ORIGINAL ARTICLE

Association of Serum Programmed Cell Death Protein 1 (PD-1) and Gene Polymorphism with Some Valid Predictors for Systemic Lupus Erythematosus (SLE) Patients in Basra Province, Iraq

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ABSTRACT

INTRODUCTION: Systemic Lupus Erythematosus (SLE) is a systematic autoimmune disorder characterized by the production of autoantibodies against nuclear antigens and inflammation initiation. We aimed to examine the correlation between IL-18, IL-37, and PD-1, and the potential link between polymorphisms in the PD-1 gene located in intron-4 and the susceptibility to SLE. **MATERIALS AND METHODS:** This cross-sectional study included 43 SLE and 53 healthy individuals. Blood samples were obtained and underwent biochemical examination. The polymorphisms were screened by amplifying the intron-4 of the PD-1 gene using particular primers and then verified through sequencing. **RESULTS:** Our findings demonstrated statistically significant positive correlations between IL-18, IL-37, and PD-1, while the AUC of the ROC curve is 0.985, 0.968, and 0.940, and cut-off concentration is ≥132.87, ≥62.98, and ≥169.02, respectively. Moreover, two separate SNPs (rs6705653 and rs41386349) were discovered within intron-4 of the PD-1 gene. The genotype AA of the +7499 (G/A) SNP was significantly related with an increased risk of SLE (OR=3.11, 95%CI=1.52–5.94, p-value=0.031). Additionally, the A allele was identified as a risk allele (OR=1.59, 95%CI=1.09–2.31, p-value=0.043). Nevertheless, our study didn't find any noteworthy connection between the allele and genotype of the +7209 (C/T) polymorphism region of the PD-1 gene frequencies and the susceptibility to SLE. **CONCLUSION:** IL-18, IL-37, and PD-1 may play significant roles in SLE immune responses and processes. Furthermore, the sequencing examination of intron-4 within the PD-1 gene demonstrated a noteworthy correlation between the A allele and the AA genotypes of PD-1 +7499 (G/A) SNP presence with the increased SLE susceptibility.

Keywords
Interleukin-18, Interleukin-37, PD-1, genetic polymorphism, SLE

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INTRODUCTION

Systemic lupus erythematosus (SLE) is characterized by its heterogeneity, inflammatory nature, and ability to affect multiple systems inside the body. Multiple genetic variables have been associated with this disease, suggesting that both the loss of self-tolerance and genetic abnormalities contribute significantly to SLE pathogenesis.[1]

Interleukin-18 (IL-18) is a cytokine with several functions, vital in regulating innate and adaptive immunity. It is a highly effective inducer of interferon-gamma (IFN-γ) production, exhibiting this stimulatory influence on both natural killer (NK) cells and T-helper 1 (TH1) lymphocytes. Consequently, it is likely to have a role in SLE development.[2]

Interleukin-37 (IL-37) belongs to the IL-1 cytokine family. Its expression is significantly elevated in tissues experiencing inflammation.[3] The main function of IL-37 is to suppress both innate and acquired immune responses by inhibiting intracellular and extracellular processes, namely, by reducing the release of proinflammatory chemokines. IL-37 shows potential in mitigating autoimmune diseases, such as SLE, by counteracting tissue damage induced by inflammatory cytokines.[4]

Programmed cell death protein 1 (PD-1), a transmembrane protein, is involved in the essential regulation of immunological homeostasis required for the maintenance of self-tolerance. The aforementioned molecular structure functions as a negative costimulatory agent.[5] The PD-1 gene is situated on chromosome 2q37.3 and comprises six discrete introns.[6] The development and propagation of numerous autoimmune illnesses, such as SLE, have been linked to PD-1 gene variants and their dysregulated manifestations. Intron-4 is a highly significant polymorphic area within the PD-1 intronic locus.[7] The presence of single nucleotide polymorphisms (SNPs) within intron-4 has the potential to impact the binding locations of these factors, hence modifying the transcription process and potentially influencing the expression of PD-1.[8]

Nevertheless, there remains a dearth of information elucidating the correlation between blood concentrations of IL-18, IL-37, and PD-1, and the involvement of PD-1 gene variants, namely, within intron-4, in SLE subjects. The objective of the current research was to explore the possible connection between IL-18, IL-37, and PD-1. Additionally, the study aimed to examine the potential relationship between PD-1 gene polymorphisms located in intron-4 and the susceptibility to SLE in a population from Basra Province, Republic of Iraq.

MATERIALS AND METHODS

Study Design and Subject Recruitment

This cross-sectional study recruited a total of 43 patients who met the criteria of complying with at least 4 elements of the updated American College of Rheumatology (ACR) guidelines for SLE diagnosis.[9] Moreover, 53 healthy volunteers were also included in the study. Each volunteer experienced a consistent and unchanging clinical progression for a minimum duration of three months. All patients were directed to the rheumatoid clinics of teaching hospitals of Al-Mawany, Al-Sadr, Al-Fayhaa, and Al-Basra, situated in Basra Province, Iraq. The investigation was carried out from September 2022 to October 2023. Exclusion criteria included patients or controls who had been diagnosed with or reported an active inflammatory condition, pregnant participants, and persons with a family history of any other autoimmune diseases.[10] The participants were duly informed about the study's objectives and potential outcomes, and their informed consent was obtained following the guidelines specified in Helsinki Declaration. The current research using human specimens has been approved by the Ethics and Behavioral Research Committee (Scientific Committee) of the Department of Chemistry, College of Science, University of Basrah (Code of Ethics: 7/54/732 in 11/09/2022).

Samples Collection

Blood samples were obtained within the specified time interval of 09:00 AM and 10:00 AM, following a preceding period of 30 minutes dedicated to relaxation. A 10 mL volume of venous blood, which had been recently collected, was obtained from the subjects' veins. Subsequently, the blood sample was divided into two portions. The first portion, consisting of 5 mL, was transferred into an EDTA tube to extract genomic DNA. The second portion, also 5 mL in volume, was transferred into a tube without any anticoagulant to extract serum for biochemical analysis.

Measurement of C3, C4 , MDA, Urea, and Creatinine with UV-Vis Spectrophotometer

Laboratory kits from Linear Chemicals S.L.U., Barcelona, Spain, were utilized for determining C3, C4, urea, and creatinine, while a kit from Bioassay Systems, California, USA, was utilized for estimating MDA levels using UV-Vis Spectrophotometer (UV-EMC-LAB, Germany). Specimens were exposed to a light source with a wavelength ranging from 190 to 900 nm, spanning the ultraviolet (UV) to the visible spectrum. The assessment was conducted to ascertain the extent of light absorption, transportation, or scattering throughout distinct spectral ranges.[11]

Measurement of CH50, TAC, CRP, ANA, Anti-dsDNA, IL -18, IL-37, and PD-1 with Enzyme Linked Immunoabsorbance Assay (ELISA)

Sandwich human ELISA laboratory kits from BT Laboratory, Shanghai, China, were utilized for measuring the levels of serum CRP, IL-18, IL-37, and PD-1, while

kits from AMSBIO, Milton, UK; Cell Biolabs, California, USA; LS Bio, Washington, USA; and MyBioSource, California, USA, were utilized for determining the levels of CH50, TAC, ANA, and anti-dsDNA, respectively, using ELISA (Humareader/Human/Germany). All procedures were performed according to the instructions specified in the kit methodology. The plate was analyzed via a Varioscan® ELISA reader produced by Thermo Fisher Scientific, with the absorbance being measured at a wavelength of 450 nm. The quantification of CH50, TAC, CRP, ANA, anti-dsDNA, IL-18, IL-37, and PD-1 levels was carried out using a standard curve for quantification.[12]

Measurements of BMI, GFR, and SLEDAI-2K

Body mass index (BMI), a measure of body fat based on height and weight, was calculated to ensure that all the participants were under the same conditions using the following equation: BMI (kg/m^2)=weight(kg)/(height)^2(m). Meanwhile, glomerular filtration rate (GFR) was calculated to evaluate renal function using the equation of the Modification of Diet in Renal Disease Study (MDRD): GFR ($mL/min/1.73\ m^2$)$=186 \times \text{Serum Cr}^{-1.154} \times \text{age}^{-0.203} \times 1.212$ (if the subject is black) × 0.742 (if the subject is a woman).

Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI-2K), a comprehensive measure created and introduced in 1985 as a clinical tool for evaluating the overall activity of SLE, was measured and evaluated by clinicians as per SLEDAI-2K variables.[13]

Genomic DNA Extraction and Screening PD-1 SNPs within Intron-4

The genomic DNA was isolated from peripheral blood leukocytes using the NucleoSpin® Blood micro spin DNA preparation kit (Geneaid, UK) following the methods provided by the manufacturer. The obtained DNA was preserved at a temperature of -70°C until the polymerase chain reaction (PCR) amplifications were performed. The locus was subjected to amplification, and sequencing was selected as the preferred method for examining the polymorphisms present within the intron-4 of the PD-1 gene. The intron-4-specific primers were provided in the following sequences. The forward primer sequence is 5'-ACAATAGGAGCCAGGCGCA-3', while the reverse primer sequence is 5'-GGGTCCTCCTTCTTTGAGG-3'.[14] The PCR amplifications were conducted using a 25 µL reaction mixture (Intron, Korea) consisting of the following components: 100 ng of genomic DNA, 10 pmol of each primer, 1 unit of Taq DNA polymerase, 10X PCR buffer, 2 mM $MgCl_2$, and 0.2 mM dNTP mixture. The amplification of the target region was performed using an Eppendorf Thermal Cycler (CBS Scientific, USA). The amplification process involved the initial denaturation stage at 95°C for five minutes. This was followed by a total of 35 cycles of denaturation at 95°C for 30 seconds, followed by annealing at 61°C for 40 seconds, then extending at 72°C for 40 seconds.[15] The amplification process concluded with a final extension step at 72°C for 5 minutes. The amplified PCR products were seen under UV radiation following electrophoresis on a 0.8% agarose gel with the SYBR-safe DNA dye (Labnet, USA). A single band with a length of 695 base pairs was detected, as illustrated in Figures 1 and 2.

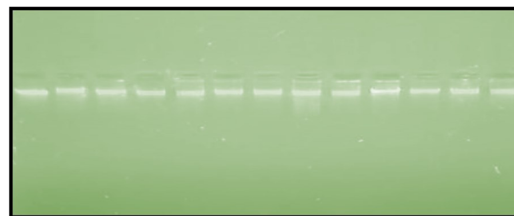

Figure 1: The integrity of genomic DNA extracted from the blood of representative samples. The DNA run on a 0.8% agarose gel at 75 voltages for 60 min, stained in 500ml of 1X TBE buffer containing 0.6 µ of ethidium bromide, then visualized by a UV transillumninator. Lane order: 1-5, DNA extracted from control individuals and lanes 6-13, DNA from patient samples with SLE.

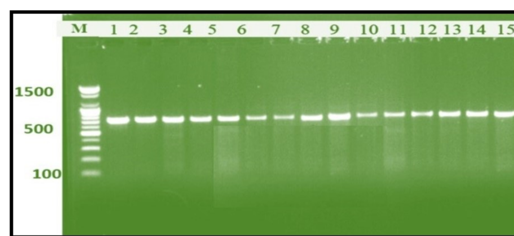

Figure 2: PCR product of amplified 695 bp fragment of intron-4 of PD-1 gene. The PCR product obtained with samples from healthy individuals (lanes order 1-5) and PCR product from SLE patient samples (lanes 6-15) and (M lane is a 100bp ladder). The PCR product was electrophoresis by a standard 1.9% (w/v) agarose gel that is pre-stained with ethidium bromide (0.6 µg/ml) in TBE buffer, using a 100bp ladder as a molecular weight marker at 5v/cm2 for 60 min.

STATISTICAL ANALYSIS

Statistical analyses were conducted using version no. 26 of the Statistical Package for the Social Sciences (SPSS), developed by IBM Corporation (Armonk, NY, USA). The data was distributed in a typical manner, and the groups

were compared using the analysis of variances before conducting Dunnett's t-test to ascertain the statistical significance. The Pearson correlation method was employed to establish the correlations. The level of significance of $p<0.05$ was considered statistically significant, whereas the level of significance of $p<0.01$ was considered highly significant. Sensitivities and specificities, together with the corresponding 95% confidence interval (95% CI), were determined using the ROC curve (receiver operating characteristics), and the AUC (area under the ROC curve) was measured (the value of AUC approaching 1 or 0 demonstrated a strong diagnosis significance).

Frequencies of categorical data, specifically genotypes and alleles, were assessed to determine whether they conformed to the HWE (Hardy-Weinberg equilibrium). The PD-1 gene genotyping and frequency of alleles were evaluated in comparison among several groups using a chi -square (X2) test. The present study examines both 95% CI and OR (odds ratio). HWE is a mathematical equation utilized for estimating genotypes and allele frequencies: $p^2+2pq+q^2=1$.

The variable p denotes the frequency of the major allele, while the variable q denotes the frequency of the minor allele. The HWE is a statistical model allowing for the estimation of genotype and allele frequencies within a population. It also enables the assessment of the degree to which a population deviates from HWE. This assessment is often conducted by comparing the observed genotypes with the expected genotypes using a chi-square test. A p-value less than 0.05 indicates a significant divergence from the HWE, whereas a p-value greater than 0.05 indicates no significant deviation from the HWE for the mean.

RESULTS

Subjects Characteristics and Biochemical Analysis

The current study had a cohort of 43 SLE female subjects. A cohort of 53 women who were in good health were selected as control participants and matched with the corresponding subjects for the research investigation. No statistically significant differences were observed in the mean age (35.44±4.85 vs. 34.29±5.19 years) and BMI (23.92±0.13 vs. 23.99±0.86) between SLE subjects and the control group. The mean SLE duration is 0.99±0.24 whereas the mean SLEDAI (systemic lupus erythematosus disease activity index) is 2.75±1.22 for SLE subjects.

On the contrary, the data acquired from Table I indicates a statistically significant increase ($p<0.01$) in the serum levels of MDA, CRP, ANA, anti-dsDNA, urea, creatinine, IL-18, IL-37, and PD1, and a statistically significant decrease ($p<0.01$) in the serum levels of C3, C4, CH50, TAC, and GFR in SLE subjects compared to controls.

Moreover, the data obtained from the AUC analysis suggests that several biomarkers, namely, IL-18, IL-37, and PD-1, may have the potential to be more accurate predictive indicators in individuals with SLE. The cut-off concentration, sensitivity, specificity, and AUC values for IL-18, IL-37, and PD-1 are depicted in Table II and Figure 3.

Table I: Total parameters levels in the current study for both SLE patients and control group.

The Characteristics	SLE Patients N = 43 (Women)	Healthy Control N = 53 (Women)	P-Value
	Mean ± SD	Mean ± SD	
C3 (mg/mL)	0.85 ± 0.39	1.63 ± 0.20	<0.01
C4 (mg/mL)	0.29 ± 0.12	0.44 ± 0.17	<0.01
CH50 (IU/mL)	51.46 ± 15.24	81.69 ± 8.99	<0.01
MDA (μmol/L)	2.49 ± 0.75	0.87 ± 0.15	<0.01
TAC (pg/mL)	1.62 ± 0.40	2.19 ± 0.46	<0.01
CRP (mg/dL)	10.77 ± 4.83	0.17 ± 0.04	<0.01
ANA (IU/mL)	2.39 ± 0.73	0.98 ± 0.31	<0.01
Anti-dsDNA (IU/mL)	28.22 ± 6.38	15.63 ± 2.47	<0.01
Urea (mg/dL)	54.98 ± 12.60	28.27 ± 7.40	<0.01
Creatinine (mg/dL)	1.07 ± 0.18	0.70 ± 0.10	<0.01
GFR (mL/min/1.73 m²)	61.89 ± 18.66	99.53 ± 18.77	<0.01
IL-18 (pg/mL)	301.61 ± 72.51	92.83 ± 23.78	<0.01
IL-37 (pg/mL)	209.42 ± 59.50	51.28 ± 6.95	<0.01
PD-1 (pg/mL)	779.14 ± 346.89	127.68 ± 24.56	<0.01

Data are presented as mean ± SD; SD: standard deviation; N: no. of subjects; SLE: Systemic Lupus Erythematosus, C3: complement component 3; C4: complement component 4; CH50: complement hemolytic 50; MDA: malondialdehyde; TAC: total antioxidant capacity; ANA: antinuclear antibodies; Anti-dsDNA: anti–double-stranded DNA; GFR: glomerular filtration rate; PD-1: Programmed Cell Death 1; IL: interleukin.

Table II: Receiver operating characteristic (ROC) and area under curve (AUC) analysis of the measured biomarkers for the diagnosis of SLE

Parameter	Cut-off concentration	Sensitivity %	Specificity %	AUC	95% CI of AUC	P-value
IL-18 (pg/mL)	≥ 132.87	93.02	94.71	0.985	0.936 - 0.999	<0.01
IL-37 (pg/mL)	≥ 62.98	90.70	89.14	0.968	0.911 - 0.993	<0.01
PD-1 (pg/mL)	≥ 169.02	91.43	88.65	0.940	0.872 - 0.978	<0.01

AUC: Area Under the Curve, CI: Confidence Interval

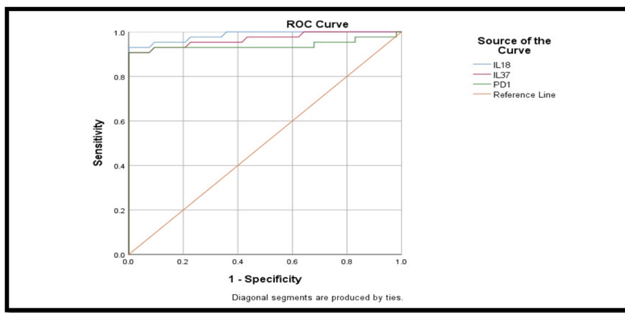

Figure 3: The ROC curve for serum IL-18, IL-37 and PD-1 in SLE and healthy control subjects.

Furthermore, the findings depicted in Table III demonstrate a strong and statistically significant positive correlation (p<0.01) between the concentrations of IL-18, IL-37, and PD-1 and the biomarkers MDA, CRP, ANA, anti-dsDNA, urea, and creatinine. Conversely, a highly significant inverse correlation (p<0.01) is observed between the levels of IL-18, IL-37, and PD-1 and the biomarkers C3, C4, CH50, TAC, and GFR. The results of the current study indicate that these markers possess potential value in identifying individuals with SLE. On the contrary, our analysis did not reveal any statistically significant positive correlations (p>0.05) between IL-18, IL-37, and PD-1 with BMI.

Table III: Correlation between serum IL-18, IL-37 and PD-1 levels with biochemical parameters in SLE patients group.

Parameter	IL-18		IL-37		PD-1	
	r	P-Value	r	P-Value	r	P-Value
BMI	0.229	0.141	0.194	0.212	0.012	0.942
C3	-0.925	<0.01	-0.526	<0.01	-0.500	<0.01
C4	-0.581	<0.01	-0.501	<0.01	-0.501	<0.01
CH50	-0.983	<0.01	-0.660	<0.01	-0.523	<0.01
MDA	0.923	<0.01	0.544	<0.01	0.528	<0.01
TAC	-0.694	<0.01	-0.533	<0.01	-0.504	<0.01
CRP	0.974	<0.01	0.638	<0.01	0.550	<0.01
ANA	0.871	<0.01	0.524	<0.01	0.513	<0.01
Anti-dsDNA	0.901	<0.01	0.528	<0.01	0.509	<0.01
Urea	0.924	<0.01	0.527	<0.01	0.507	<0.01
Creatinine	0.908	<0.01	0.508	<0.01	0.532	<0.01
GFR	-0.843	<0.01	-0.500	<0.01	-0.517	<0.01
IL-18	-	-	0.672	<0.01	0.543	<0.01
IL-37	0.672	<0.01	-	-	0.502	<0.01
PD-1	0.543	<0.01	0.502	<0.01	-	-

r=Pearson correlation coefficient, SLE: Systemic Lupus Erythematosus, C3: complement component 3; C4: complement component 4; CH50: complement hemolytic 50; MDA: malondialdehyde; TAC: total antioxidant capacity; ANA: antinuclear antibodies; Anti-dsDNA: anti–double-stranded DNA; GFR: glomerular filtration rate; PD-1: Programmed Cell Death 1; IL: interleukin.

The Distinguished Alleles and Genotypes Association with the SLE Susceptibility

The examination of the sequencing outcomes obtained from SLE subjects in relation to 69 well-established polymorphic loci situated within the intron-4 of the PD-1 gene unveiled the presence of two discernible SNPs, namely, rs6705653 and rs41386349. Three distinct inheritance models were used to examine the correlation between reported PD-1 gene polymorphisms and SLE, as well as to assess the distribution of associated alleles and genotypes. Our study revealed a higher prevalence of the GG genotype of PD-1 +7499 (G/A) in the healthy control group. When considering the GG genotype as the baseline, it was observed that those with AA genotypes had a significantly increased risk of SLE according to the co-dominant model (OR=3.11, 95% CI=1.52–5.94, p-value=0.031). Similarly, the A allele exhibited a significant association with susceptibility to SLE (OR=1.59, 95% CI=1.09–2.31, p-value = 0.043). Table IV presents the frequencies of genotypes of PD-1 +7499 (G/A) across several inheritance models.

Table IV: Genotype and Allele Frequency for SNP +7499 G/A (rs6705653) in PD-1 gene.

SNP +7499 G/A Polymorphism	Control N = 53 (%)	SLE N = 43 (%)	OR (95% CI)	P-Value
Codominant				
AA	5 (9.4)	13 (30.2)	3.11 (1.52-5.94)	0.031
GA	22 (41.5)	14 (32.6)	1.29 (0.78-2.64)	0.03
GG (Reference)	26 (49.1)	16 (37.2)	-	-
Dominant Genetic Model				
GA + AA	27 (50.9)	29 (67.4)	1.68 (1.05-3.19)	0.054
GG (Reference)	26 (49.1)	16 (37.2)	-	-
Recessive Genetic Model				
AA	5 (9.4)	13 (30.2)	2.71 (1.42-4.89)	0.026
GA + GG (Reference)	48 (90.6)	30 (69.8)	-	-
Allele Frequency				
G (Wild Allele)	67	58	-	-
A (Mutant Allele)	33	42	1.59 (1.09-2.31)	0.043
X^2	4.07	3.23	-	-
HWE*	0.29	0.062	-	-

N: no. of subjects, SLE: Systemic Lupus Erythematosus, OD: odd ratio, SNP: single nucleotide polymorphism, X2: chi-square, HWE: Hardy-Weinberg equilibrium, the level of significance is 0.05.

The reference genotype for the PD-1 +7209 (C/T) polymorphism region was established as the CC genotype, exhibiting the highest incidence among healthy individuals. Nevertheless, our study did not find any noteworthy correlation between TT (OR=1.07, 95% CI=0.43–3.02, p-value=0.58) or CT (OR=1.21, 95% CI=0.63–2.03, p-value=0.79) genotypes with the vulnerability to SLE when considering the co-dominant model. Furthermore, our analysis did not reveal any substantial correlation between PD-1 +7209 (C/T) genotypes and SLE when considering alternative inheritance models, as presented in Table V.

Table V: Genotype and Allele Frequency for SNP +7209 C/T (rs41386349) in PD-1 gene.

SNP +7209 C/T Polymorphism	Control N = 53 (%)	SLE N = 43 (%)	OR (95% CI)	P-Value
Codominant				
TT	2 (3.7)	3 (7)	1.07 (0.43-3.02)	0.58
CT	11 (20.8)	11 (25.6)	1.21 (0.63-2.03)	0.79
CC (Reference)	40 (75.5)	29 (67.4)	-	-
Dominant Genetic Model				
CT + TT	13 (24.5)	14 (32.6)	1.31 (0.84-2.36)	0.27
CC (Reference)	40 (75.5)	29 (67.4)	-	-
Recessive Genetic Model				
TT	2 (3.7)	3 (7)	1.19 (0.42-3.55)	0.69
CT + CC (Reference)	51 (96.3)	40 (93)	-	-
Allele Frequency				
C (Wild Allele)	84	83	-	-
T (Mutant Allele)	16	17	1.43 (0.68-2.37)	0.48
X^2	1.49	0.42	-	-
HWE*	0.27	0.61	-	-

N: no. of subjects, SLE: Systemic Lupus Erythematosus, OD: odd ratio, SNP: single nucleotide polymorphism, X2: chi-square, HWE: Hardy-Weinberg equilibrium, the level of significance is 0.05.

DISCUSSION

The results of our investigation unveiled a statistically significant positive correlation between IL-18 concentration and MDA, CRP, ANA, anti-dsDNA, urea, creatinine, IL-37, and PD-1, whereas a strong negative correlation was observed between IL-18 and C3, C4, CH50, TAC, and GFR in individuals with SLE in comparison with the control group. The observed impact may potentially be ascribed to the SLE inflammation reaction. IL-18 elevated concentrations could result in a consequence of proinflammatory state observed in SLE, as immune cells are known to secrete IL-18 in response to inflammatory signals.[16-17] Moreover, it is essential to acknowledge that SLE often manifests with renal involvement, commonly known as lupus nephritis (LN). Elevated concentrations of IL-18 may manifest as a result of renal tissue damage, as the kidneys are a prominent location for producing IL-18.[18]

The findings from our data analysis revealed a statistically significant positive correlation between IL-37 and MDA, CRP, ANA, anti-dsDNA, urea, creatinine, IL-18, and PD-1, while a notable inverse correlation is shown between IL -37 and C3, C4, CH50, TAC, and GFR in individuals with SLE in comparison with the control group. The behavior under observation can be attributed to the existence of inflammation, as IL-37 has the capacity to function as a compensatory mechanism used by the organism to alleviate the increased inflammation and immune response typically associated with the disease.[19] In addition, the presence of endothelial dysfunction can result in inflammation and consequent compromise of blood vessel integrity. IL-37 has the capacity to be synthesized as a protective response to the manifestation of endothelial dysfunction.[20-21]

Based on our data analysis, a statistically significant positive connection was noticed between PD-1 and MDA, CRP, ANA, anti-dsDNA, urea, creatinine, IL-18, and IL-37. On the contrary, a notable inverse relationship was identified between PD-1 and C3, C4, CH50, TAC, and GFR in individuals with SLE in comparison with healthy control. Serum PD-1 elevated levels found in individuals with SLE can perhaps be ascribed to the presence of chronic inflammation and an amplified immunological response. The chronic inflammatory state may lead to the induction of immunological checkpoints, including PD-1, which function as regulatory mechanisms to attenuate excessive immune responses.[22] The immune system may utilize increased levels of PD-1 as a potential compensatory mechanism to alleviate the inflammatory reactions and inflammation commonly observed in SLE.[23] Furthermore, the presence of self-antigens in SLE is accompanied by the simultaneous occurrence of autoantibodies and immune complexes. Extended exposure to these naturally occurring antigens can cause T cells to increase the production of PD-1 during their interaction with autoantigens, as a means to reduce the immune response targeted at self-antigens.[24] This finding was further corroborated in the current investigation, wherein hospitalized individuals exhibited diminished levels of C3, C4, and CH50, heightened oxidative stress activity (elevated levels of MDA and reduced levels of TAC), elevated levels of other inflammatory markers (CRP), increased levels of autoantibodies (ANA and anti-dsDNA), and impaired kidney function (elevated levels of urea and creatinine with reduced levels of GFR). Elevated levels of cytokines in the current study data indicated early detection of organ damage or morbidity-related consequences.[25]

The intron-4 of the PD-1 gene consists of several transcription-binding sites that interact with regulatory

elements. The presence of polymorphisms in these specific loci has the potential to cause modifications in gene expression.[25-26] In the current study, we investigated the PD-1 gene polymorphisms located within intron-4 among SLE subjects and a control group of healthy participants. The polymorphic loci were identified using PCR-sequencing, resulting in the recognition of two different loci, namely, rs6705653 and rs41386349.[27-28] In relation to the allelic frequencies and genotype distributions of PD-1 +7499 (G/A), the AA genotype showed a statistically significant increase among SLE subjects.[29-30] Consistent with prior research, the homozygous AA and heterozygous GA genotypes exhibited a higher frequency among SLE subjects, while the GG genotype was more commonly observed among individuals without the disease.[31-32] Therefore, the heightened risk of SLE can be related to the presence of the A allele and, subsequently, the AA genotype at this location.[33-34] Additionally, it has been demonstrated that the presence of the G allele and GG genotype at the +7499 region of the PD-1 gene may confer protective effects against SLE. The examination of the allelic frequencies of PD-1 +7209 (C/T) polymorphisms in individuals with SLE in comparison with individuals without the condition revealed no statistically significant disparity.[35] The findings of the current investigation revealed that the T allele could be considered a potential risk allele for SLE, while the C allele may have a protective effect.[36] We were limited in our investigation because most of our patients had a modest period of SLE, and the cytokine profiles may differ in individuals with early SLE who later developed nephritis.[37] Additionally, another potential limitation of our study was that all SLE patients were currently being treated with glucocorticoids. We cannot disregard the impact of glucocorticoids on these compounds.[38] The present study is the first to try to establish a significant connection between the +7499 locus and SLE in Basra Province, Iraq, due to the scarcity of experimental findings on this correlation. Further investigations are required to clarify the functional implications of these SNPs at this location. The results of this study could be utilized to create therapeutic approaches for the early identification, treatment, or control of SLE patients in Basra Province, Iraq.

CONCLUSION

The strong correlation between IL-18, IL-37, and PD-1 and C3, C4, CH50, MDA, TAC, CRP, ANA, anti-dsDNA, urea, creatinine, and GFR suggests that these inflammatory biomarkers may play remarkable roles in SLE immune responses and inflammatory processes when analyzed using a multivariable approach. An analysis of the sequencing results of intron-4 in the PD-1 gene indicated a significant association between the A allele and AA genotypes of PD-1 +7499 (G/A) SNP with a higher risk of SLE. On the other hand, the G allele was shown to be more common in healthy persons. However, no significant connection was observed between the PD-1 +7209 (C/T) SNP and SLE. This work proposes a new theory suggesting that the combined quantities of these compounds may serve as crucial markers in SLE.

CONFLICT OF INTEREST

There is no conflict of interest.

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