The Egyptian Journal of Hospital Medicine (October 2022) Vol. 90, Page 93- 98

Evaluation of Interleukin-9 Serum Level and Gene Polymorphism in A Sample of Systemic Lupus Erythematosus in Iraqi Female's Patients

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ABSTRACT

Background: Immunological gene and serum level for interleukin- 9 rs 17317275 have been established to have linked to predisposition systemic lupus erythematosus (SLE) and its severity**.** SLE is a severe, systemic autoimmune disease characterized by autoantibody generation, complement activation, and immune complex deposition. In the pathophysiology of SLE, cytokines have a pleiotropic function. Recently, IL-9 was discovered to mediate strong anti-inflammatory effects in numerous cells or experimental autoimmune models.

Objective: This study aimed to determine the role of age, IL-9 serum level and genetic polymorphism, C-reactive protein (CRP), Anti-nuclear antibody (ANA) and Anti- double-stranded DNA (anti-dsDNA) to recognize SLE pathogenesis.

Materials and Methods: This case-control study was carried out in Baghdad Teaching Hospital and Typical Rheumatology Unit through the period from October 2021 to January 2022.103 Iraqi patients with SLE illness and 50 healthy Iraqis were included. Blood samples were taken. Serum IL-9 levels measured using the sandwich enzyme-linked immune-sorbent assay technology (ELISA) and cytokine genotyping by using allele-specific PCR technique

Results: SLE groups had greater levels of IL-9 than did healthy volunteer. Furthermore, both autoantibodies (ANA, Ant-dsDNA) were positive in SLE patients. The findings indicated that CRP was much higher in SLE subjects than those of healthy controls. On the other hand, the results showed that there was non-significance (p-value > 0.05) in age of studied groups. Concerning genotyping frequencies, the GG and AG genotyping were greater in the SLE group compared to the healthy subjects, while the AA genotyping frequency was significantly lower in the SLE group compared to the healthy people.

Conclusion: This study indicated the major roles of the serum level and genetic polymorphism of IL-9, CRP, ANA and anti-ds DNA in pathogenesis and severity of SLE.

Keywords: SLE, IL-9, Polymerase chain reaction, ANA.

INTRODUCTION

SLE is a complex and heterogeneous autoimmune condition that usually affects women throughout their reproductive years. It is caused by a breakdown in immunological tolerance and the interaction of SLE susceptibility genes with diverse environmental variables, which results in the creation of pathogenic autoantibodies [(1)]. SLE has an etiology that is unknown. Several potential factors have been identified and demonstrated to increase disease susceptibility or to stimulate the immune system resulting in an inflammatory response that eventually causes the illness to develop. Predisposition to SLE is influenced by genetic factors [(2)]. Autoantibody generation, inflammation, and endorgan damage emerge from a complex combination of genetics, environment, and hormones that cause immunological dysregulation and the lss of self-antigen tolerance [(3)].

Besides, immune dysfunction, inflammation, and organ damage are all caused by cytokine abnormalities. Cytokine production is increased when both the innate and adaptive immune systems are disordered [(4)]. They are small, soluble proteins made by immune system cells and mediate the activation or functional direction of neighboring cells by attaching to their surface receptors. Communication between immune cells is mediated by cytokines, which is essential for organizing defenses against infections [(5)]. Secreted cytokines can be seen in the bloodstream, saliva, urine, as well as in the tissues of target organs such as the skin, kidneys, and synovia. Most of the cytokines promote inflammation, although some also have immunoregulatory or anti-inflammatory activities [(6)].

In SLE patients, the chronic inflammatory condition can cause mortality and morbidity. Many immunological lineages' development, maturation, and activation depend greatly on cytokines. These cytokines accelerate the inflammatory process in several organs, affect the immune cells' gene expression, and the pathophysiology of SLE is influenced by many cytokines [(7)].

In this study, we aimed to understand the role of interleukin-9 (IL-9) in the pathogenicity of SLE. It is a 144-amino acid protein with an 18-amino acid secretory signal sequence that belongs to the gamma-chain cytokine family. It was first identified as one of a growing number of cytokines that play critical roles in the growth, survival, proliferation, and differentiation of various types of cells [(8)].

Mast cells, NKT cells, Th2, Th17, Treg, and ILC2 cells were among the cells that generated it, although Th9 cells are the main ones [(9)].

The human IL-9 gene can be identified on chromosome 5 long arm [(10)]. A wide spectrum of immunological functions has been attributed to IL-9 in promoting cellular and humoral immune responses [(11)]. IL-9 has been linked to a variety of disease pathogenic processes, most notably allergic disorders such as

Received: 26/6/2022
Accepted: 2/9/2022

asthma and atopic dermatitis. [12].

Its serum levels are raised in people with autoimmune diseases such as systemic lupus erythematosus [13] and rheumatoid arthritis[14-15].

MATERIALS AND METHODS

From October 2021 to January 2022, a case-control study of 50 healthy control individuals with an average age of 41.09 ± 2.38 and 103 SLE female Iraqi volunteers with an average age of 35.74 ± 1.59 was done. C-RP levels in SLE and control group sera were measured using an automated technique (Roche, Germany). ANA and anti-dsDNA information was collected from Baghdad Teaching Hospital and Typical, Rheumatology Unit.

Using a commercial sandwich ELISA kit, serum from SLE patients and control groups were quantitatively examined for IL-9 serum level evaluation (Al-shkairate institution for medical supply, Jordan). The polymerase chain reaction (PCR) was used to identify the IL-9 gene in SLE patients.

The DNA was extracted from the 300 µl blood samples of all participants using the FavorPrep TM /Cultured cells Genomic DNA extraction small kit according to the manufacturer's procedure (FAVORGEN; USA).

The sample's purity and concentration were evaluated. The concentration ranged from 60 to 95 g/mL, while the purity ranged from 1.6 to 2. IL-9 genotyping was accomplished by electrophoresis on a 1.5% agarose gel at 100 V/cm2 for 30 minutes and staining with Red Safe dye (Intron Biotechnology Inc., Korea) using UV trans illuminator at 350 nm. The size of the PCR result was determined using a Universal DNA Ladder (Intron Biotechnology Inc., Korea).

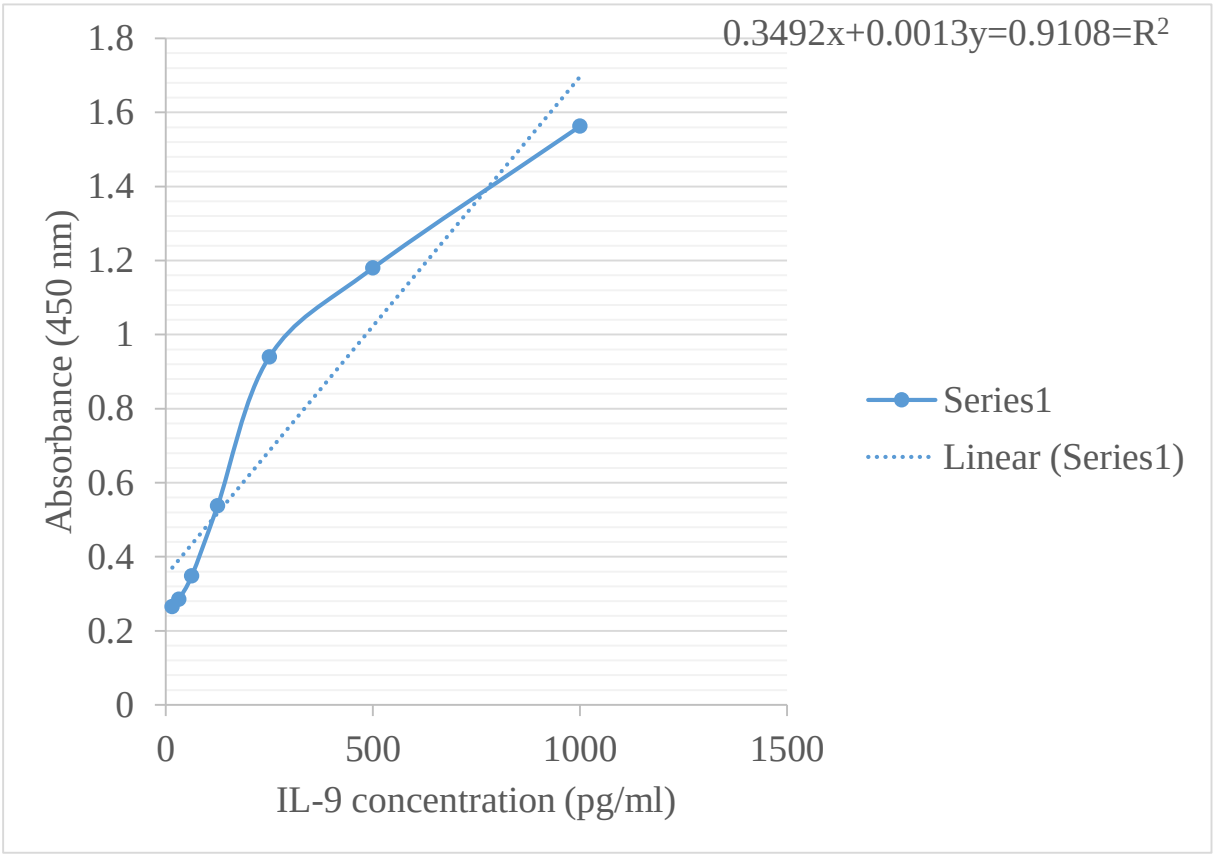

Figure (1): Standard curve of IL-9 concentration (pg/ml)

Ethical approval: An approval of this study was obtained from University of Baghdad Academic and Ethical Committee. Informed consents of all patients were taken. This study was carried out in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki) for studies involving humans**.**

Statistical Analysis

The linearity, homogeneity, and normal distribution tests for the research groups' IL-9 serum level, age, CRP, ANA, and anti-ds DNA were performed using IBM SPSS version 26.0 (IBM Corp., released 2019). Aside from the mean, standard error, and probability, the WinPepi program version 11.65 was used to compute the odd ratio, Pearson's chi-square test, and Fishers' exact probability of genotyping and allele frequency. Abramson (2011) was used to estimate genotyping and allele frequency probability. The Hardy-Weinberg online calculator was also employed. $P \leq 0.05$ was significant when the p-value was adjusted using a website called false discovery rate.

RESULTS

SLE disease according to Age.

Table (1) showed that there was no significant difference (P>0.05) in age between the SLE group and the controls.

Table (1): SLE group demographic statistics against control group

Groups	Age mean ± SE (Years)	Probability
Patients group	35.74 ± 1.59	0.06
Control group	41.09 ± 2.38	

CRP level according studied groups

With reference to C-RP level our study documented that C-RP serum level was raised significantly (P <0.05) in SLE patient (11.61 ± 1.31 mg/dl) in comparison with control (2.67 ± 0.19 mg/dl) as shown in table (2).

Table (2): C-RP levels distribution among the studied groups

Groups	CRP level mean ± SE (mg/ dl)	Probability
Patients group	11.61 ± 1.31	0.000004
Control group	2.67 ± 0.19	

Autoantibodies (ANA and ant-ds DNA) frequency (%) in SLE group compared to controls

Ant-nuclear antibody (ANA), Ant-dsDNA were increased significantly (P<0.05) in SLE patients compared to the control group (ANA 43.2 % and Ant-dsDNA 31.3%) (Table 3).

Table (3): Autoantibodies frequency (%) among studied groups

Groups	ANA frequency (%)		Probability
	Positive	Negative	
Patients group	41 (43.2)	62 (56.8)	1.85×10^{-7}
Control group	0 (0.0)	50 (100.0)	
	Anti- dsDNA frequency (%)		
	Positive	Negative	
Patients group	32 (31.3)	71 (68.9)	0.000009
Control group	0 (0.0)	50 (100.0)	

IL-9 serum level among studied groups

IL-9 serum levels were considerably higher (*P*< 0.05) in SLE patients compared to the control group (16.44 0.19 vs. 7.13 0.26 Pg/ml) as seen in table (4).

Table (4): The distribution of IL-9 serum levels in the SLE group against the control group

Groups	IL-9 mean ± SE (Pg/ml)	Probability
Patients group	16.44 ± 0.19	7.54×10^{-47}
Control group	7.13 ± 0.26	

IL9 gene polymorphisms rs 17317275 in studied groups

Regarding genotyping and allele frequencies, table (5A) showed that SLE group had a higher prevalence of the GG, AG/G allele (Genotyping /allele) than the control group. The AA/A allele (Genotyping /allele) was less common in SLE patients compared to controls.

Table (5A): The genotyping and alleles frequencies of IL9 gene polymorphisms rs 17317275 in studied groups

Genotyping of rs17317275	Patients group frequency (%)		Control group frequency (%)	
	Observed	Expected	Observed	Expected
GG	22 (21.36)	32.10 (31.16)	5 (10.0)	8 (16.0)
AG	71 (68.93)	50.80 (49.32)	30 (60.0)	24 (48.0)
AA	10 (9.71)	20.10 (19.51)	15 (30.0)	18 (36.0)
Total	103 (100.0)	103 (100.0)	50 (100.0)	50 (100.0)
P-HWE	0.0001		0.0771 NS	

Furthermore, table (5B) showed that the GG, AG/ G (Genotyping /allele) had higher odds ratio (OR) values (2.44 and 1.48, respectively), indicating that it could be considered a risk factor, whereas the AA/ A (Genotyping /allele) had a lower OR value (0.25), indicating that this genotyping and allele had a protective property.

Table (5B): The genotyping, odd ratio and alleles frequencies of IL9 gene polymorphisms rs 17317275 in studied groups

Genotyping of rs17317275	Patients group frequency (%)	Control group frequency (%)	OR (95% CI)	EF or PF %	Fisher's exact probability
GG	22 (21.36)	5 (10.0)	2.44 (0.87 – 6.83)	12.6	0.113 NS
AG	71 (68.93)	30 (60.0)	1.48 (0.74 – 2.97)	22.3	0.281 NS
AA	10 (9.71)	15 (30.0)	0.25 (0.10 – 0.61)	22.5	2.0×10^{-3}
Total	103 (100.0)	50 (100.0)			
Alleles frequencies					
G	115 (56)	40 (40)	1.90 (1.17 – 3.07)	26.4	0.01
A	91 (44)	60 (60)	0.53 (0.33 – 0.86)	28.3	

IL-9 serum level and genotyping

Table (6) showed a significant increase in IL-9 level GG, AG of patients' group compared to control groups (16.28 ± 0.16^{A} vs. 6.34 ± 0.51^{A} and 16.68 ± 0.23^{A} vs. 6.62 ± 0.30^{A}, respectively). These results were reported for the first time.

Table (6): The genotyping and alleles frequencies in studied groups

Genotyping	IL-9 level mean ± SE (pg/ml)		Probability
	Patients	Control	
GG	16.28 ± 0.16^{A}	6.34 ± 0.51^{A}	6.54×10^{-15}
GA	16.68 ± 0.23^{A}	6.62 ± 0.30^{A}	6.09×10^{-28}
AA	15.65 ± 0.89^{A}	7.87 ± 0.43^{A}	2.09×10^{-8}
The similar letters referred to non-significant differences			

CRP and genotyping

Table (7) showed an increasing in CRP in AG and AA genotyping of patients compared to control group. While GG showed non-significant differences in patients compared to control group. In addition, non-significant differences between genotype in respect to CRP level (similar letters referred to non-significant differences).

Table (7): CRP distribution in SLE according to the genotyping of the studied groups

Genotyping	CRP level mean ± SE (mg/dl)		Probability
	Patients	Control	
GG	10.76 ± 2.28^{A}	3.17 ± 0.35^{A}	0.116 NS
AG	11.25 ± 1.57^{A}	2.51 ± 0.25^{A}	0.0004
AA	15.81 ± 5.83^{A}	2.81 ± 0.39^{A}	0.011
The similar letters referred to non-significant differences			

Autoantibodies (ANA and ant-dsDNA) and genotyping

In this study, these genotyping results were published for the first time showed ANA increasing in the frequency percentage of AG 63.4% and GG 22.0% as shown in table (8A)

Table (8A): ANA frequency distribution in SLE according to the genotyping of the studied groups

Genotyping	ANA frequency (%)				Probability
	Patients		Control		
	Positive	Negative	Positive	Negative	
GG	9 (22.0)	13 (21.0)	0 (0.0)	5 (10.0)	0.08 NS
AG	26 (63.4)	45 (72.6)	0 (0.0)	30 (60.0)	1.2×10^{-4}
AA	6 (14.6)	4 (6.5)	0 (0.0)	15 (30.0)	0.001
Probability	0.365 NS		-		

The result of ant-ds DNA showed increasing in the frequency percentage of AG genotyping (78.1%). While GG showed non- significant improvement in level of ant-dsDNA in patients compared to control ($P>0.05$) as shown in table (8B).

Table (8B): Ant-dsDNA frequency distribution in SLE according to the genotyping of the studied groups

Genotyping	dsDNA frequency (%)				Probability
	Patients		Control		
	Positive	Negative	Positive	Negative	
GG	6 (18.8)	16 (22.5)	0 (0.0)	5 (10.0)	0.067 NS
AG	25 (78.1)	46 (64.8)	0 (0.0)	30 (60.0)	1.8×10^{-4}
AA	1 (3.1)	9 (12.7)	0 (0.0)	15 (30.0)	0.211 NS
Probability	0.248 NS		-		

DISCUSSION

The present findings aligned with those of a prior inquiry. There were no statistically significant variations in age between SLE patients and controls ($p>0.05$) [16]. Recent research has confirmed previous findings that SLE may arise at any age [17]. In around 10-20% of cases, it begins before the age of 16. Although it is more prevalent in women of reproductive age, it can begin at any age. However, it is more common in the first five years of life, and especially in the next ten years [18] .

Several studies have demonstrated that serum CRP levels increase as the duration of SLE disease increases, and this study revealed that CRP levels in SLE patients are greater than in the control group. C-reactive protein is an important and sensitive diagnostic for predicting lupus diseases. CRP is a nonspecific measure of systemic inflammation that is higher in patients with active SLE. These findings support the first use of these assays to examine individuals with suspected inflammation [19].

Antibodies to numerous nuclear antigens are the immunological characteristic of SLE and are seen in more than 95% of patients. As a result of its great sensitivity and ability to predict the clinical start of SLE, ANA testing is commonly employed for SLE early diagnosis [20]. Furthermore, the current investigation found that all patients were positive for these autoantibodies, indicating that autoantibodies have diagnostic value as early indicators of SLE development [21]. It has been proposed that these antibody deposits in the kidney may induce lupus nephritis [22]. Anti-dsDNA IgG is a highly specific illness marker in SLE that may be used to explore the relationship between autoimmune disease-specific pathogenic autoantibodies and IgG isotype [23].

Higher levels of IL-9 in the serum of SLE patients compared to healthy controls, particularly in active SLE patients, may stimulate the inflammatory process in SLE [24]. Furthermore, the IL-9 pathway might be seen as a possible method for treating several types of inflammatory illnesses [25] .Since IL-9 plays a role in allergic processes such as asthma by promoting the proliferation of mast cells and T cells, it has been linked to immunopathological disorders [26] . According to one of these recent studies, IL-9 serum levels raised dramatically in rheumatoid arthritis and type 2 diabetes patients [15, 27] .

The genotyping and allele frequencies in the research groups revealed that T2DM group had a greater AA, AG/A (genotyping/allele) than healthy persons. T2DM was lower in the GG/ G (genotyping/ allele) group than in the control group. Furthermore, in T2DM group, the elevated OR value of AA, AG/A (genotyping/allele) was deemed as a risk factor, particularly in genetically predisposed people. While the greatest OR value of GG/ G (genotyping/allele) in healthy persons was predicted to be a protective factor, this was not the case. [27]. Other study indicate that IL-9 SNP rs1313970720 showed associations (positive and negative) with Bacterial vaginitis (BV) patients and may be having a role in the mechanism of etio-pathogenic of BV in the samples of Iraqi patients [28]. The HLA-DR3 link with SLE SNP, rs2187668, is considerably a higher influence on the propensity to manufacture autoantibodies [29].

CONCLUSION

In conclusion, this study indicated the major roles of serum level and genetic polymorphism of IL-9, CRP, ANA and anti-ds DNA in pathogenesis and severity of SLE.

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