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ORIGINAL ARTICLE

Association between interleukin -4 gene polymorphism with the risk and disease activity of Egyptian patients with systemic lupus erythematosus

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ABSTRACT

Background: Cytokines are a heterogeneous group of molecules organizing different processes of the immune response. Interleukin -4 (IL-4) modulates various immune functions. It plays a vital role in the development of systemic lupus erythematosus (SLE). We aimed to explore the correlation of IL-4 C-589T (rs2243250) gene polymorphism with SLE and its association with disease activity.

Method: This study included 100 SLE patients and 90 healthy controls. Disease activity was evaluated by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Samples from all participants were analyzed for IL-4 589 genotyping by restriction fragment length polymorphism (RFLP) and serum IL-4 level by enzyme linked immunosorbent assay (ELISA).

Results: IL-4 serum level was significantly lower in patients with SLE (3.14±0.782 pg/ml) compared to control (13.82±4.85 pg/ml) ($p < 0.001$), there was a significant negative correlation between IL-4 serum level and SLEDAI score. There was no significant difference in distribution of different genotypes among studied groups. However, the frequency of the IL-4 589 T allele was 87% (174 out of 200) in the SLE group compared to 73.9% (133 out of 180) in the controls [OR (2.365 (1.392-4.016), P= 0.001.

Conclusion: T allele of IL-4 C-589T (rs2243250) gene can be a risk factor for SLE. Serum IL-4 level significantly correlated with SLEDAI score indicating its effect on disease activity.

Keywords: systemic lupus erythematosus, SLEDAI, IL-4 589.

INTRODUCTION

Accumulating studies have reported that SLE is a multisystem autoimmune disorder mainly affecting young females. It presents by a range of different symptoms some are just mild lesions e.g., cutaneous rash others are more severe lesions e.g., glomerulonephritis [1]. SLE is a chronic disorder characterized by immunological dysfunction, abnormal autoantibody production and multiorgan damage [2].

It has been widely described that many cytokines play a noteworthy role in SLE pathogenesis. Cytokines play a role in all stages of immune cell production. Moreover, they are involved in immune dysregulation of SLE leading to tissue injury[3, 4] .

Current evidence confirmed that interleukin -4 (IL-4) is a key player molecule in the immune response. It is secreted by T helper 2 (Th2) lymphocytes, and other immune cells [5, 6]. Interestingly, IL-4 has pleiotropic functions particular Th2 cell development, T and B cell growth factor, as well as induction and maintenance of allergy. Emerging scientific evidence has disclosed several polymorphisms in the IL-4 gene which has been linked to dysregulation of IL-4 expression and total immunoglobulin-E levels that might increase the risk of infection, autoimmunity.[7-9]

SLE is a complex autoimmune disease of unknown etiology, although it is believed that both genetic susceptibility and environmental factors can contribute to the disease. However, all

previously studied biomarkers are not considered reliable for prediction of the risk of SLE. Therefore, more sensitive biomarkers are required for early diagnosis of SLE and prevention of its related complications. To address these needs, we conducted this study and to the best of our knowledge, this is the first Egyptian study to explore the correlation of IL-4 C-589T (rs2243250) gene polymorphism with susceptibility and disease activity of SLE and to clarify the impact of this polymorphism on clinical and laboratory characteristics of SLE.

Methods

This case-control study was conducted on 100 patients diagnosed with SLE years according to the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria [10], in addition to age and sex-matched 90 healthy volunteers. Written informed consent was obtained from all participants, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. This study was carried according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. SLE disease activity index (SLEDAI) was used for assessment of disease activity [11]. All study subjects voluntarily participated and were clinically evaluated. Inclusion criteria included: age > 18, provide written informed consent and cases fulfill the SLICC criteria, The exclusion criteria were patients with a history of myocardial infarction, angina, stroke, drug-induced SLE, pregnancy, diabetes. In addition to the patients with hepatitis C virus, hepatitis B virus, and other connective tissue diseases.

Sample collection:

Samples obtained from SLE patients included: 2 ml of blood on citrate anticoagulant for ESR measurement and 4 ml of blood in plain tube for measurement of C3, C4, CRP and autoantibodies (ANA & Anti-dsDNA). Additional samples from both cases and controls included: One ml of blood on EDTA anticoagulant for DNA extraction and 2 ml in plain tubes for serum IL4 measurement.

Laboratory assessments

We measured antinuclear antibodies (ANA) and anti-double-stranded DNA antibody (anti-dsDNA). The ANA was tested using the Indirect Immunofluorescent Kit INOVA Lite®HEp-2 ANA kit (INOVA Diagnostics, Inc, San Diego, USA). For anti-dsDNA, we used the anti-dsDNA indirect immunofluorescence Kit INOVA Lite ®dsDNA Crithidia luciliae kit (INOVA Diagnostics, Inc, San Diego, USA). The Erythrocyte sedimentation rate (ESR) was determined manually. High sensitive C- reactive protein (Hs-CRP), complement C3, C4 were measured using immunoturbidimetric assay on Roche/Hitachi Cobas system (c501) autoanalyzer (Roche Diagnostics, Mannheim, Germany).

Measurement of IL-4 serum level

An enzyme-linked immunosorbent assay (ELISA) kit from (Maptech, USA) was used to measure serum levels of IL-4 according to manufacturer protocol. All samples were measured in duplicate.

DNA Extraction

Genomic DNA extraction was done from EDTA whole blood. A spin-column method was used according to the manufacturer protocol (QIAamp Blood Kit; Qiagen, GmbH, Hilden, Germany).

Genotyping of IL-4 589 C/T SNPs

Gene amplification and resection enzyme digestion

The purified genomic DNA was used to amplify IL-4/589 gene using the specific primer sequences F: 5'-ACTAGGCCTCACCTGATACG-3', R: 5'-GTTGTAATGCAGTCCTCCTG-3'. We performed PCR in an Amp Gene DNA thermal cycler and the BsmFI (New England Bio Labs, Ipswich, UK) was used to digest IL-4-C 589.

Statistical analysis

Analysis was carried out using the IBM® SPSS® Statistics software (version 22.0.0.0 IBM Corporation, Armonk, NY, USA. Quantitative data are expressed as mean and SD. Comparison of continuous variables among groups was made using the student's t-test. Associations between two categorical variables were tested using the Likelihood ratio χ 2 test, as appropriate. Statistical correlation between continuous variables was tested using the Pearson’s product-moment coefficient of correlation (r). Genotype frequencies in patients and controls were tested for Hardy–Weinberg equilibrium and difference between observed and expected frequencies were tested for significance. To identify independent relationships linear regression analysis was performed**.** Receiver operating characteristic (ROC) analysis was performed to assess the potential diagnostic accuracy of IL-4 levels. Statistical significance was considered accepted at $P < 0.05$.

RESULTS

Patients and controls were age, gender, and ethnicity matching**.** All studied patients were females with a mean age of (33.2±5.96 years), disease duration of 5.7 ± 4.6 years, and SLEDAI of 18.7 ± 8.8. All SLE were positive for ANA & Anti-ds-DNA Clinical assessment of the patients showed: malar rash (33%), alopecia (32.5%), oral

ulcer (30%), pleurisy (27%), arthritis (36%), fever (21%), pericarditis (28%), vasculitis (14.7%), myositis (16.7), seizures (5.9%) retinal change (2.1%), and psychosis (3.6%). Laboratory data of the studied groups are summarized in table 1.

Table1: Clinical characteristics and laboratory parameters of studied groups.

Variable	Healthy controls *n*=90	SLE patients *n*=100	P value
Age (y)	32.61± 5.89	33.2±5.96	0.510
WBC count (cell × 10^3µl)	4.89±0.48	10.9±4.313	<0.001*
Hb (g/dl)	12.44± 1.215	10.11± 1.09	<0.001*
hs-CRP (µg/ml)	0.73±0.097	1.527±0.588	<0.001*
ESR (mm/h)	12.24± 1.21	84.47± 11.54	<0.001*
Cr (mg/dl)	0.93±0.097	2.07±0.399	<0.001*
C4 (mg/dl)	23.94± 2.33	21.4±3.276	<0.001*
C3 (mg/dl)	95.78±9.35	85.82±13.10	<0.001*
IL-4(pg/ml)	13.82±4.85	3.14±0.782	<0.001*

SLE; systemic lupus erythematosus, SLEDAI; systemic lupus erythematosus disease activity index, ESR; erythrocyte sedimentation rate, CRP: C-reactive protein, C3: complement 3, C4: complement 4; Interleukin-4; IL-4* statistically significant (*P*<0.05)

Comparison of IL-4 serum levels (pg/ml) in studied groups

In the SLE group there were significantly lower values of IL-4 serum levels (3.14±0.782), compared to the control group (13.82±4.85), P < 0.001* as shown in figure1.

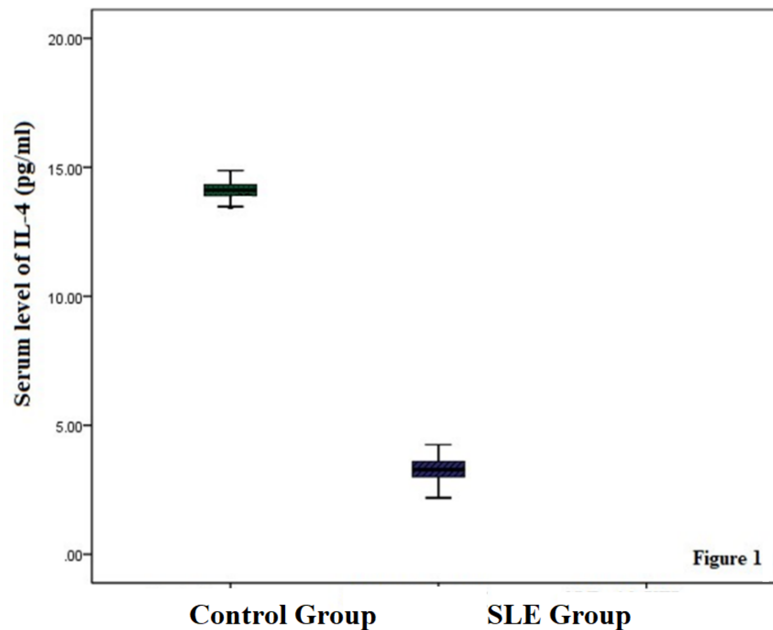

Figure 1 *Comparison of IL-4 serum levels (pg/ml) in studied groups*

Distribution of Genotype and Allele Frequencies of IL-4 C/T 589 in healthy controls and SLE patients.

Genotype and allelic frequencies of IL-4 C-589T (rs2243250) gene polymorphism in patients with SLE and healthy volunteers are presented in Table2. The genotype distribution was in Hardy–Weinberg equilibrium in both studied group. there was no significant difference in genotypes frequency between SLE patients and healthy controls. However, in Allele distribution, the frequency of the IL-4 589 T allele was 87% (174 out of 200) in the SLE group compared to 73.9% (133 out of 180) in the controls [OR (2.365 (1.392-4.016), P=0.001*]. table 2.

Table2: Distribution of IL-4 C-589T (rs2243250) genotypes and allele frequencies in healthy controls and SLE patients.

		Healthy controls *n*=90, *n* (%)		SLE patients *n*=100,*n* (%)		OR (95% CI)	*P*
IL-4 589	CC®	2	(2.2)	4	(4)		
	CT	43	(47.8)	18	(18)	0.209 (0.035- 1.246)	0.09
	TT	45	(50)	78	(78)	0.866 (0.152-	0.85717
	C allele®	47	(26.1)	26	(13)		
	T allele	133	(73.9)	174	(87)	2.365 (1.392- 4.016)	0.001*

® reference * Statistically significant (P<0.05*)

Impact of IL-4 C-589T (rs2243250) polymorphism on laboratory manifestations of SLE.

Patients carrying the TT genotype of IL-4 589 had significantly lower values of C4, C3 (P <0.001 for both) and IL-4 serum levels (P = 0.02), compared to patients carrying CT genotype. On the other hand, patients carrying CT genotype had statistically significant higher values of SLEDAI score and Hs-CRP compared to patients carrying TT genotype, (P < 0.001*). While, WBCs count was highest among CC genotype (14.58±2.6 cell × 10^3µl) followed by CT genotype (13.12±5.7 cell × 10^3µl) then TT genotype (10.3±3.81 cell × 10^3µl) table 3.

Table3: Impact of IL-4 589 polymorphism on laboratory manifestations and disease activity of SLE
SLEDAI ; systemic lupus erythematosus disease activity index; Hb, hemoglobin; WBC, white blood cells ;hs-CRP, high-sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; Cr,

	IL-4 589 N=100					
	CC N=4	CT N=18	TT N=78	P1	P2	P3
SLEDAI	19.7±4.974	21.67±1.80	17.3±3.52	0.176	0.195	<0.001*
WBC count (cell × 10^3µl)	14.58±2.6	13.12±5.7	10.3±3.81	0.646	0.03*	0.01*
Hb (g/dl)	9.65±0.848	9.57±0.84	10.25±1.12	0.865	0.295	0.02*
hs-CRP (µg/ml)	1.8±0.77	1.98±0.62	1.4±0.52	0.642	0.135	<0.001*
ESR (mm/h)	82.93±21.07	90.08±7.41	83.3±11.51	0.236	0.948	0.02*
Cr (mg/dl)	1.9±0.15	2.12±0.37	2.01±0.415	0.544	0.715	0.575
C4 (mg/dl)	22.9±5.26	24.7±1.852	20.6±2.950	0.235	0.148	<0.001*
C3 (mg/dl)	91.93±21.07	99.01±7.41	82.66±11.8	0.241	0.146	<0.001*
IL-4 (pg/ml)	3.07±1.21	3.2±0.93	2.72±0.73	0.812	0.368	0.02*

Creatinine;C4, complement4.P[1], significant among IL-4 589 genotype; CT versus CC. P[2], significant between TT versus CC.P[3], significant between TT versus CT** Statistically significant (P<0.05*)

Correlation between IL-4(pg/mg) with characteristics and disease activity of SLE patients:

In SLE patients, there was a statistically negative correlation between IL-4 and WBC count P < 0.001*, Hb P 0.003*, CRP P < 0.001*, Cr P < 0.001*, in addition to SLEDAI score, P 0.002*. Our results revealed a significant positive correlation between IL-4 serum level and C3, C4, P < 0.001*. table 4.

Table 4: Correlation between serum IL-4(pg/mg) and laboratory markers as well as disease activity of SLE

Variables	Serum IL-4 (n=100)	
	r	P value
SLEDAI	-0.788	0.002*
WBC count (cell × 10^3µl)	-0.500	<0.001*
Hb (g/dl)	-0.938	0.003*
CRP (mg/l)	-0.459	<0.001*
ESR (mm/h)	-0.033	0.741
Cr (mg/dl)	-0.408	<0.001*
C4 (mg/dl)	0.408	<0.001*
C3 (mg/dl)	0.413	<0.001*

*Significant P < 0.05.

Linear regression analysis in SLE patients to test the influence of the main independent variables against IL-4(pg/mg)

Our results observed that WBC count, and ESR were the main independent variables against IL-4 among SLE patients, P < 0.001*. Table 5

Table 5: linear regression analyses in SLE patients to test the influence of the main independent variables against IL-4(pg/mg) .

Model		Unstandardized Coefficients		Standardized Coefficients	t	p	95% C.I.	
		B	SE	Beta			Lower Bound	Upper Bound
1	(Constant)	3.260	0.859		3.796	<0.001*	1.555	4.965
	ESR (mm/h)	0.028	0.010	0.303	2.765	<0.001*	0.008	0.048
	C3 (mg/dl)	-0.021	0.012	-0.25	1.750	0.083	-0.045	-0.003
	SLEDAI	-0.037	0.033	-0.130	-1.150	0.253	-0.102	0.027
	WBC count	-0.113	0.055	0.454	2.036	<0.05*	0.003	0.222

* P < 0.05

ROC curve for an estimate of the diagnostic power of IL-4 serum level in differentiating SLE patients from the control group.

We further investigated our results by ROC test, we found that among SLE patients, The AUC of IL-4 was 0.983 (95% CI = 0 .959–1.000) with sensitivity =98 %, specificity = 97.5%, at the cutoff values (4.62 pg/ml), P < 0.001* figure2.

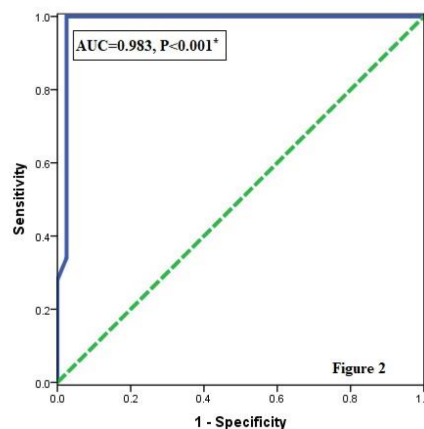

Figure 2 : Receiver operating characteristic (ROC) analysis of circulatory IL-4 serum level for differentiation between SLE patients and healthy controls.

DISCUSSION

There have been tremendous advances over the last decades in understanding the processes that regulate the immune system in SLE. On these grounds, cytokines have either pro or anti-inflammatory properties, or both, that is contributing to SLE pathogenesis [12]. The disturbed balance between Th1 and Th2 responses leads to various disorders, including autoimmune and allergic diseases. Interestingly, IL-4 is considered as a characteristic cytokine secreted from Th2[12, 13].

Emerging evidence demonstrated that IL-4 could favor the initiation and progress of inflammatory reactions mediated by Th2[12]. However, the precise molecular mechanisms of IL-4 polymorphisms in the initiation and progression of autoimmune diseases remain unclear. A study showed that IL-4 gene polymorphism and its signaling molecule, STAT6, are associated with SLE susceptibility [14].

Conflicting data have been reported about the role of IL-4 in autoimmunity. IL-4 is considered the key Th2 deviator for initiation and exponential progression of humoral immunity. In spite of its importance for B cell maturation and survival, the antagonistic role of IL-4 on Th1 activation makes it a possible therapeutic target [13]

Patients with SLE exhibit alterations in cytokine production that may be relevant to SLE pathogenesis. Intriguing reports observed that there are several common polymorphisms of the IL-4 gene. The impact of single nucleotide polymorphisms IL4 genes on the expression of inflammatory and anti-inflammatory cytokines in SLE has not been sufficiently studied [15,16]. To our knowledge, this is the first study conducted to explore the correlation of IL-4 589 gene polymorphism with susceptibility and disease activity of SLE in Egyptians.

The interesting finding of the present study is that patients with SLE had statistically significant lower values of IL-4 compared to the control group. Our results investigated the diagnostic power of serum IL-4 in the differentiation of SLE among controls by ROC test, we found that among SLE patients AUC of IL-4 was 0.998 (95% CI = 0 .994–1.000) with sensitivity =98.2%, specificity = 99.8%. Moreover, there were significant negative correlations between serum IL-4 and laboratory manifestations of SLE as well as a disease activity marker. Linear regression analyses observed that WBC count, and ESR were the main independent variables against IL-4 among SLE patients.

Similar results observed in another Egyptian study as they found that the serum levels of IL-4 were significantly lower in patients with SLE while. On the other hand, others studied cytokines IL-17 and IFN-gamma levels were significantly higher in SLE patients compared to the control group [4].

Similar findings observed by Yu et al. who conducted their study on Chinese patients with SLE and they found that the plasma levels of IL-4 were significantly lower in SLE patients versus controls [14].

Our findings were in concordance with Lit et al. they found lower levels of IL-4 in SLE compared to healthy controls [13]. Similar findings observed in Sugimoto et al. study detected lower values of IL-4 in flaring SLE patients compared to controls as the absolute count of IL-4 producing CD4 + T cells was significantly low [15] .

An interesting study from Kawamoto et al. identified that Inducible co-stimulator (ICOS) is vital for the proliferation and activation of T cells. Based on these observations, the IL-4 level was increased in inactive SLE patients after ICOS co-stimulation compared to patients with flaring SLE and normal controls [16].

Other study have indeed demonstrated that IL-4 level was higher in SLE compared to control and they suggested that SLE is associated with elevation of both Th1 and Th2 cytokines and this imbalance of cytokine profile contributed to inflammatory reactions in SLE [17] .The diverse results summarized above could be attributed to the fact that Th1 and Th2 cytokines are both involved in SLE pathogenesis. However, there is Th1 predominance in SLE patients, especially in active SLE, and this Th1/Th2 imbalance may limit the secretion of IL-4 levels [15].

Similar results observed by Sugimoto et al detected that the ratio of IFNγ /IL-4 production by CD4 + T cells had a positive correlation with SLEDAI and was significantly increased in patients with lupus nephritis. Thus, the imbalance of IFNγ /IL-4 producing helper T cells was due to the reduction in IL-4 producing T cells and may have a vital role in the progression of active SLE [18].

All genotype and allele frequencies reached genetic balance by Hardy-Weinberg equilibrium method, with group representativeness; our results confirmed that IL-4 C/T 589 gene polymorphism was significantly associated with SLE. The frequency of the IL-4 589 T allele was significantly higher in the SLE group compared to controls.

In other studies, conducted to assess C-589T polymorphisms of interleukin-4 gene

promoter in asthma observed that C allele is protective against atopic asthma. These results highlight the important effect of SNPs in the promoter region of the IL-4 gene on the immune system [19,20].

In harmony with our study, Zang et al. detected that the risk of SLE development among T allele carriers was higher than that of C allele. Also, the risk of SLE development among TT+CT genotype carriers was higher than that of CC gene carriers (P<0.05). The IL-4rs2243250 gene polymorphism was associated with SLE and LN susceptibility. Homozygous TT genotype may be a susceptibility factor of SLE. The TT+CT genotype was a susceptibility factor of LN [21].

This is in line with the findings of immunological studies that investigated IL-4 C-589T single nucleotide polymorphism in atopy and they confirmed that the C allele is linked to increased binding of transcription factors. Therefore, it supports the hypothesis that this SNP may lead to increased expression of the IL-4 gene and thus up regulation of IL-4 based immune reactions [22].

In contrast to the previous results, Wu et al. found no association between SLE development and IL-4 third intron polymorphisms in Chinese patients with SLE compared to the healthy controls [23]

Assessment of the impact of IL-4 C/T 589 polymorphism on laboratory manifestations of SLE revealed that patients carrying the TT genotype of IL-4 589 had significantly lower values of SLEDAI and inflammatory markers compared to patients carrying CT genotype.

Similar to our results, Wu et al. 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 [23].

Study Limitations

Some limitations should be considered. First, the small sample size of the study and further studies with larger sample size should be performed in the future to validate our results. Third, the relatively insufficient data about the impact of IL-4 gene polymorphisms on SLE medications.

CONCLUSIONS

Patients with SLE had lower values of serum IL-4 than healthy controls, and serum IL-4 level significantly correlated with SLEDAI score. Interestingly, there were significant differences between studied groups as regard frequencies of T allele of IL-4 C-589T (rs2243250) . Thus, the T allele of IL-4 C-589T (rs2243250) gene polymorphism can be a risk factor for SLE.

+Conflicts of interest: The authors report no conflicts of interest.

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Authors' contributions: NMR, AME, NFI collected patients' samples and clinical data. NMS, HMK and RSA prepared sample for laboratory investigations. NMR wrote the paper. Statistical analysis, interpretation of data and preparation the paper for submitting international was done by NMR. Critical revision of the manuscript was performed by all of the authors. All the authors have read and approved the manuscript.

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