيئات بهدف تحسين الرعاية والنتائج لمرضى الذئبة الحمراء في العراق.

1. Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune illness that can affect multiple organs of the body [1], characterised by the generation of many autoantibodies [2], multi-organ involvement, and chronic inflammation [3]. Numerous autoantibodies are linked to the SLE symptoms, resulting in the formation and deposition of immunological complexes [4]. The clinical signs of SLE are quite varied [5], ranging from minor symptoms to significant organ damage. Although the precise aetiology of SLE is still unknown [6], its pathogenesis is thought to be influenced by several genetic, epigenetic, immunological and hormonal variables [7]; hyperprolactinemia (HPRL), for example, has been reported in SLE Iraqi individuals [8]. The development of SLE is mostly influenced by genetic vulnerability; many single nucleotide polymorphisms (SNPs) in immune-related genes have been implicated as possible causes [9, 10]. Among these genetic factors, studies have focused on cytokine dysregulation and specific SNPs associated with immune responses.

Cytokines, which are small proteins secreted by immune cells, play essential roles in immune response modulations [11]. Dysregulation of cytokine signaling is a hallmark of autoimmune diseases [12, 13], including SLE. Interleukin-18 (IL-18) and Interleukin-22 (IL-22) are two key cytokines that have garnered interest [14, 15] due to their involvement in the inflammatory processes of SLE.

The pro-inflammatory cytokine IL-18 is primarily produced by macrophages and dendritic cells [16], which promotes Th1-type immune responses by inducing the production of interferon-gamma (IFN-γ) [17]. Th1 responses are known to contribute to the pathogenesis of SLE through the activation of autoreactive T cells [18] and subsequent antibody production. Elevated serum levels of IL-18 have been consistently observed in patients with SLE [19] and are associated with increased disease activity, organ damage, and poor prognosis. IL-18 also promotes tissue damage [20] in key organs affected by SLE, including kidneys and skin. This

suggests that IL-18 not only serves as a marker of disease severity but also as a potential therapeutic target in managing SLE. Although IL-18 involves many other tissue damages like systemic juvenile idiopathic arthritis (s-JIA) [21].

On the other hand, IL-22 is primarily produced by Th17 cells [22] and innate immune cells such as natural killer (NK) cells [23]. It maintains epithelial barrier integrity [24] and promotes tissue repair [24], particularly in the skin, lungs, and gastrointestinal tract. However, its role in SLE is more nuanced. While IL-22 contributes to the protective immune responses in epithelial tissues, its overexpression may exacerbate autoimmune conditions [25]. Studies have shown increased IL-22 levels in patients with SLE, suggesting that IL-22 could contribute to inflammation and tissue damage in certain organs, particularly skin [26].
In addition to cytokine dysregulation, genetic polymorphisms are known to influence the risk and severity of SLE [27]. Two SNPs, rs360719 and rs1012356, have been highlighted for their potential roles in SLE pathogenesis. The rs360719 SNP is located in the *IL18* gene, potentially affecting the transcription and expression of *IL-18* [28]. Variants of rs360719 have been associated with altered IL-18 production, which could influence the pro-inflammatory responses seen in SLE. Polymorphisms in the *IL18* gene may, therefore, modulate the severity of immune activation in SLE, providing a genetic link to elevated IL-18 levels observed in patients.

The rs1012356 SNP is located in a region that may regulate immune-related genes [29], including those associated with *IL-22* signaling pathways. While the exact functional consequences of rs1012356 remain under investigation, it is hypothesized that this polymorphism may affect immune cell function [30], contributing to the dysregulated immune responses seen in SLE. Understanding how rs1012356 impacts *IL-22* could help clarify its role in SLE susceptibility and progression.

This study has tried to search the relationship between the dysregulations in Interleukins 18 and 22 and genetic polymorphisms rs360719 and rs1012356, which offers valuable insights into the pathogenesis of SLE.

2. Materials and Methods

2.1. Study Design

The study included 63 SLE patients (58 females and 5 males) and 42 apparently healthy controls (38 females and 4 males). The age range for the patients was 34.1 ± 1.6 years (31.1 ± 1.2 for males and 33.9 ± 1.7 for females), and for the controls, it was 31.1 ± 1.2 years (30.5 ± 5.4 for males and 31.1 ± 1.2 for females) with no statistically significant difference between any of the corresponding groups. The patients were visiting CMC hospital and Norin Polyclinic, Erbil, Iraqi Kurdistan Region, for their periodical review from April to December 2023.

2.2. Blood Samples Collection

Venous blood samples were obtained from each patient and healthy volunteers and each sample was divided immediately into two aliquots. The first aliquot (2 ml) was transferred to a sterile EDTA tube, from which 0.3 ml of blood was transferred to two Eppendorf tubes each; one was closed tightly and stored at -70ºC until assayed for detection of interleukins SNPs, and the other for gene expression detection as it is clarified in the next section.

2.3. ANA and Anti-dsDNA

The studied groups were screened for ANA and anti-dsDNA autoantibodies by using a commercial kit (IMTEC-ANA-LIA MAXX, Human company, Germany) that is based on indirect membrane-based enzyme immunoassay for the quantitative measurement of IgG class antibodies and instructions of the manufacturer were followed.

2.4. Real Time-PCR (RT–PCR)

Up to 200 µl of blood sample was added to 600 µl of GENEzol™ Reagent, and the subsequent steps were followed to isolate total RNA using the components of the GENEzol TriRNA Pure Kit (Geneaid Biotech/ Taiwan). The steps included homogenization and lysis of the blood, RNA binding to RB columns, washing and elution. Nano-drop was used to assess the RNA quantity. Then, qMAXSen™ One-Step Green RT-qPCR Kit (Canvax/ Spain) was used to estimate the expression levels of *IL-18* (accession number: NC_000011.10) [31] and *IL-22* (accession number: NC_000012.12) [32] genes by RT-qPCR and the *GAPDH* gene was used as a housekeeping gene. The kit condition starts with reverse transcription of cDNA synthesis, followed by initial activation, denaturation and annealing/extension.

The sequences of the forward and reverse primers of the genes which were used in the present study were designed as follows, respectively: *IL-18:* 5′-GGTTTTGGAAGGCACAGAGC-3′ and 5′-AAGCCCCAGAGTAATGCTTGA-3′, *IL-22:* 5′-GCTCCTGTGGTGGTTAGGTC-3′ and 5′-CCTGGTCGAAGACAACGTGA-3′. *GAPDH* primer sequences were picked up from previous studies and were forward 5′-CAATGACCCCTTCATTGACC-3′ and reverse 5′-TTGATTTTGGAGGGATCTCG-3′ [33].
To each well of the PCR plate, the following components were added: 5 µl of Master Mix (Green one step low ROX), 1 µl of each of forward and reverse primers and RT mix, 2 µl of RNA template and 10 µl of nuclease-free water. The final volume of the mixture in each well was 20 µl.

2.5. Genomic DNA Extraction and Genotyping:

DNA was isolated using 2 mL of whole blood collected in EDTA tubes using a genomic DNA purification kit (Blood DNA Preparation - Solution Kit, Jena Bioscience GmbH/ Germany). Blood cells were lysed, their protein contents were precipitated, then the DNA material was precipitated, and lastly, it was hydrated using a DNA hydration solution.
The amplification of DNA is represented in fragments of *IL-18* and *IL-22* genes (forward and reverse). Then, the polymorphisms of the *IL-18* and *IL-22* genes were detected using the PCR-sequencing method. PCR amplifications were detected in a total volume of 25 µl consisting of 5 µl genomic DNA (30 to 53.3 µg/ml), 13 µl D.W., 5 µl master mix [1 U DNA polymerase, 1000 µM dNTP, Reaction Buffer with 1.5 mM $MgCl_2$ (1x)] and 1 µl of each of the primers, and the PCR process was managed according to Table-1.

Table 1: The PCR program for *IL-18* and *IL-22* polymorphism detection.

No.	Steps	Temperature (°C)	Time	Number of Cycles
1	Initial denaturation	94	5 min.	1
2	Denaturation	94	45 sec	
3	Annealing	58	45 sec	35
4	Extension	72	2 min	
5	Final extension	72	5 min	1

The forward primer sequence of the *IL-18* gene SNP rs360719 was 5′-TAGGAAGGGAATTAGCAAGG-3′, and that of the reverse was 5′-GGGTAGGAATAAGTGAGATG-3′ and showed a single band of 657 bp molecular size. While the sequence of the forward primer of *IL-22* gene SNP rs1012356 was 5′-CCATGGGTGTGGAGGTTCATAAAG-3′ and that of the reverse was 5′-GAATGGAAGGTCTTGATGGGAGAG-3′ and showed a single band of 876 bp molecular size. All the primers were designed by previous studies [34]. PCR fragments for *IL-18* and *IL-22* were confirmed based on their separation on a 2% agarose gel with ethidium bromide. Then, a sequencing technique was used to identify the *IL-18* and *IL-22* genes polymorphisms.

2.6. DNA Sequencing

After amplification, the PCR products were analyzed for *IL-18* and *IL-22* genes (forward and reverse) of all SLE patients and control groups. The 105 samples (63 patients and 42 controls) were subjected to sequencing. Sanger's sequencing was performed on the samples of PCR-amplified products on *IL-18* and *IL-22* genes. The sequences were blasted to a reference sequence of *IL-18* and *IL-22* genes in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov). The process was executed in ExoGene Laboratory at Zheen International Hospital, Erbil, Iraqi Kurdistan Region, using Applied Biosystems SeqStudio Genetic Analyzer.

3. Ethics Approval

This study was authorized by the Ethics Committee, University of Baghdad, College of Science (Ref.: CSEC/1024/0072, October 20, 2024).

4. Statistical Analysis

The results of genomic DNA amplification were analyzed using Unipro UGENE software (v.50.0, 64-bit version, April, 11, 2024). Online Hardy-Weinberg equilibrium (HWE) was used to estimate the number of homozygous and heterozygous variant carriers based on its allele frequency in the groups. WINPEPI software was used to calculate the significance and odds ratios of genotyping and allele frequencies of the studied genes.

5. Results

5.1. Gene expression of IL-18

All blood samples from the 63 SLE patients and 42 controls were subjected to RT-qPCR for the *IL-18* gene expression.

The folding expression ($2^{-\Delta\Delta Ct}$) of *IL-18* mRNA was increased by 1.035 ± 0.176 folds in total SLE patients, but such increase was not significantly different between male and female groups (1.087 ± 0.450 vs. 1.031± 0.188) (Figure. 1).

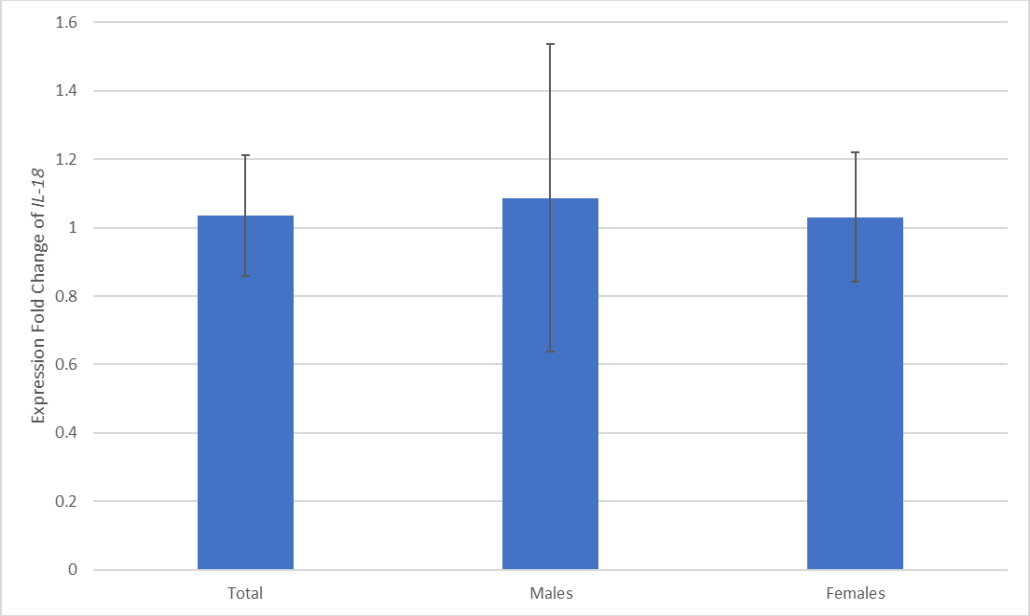

Figure1: Comparison among total male and female SLE patients' groups expression fold of *IL-18* gene.

The patients were distributed according to the duration of the disease. The expression fold ($2^{-\Delta\Delta Ct}$) of *IL-18* in SLE patients for <10 years showed a mean expression of 0.864 ± 0.279, and those with 10-20 years exhibited a higher mean of expression (1.178 ± 0.309), while patients with >20 years revealed a lower mean of 0.745 ± 0.235. However, no significant differences were found between these groups (Table 2).

The patients were also distributed according to the ANA and ani-dsDNA test results. The results of the ANA test revealed that all the patients were positive for ANA, so there is no way to compare the results statistically. While anti-dsDNA-positive patients (n = 43) had a mean expression fold of 1.051 ± 0.198, and the anti-dsDNA-negative patients (n = 20) had a lower mean of 0.782 ± 0.361. However, the difference was not statistically significant (Table 3).

Table 2: Expression fold ($2^{-\Delta\Delta Ct}$) of *IL-18* mRNA in SLE patients distributed by laboratory and clinical findings**.**

Groups		Number	Fold Change (Mean ± SEM)	LSD *p*
Duration of Disease	< 10 years	26	0.864 ± 0.279	0.628 (NS)
	10 – 20 years	25	1.178 ± 0.309	
	> 20 years	12	0.745 ± 0.235	
ANA	Positive	63	0.966 ± 0.176	SCNP
	Negative	0	0	
Anti-dsDNA	Positive	43	1.051 ± 0.198	0.516 (NS)
	Negative	20	0.782 ± 0.361	

NS: Not significant (p <0.05)
SCNP: Statistical comparison not possible

5.2. Gene expression of IL-22

The present study result showed that the fold changes in the *IL-22* gene for total male and female patients were 1.173 ± 0.2190.610 ± 0.239, and 1.221 ± 0.237, respectively, with no significant difference between males and females (Fig. 2).

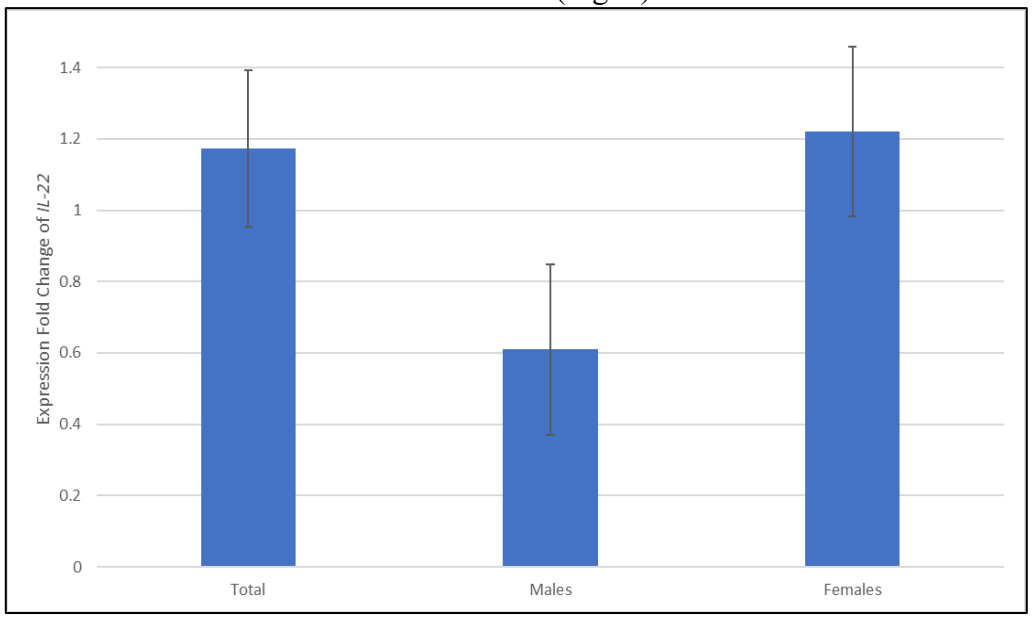

Figure-2 Comparison among total, male and female SLE patients' groups expression fold of *IL-22* gene.

The patients were distributed according to the duration of the disease, and the expression folds ($2^{-\Delta\Delta Ct}$) of *IL-22* in SLE patients for <10 years with a mean expression of 0.768 ± 0.155, and those with 10-20 years showed a higher mean of 1.204 ± 0.293, while patients with >20 years recorded with a higher mean of 2.055 ± 0.971. Overall, no significant differences were found between these groups.

The expression fold ($2^{-\Delta\Delta Ct}$) of *IL-22* in SLE patients with <10 years of disease duration had a mean expression of 0.768 ± 0.155, while those with 10–20 years had a higher mean of 1.204 ± 0.293, and those with > 20 years had a higher mean of 2.055 ± 0.971. Overall, no significant differences were found between these groups.

The patients were also distributed regarding the results obtained from ANA and ani-dsDNA tests. All the patients were positive ANA, so there is no way to compare the results statistically. While anti-dsDNA-positive patients (n = 43) had a mean expression fold of 1.339 ± 0.308, and the anti-dsDNA-negative patients (n = 20) had a lower mean of 0.814 ± 0.184, but the difference was also not significant (Table 3).

Table 3: Expression fold change ($2^{-\Delta\Delta Ct}$) of *IL-22* mRNA in SLE patients distributed by laboratory and clinical findings.

Groups		Number	Fold Change (Mean ± SEM)	LSD *p*
Duration of Disease	< 10 years	26	0.768 ± 0.155	0.151 (NS)
	10 – 20 years	25	1.204 ± 0.293	
	> 20 years	12	2.055 ± 0.971	
ANA	Positive	63	1.173 ± 0.219	SCNP
	Negative	0	0	
Anti-dsDNA	Positive	43	1.339 ± 0.308	1.462 (NS)
	Negative	20	0.814 ± 0.184	

NS: Not significant (p <0.05)

SCNP: Statistical comparison not possible.

5.3. Polymorphism of the genes

The present study examined the *IL-18* gene polymorphism rs360719 and *IL-22* gene polymorphism rs1012356 by sequencing method in SLE patients and healthy controls. The SNP rs360719 of the *IL-18* gene was observed to have three genotypes (TT, TC and CC), as shown in Figure 3, that were correspondent to two alleles, A and G, with significant variation in AA and GG between patients and controls, but the variation was lost after correction (Table-4).

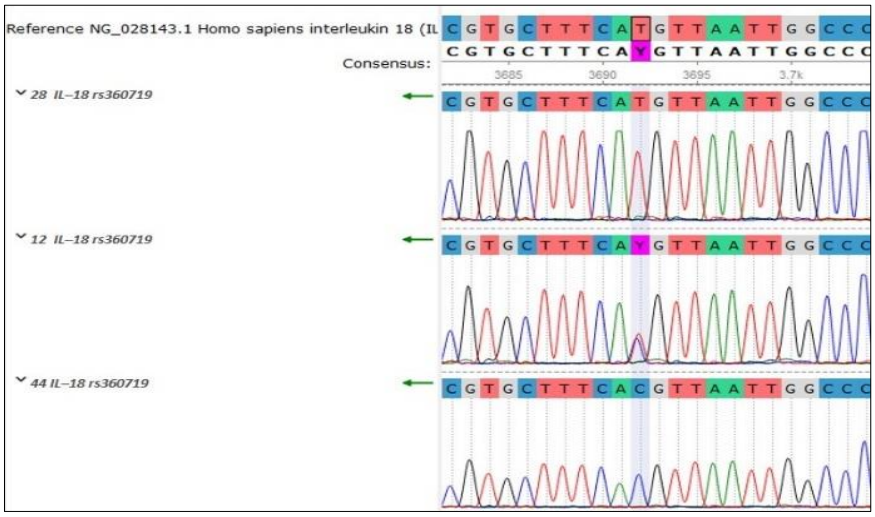

Figure 3: DNA sequence chromatogram of *IL-18* gene SNP rs360719, showing three genotypes: TT (sample 28), CT (sample 12; Y) and CC (sample 44). The reference sequence (rs1946518) is shown on top.

Table 4: Statistical analysis of association between *IL-18* gene polymorphism rs360719 genotypes and alleles, their frequencies and SLE.

Genotype or Allele	Patients (No. = 63)	Controls (No. = 42)	Odds Ratio	95% Confidence Interval	EF or PF	*p*-value	*pc*-value
TT	45	21	2.500	1.106 - 5.649	0.429	**0.039**	NS
TC	12	17	0.329	0.134 - 0.812	1.978	**0.025**	NS
CC	6	4	0.700	0.178 - 2.747	1.248	NS	NS
T	102	59	1.801	0.944 - 3.434	0.360	NS	NS
C	24	25	0.555	0.291 - 1.059	1.397	NS	NS

EF: etiological fraction; PF: preventive fraction; *p*: Fisher's exact probability; *pc*: Corrected *p*; NS: not significant.

The SNP rs1012356 of *IL-22* was also observed to have three genotypes (TT, TA and AA) (Figure 4), that correspond to two alleles, T and A, with no significant variation (Table 5).

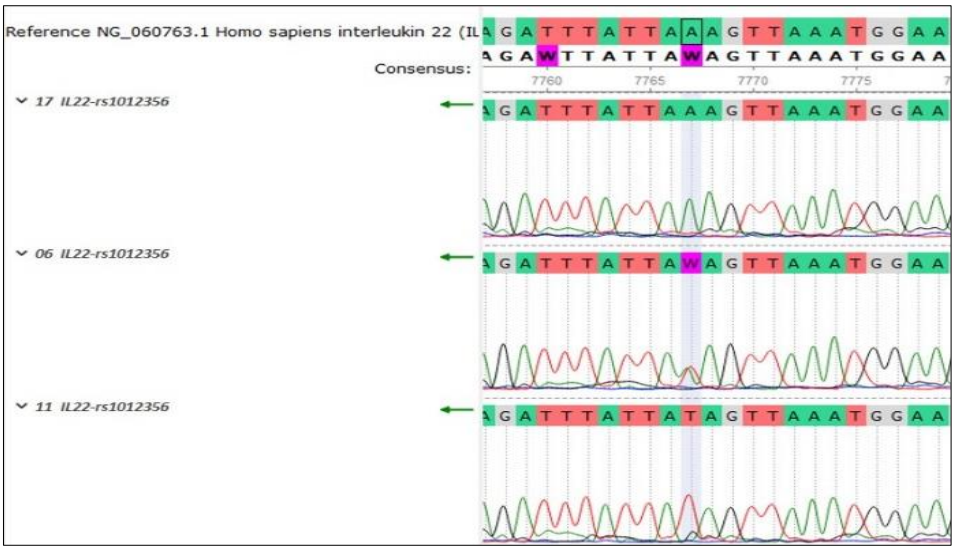

Figure 4: DNA sequence chromatogram of *IL-22* gene SNP rs1012356, showing three genotypes: AA (sample 17), TA (sample 6; W) and TT (sample 11). The reference sequence rs1012356 is shown on top.

Table 5: Statistical analysis of association between *IL-22* gene polymorphism rs1012356 genotypes and alleles, their frequencies and SLE.

Genotype or Allele	Patients (No. = 63)	Controls (No. = 42)	Odds Ratio	95% Confidence Interval	EF or PF	*p*-value	*pc*-value
TT	22	14	1.073	0.471 - 2.448	0.024	NS	NS
TA	26	18	0.919	0.374 - 2.261	0.521	NS	NS
AA	15	10	0.955	0.336 - 2.711	0.630	NS	NS
T	70	46	1.033	0.593 - 1.799	0.018	NS	NS
A	56	38	0.968	0.556 - 1.687	0.580	NS	NS

EF: etiological fraction; PF: preventive fraction; *p*: Fisher's exact probability; *pc*: Corrected *p*; NS: not significant.

5.4. Correlation between Gene Expression and Genotypes

IL-18 expression fold ($2^{-\Delta\Delta Ct}$) levels in SLE patients and controls were analyzed by genotypes of the *IL-18* SNP rs360719, showing that TT genotype mean expression in controls was 0.979 ± 0.152, not significantly different from that of TC genotypes in control and corresponding SLE group (1.284 ± 0.258 and 0.663 ± 1.166, respectively). Meanwhile, TC genotype mean expression in control was 1.284 ± 0.258, and the value shows a significant difference from that of CC in controls and not significant to corresponding SLE patients (3.608 ± 0.378 and 1.352 ± 0.575, respectively). While CC genotype mean expression in the control was 3.608 ± 0.378, which differs significantly from that of SLE patients in the corresponding group (2.463 ± 0.359) and from the other two genotypes in the controls (Table 6).

Table 6: *IL-18* expression fold ($2^{-\Delta\Delta Ct}$) in SLE patients and controls distributed by *IL-18* SNP rs360719 genotypes.

Groups	Number	Polymorphisms (Mean ± SEM)		
		TT	TC	CC
Controls	42	0.979 ± 0.152^{AF}	1.284 ± 0.258^{AG}	3.608 ± 0.378^{B}
SLE patients	63	0.663 ± 1.166^{CF}	1.352 ± 0.575^{DG}	2.463 ± 0.359^{E}

Different letters: Significant difference ($p \leq 0.05$) between means (Student t-test)
Similar letters: No significant difference ($p > 0.05$) between means (Student t-test).

Regarding *IL-22* SNP rs1012356 genotypes, expression folds for TT, TA and AA in controls were 0. 958 ± 0.104, 1.042 ± 0.108 and1.351 ± 0.130, respectively, and those of SLE patients were 0.892 ± 0.336, 1.299 ± 0.283 and 1.364 ± 0.621, respectively. The results showed no significant difference between the TT genotype expression fold in control and that of TA and the corresponding patient group. The expression fold of the AA genotype was significantly different from those of the other control genotypes but not with that of the corresponding patient group. The values of fold expression in patients showed no significant difference from each other (Table 7).

Table 7: *IL-22* expression fold ($2^{-\Delta\Delta Ct}$) in SLE patients and controls distributed by *IL-22* SNP rs1012356 genotypes.

Groups	Number	Polymorphisms (Mean ± SEM)		
		TT	TA	AA
Controls	42	$0. 958 \pm 0.104^{AD}$	1.042 ± 0.108^{AE}	1.351 ± 0.130^{BF}
SLE patients	63	0.892 ± 0.336^{CD}	1.299 ± 0.283^{CE}	1.364 ± 0.621^{CF}

Different letters: Significant difference ($p \leq 0.05$) between means (Student t-test)
Similar letters: No significant difference ($p > 0.05$) between means (Student t-test).

6. Discussion

This study examined the expression levels of the SNPs rs360719 in the *IL-18* gene and rs1012356 in the *IL-22* gene in both SLE patients and control groups. Three genotypes (TT, TC, CC for *IL-18*; TT, TA, AA for *IL-22*) were detected by Sanger sequencing, and each genotype corresponded to two alleles (T and C for *IL-18*; T and A for *IL-22*).
Despite the fact that the control group had higher mean levels, there was no statistically significant difference in *IL-18* expression between the SLE patients and controls across all subjects. This implies that *IL-18* expression levels are comparable in both groups when examining the total group without considering the gender differences.

There were no significant differences in the *IL-18* expression of male participants with SLE and that of male controls. This suggests no gender-specific difference in IL-18 expression exists between male SLE patients and male controls.

Our results implied that the duration of the SLE disease in the patients did not appear to have a significant impact on *IL-18* expression, as there were no significant differences when the duration of the disease was considered, which was consistent with a previous study [35].
Since all patients in this investigation were ANA-positive, it is not possible to compare the levels of *IL-18* expression. Therefore, there is no information in this sample about how ANA status may affect *IL-18* expression in SLE patients, and this was the same issue for *IL-22*.
In this study, *IL-18* expression did not significantly differ between anti-dsDNA-positive and anti-dsDNA-negative patients. This may reveal that *IL-18* mRNA expression levels in SLE patients are not significantly impacted by the presence of anti-dsDNA antibodies. However, it had been found in a previous study that IL-18 levels were significantly elevated in SLE patients with anti-dsDNA antibody titers [36].
Before the correction, the *IL-18* gene polymorphism rs360719 was found to be substantially linked with SLE. With an odds ratio (OR) of 2.500 and a *p*-value of 0.039, the TT genotype was more common in patients than in controls (45 vs. 21), indicating a greater risk of SLE in those carrying the TT genotype. This was proved to be true in Polish SLE patients, as a study indicated that patients carrying the allele C and CC genotype had a decreased risk of the disease [37]. Similarly, the TC genotype appeared to have a protective impact (OR = 0.329, *p*

= 0.025), which might indicate a lesser chance of having SLE in individuals with this genotype. Nevertheless, these relationships ceased to be significant when multiple comparisons were taken into account (*pc*-value).

The lack of significance following adjustment emphasises the need for care in how these data are interpreted [38]. Despite that, the uncorrected data indicated that the TT genotype may increase the risk of developing SLE, and the TC genotype may provide protection; the lack of significance after correction may indicate that sample size restrictions or other confounding variables are at play. It's probable that more extensive researches are needed to verify rs360719 could be involved in the development of SLE.

In contrast to rs360719 of *IL-18*, the rs1012356 polymorphism in the *IL-22* gene did not significantly correlate with SLE. Similarity distribution was observed between patients and controls in the genotypic distributions of TT, TA, and AA as well as the allelic frequencies of T and A. This might elucidate that SLE susceptibility in this population is not likely to be influenced by the *IL-22* rs1012356 polymorphism. These results coincided with those reported by Attia and her co-workers, who reported no clear correlation between *IL-22* gene polymorphisms and autoimmune disorders, such as SLE [29]. Considering the significance of *IL-22* in immunological control and inflammation, more research may be conducted to investigate whether additional variations in this gene or associated signalling pathways may be linked to SLE.

This study examined the expression levels of the *IL-18* and *IL-22* genes in SLE patients and healthy controls. This might allow us to investigate the effects of sex-specific patterns in the expression of these genes and their relation to SLE. As observed from the results, among all SLE cohorts, there were changes in the expression levels of *IL-18*, but they were not significant, which revealed no significant difference in *IL-18* gene expression between controls and patients.

When analysed by sex, there was no statistically significant difference between the male and female groups. On the other hand, *IL-18* expression in female patients was considerably higher than in female controls ($p < 0.05$), indicating that female SLE patients may have sex-specific overexpression of *IL-18*. This finding is consistent with the prevailing knowledge of the disease's female preponderance, as SLE is known to disproportionately impact women. The limited sample size could influence the absence of significant differences in males, which reduces statistical power. Additionally, biological differences in immune regulation between males and females may play a role, as sex hormones and genetic factors have been shown to influence immune responses. Though *IL-18* expression may not be a reliable biomarker for SLE in the general population, it may be more significant in elucidating sex-based differences in SLE pathogenesis, as evidenced by the lack of substantial expression changes in the community as a whole and in male patients. However, previous studies have shown that IL-18 plays a role in developing SLE in people [39] and mice [40].

The research found that there are significant variations in the expression of the *IL-22* gene between SLE patients and controls. There was a slight elevation in *IL-22* expression in people with SLE, and this may indicate that IL-22 plays a role in the inflammatory processes linked to SLE [41].
When the results were broken down by sex, the expression of *IL-22* in male patients was not significantly different from that of the male controls. On the other hand, *IL-22* expression in female patients was significantly higher than in female controls ($p = 0.004$). The expression

of *IL-22* can be influenced by various variables, especially estrogen [42]. Numerous studies have demonstrated that sex hormones, such as progesterone and androgens, control the generation of cytokines and other immune responses [43-45]. Despite hormones' roles, genetic and epigenetic factors amplify cytokine dysregulation that may influence immune response [46, 47]. This pattern is consistent with the findings regarding *IL-18*, where the overexpression observed in women elucidated that these cytokines may be more important in the pathophysiology of SLE in women than in men.

The longer the condition is present, the higher the expression of *IL-22*. *IL-22* expression levels were lower in patients with a shorter disease duration (less than 10 years) and higher in patients with a longer disease duration (more than 20 years). Though *IL-22* expression may be increased over time in SLE patients, these differences were not statistically significant, indicating that this elevation was not significant enough to support a strong correlation with disease duration in this group.

In patients with anti-dsDNA status, *IL-22* expression seems to be marginally higher than in those with anti-dsDNA positivity. The fact that this difference is likewise not statistically significant may suggest that the presence of anti-dsDNA has no discernible effect on the expression of *IL-22* mRNA in SLE patients in our dataset.

Across particular genotypes of *IL-18* SNP rs360719, there was a significant difference in the IL-18 expression fold ($2^{-\Delta\Delta Ct}$) levels between SLE patients and controls. When compared to other genotypes, the SLE group's drop in IL-18 expression is noticeable, although it is not a significant difference. This suggests that the TT genotype has a negligible impact on the control of IL-18 in SLE. The absence of a statistically significant difference in expression between TC genotype groups implies that TC genotype may not significantly impact *IL-18* expression levels in SLE. Nonetheless, the overall increase in IL-18 in controls draws attention to possible immunological modulation linked to this genotype. The significant decrease in IL-18 levels seen in SLE patients with the CC genotype may indicate that this genotype may have a part in reducing *IL-18* expression, which could lead to disease pathogenesis or susceptibility.

SLE patients and controls also showed significant differences in the *IL-22* expression fold ($2^{-\Delta\Delta Ct}$) levels, especially for the TA and AA genotypes of *IL-22* SNP rs1012356. The expression level of the TT genotype was marginally higher in controls than in SLE patients. The TT genotype may not have a significant effect on *IL-22* dysregulation in SLE patients based on this slight decrease in *IL-22* expression. The *IL-22* expression level in controls with the TA genotype was noticeably lower than that of SLE patients. The TA genotype may contribute to *IL-22* overexpression in the illness environment, as seen by the increased *IL-22* expression in SLE patients with this genotype. However, controls for the AA genotype did not differ significantly from SLE patients, suggesting that the AA genotype may not significantly influence *IL-22* expression differences between healthy individuals and SLE patients.

Our results contributed to the increasing amount of data indicating that *IL-22* may play a part in the pathophysiology of SLE. Previous investigations have linked *IL-22* to a number of inflammatory and autoimmune illnesses due to their function in tissue repair, protection, and epithelial cells [48-50].

Our study's primary limitation is its remarkably small sample size, which would have decreased the statistical power required to identify weaker associations—especially once multiple comparisons were taken into account. A more thorough understanding of the genetic

factors influencing SLE susceptibility may be possible with more studies, including larger cohort studies and investigating additional SNPs or haplotypes.

Conclusion

In the present study, it has been concluded that the *IL-18* rs360719 polymorphism may be linked to susceptibility to SLE. However, this correlation was not statistically significant when multiple comparisons were considered. No correlation was discovered between SLE and *IL-22* rs1012356. To better understand the possible involvement of these cytokine genes in the pathophysiology of SLE, more extensive research and functional analyses are required. Despite not reaching significance, the expression levels of both genes, *IL-18* and *IL-22,* were higher in female SLE patients than in female controls, indicating that these cytokines might have a role in the disease's preponderance in females. These results emphasise the need to take sex differences into account when studying autoimmune diseases and point to *IL-22* as a possible target for further research on the pathophysiology of SLE.

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9. Conflict of Interest

The authors declare that they have no conflict of interest.

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