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Study of Interleukin-4 Gene Polymorphism in Systemic Lupus Erythematosus and Lupus Nephritis

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ABSTRACT

Background: The anti-inflammatory cytokine interleukin 4 (IL-4) controls the ratio of Th1 to Th2 immune responses.
Objective: The goal of this research was to study the interleukin-4 gene polymorphisms rs2243250 in individuals with Systemic Lupus Erythematosus (SLE) and lupus nephritis (L.N) and the association among the gene polymorphism and illness susceptibility. **Subjects and Methods:** This study enrolled 120 subjects separated into two groups, 60 SLE cases and sixty matched age and gender healthy controls. Cases have been separated into 2 groups: L.N (30 patients) and non-L.N (30 patients). Analysis of IL-4 rs2243250 gene polymorphism was performed by real time PCR.
Result: Compared to controls, SLE cases had a considerably greater prevalence of the TT+ CT genotype of the IL-4 gene polymorphism. Healthy controls had a higher representation of the CC genotype. There was a significant variance (P-value under 0.05) in the incidence of the rs2243250 locus and allele between the control and case groups. The polymorphism in the interleukin-4 rs2243250 gene was linked to a higher risk of LN. A risk factor for SLE and L.N. may be the T allele of the interleukin-4 gene (rs2243250). **Conclusion:** The TT+ CT genotype of the interleukin-4 gene polymorphism could be a susceptibility factor for developing L.N. T allele of interleukin-4 (rs2243250) gene could be a risk factor for SLE (non-L.N and L.N).
Keyword: Single nucleotide polymorphism, IL-4 gene, rs2243250, SLE, Lupus nephritis.

INTRODUCTION

A persistent autoimmune illness that affects several organs is called systemic lupus erythematosus (SLE) [(1)]. Immune dysregulation and a breakdown in tolerance to self-antigens are caused via a complex interaction among environmental and genetic variables. This leads to the generation of autoantibodies, inflammation, and organ death [(2)]. The ratio of female to male for SLE is 9 to 1 with the majority of affected women being of reproductive age [(3)]. The signs and severity of SLE are shown to be significantly influenced by ancestry, race, and ethnicity [(4)]. Patients who are Black, Asian, or Hispanic have a greater incidence and prevalence of SLE, and they also have more severe and active illness and a tendency to develop lupus earlier [(5,6)].

One of the worst organ presentations of SLE is lupus nephritis, a kind of glomerulonephritis that is also a leading cause to morbidity and death in SLE cases [(7)]. One nucleotide variant that arises at a particular location in the genome is known as a single-nucleotide polymorphism (SNP). Individual variances in human genomes are caused by these genetic variants, which are also at the center of numerous research on the relationship between genes and disease [(8)]. Type 2 inflammation, which is driven by IL-4 released by T helper 2 (Th2) cells, defends the host against big multicellular infections such parasitic helminth worms and controls immune responses to allergens [(9)]. Additionally, different levels of the cytokine can be generated by basophils, mast cells, eosinophils, macrophages and natural killer cells (NK) [(10)].

Furthermore, a variety of adaptive and innate immune cells as well as non-hematopoietic cells are stimulated by IL-4 to coordinate the generation of antibodies, fibrosis and immunological control among other tasks [(11)].

Thus, this investigation aimed to elucidate the association among the IL-4 rs2243250 polymorphism and the vulnerability to SLE and L.N.

SUBJECTS AND METHODS

Subjects: This research has been conducted in the Departments of Internal Medicine and Clinical Pathology at Menoufia University Hospital and Shebin Elkom Teaching Hospital between February 2023 and February 2025.

Sample size estimation: Participants were recruited from all patients with SLE coming to the Internal Medicine outpatient clinic, Menoufia University Hospitals for at least 6 months. By rate estimation, it's about 10 patients per month. So, it was expected to include at least 60 patients in this study. And 60 healthy controls were included as well.

120 subjects have been involved in this research. The subjects were separated into: **Group Ⅰ:** this group involved 30 SLE (non-L.N) cases, **Group Ⅱ:** involved 30 L.N cases, **Group Ⅲ:** involved 60 apparently healthy people, age and gender matched with cases. SLE cases were diagnosed according to criteria for the categorization of SLE, which have been updated by the American College of Rheumatology (ARC) in 1997[(12)] which demanded 4 out of 11 criteria for the categorization of SLE. The 11 criteria involved were discoid rash, malar rash, nasal / oral ulcers, photosensitivity, on-erosive

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arthritis comprising 2 or more peripheral joints, renal illness, serositis (pericarditis or pleurisy), hematologic disease, neurological disorders, immunological disorders (Positive LE cell prep, anti-Smith antibodies, anti-dsDNA antibodies, or false positive syphilis test), and positive antinuclear antibodies. The research did not include patients with rheumatoid arthritis, a family history of immunological disorders, severe liver or renal illness, or infectious infections.

METHOD

Sample collection: under completely sterile circumstances, 6 ml of venous blood has been collected, then separated as follows : **Tube A :** 2 ml has been added in plain tube for urea, creatinine, CRP, Complement 4 (C4), Complement 3 (C3), antinuclear antibody(ANA), anti dsDNA (anti dsDNA).**Tube B:** 2 milliliters of blood has been gathered into an EDTA tube then aliquoted in 2 Eppendorf' tubes for complete blood count (CBC) assay and IL-4 rs2243250 genotyping. **Tube C:** 1.6 ml of whole blood was added to Na^{++} citrate sterile tube at room temperature for erythrocyte sedimentation rate. Urine sample was collected to detect proteinuria.

Laboratory tests: CBC was done using Sysmex XN-1000 Automated Hematology Analyzer (Sysmex, Kobe, Japan), urea, creatinine, CRP, C3 and C4 were measured on (Au 680 automatic auto analyzer Beckman Coulter, USA), ESR was measured by the Westergren method. ANA and anti dsDNA were done by immunofluorescence technique (Inova Diagnostics, USA).

Genotyping of rs2243250 C/T polymorphism of IL-4 gene: Venous blood was extracted on EDTA anticoagulant for DNA extraction and stored at-80 °C for the study. Following the instructions, a genomic DNA extraction kit (Trans Gen Biotech, China) has been utilized to extract the DNA from peripheral blood cells. A protein nucleic acid analyzer (Implant Nano Photometer N60 UV/VIS spectrophotometer, Implen GmbH, Schatzbogen, München, Germany) was used to evaluate the concentration and purity of DNA. Using fluorescently labeled probes, real-time PCR has been utilized to genotype the IL-4 gene's rs2243250 locus. The ABI TaqMan Allelic Discrimination Kit was used to identify the gene. The reaction system contained 7µl DNA, 10µl TaqMan master mix (Applied biosystem, USA), 2.5µl nuclease free water, 0.5µl SNP Assay (Applied biosystem, USA). The sequences for the probes were **(**VIC dye for alle C, FAM dye for alle T**),** TAAACTTGGGAGAACATGGT(**C/T**) TGGGGAAAGATAGAGTAATA.

After gently mixing the reaction, appropriate volume was added to each PCR plate well. Once the PCR tubes were in the cycler, the PCR cycling program began with an initial denaturation at ninety-four degrees Celsius for fifteen seconds (s) followed by fifty cycles of denaturation at 95-degrees Celsius for fifteen s and annealing at sixty degrees Celsius for 30 seconds. The sequence detection systems (SDS) automation controller software v2.0.6 (ABI) has been used to read all well plates. Allelic discrimination data were plotted on a scatter plot of allele 1 vs allele 2 by the real-time PCR instrument's software. Detection of amplification products was made by Rotor-Gene Q. In real-time PCR, DNA was measured following each cycle utilizing fluorescent dyes that produce a signal that increases in direct proportion to the quantity of PCR product molecules produced.

Statistical analysis

IBM SPSS software package version 20.0 (IBM Corp., Armonk, NY) has been utilized to examine the information that was input into the computer. Percentages and numbers have been utilized to represent the qualitative information, which were compared by Chi-square test or Fisher's Exact test when over twenty percent of the cells have predicted count under five. The normality of the distribution has been confirmed utilizing the Kolmogorov-Smirnov test. Range (maximum and minimum), standard deviation (SD), mean, median and interquartile range (IQR) have been utilized to characterize quantitative information, which were compared by one-way ANOVA (F) test for normally distributed quantitative parameters with a Post Hoc test (Tukey) or by Kruskal Wallis test for abnormally distributed quantitative parameters with Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons. At the five percent level, the outcomes' significance has been evaluated. The OR is used to calculate the ratio of the odds and 95% CI of an event occurring in one risk group to the odds of it occurring in the non risk group. Logistic regression analysis was used. Univariate analysis was first applied and variables with $p<0.05$ were entered into multivariate model to determine independent predictors.

Ethical considerations:

The authors affirmed that the work presented has been performed according to the World Medical Association's 2013 revision of the Declaration of Helsinki for human experimentation. The research has been authorized by the Medical Research Ethics Committee of Menoufia University (2/2023 CPATH40). Department of Clinical Pathology, accepted all study protocols. After outlining the purpose of the research, all participants provided written informed consent.

RESULTS

This study included 120 subjects (94 females and 24 males). The case and the control were age and gender matched **(Table 1)**.

Table (1): baseline characteristics of the examined groups

	Parameters	Group I (No= 30)		Group II (No= 30)		Group III (No = 60)		p
		No.	%	No.	%	No.	%	
Gender	Male	7	23.3	2	6.7	15	25.0	χ^2**p**=
	Female	23	76.7	28	93.3	45	75.0	0.107
Age (years)	Min. – Max.	18.0 – 56.0		17.0 – 52.0		18.0 – 45.0		^{F}p=
	Mean ± SD.	31.93 ± 8.03		35.77 ± 9.08		33.40 ± 7.20		0.089

F: One-way ANOVA test, χ^2: Chi square test, Group I: non-L.N, Group II: LN, Group III: control

There was statically significant variance among case and control group with regard to diabetes, hypertension (according to medical history), hemoglobin (HGB), platelet (PLT), C reactive protein (CRP), erythrocyte sedimentation rate (ESR), proteinuria, total leukocyte count (TLC), complement 3, complement 4, ANA ab and anti dsDNA ab. There was statically significant difference between SLE (non-L.N) and L.N cases according to urea, creatinine, proteinuria and PLT **(Table 2)**.

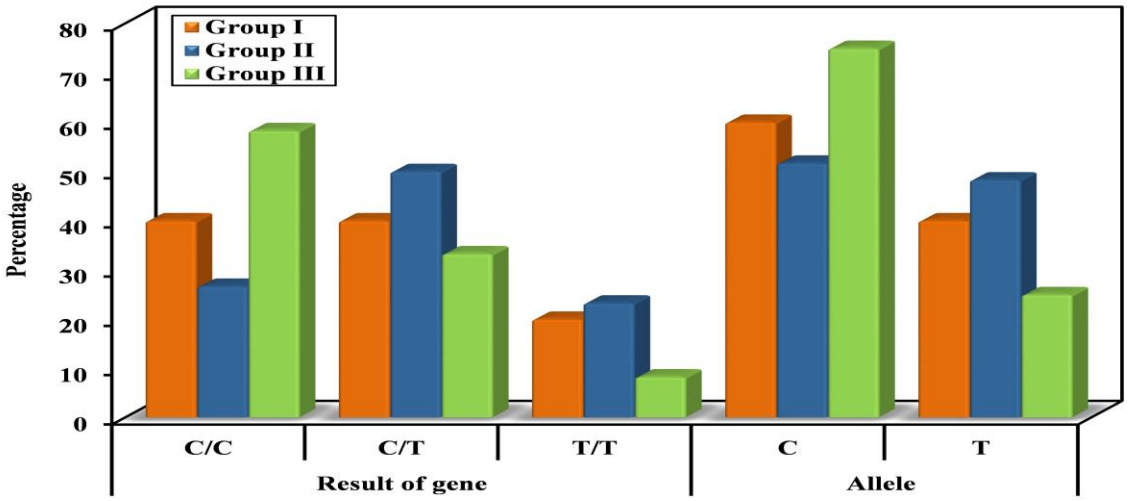
Table (2): Comparative analysis between examined groups with regard to clinical and laboratory investigations

Parameters	Group I (Number = 30)		Group II (Number = 30)		Group III (Number = 60)		P	Significance between groups		
								I versus II	I versus III	II versus III
	No.	%	No.	%	No.	%				
DM or HTN										
No	27	90.0	23	76.7	60	100.0	^{FET}p <0.001*	0.166	^{FE}p= 0.035*	^{FE}p <0.001*
Yes	3	10.0	7	23.3	0	0.0				
Urea (mg/dl)										
Min. – Max.	11.0 – 74.0		41.0 – 260.0		15.0 – 45.0		^{H}p<0.001*	<0.001*	0.014*	<0.001*
Median (IQR)	19.0(15.0 –29.0)		88.5(69.0–185.0)		30.0(23.5 –36.0)					
Creatinine (mg/dl)										
Min. – Max.	0.40 – 1.80		1.80 – 12.70		0.50 – 1.20		^{H}p<0.001*	<0.001*	0.217	<0.001*
Median (IQR)	0.60(0.50 –0.90)		2.90 (2.50 –4.0)		0.70(0.60 –0.90)					
Proteinuria										
Negative	30	100.0	0	0.0	60	100.0	c^2<0.001*	<0.001*	1.000	<0.001*
Positive	0	0.0	30	100.0	0	0.0				
Hemoglobin (g/dl)										
Mean ± SD.	9.56 ± 1.59		9.33 ± 1.54		13.79 ± 1.39		^{F}p<0.001*	0.819	<0.001*	<0.001*
TLC										
Min. – Max.	2.70 – 17.30		2.80 – 13.40		4.0 – 10.60		^{H}p=0.024*	0.240	0.179*	0.007*
Median (IQR)	6.45(3.80 – 10.50)		10.85(4.0 – 12.0)		5.35(4.95 – 5.90)					
PLT										
Min. – Max.	100.0 – 426.0		60.0 – 399.0		150.0 – 500.0		^{H}<p 0.001*	0.033*	<0.001*	<0.001*
Median (IQR)	175 (120 –307)		132 (124 –156)		260(215 –348.5)					
ESR										
Min. – Max.	15.0 – 110.0		10.0 – 150.0		5.0 – 20.0		^{H}<p 0.001*	0.241	<0.001*	<0.001*
Median (IQR)	40.0(30.0 –50.0)		55.0(40.0 –80.0)		10.0 (5.0 –15.0)					
CRP										
Min. – Max.	4.0 – 36.0		10.0 – 90.0		1.0 – 5.0		^{H}p< 0.001*	0.097	<0.001*	<0.001*
Median (IQR)	15.50(12.0 – 18.0)		20.0(16.0 – 25.0)		3.50(3.0 – 4.0)					
C3 level					66.0 – 160.0		^{F}p< 0.001*	0.847	<0.001*	<0.001*
Mean ± SD.	37.30 ± 9.01		34.90 ± 10.11		116.4 ± 21.89					
C4 level					23.0 – 41.0		^{F}p< 0.001*	0.714	<0.001*	<0.001*
Mean ± SD.	10.20 ± 2.99		9.33 ± 2.47		31.10 ± 5.38					
ANA ab Negative	0	0.0	0	0.0	60	100.0	χ^2<0.001*	–	<0.001*	<0.001*
Positive	30	100.0	30	100.0	0	0.0				
ANTIdsDNAab										
Negative	0	0.0	0	0.0	60	100.0	χ^2<0.001*	–	<0.001*	<0.001*
Positive	30	100.0	30	100.0	0	0.0				

SD: Standard deviation, IQR: Inter quartile range, FET: Fisher Exact test, H: Kruskal Wallis test, F: One-way ANOVA test, χ^2: Chi square test, Group I: non-L.N, Group II: LN, Group III: control, *: Significant. TLC: total leukocyte count, PLT: platelet, ESR: erythrocyte sedimentation rate; CRP: C- reactive protein; C3, C4: complement 3 and 4; ANA: antinuclear antibody, Anti dsDNA: anti-double stranded DNA.

The genotyping and allele distribution of IL4 rs2243250 among studied groups were demonstrated in **(Table 3)**. The T/T and C/T genotype and T allele were more represented in the case group. However, the CC genotype and C allele were more represented in the healthy group **(Figure1).**

Figure (1): Comparative analysis between the three examined groups with regard to result of genotyping



Group I: non-L.N, Group II: LN, Group III: control

The C/T and T/T genotype displayed significantly higher risk for L.N patients. The T allele was considered significantly higher risk for SLE (non-L.N) and L.N. Dominant genotypes CT + TT displayed significant risk for L.N cases **(Table 3)**.

Table 3: **The risk of development of SLE and L.N with different genotypes and alleles.**

	Group I ® versus. Group II		Group I versus. Group III®		Group II versus. Group III®	
	p	OR (95% C.I)	P	OR (95% C.I)	p	
Result of gene (rs22-43250)						
C/C		1.000		1.000		1.000
C/T	0.294	1.875 (0.580 – 6.061)	0.258	1.750(0.663 – 4.619)	0.022*	3.281(1.185 – 9.089)
T/T	0.437	1.75 0(0.427 – 7.171)	0.070	3.500(0.902 – 13.581)	0.010*	6.125(1.539 – 24.369)
Allele						
C		1.000		1.000		1.000
T	0.359	1.403(0.681 – 2.892)	0.040*	2.0(1.032 – 3.875)	0.002*	2.807(1.460 – 5.395)
Dominant						
CC		1.000		1.000		1.000
CT + TT	0.276	1.833(0.616 – 5.453)	0.103	2.100(0.860 – 5.128)	0.006*	3.850(1.477 – 10.037)
Recessive						
C/C + C/T		1.000		1.000		1.000
T/T	0.754	1.217(0.355 – 4.170)	0.121	2.750(0.765 – 9.891)	0.057	3.348(0.962 – 11.645)
Over dominant						
CC + TT		1.000		1.000		1.000
CT	0.276	1.833(0.616 – 5.453)	0.103	2.100(0.860 – 5.128)	0.437	1.500(0.539 – 4.171)

®: Reference group, OR: Odd's ratio**,** LL: Lower limit, CI: Confidence interval, UL: Upper Limit, Group I: non-L.N, Group II: LN, Group III: control, p: for Univariate regression analysis for comparing with the reference genotype, *: Statistically significant

According to univariate and multivariate logistic regression: univariate analysis illustrated that ESR, urea, and IL4 rs2243250 (CT+ TT) were considered dependent factors affecting SLE (non-L.N), ESR and IL4 rs2243250 polymorphism were considered risk factors for L.N. Multivariate analysis demonstrated that ESR was the only dependent factor affecting SLE (non-L.N) and L.N **(Table 4).**

There was no significant relationship between allele of gene distribution and lab investigations.

Table (4): Univariate and multivariate Logistic regression analysis for the parameters affecting non-LN and LN patients vs control (n= 30 vs 60)

	Non-L.N vs control				LN vs control			
	Univariate		#Multivariate		Univariate		#Multivariate	
	P	OR (LL – UL ninety-five percent C.I)	P	OR (LL – UL ninety-five percent C.I)	p	OR (LL – UL ninety-five percent C.I)	p	OR (LL – UL ninety-five percent C.I)
Gender	**0.862**	1.095(0.392 – 3.062)			**0.051**	4.667(0.991–21.965)		
Age (years)	**0.379**	0.974(0.917 – 1.033)			**0.182**	1.040(0.982 – 1.102)		
DM or HTN	**0.999**	NA			**0.999**	NA		
Urea (mg/dl)	**0.035***	0.952(0.909 – 0.997)	0.537	1.026(0.946 – 1.113)				
Creatinine	**0.855**	0.983(0.821 – 1.178)			**0.989**	NA		
CRP	**0.086**	6.539(0.767–55.770)			**0.989**	NA		
ESR	**0.009***	1.630(1.130 – 2.349)	0.009*	1.610(1.127 – 2.302)	**0.005***	1.388(1.106 – 1.742)	0.007*	1.492(0.117–19.014)
C3 level	**0.985**	NA			**0.984**	NA		
C4 level	**0.990**	NA			**0.994**	NA		
rs22-43250 CC vs CT + TT	**0.103**	2.100(0.860 – 5.128)			**0.006***	3.850(1.477–10.037)	0.758	1.393(1.095–1.771)

#: All variables with p-value under 0.05 was included in the multivariate .

DISCUSSION

SLE is an autoimmune condition marked by many molecular and cellular immune system irregularities, along with the development of numerous polyclonal autoantibodies [13]. Cytokines are soluble factors, which are mostly generated through immune cells and in turn have essential roles in the maturation and activation of various immune cells. Cytokines possess either pro-inflammatory or anti-inflammatory characteristics that contribute to the SLE [14]. An interrupted balance between Th2 and Th1 responses results in allergy and autoimmune [15]. The humoral immune response is mediated through the anti-inflammatory cytokine IL-4, which is additionally crucial for the immunological control of the of helper-2 subset of lymphocytes. IL-4 is a pivotal cytokine that stimulates the differentiation and activation of B cells and plays a role in T cell development. Interleukin-4 is a pivotal cytokine that stimulates the differentiation and activation of B cells and plays a role in T cell improvement [16]. IL-4 contributes to the rescue of B cells from apoptosis, increasing the survival of autoreactive B lymphocytes, causing the switching of antibody isotype class, leading to higher affinity and pathogenic autoantibodies [17]. The exact molecular mechanisms of interleukin-4 polymorphisms in the initiation and development of autoimmune disorders are still unclear.

In our study, there were 120 participants (94 women and 24 men). The patient group included 51 females and 9 men with an average age of 31.93 ± 8.03 years. The control group consisted of 15 men and forty-five females, with an average age of 33.40 ± 7.20 years. We found that a statistically insignificant variance with regard to sex and age among cases and controls. This came in line with **Trentin *et al.*** [18] and **Gui *et al.*** [19] who obtained that no significance in sex but **Mahmood *et al.*** [20] demonstrated that male had severe clinical manifestation and more prone to L.N. With the favour of our study, **Massias *et al.*** [21] found no significant difference in age.

This research demonstrated that there was statistically significant reduction in HGB, TLC, PLT in all cases of SLE compared to control. This result agreed with **Abira and Akhter** [22] and **Skare *et al.*** [23] who clarified that anemia, leukopenia, and thrombocytopenia were frequent in SLE.

In this research, the concentration of C4 and C3 were significantly reduced in the SLE cases illustrating consumption of these complements. In line with these outcomes, **Ayano and Horiuchi** [24] observed low complement concentrations.

Also, in this study, there was higher levels of CRP and ESR, which is in agreement with **Aringer** [25] and **Qu *et al.*** [26] who also found that all cases had positive ANA Ab and anti dsDNA Ab, this result agreed with our results.

In this study, we revealed significant difference in IL4 rs2243250 gene variant distribution between cases (Non-L.N and L.N) and control as CT+TT genotype were higher in cases (66.7%) compared to control (41.7%), whereas the CC genotype was greater in control (58.3%) compared to cases (33.3%). Finally, we demonstrated that IL-4 gene polymorphism rs2243250 (CT+TT) associated with L.N and T allele is a risk factor for SLE (non-L.N and L.N). In agreement with this study, **Zhang *et al*.** [(27)] observed that carriers of the T allele had a greater chance of developing SLE than those carrying the C allele. TT+CT genotype carriers had a greater chance of developing SLE than CC gene carriers (P-value under 0.05); however, the IL-4 rs2243250 gene polymorphism has been linked to both LN and SLE susceptibility.

In harmony with our research, **Rashad *et al*.** [(28)] and **Mohammadoo-Khorasani *et al*.** [(29)], found that there was association among interleukin-4 gene polymorphism and the risk and illness activity of Egyptian cases with SLE.

Yu *et al*. [(30)] found that the polymorphisms were significantly associated with certain clinical findings in the Chinese SLE patients. They implied that the IL-4 gene polymorphisms might not affect the onset of disease, but it may have an impact on variation in clinical presentation and progress of SLE patients**,** this came in disagreement with our study as we demonstrated that no significant association was found between allele of gene distribution and clinical and lab investigation.

In contrast to our outcomes, **Wu *et al***. [(31)] and **Qiu *et al*.** [(32)] observed no association between SLE development and IL-4 rs2243250 polymorphisms in cases with SLE compared to the healthy controls**.**

Depending on the above-mentioned outcomes, it can be concluded that IL-4 rs2243250 gene polymorphism (CT+TT) could be used as a predictor for developing LN susceptibility. T allele of IL-4 (rs2243250) gene could be a risk factor for SLE (non-L.N and L.N).

The conflicts among the outcomes could be because of the complexity of genetic factors, pathogenesis, and environmental factors of SLE and may be due to small sample size.

Further studies, including measurement of serum IL-4 and its correlation with gene polymorphism and larger sample size should be performed in the future to validate our result with influence of environmental conditions and various ethnic groups are vital to validate the present result.

CONCLUSION

The TT+ CT genotype of the interleukin-4 gene polymorphism could be a susceptibility factor for developing L.N. T allele of interleukin-4 (rs2243250) gene could be a risk factor for SLE (non-L.N and L.N).

DECLARATIONS

Consent for publication: I certify that each author granted permission for the work to be submitted.
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Conflicts of interest: None
Competing interests: None.

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