

# Effects of interleukin-10 gene polymorphism on clinical diversity and activity of systemic lupus erythematosus

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## Aim

This study was carried out to determine the effects of interleukin (IL)-10 gene polymorphism 1082A/G on clinical diversity and activity of systemic lupus erythematosus (SLE), as it was shown to be associated with E26 transcription factor binding site, and to assess IL-10 concentration in SLE patients.

## Patients and methods

Blood was drawn from 54 SLE patients and 27 healthy controls for DNA extraction. The single nucleotide polymorphism was identified using the PCR-restriction fragment length polymorphism, and serum sample was collected to assay IL-10 concentration.

## Results

There was an increase in IL-10 concentration in SLE total patients (mean 49.5 pg/ml). Mutant allele A (76.9%) was found more frequently in SLE patients compared with allele G (23.1%). Genotype AA (66.7%) was found more frequently, followed by AG (20.4%) and GG (12.9%).

## Conclusion

IL-10 concentration was elevated in SLE patients and was shown to be associated with disease activity. IL-10 gene polymorphism is not a strong indicator to show susceptibility of the disease.

## Keywords:

interleukin-10, single nucleotide polymorphism, systemic lupus erythematosus

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## Introduction

Systemic lupus erythematosus (SLE) is the prototypical autoimmune disease that is characterized by autoantibody production, complement activation, and immune complex deposition, leading to diverse clinical manifestations and target tissue damage [1].

SLE can vary widely in its severity, both at onset and during its course; some patients may have very mild disease with mild symptoms and no serious organ involvement, whereas others may be very ill with a number of organs affected [2].

Cytokines have been suggested to be involved in the pathogenesis of SLE, as they are fundamental components in the regulation of immune response, intervening in both cellular and humoral responses [3].

Interleukin (IL)-10 is a key immunoregulatory cytokine that can be produced by almost all leukocytes, including innate immune cells such as monocytes, macrophages, dendritic cells, mast cells, natural killer cells, eosinophils, and neutrophils, and adaptive immune cells such as Th1, Th2, Treg, Tr1, Th3, T cells, and B cells [4,5].

The role of IL-10 in the pathogenesis of SLE is unknown, but the increased production of IL-10 in sporadic cases

of SLE [6,7] and the clinical improvements in SLE patients resulting from administration of anti-IL-10 monoclonal antibody support a central role for IL-10 in the pathogenesis of SLE [8].

The human IL-10 gene located on 1q31-32 is a susceptible region for SLE [9]. The individual variation in IL-10 production may be due to the genotypic variations in the human IL-10 promoter. Three single nucleotide polymorphisms (SNPs) at 1082A/G, 819T/C, and 592A/C in the promoter region have been demonstrated [10].

Our study was carried out to determine the effects of IL-10 gene polymorphism 1082A/G on clinical diversity and activity of SLE and to assess the IL-10 concentration in SLE patients.

## Patients and methods

Eighty-one participants were selected in this study. They were divided into two groups: the control group and the systemic lupus patient group. The control group included 27 healthy participants (17 female and 10 male participants), whose ages ranged from 19 to 60 years (mean 31.3 years). They were clinically free from any disease and were not receiving any drugs.

The systemic lupus patient group included 54 systemic lupus patients (diagnosed according to the American Rheumatism Association Criteria for the Classification of SLE [11,12]). Patients were divided on the basis of the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score into two groups: SLE group I (mild and moderate cases), which included 27 patients whose SLEDAI score was less than 12, and SLE group II (severe cases), which included 27 patients whose SLEDAI score was 12 or greater [13].

#### Ethical considerations

The nature of the present study was explained to all patients. Verbal and written consent was obtained from all patients and controls. Research protocol was approved by the Ethical Committee of faculty of medicine, Zagazig University.

#### Methods

All patients were subjected to the following investigations:

- (1) Complete blood picture.
- (2) Erythrocyte sedimentation rate using the Westergren method.
- (3) C-reactive protein using the latex agglutination method.
- (4) Autoantibodies measurement with antinuclear antibody (ANA) and anti-dsDNA.
- (5) Kidney function tests including evaluation of creatinine and blood nitrogen urea (BUN), complete urine analysis, and protein/creatinine ratio.

Blood samples were collected from all participants in two tubes: a plain tube to obtain serum, and a sodium heparin anticoagulant tube for whole blood.

Serum samples were assayed for IL-10 concentration using Human IL-10 Immunoassay (R&D Minneapolis, Minnesota, USA). This assay uses the quantitative sandwich enzyme immunoassay technique (ELISA). A microplate precoated with a monoclonal antibody specific for IL-10 is used. IL-10 standards and samples are pipetted into the wells, and any IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for IL-10 is added to the wells. Following a wash to remove any unbound antibody enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-10 bound in the initial step. The color development is stopped, and the intensity of the color is measured.

#### DNA extraction for PCR amplification

DNA extraction was performed on all blood specimens using Gene JET whole-blood genomic DNA

purification mini kit (Qiagen, Hilden, Germany). The purified DNA was stored at  $-20^{\circ}\text{C}$  for PCR amplification.

#### PCR amplification

PCR reactions were all performed on DNA extracts using the PCR Gold Master Mix Kit (BIORON, Ludwigshafen, Rhein, German).

The primer pair specific for the IL-10 gene promoter as used in the study by Mavrothalassitis and Ghysdael [14] (Sigma, St. Louis, Missouri, USA) was selected.

A forward primer, whose sequence was 5'-AAG ACA ACA CTA CTA AGG CTT CCT T-3' for genotyping of SNP in the promoter region of the human IL-10 gene (-1082G>A) to produce a segment of 584 bp, and a reverse primer, whose sequence was 5'-TAA ATA TCC TCA AAG TTC C-3', were used. Reaction mixtures were subjected to 35 cycles of amplification: initial denaturation at  $94^{\circ}\text{C}$  for 5 min; denaturation at  $95^{\circ}\text{C}$  for 30 s; annealing at  $45^{\circ}\text{C}$  for 30 s; extension at  $72^{\circ}\text{C}$  for 1 min; and final extension for 7 min.

#### Restriction enzyme digestion of DNA

*Eco*NI (Thermo Scientific Fast Digest Enzyme; Thermo Scientific) (Thermo Scientific, Waltham, Massachusetts, USA) recognizes CCTNN<sup>N</sup>NNNAGG sites and cuts best at  $37^{\circ}\text{C}$  in 5–15 min.

#### Conventional agarose gel electrophoresis

Conventional agarose gel electrophoresis was performed using ethidium bromide and 100 bp step ladder as a marker to determine the band size of genotypes.

#### Statistical analyses

Statistical analyses were carried out using SPSS (version 19; SPSS Inc., Chicago, Illinois, USA) for Windows. Data were statistically described in terms of mean  $\pm$  SD, range or frequencies, and percentages. The  $\chi^2$ -test was used to compare qualitative variables. A *t*-test was used to compare two independent groups as regards quantitative variables. Spearman's correlation coefficient and the Kruskal–Wallis test were used to differentiate variables against each other positively or inversely. A *P* value of less than 0.05 was considered to be significant.

## Results

The demographic data of SLE patients and controls are shown in Table 1.

**Table 1 Demographic data of the studied groups**

Group	N (%)			$\chi^2$	P value
	Control	SLE group I	SLE group II		
Sex					
Female	17 (63.0)	25 (92.6)	24 (88.9)	9.33	0.009 (S)
Male	10 (37.0)	2 (7.4)	3 (11.1)		
Total	27 (100.0)	27 (100.0)	27 (100.0)		
Age [mean $\pm$ SD (range)] (years)	31.3 $\pm$ 9.3 (19–60)	33.9 $\pm$ 7.2 (19–48)	33.5 $\pm$ 8.2 (18–50)	F = 0.801	0.452 (NS)

SLE, systemic lupus erythematosus;  $P < 0.05$ , significant.

In our study we found that there was an increase in IL-10 concentration in SLE group I, SLE group II, and SLE total patients compared with that in controls (Table 2). Moreover, IL-10 concentration correlated directly to the scores of SLEDAI in SLE total patients (Table 3).

Mutant allele A was found more frequently in SLE patients compared with allele G. Genotype AA was found more frequently, followed by AG and GG (Tables 4 and 5).

Genotype AA and AG were found to be associated with higher IL-10 concentration compared with GG, which means that allele A is suspected to affect IL-10 production (Table 6).

There was no significant association between IL-10 gene promoter and SLEDAI (Fig. 1).

## Discussion

SLE is a potentially severe autoimmune disease, which demonstrates variations in incidence, prevalence, disease activity, and prognosis based on race and ethnicity [15]. SLE is a chronic systemic disease with variable clinical presentation. The exact pathological mechanisms of SLE remains elusive, and the etiology of SLE is known to be multifactorial, involving genes, sex hormones, and environmental factors including sunlight, drugs, and infections [16]. This study included 81 participants, and 54 of them were suffering from SLE [11,12]. An overall 90% of SLE patients were female and 10% were male. This finding indicates that female sex is considered as one of the predisposing factors of the disease and that hormones contribute through unknown mechanisms to increase the prevalence of SLE among women. The X chromosome may contribute to increasing the severity of the disease, as the gene known to contribute to the pathogenesis of SLE is CD40, which is located on chromosome X [17]. In our study, the most frequent clinical manifestations among SLE patients were malar rash (80%), arthritis (75%), renal involvement (55%), and fever (50%), and the least frequent clinical variables were cranial nerve affection and organic brain syndrome (0% for both).

Moreover, the laboratory findings among SLE patients according to SLEDAI showed positivity of ANA (100%) and anti-dsDNA (80%).

However, in a study by Akbarian *et al.* [18], which was conducted in Iran, the prevalence of manifestations was as follows: musculoskeletal, 83.2%; renal, 75%; hematologic, 66.4%; cutaneous, 55%; neuropsychiatric, 23.4%; pulmonary, 21.5%; and cardiac, 17.2%. There were positive ANAs in 86.4% of cases and positive anti-DNA in 82.3% of cases. These differences in clinical and laboratory findings indicate that genetic and climatic factors may lead to different presentations of lupus.

Cytokines are important components of immune response and regulation, and play an active role in activating, differentiating, and maturing immune cells. An imbalance between proinflammatory cytokines such as IL-6, tumor necrosis factor (TNF)- $\alpha$ , and IL-1, and anti-inflammatory cytokines such as IL-10, IL-4, and IL-13 is a well-known characteristic of SLE. Cytokines are heavily integrated in T-cell and B-cell signaling systems, and abnormal proinflammatory cytokines levels, particularly ILs, interferons, and TNFs, are important hallmark in hyperactivity of lymphocytes [19].

IL-10, primarily produced by monocytes and lymphocytes, is a multifunctional cytokine in immunoregulation and inflammation. It enhances B-cell proliferation, differentiation, and antibody production and thus plays a role in B-cell hyperactivity and in increasing production of autoantibodies in SLE. It also inhibits functions of T cells and antigen-presenting cells, which in SLE may contribute to impaired cell-mediated immunity [20]. In our study, there was an increase in IL-10 concentration in total SLE patients (mean  $\pm$  SD 49.5  $\pm$  79.8 pg/ml), and the difference was found to be statistically significant when compared with that of the control group ( $P = 0.012$ ). Moreover, a higher concentration of IL-10 was found in SLE group II (severe cases), with a mean  $\pm$  SD of 81  $\pm$  104.2 pg/ml, compared with SLE group I (mild and moderate cases), which had a mean  $\pm$  SD of 17.9  $\pm$  7.7 pg/ml. On comparing SLE group I (mild and moderate cases)

**Table 2 Interleukin-10 concentration in different studied groups**

Groups	N	Range (pg/ml)	Mean (pg/ml)	SD
Control group	27	2.2–30	9.9	6.3
SLE group I	27	1–33.1	17.9	7.7
SLE group II	27	32–574.5	81	104.2
SLE total	54	1–574.5	49.5	79.8

SLE, systemic lupus erythematosus.

**Table 3 Correlation of interleukin-10 concentration with scores of Systemic Lupus Erythematosus Disease Activity Index**

Group	r value	P value	Significance
SLE group I	-0.046	0.824	NS
SLE group II	0.503	<0.001	HS
SLE total	0.383	0.044	S

HS, highly significant; S, significant; SLE, systemic lupus erythematosus;  $P < 0.05$ , significant;  $P < 0.001$ , highly significant.

**Table 4 Interleukin-10 promoter alleles' distribution in different studied groups**

Groups	Total number of IL-10 alleles	Allele A [N (%)]	Allele G [N (%)]
Control group	54	38 (70.4)	16 (29.6)
SLE group I	54	39 (72.2)	15 (27.8)
SLE group II	54	44 (81.5)	10 (18.5)
SLE total	108	83 (76.9)	25 (23.1)

IL, interleukin; SLE, systemic lupus erythematosus.

**Table 5 Interleukin-10 promoter genotypes distribution in different studied groups**

Groups	Total (n)	[N (%)]		
		Genotype AA	Genotype AG	Genotype GG
Control group	27	17 (63.0)	4 (14.8)	6 (22.2)
SLE group I	27	18 (66.7)	3 (11.1)	6 (22.2)
SLE group II	27	18 (66.7)	8 (29.6)	1 (3.7)
SLE total	54	36 (66.7)	11 (20.4)	7 (12.9)

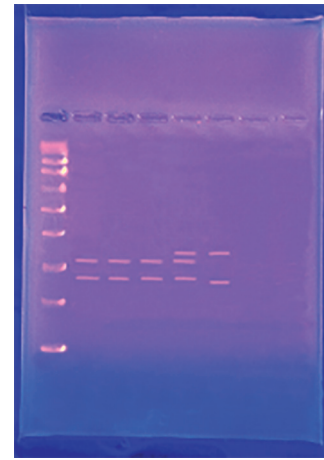
SLE, systemic lupus erythematosus.

**Table 6 Association of interleukin-10 promoter genotypes with interleukin-10 concentration**

Group	IL-10 concentration (median) (pg/ml)			KW	P value
	Genotype AA	Genotype AG	Genotype GG		
Control group	9.1	9.1	8.4	0.036	0.82
SLE group I	20.1	16.6	12.4	7.662	0.022 (S)
SLE group II	60.6	46.5	47.0	2.437	0.296
SLE total	33.1	38.0	13.6	7.066	0.029 (S)

IL, interleukin; KW, Kruskal–Wallis; S, significant; SLE, systemic lupus erythematosus;  $P < 0.05$ , significant.

and SLE group II (severe cases), the difference was statistically highly significant ( $P = 0.003$ ). We also found that there is a highly significant correlation (directly proportionate) between IL-10 concentration in SLE total patients and SLEDAI ( $r = 0.383$ ). Other studies [21,22] found that IL-10 concentration in SLE patients was increased ( $P < 0.001$ ) and correlated positively with SLEDAI. Another study by El-Sayed

**Figure 1**

Gel electrophoresis representing the restriction fragment length polymorphism patterns obtained following digestion with the restriction enzyme *Eco*NI. Lane 1 represents 100 bp ladder marker; lanes 2, 3, and 4 represent two bands of homozygous mutant alleles AA with size 278 and 306 bp; lane 5 represents three bands of heterozygous alleles AG with size 272, 306, and 317 bp; and lane 6 represents two bands of homozygous wild alleles GG with size 267 and 317 bp.

*et al.* [23] which was conducted in Egypt found that IL-10 concentration was higher in SLE patients than in controls, but did not reach the significant level. The overproduced IL-10 in SLE patients may be ascribed to B cells and monocytes [24,25]. Moreover, B-cell secretion of IL-10 could regulate dendritic cells and T-cell function to promote Th2 cell deviation of the immune response [26]. Moreover, the enhanced excretion of IL-10 may be due to an increasing number of the earlier peripheral B-cell abnormalities including plasma cell expansion [27].

In our study, we found that in SLE group I (mild and moderate cases) the IL-10 concentration was increased, but negatively correlated with SLEDAI ( $r = -0.046$ ). Arora *et al.* [28] also reported that IL-10 concentration was higher in SLE patients than in controls, but negatively associated with SLEDAI scores. Other studies [29,30] found that there was no difference in serum IL-10 level between patients and controls. These conflicts may arise probably from several potential factors such as sample size, patients with different demographics, clinical characteristics, or types of therapy. In addition, in some indices measured for SLE activity, qualitative data with high heterogeneity between studies can also contribute to this discordance.

The human IL-10 gene that is located on chromosome 1q31–32 is a susceptible region for SLE. The individual variation in IL-10 production may be due to the genotypic variations in the human IL-10 promoter [31]. The 1082A/G polymorphism is located at putative



regulatory regions in the promoter of the IL-10 gene [32]. As regards IL-10 gene 1082A/G polymorphism, in our study, we found that the mutant allele A (76.9%) was found more frequently compared with allele G (23.1%) in SLE total patients. Moreover, genotype AA distribution was found more frequently in SLE total patients (66.7%), followed by genotype AG (20.4%) and GG (12.9%). A study conducted on the southern Chinese population by Mok *et al.* [33] found that the frequency of allele A was 96% and that of allele G was 4%. A study conducted in Malaysia by Hee *et al.* [34] found that the frequency of allele A was 91% and that of allele G was 9% with genotype frequency (AA, 81.82%; AG, 18.18%; and GG, 0%). Other studies reported that there is a significant association with allele G and SLE risk, as reported in studies conducted in Poland by Sobkowiak *et al.* [35], in Vietnam by Khoa *et al.* [10] and in Sweden by Fei *et al.* [36]. Another study [37] showed that the IL-10 G allele was associated with Asian but not with White SLE patients. However, no association was observed between 1082A/G and SLE patients in other studies [38,39]. These distinctions between the effects of the 1082A/G polymorphism in SLE risk may result from genetic heterogeneity, ethnicity, different exposure to environmental factors, and different sample size of population.

In our study we also found that there was an increase in IL-10 concentration in association with genotype AA in SLE patients, especially in SLE group I (mild and moderate cases), which was more significant ( $P = 0.022$ ). However, in SLE group II (severe cases) there was no statistically significant association ( $P = 0.296$ ), but genotype AA was more associated with increased IL-10 concentration compared with AG and GG (Table 6). In SLE total patients there was a statistically significant association of genotype AA with IL-10 concentration ( $P = 0.029$ ). The SNP 1082 was considered to be in the proximal end of the IL-10 promoter, upstream of the transcription start site. It has been shown to be associated with the transcription binding site, which in turn may affect the production of IL-10 [40].

In our study when we compared IL-10 gene promoter genotype with SLEDAI, we found no relation or association. López *et al.* [41] stated that increased circulating levels of IL-10 have been reported in patients with SLE. However, there were no definitive data on the association of IL-10 polymorphisms and SLEDAI, probably due to heterogeneity of the disease.

## Conclusion

From this study we conclude that IL-10 concentration is elevated in SLE patients and is shown to be

associated with disease activity, and so it can be selected as a biomarker of the disease. IL-10 gene polymorphism 1082A/G is not a strong indicator to show susceptibility of the disease. Allele A is suspected to affect IL-10 production more than allele G.

## Recommendations

Further studies with large sample size to provide us with a sufficient data to help in gene study are needed to confirm these findings. A study should be performed on gene haplotype frequencies in SLE patients, as it may have a role in disease susceptibility. Moreover, there could be some other uncovered mechanisms involved in the susceptibility to SLE. Therefore, further studies are needed to explore the exact mechanism. Other studies should be performed on other cytokines and their receptors such as IL-6, IL-4, TNF, TGF, and interferon to assess their role in the pathogenesis of the disease.

## Acknowledgements

### Conflicts of interest

There are no conflicts of interest.

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