ORIGINAL ARTICLE

Polymorphisms of Interleukin-1β and Cytotoxic T lymphocyte Associated Antigen-4 Genes in Systemic Lupus Erythematosus

[1]Hassan Elbanna M. Younes, [1]Mabrouk M. Ghonaim, [1]Amira H. Elkhyat, [2]Alaa A. Labeeb, [3]Sally M. El-Hefnawy and [1]Amal M. Dawoud*

[1]Department of Microbiology and Immunology, Faculty of Medicine, Menoufia University
[2]Department of Physical Medicine-Rheumatology and Rehabilitation, Faculty of Medicine, Menoufia University,
[3]Department of Biochemistry, Faculty of Medicine, Menoufia University

Key words:
CTLA-4, IL-1β, polymorphism, SLE

Corresponding Author:
Amal Mohamed Abd El-Hameed Dawoud
Department of Microbiology and Immunology, Faculty of Medicine, Menoufia University
Tel.: 01229688245
amalmohamed549@yahoo.com

ABSTRACT

Background: *SLE is an autoimmune disease with complex etiology. Genetic aberrations disrupting the immune regulatory mechanisms may initiate autoimmune disease development. As CTLA-4 is a negative regulator of T-cell immune response and IL-1β is a potential pro-inflammatory cytokine, their allelic polymorphisms might have an impact on SLE susceptibility.* ***Objectives:*** *To investigate a possible association between the polymorphisms of interleukin-1β (IL-1β) and cytotoxic T lymphocyte associated antigen-4 (CTLA-4) genes and increased susceptibility and activity of systemic lupus erythematosus (SLE).* ***Methodology:*** *This study was conducted on 50 SLE patients and 25 age- and sex-matched healthy individuals. All patients were subjected to full clinical evaluation and laboratory investigations. The studied groups were genotyped for CTLA-4 -318 C/T and IL-1β -31 T/C polymorphisms by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.* ***Results:*** *The TT genotype and T allele frequencies of the IL-1β -31 T/C polymorphism were significantly (P <0.05) higher in SLE patients than controls. In SLE patients, significant (P <0.05) association of IL-1β -31 T/C polymorphism and SLE activity was observed in TT genotype. There was an increased frequency of TT genotype of IL-1β -31 T/C polymorphism in SLE patients with arthritis and vasculitis compared to those without these manifestations. SLE patients with TT genotype had higher SLEDAI score, anti-dsDNA titer and ESR compared to those with C/T or CC genotypes. On the other hand, the disease susceptibility and activity, demographic characters, clinical data, SLEDAI score, clinical manifestations, autoantibody profile and laboratory characteristics had insignificant association with different genotypes of CTLA-4 -318 C/T polymorphism (P >0.05).* ***Conclusion:*** *IL-1β -31 T/C but not CTLA-4 -318 C/T polymorphisms are associated with increased SLE susceptibility and activity.*

INTRODUCTION

SLE is an autoimmune disease which is characterized by autoantibodies production against nuclear antigens. Genetic and environmental factors have an essential role in determining disease susceptibility and prognosis [1]. Gene polymorphisms were suggested to be involved in the disease etiology and pathogenesis [2].

CTLA-4 is an essential negative regulator of T-cell responses; its dysregulation could affect SLE pathogenesis by altered T cells activation to self-antigens. CTLA-4 polymorphisms were reported to be associated with various autoimmune diseases such as rheumatoid arthritis, type I diabetes, Graves' disease, and SLE [3].

IL-1β is a potent pro-inflammatory cytokine *via* stimulating the expression of genes associated with inflammation and immune response, stimulation of B cell proliferation and differentiation, co-stimulation of T cells and natural killer cell activation [4]. Because of the important role of IL-1 in inflammation, it appears that allelic polymorphisms in *IL-1* might have an impact on SLE susceptibility [5].

This study aimed to determine the polymorphisms of IL-1β and *CTLA-4* genes in SLE patients and a possible relation between these polymorphisms and increased susceptibility and activity of SLE.

METHODOLOGY

Study population and selection of patients

This study was conducted during the period from January 2017 to June 2018 at Menoufia University Hospitals. The study was approved by the local ethics committee of the Menoufia University and informed consents were obtained from all the participants.

The study involved two groups of females: group I included 50 SLE patients diagnosed according to the SLICC classification criteria [6,7] and group II included 25 age- and sex-matched healthy females. Patients were recruited from Outpatient Clinics or Inpatient Wards of Internal Medicine and Rheumatology and Rehabilitation Departments.

Patients with hepatic, renal (other than SLE) or malignant diseases, patients with drug-induced lupus cases, SLE patients with pregnancy and SLE patients with other connective tissue disorders were excluded from the study.

Methods:

Patients were subjected to history taking, complete medical examination and thorough laboratory investigations.

About 15 ml of venous blood were aseptically withdrawn from all the studied subjects. Each sample was divided into 5 aliquots. The first part (2 ml) was collected in sterile vacutainer tubes with EDTA for molecular testing of polymorphism by PCR-RFLP. The blood was stored at - 20°C for later use. The other parts were collected in the suitable vacutainer tubes for hematology and biochemistry analyses

Hematology and biochemistry analyses were performed at the local laboratory of the Menoufia University Hospitals. Laboratory investigations included the following: renal function tests (serum urea and creatinine), complete blood picture (CBC), erythrocyte sedimentation rate, autoantibody profile (ANA and anti-dsDNA), C3 and C4 levels.

DNA amplification and polymorphism analysis:

PCR-RFLP was used to analyze the _318C/T polymorphism at the promoter region of *CTLA-4* and *IL-1β* -31 T/C genes.

Polymerase chain reaction (PCR):

Total DNA was extracted from EDTA treated blood sample using Zymo Research Corp. Quick-gDNA™ MiniPrep Genomic DNA Purification Kit (Catalog D3024 and D3025) (Zymo Research Corp., USA) following the Manufacturer`s instructions. The isolated DNA was stored at -20°C until analysis of *CTLA-4* and *IL-1β* genes polymorphisms was performed.

Genotypes of *IL-1β* and *CTLA-4* polymorphisms were detected using PCR-RFLP method. The sequence of primers were as follows: for *IL-1β-31 T/C* polymorphism, forward:
5'-AGAAGCTTCCACCAATACTC-3' and reverse
5'-ACCACCTAGTTGTAAGGA-3';
for *CTLA-4* _318C/T polymorphism, forward:
5′-AAATGAATTGGACTGGATGGT-3′ and reverse
5′-TTACGAGAAAGGAAGCCGTG-3′ .

PCR procedure was done according to the manufacture's instructions.

The amplification products were separated by agarose gel electrophoresis through 2% agarose gel stained with ethidium bromide for 20 minutes at 150 volt. Detection of positive bands was confirmed by visualization of the specific bands under ultraviolet light which correspond to 239 bp bands for *IL-1β* and 247bp bands for *CTLA-4*.

Restriction fragment length polymorphism (RFLP):

a) The 239bp PCR product of *IL-1β* -31 T/C polymorphism was digested with Fast Digest® *AluI* enzyme (New England BioLabs, Hitchin, UK) for 1.5 hrs at 37°C. The T allele had *Alu I* cleavage site and digested to 152 and 87bp fragments; the C allele had no cleavage site for *Alu I* and only produced 239bp fragment. Digested products were separated using electrophoresis on a 2% agarose gel. Samples yielding 152 and 87 bp fragments were considered as genotype homozygote T/T, those with single 239 bp fragments were considered as genotype homozygote C/C, while the presence of 239, 152 and 87 bp were considered as genotype heterozygote C/T (Figure 1).

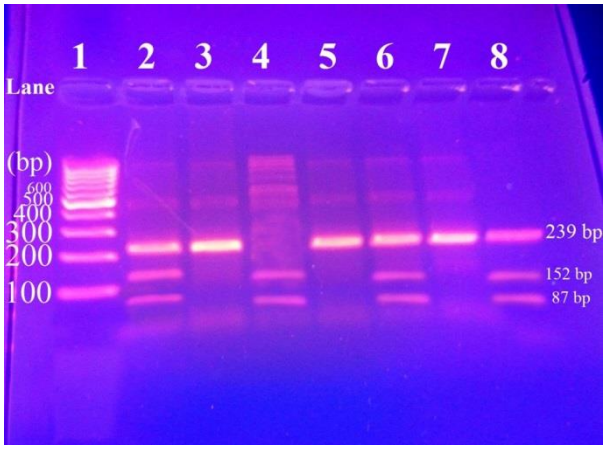

Figure (1): Genotyping of the *IL-1β* gene by Alu I restriction enzyme.

Lane (1) shows 100 bp ladder. Lanes (2, 6 and 8) show C/T heterozygote genotype at (87, 152, 239 bp). Lane (4) shows TT homozygote genotype (87, 152 bp). Lanes (3, 5 and 7) show CC homozygote genotype (239 bp).

b) The 247bp PCR product of *CTLA-4* _318C/T polymorphism was digested with Fast Digest® *MseI* enzyme (New England BioLabs, Hitchin, UK) for 1.5 hrs at 37°C. The PCR products with C allele at the polymorphic site were digested into two fragments; 226 and 21 bp while those with T allele were digested into three fragments; 21, 96 and 130 bp fragments. Samples yielding 226 and 21 bp fragments were considered as C/C and those with 130, 96 and 21 bp fragments were considered as T/T, while those with 226, 130, 96 and 21 bp fragments were considered as C/T. The 21 bp fragments could not be seen on the agarose gel **(Figure 2).**

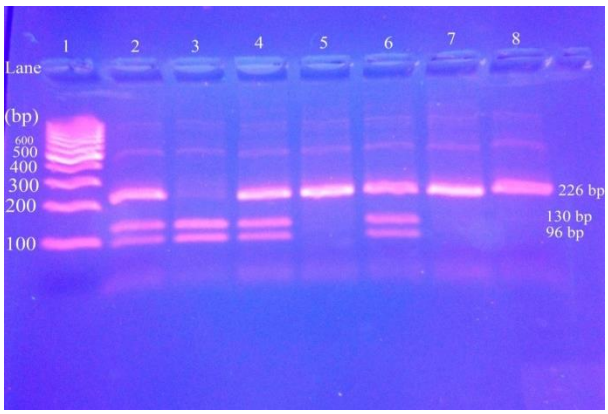

Figure (2): Genotyping of the *CTLA-4* gene by MseI restriction enzyme.
Lane (1) shows 100 bp ladder. Lanes (2, 4 and 6) show C/T heterozygote genotype at (96, 130, 226 bp). Lane (3) shows TT homozygote genotype (96, 130 bp). Lanes (5, 7 and 8) show CC homozygote genotype (226 bp).

Statistical analysis:

The collected data were tabulated and analyzed by SPSS statistical package version 20 on IBM compatible computer. The results were analyzed using Chi-square, Fisher`s Exact, Mann-Whitney and Student`s t tests wherever appropriate. Chi-square was used to analyze the genotypes and alleles frequencies between SLE patients and controls. P value <0.05 was considered to be statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) was used to estimate the strength of the association of alleles and genotypes of polymorphism between different groups

RESULTS

Demographic and clinical history of the studied groups are shown in table (1). A significant difference ($P < 0.05$) was detected between patients and controls regarding residence and family history of SLE. Organ involvement and clinical manifestations of SLE activity among patients are shown in table (2).

Routine and specific laboratory data are shown in table (3). Hemoglobin level, WBCs count, platelet count, serum complement C3 and complement C4 were significantly ($P < 0.05$) lower in SLE patients compared to controls. Serum creatinine, anti-dsDNA titers, ANA and ESR were significantly ($P < 0.05$) higher in SLE patients compared to controls.

Regarding the genotype and allele frequencies of *IL-1β* -31 T/C polymorphism, the risk of SLE was higher in individuals with TT genotype compared to those with CC genotype [OR, 8.27 (95% CI, 1.53 to 44.61); P <0.01]. The risk of SLE activity was 8.88 fold higher in individuals carrying TT genotype [OR, 8.88 (95% CI, 1.39 to 56.57); P <0.05]. However, there was no significant (P >0.05) difference between SLE patients and control regarding genotype and allele frequencies of *CTLA-4* _318C/T polymorphism as shown in table (4).

Both arthritis and vasculitis were more frequent in patients having the TT genotype at −31 site in *IL-1β* gene ($P < 0.05$). However, genotypic pattern of _318 C/T *CTLA-4* polymorphisms in SLE patients didn`t have significant association with clinical manifestations and organ involvement in our patients ($P > 0.05$). SLE patients with TT genotype of *IL-1β* -31T/C polymorphism had significantly higher SLEDAI score compared to those with C/T and CC genotypes. However, SLEDAI score of SLE patients didn`t show significant differences among different genotypic patterns of _318 C/T *CTLA-4* polymorphisms as shown in table (5).

Table (1): Demographic and clinical history of the studied groups

Demographic and clinical characters	Studied groups				Test of sig.	P value
	SLE patients (n=50)		Controls (n=25)			
Age (years)						
Mean±SD	31.70±7.37		30.56± 6.86		**t**=	>0.05 NS
Range	20.0–49.0		18.0–44.0		0.64	
Residence:	**NO.**	**%**	**NO.**	**%**		
Urban	19	38.0	17	68.0	χ^2 =	<0.05
Rural	31	62.0	8	32.0	6.01	S
Family history of SLE:						
Positive	9	18.0	0	0.0	χ^2 =	<0.05*
Negative	41	82.0	25	100.0	5.11	S
Diabetes Mellitus:					χ^2 =	>0.05*
Present	5	10.0	0	0.0	2.67	NS
Absent	45	90.0	25	100.0		
Hypertension:						
Present	10	20.0	0	0.0	χ^2 =	<0.05*
Absent	40	80.0	25	100.0	5.76	S
Disease duration (years):						
Mean±SD	5.97±3.97		_____		____	____
Range	0.5–15.0					
Age of onset (years):						
Mean±SD	25.61±4.53		_____		____	____
Range	17.0–36.0					
SLEDAI category						
No activity	25	50.0	_____		____	____
Mild activity	7	14.0				
Moderate activity	9	18.0				
High activity	9	18.0				

t: Student`s t test $\chi^2$: Chi-Square test *: Fisher`s Exact test

Table (2): Comparison between SLE patients with different activity regarding clinical manifestations and organ involvement:

Parameter	Total N=25	Activity categories of SLE patients			χ^2	P value
		Mild activity N=7	Moderate activity N=9	High activity N=9		
	N (%)	N (%)	N (%)	N (%)		
Arthritis						
Present	13 (52.0)	1 (14.3)	5 (55.6)	7 (77.8)	6.43	<0.05
Absent	12 (48)	6 (85.7)	4(44.4)	2(22.2)		S
Malar rash						
Present	19 (76.0)	3 (42.9)	8 (88.9)	8 (88.9)	5.85	0.05
Absent	6 (24.0)	4 (57.1)	1(11.1)	1(11.1)		S
Fever						
Present	18 (72.0)	3 (42.9)	7 (77.8)	8 (88.9)	4.37	> 0.05 NS
Absent	7 (28.0)	4 (57.1)	2(22.2)	1 (11.1)		
Nephritis						
Present	13 (52.0)	0 (0.0)	6 (66.7)	7 (77.8)	10.75	<0.01
Absent	12 (48.0)	7 (100)	3 (33.3)	2(22.2)		S
Alopecia						
Present	12 (48.0)	3 (42.9)	4 (44.4)	5 (55.6)	0.32	> 0.05 NS
Absent	13 (52.0)	4 (57.1)	5 (55.6)	4 (44.4)		
Vasculitis						
Present	8 (32.0)	0 (0.0)	3 (33.3)	5 (55.6)	5.59	> 0.05 NS
Absent	17 (68.0)	7 (100)	6 (66.7)	4 (44.4)		
Serositis						
Present	4 (16.0)	1 (14.3)	2 (22.2)	1 (11.1)	0.43	> 0.05 NS
Absent	21 (84.0)	6 (85.7)	7 (77.8)	8 (88.9)		
Oral ulcers						
Present	11 (44.0)	1 (14.3)	4 (44.4)	6 (66.7)	4.38	> 0.05 NS
Absent	14 (56.0)	6 (85.7)	5 (55.6)	3 (33.3)		
Photosensitivity						
Present	12 (48.0)	2 (28.6)	4 (44.4)	6 (66.7)	2.36	> 0.05 NS
Absent	13 (52.0)	5(71.4)	5 (55.6)	3 (33.3)		

Table (3): Routine and specific laboratory findings of the studied population

Laboratory parameters	Studied groups		Test of sig.	P value
	SLE patients (n=50)	Controls (n=25)		
Haemoglobin (gm /dl):				
Mean±SD	10.48±1.37	12.12 ±0.73	**t**=	<0.001 HS
Range	8.0 –14.10	10.50 –14.0	6.71	
WBCs count (×10³)**:**				
Mean±SD	5.82±1.65	7.33±1.67	**t**=	<0.001 HS
Range	2.80–9.80	4.30–10.50	3.69	
Platelets count (×10³):				
Mean±SD	232.86±67.15	287.40±71.28	**t**=	<0.01
Range	100.0 –381.0	155.0–430.0	3.24	S
Blood urea (mg/dl)**:**				
Mean±SD	40.18±22.84	30.64 ±6.29	**U** =	>0.05
Median	29.0	31.0	0.61	NS
Range	22.0 –99.0	20.0 –40..0		
Serum creatinine (mg/dl):				
Mean±SD	1.45±0.70	1.08±0.19	**U**=	<0.05
Median	1.25	1.10	2.54	S
Range	0.50–3.80	0.69–1.40		
ESR mm/hour				
Mean±SD	63.32±31.81	13.64±5.19	**U**=	<0.001 HS
Median	59.0	13.0	6.91	
Range	20.0 –150.0	6.0–30.0		
Complement C3 (mg/dL)				
Mean±SD	52.44±17.53	131.12±23.91	**t=**	<0.001 HS
Range	24.0 - 90.0	90.0 – 172.0	16.17	
Complement C4 (mg/dL)				
Mean±SD	12.70±2.94	26.48±5.73	**t=**	<0.001 HS
Range	7.0 - 61.0	13.0 – 35.0	11.28	
Anti-dsDNA titer IU/ml				
Mean ± SD	103.94 + 74.61	15.34 + 4.63	**U**=	<0.001
Median	86.0	15.50	55.63	HS
Range	28.0 - 315.0	8.0 - 22.0		
ANA	**NO.** **%**	**NO.** **%**		
Positive	50 100	0 0.0	**χ2**= 75.0	<0.001
Negative	0 0.0	25 100		HS

U: Mann-Whitney

Table (4): Genotypic and allelic frequencies of *CTLA-4 -318 C/T* and *IL-1β* -31 T/C polymorphisms in SLE patients and healthy controls

Genotypic and allelic frequencies	Studied groups		χ2 P value	OR	95% CI (LL – UL)	Studied groups		χ2 P value	OR	95% CI (LL – UL)
	SLE patients (n=50)	Controls (n=25)				Active SLE (n=25)	Inactive SLE (n=25)			
	N (%)	N (%)				N (%)	N (%)			
***CTLA-4 -318 C/T* polymorphism**										
Genotypes:										
CC	39 (78.0)	17 (68.0)	2.27	**1®**		19 (76.0)	20 (80.0)	0.16	**1®**	
C/T	7 (14.0)	3(12.0)	>0.05	1.01	0.23---4.41	4 (16.0)	3(12.0)	>0.05	1.40	0.27---7.11
TT	4 (8.0)	5 (20.0)		0.34	0.08---1.46	2 (8.0)	2 (8.0)		1.05	0.13---8.24
Allele frequency:										
C	85 (85.0)	37 (74.0)	2.65	**1®**		42 (84.0)	43 (86.0)	0.07	**1®**	
T	15 (15.0)	13 (26.0)	>0.05	0.50	0.21---1.16	8 (16.0)	7 (14.0)	>0.05	1.17	0.38---3.51
***IL-1β* -31 T/C polymorphism**										
Genotypes:										
CC	11 (22.0)	14 (56.0)	9.39	**1®**		3 (12.0)	8 (32.0)	6.19	**1®**	
C/T	26 (52.0)	9 (36.0)	<0.01	3.67	1.23---10.98	12 (48.0)	14(56.0)	<0.05	2.28	0.49---10.60
TT	13 (26.0)	2 (8.0)		8.27	1.53---44.61	10 (40.0)	3 (12.0)		8.88	1.39---56.57
Allele frequency:										
C	48 (48.0)	37 (74.0)	9.17	**1®**		18 (36.0)	30 (60.0)	5.76	**1®**	
T	52 (52.0)	13 (26.0)	<0.01	3.08	1.46---6.48	32 (64.0)	20 (40.0)	<0.01	2.66	1.18---5.98

®: Reference value for odds ratio

OR: Odds ratio, CI: Confidence interval, LL: Lower limit, UL: Upper limit

Table (5): Association between genotypic pattern of _318 C/T *CTLA-4* and T−31C *IL-1β* polymorphisms in patients with active SLE and their clinical manifestations and organ involvement:

Parameter	Genotypes of _318 C/T *CTLA-4* polymorphisms in SLE patients			χ2 P value	Genotypes of T_31C *IL-1β* polymorphisms in SLE patients			χ2 P value
	CC N=19	C/T N=4	TT N=2		TT N=10	C/T N=12	CC N=3	
	N (%)	N(%)	N (%)		N (%)	N(%)	N (%)	
Arthritis								
Present	11 (57.9)	2 (50.0)	0 (0.0)	2.43	8 (80.0)	5 (41.7)	0 (0.0)	6.90
Absent	8 (42.1)	2 (50.0)	2 (100)	>0.05	2 (20.0)	7 (58.3)	3 (100.0)	<0.05
Malar rash								
Present	14 (73.7)	4 (100.0)	1 (50.0)	2.06	8 (80.0)	9 (75.0)	2 (66.7)	0.23
Absent	5 (26.3)	0 (0.0)	1(50.0)	>0.05	2 (20.0)	3 (25.0)	1 (33.3)	>0.05
Fever								
Present	13 (68.4)	3 (75.0)	2 (100.0)	0.91	9 (90.0)	7 (58.3)	2 (66.7)	2.76
Absent	6 (31.6)	1 (25.0)	0 (0.0)	>0.05	1 (10.0)	5 (41.7)	1 (33.3)	>0.05
Nephritis								
Present	9 (47.4)	3 (75.0)	1 (50.0)	1.01	5 (50.0)	7 (58.3)	1 (33.3)	0.62
Absent	10 (52.6)	1 (25.0)	1(50.0)	>0.05	5 (50.0)	5 (41.7)	2 (66.7)	>0.05
Alopecia								
Present	9 (47.4)	2 (50.0)	1 (50.0)	0.01	6 (60.0)	4 (33.3)	2 (66.7)	2.03
Absent	10 (52.6)	2 (50.0)	1 (50.0)	>0.05	4 (40.0)	8 (66.7)	1 (33.3)	>0.05
Vasculitis								
Present	6 (31.6)	1 (25.0)	1 (50.0)	0.38	6 (60.0)	2 (16.7)	0 (0.0)	6.13
Absent	13 (68.4)	3 (75.0)	1 (50.0)	>0.05	4 (40.0)	10 (83.3)	3 (100.0)	<0.05
Serositis								
Present	3 (15.8)	0 (0.0)	1 (50.0)	2.48	1 (10.0)	3 (25.0)	0 (0.0)	1.56
Absent	16 (84.2)	4 (100)	1 (50.0)	>0.05	9 (90.0)	9 (75.0)	3 (100.0)	>0.05
Oral ulcers								
Present	7 (36.8)	3 (75.0)	1 (50.0)	1.98	5 (50.0)	4 (33.3)	2 (66.7)	1.32
Absent	12 (63.2)	1 (25.0)	1(50.0)	>0.05	5 (50.0)	8 (66.7)	1 (33.3)	>0.05
Photosensitivity								
Present	11 (57.9)	0 (0.0)	1 (50.0)	4.44	6 (60.0)	5 (41.7)	1 (33.3)	1.02
Absent	8 (42.1)	4 (100)	1 (50.0)	>0.05	4 (40.0)	7 (58.3)	2 (66.7)	>0.05
SLEDAI category								
Mild	5 (26.3)	1 (25.0)	1 (50.0)	1.56	1 (10.0)	4 (33.3)	2 (66.7)	9.78
Moderate	7 (36.8)	1 (25.0)	1 (50.0)	>0.05	2 (20.0)	6 (50.0)	1 (33.3)	<0.05
High	7 (36.8)	2 (50.0)	0 (0.0)		7 (70.0)	2 (16.7)	0 (0.0)	

DISCUSSION

This study was performed to analyze the polymorphisms of IL-1β and CTLA-4 genes in SLE patients and to investigate a possible relation between these polymorphisms and increased susceptibility and activity of SLE.

In the current study, the genotypic and allelic frequencies of −318 CTLA-4 polymorphisms didn`t have significant association with SLE susceptibility or activity. In agreement with our results, Chua et al. [8], in Malaysia, and Parks et al.[9] in USA, investigated the polymorphisms of the exon 1 (+49A/G), promoter sites (–1722T/C, –1661A/G, –318C/T), and 3´-untranslated region (3´-UTR) (+6230 A/G) of CTLA-4 gene in lupus patients. Of the five tested polymorphisms, no significant differences were found in the genotypic or allelic frequencies between SLE and healthy controls. Zhu et al.[10] discussed the relation between CTLA-4 polymorphisms (CT60A/G, -1722T/C, -1661G/A, and -318C/T) and SLE susceptibility. They reported that only -1722T/C and CT60A/G polymorphisms in CTLA-4 were associated with SLE, particularly in Asians. In accordance, Ahmed et al.[11] in Japan, Lee et al.[12] and Hudson et al.[13] in Korea, Aguilar et al.[14] in Spain, and Dong et al.[15] in China, reported no significant differences regarding the allele or genotype frequencies of the −318C/T polymorphism between lupus patients and controls. Also, Sugimoto et al.[16] reported that there was no association between lupus activity and polymorphism of exon 1 promoter region (at -318) of CTLA-4 gene. Contrary to the previous results, Shojaa et al.[17] in Iran, documented that the CC genotype was found in 94.5% of their patients and T allele was significantly ($P < 0.001$) more common in the controls. To the best of knowledge, the previous study was the first to show that −318C/T polymorphism of CTLA-4 gene might play a significant role in the development of SLE. Ligers et al.[18] and Wang et al.[19] could explain the previous result as they stated that the T allele of

_318C/T polymorphism was associated with higher promoter activity compared to the C allele. Also, they demonstrated increased expression of CTLA-4 after stimulating the cell surface and at the messenger RNA level in non-stimulated cells in individuals carrying the T allele. The contradictory results between the different studies may be caused by a variation in the genetic background and impacts of race and ethnic differences in SLE predisposition among various populations. Moreover, other risk factors may interact with CTLA-4 to cause the disease. Furthermore, the sample size, patient characters as age of onset, disease severity and organ involvement may have a role[8,11].

In this study, the −31 IL-1β TT genotype and T allele frequencies were higher in SLE patients compared to healthy controls. These findings were in agreement with Mohammadoo-Khorasani et al.[5] in Iran, who concluded that the C allele could have a protective effect on SLE susceptibility. In contrast, Muraki et al. [20] reported that the TT genotype of IL-1β gene -31 T/C polymorphism was significantly lower in SLE patients. However, Wang et al.[21] reported that this polymorphism didn`t have an association to SLE risk. Manchanda et al. [22] explained differences between these studies` results by ethnic and clinical heterogeneity between different populations.

In our study, the risk of SLE activity was 8.88 fold higher in individuals with the TT genotype for IL-1β -31 T/C polymorphism. These findings are supported by that reported by Lind et al. [23], Zienolddiny et al.[24] and Yencilek et al.[25] who stated that IL1β _31 T/C polymorphism can affect DNA-protein interactions, thus modulating the IL-1β gene expression and the T allele was associated with higher transcription of IL1β leading to higher production of IL-1β and inflammation. Our findings showed that both arthritis and vasculitis were higher among patients with the TT genotype. These results are supported by Hayashi et al.[26] and Yang et al.[27] who found that the proportion of Th17 cells was higher in patients with T allele, which correlates with higher production of IL-1β and explains some features in SLE as vascular inflammation induction, leucocytes recruitment, B cells activation and autoantibody production. Other studies of Ghanima et al. [28] in Egypt, and Shah et al.[29] in USA, showed that SLE activity had significant positive correlation with the frequency of circulating Th17 cells. Also Aml et al. [30] concluded the regulatory protective role of TH 17 in the pathogenesis of SLE.

Many SLE genetic variants are still to be discovered. This may be helpful in SLE management with much lower toxicities than the available traditional immunosuppressive drugs.

CONCLUSION

The T allele of *IL-1β* -31 T/C polymorphism is associated with SLE susceptibility and activity. However, there was no association between *CTLA-4 -318 C/T* polymorphism and SLE. Further studies that include larger sample size and populations of different ethnicities should be performed to accurately estimate the relation between polymorphisms in *IL-1β* and *CTLA-4* genes and SLE.

REFERENCES

1. Devaraju P, Mehra S, Gulati R, et al. The IRF5 rs2004640 (G/T) polymorphism is not a genetic risk factor for systemic lupus erythematosus in population from south India. Indian J Med Res. 2018; 147(6): 560-566.
2. El Guindy NM, Ghaffar HA, El Refai RM & El Hawary RE. Cytotoxic T lymphocyte antigen-4 gene polymorphism in systemic lupus erythematosus Egyptian patients. Comp Clin Path. 2015; 24(1): 41-45.
3. Shojaa M, Amoli M, Aghaie M, et al. CTLA-4 polymorphism in Iranian patients with systemic lupus erythematosus. Am J Exp Clin Res. 2014; 1(4):68-71.
4. Umare V, Pradhan V, Rajadhyaksha A, Ghosh K, Nadkarni A. Predisposition of IL-1β (-511 C/T) polymorphism to renal and hematologic disorders in Indian SLE patients. Gene. 2018; 641:41-45.
5. Mohammadoo-khorasani M, Salimi S, Tabatabai E, Sandoughi M, Zakeri Z, Farajian-Mashhadi F. Interleukin-1β (IL-1β) & IL-4 gene polymorphisms in patients with systemic lupus erythematosus (SLE) & their association with susceptibility to SLE. Indian J Med Res. 2016; 143(5):591-596.
6. Petri M, Orbai AM, Alarcon GS, et al. Derivation and validation of the systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012; 64: 2677–2686.
7. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum1992; 35: 630-40.
8. Chua KH, Puah SM, Chew CH, Tan SY, Lian LH. Study of the CTLA-4 gene polymorphisms in systemic lupus erythematosus (SLE) samples from Malaysia. Ann Hum Biol. 2010; 37(2):275-81.
9. Parks CG, Hudson LL, Cooper GS, et al. CTLA-4 gene polymorphisms and systemic lupus

erythematosus in a population-based study of whites and African-Americans in the southeastern United States. Lupus. 2004; 13(10):784-91.

10. Zhu Y, Wang J, Feng X. CTLA-4 SNPs (CT60A/G, -1722T/C, -1661G/A, and -318C/T) and systemic lupus erythematosus: a meta-analysis. Crit Rev Eukaryot Gene Expr. 2014; 24(2):89-100.
11. Ahmed S, Ihara K, Kanemitsu S, et al. Association of CTLA-4 but not CD28 gene polymorphisms with systemic lupus erythematosus in the Japanese population. Rheumatology. 2001; 40(6):662-7.
12. Lee YH, Ji JD, Sohn J, and Song GG. Polymorhisms of CTLA-4 exon I +49, CTLA-4 promoter −318 and Fas promoter −670 in spondyloarthropathies. Clin Rheumatol 2001; 20(6): 420–422.
13. Hudson LL, Rocca K, Song YW, Pandey JP. CTLA-4 gene polymorphisms in systemic lupus erythematosus: a highly significant association with a determinant in the promoter region. Hum Genet 2002; 111(4-5):452-5.
14. Aguilar F, Torres B, Sanchez-Roman J, Nunez-Roldan A, Gonzalez-Escribano MF. CTLA4 polymorphism in Spanish patients with systemic lupus erythematosus. Hum Immunol 2003; 64, 936–40.
15. Dong HR, Li HS, Wang SC, Balin QM, Chang PY. Association between PDCD1, CTLA4, and MECP2 gene polymorphisms and systemic lupus erythematosus in the Chinese Northern Han. Genet Mol Res. 2015; 14(4): 17567-73.
16. Sugimoto K, Fujita S, Yanagida H, et al. Clinical manifestations and analyses of the cytotoxic T-lymphocyte associated-4 gene in two Japanese families with systemic lupus erythematosus. Clin Exp Nephrol. 2008; 12(2): 149-54.
17. Shojaa M, Aghaie M, Amoli M, et al. Association between 318 C/T polymorphism of the CTLA-4 gene and systemic lupus erythematosus in Iranian patients. Int J Rheum Dis. 2017; 20(12):2040-4.
18. Ligers A, Teleshova N, Masterman T, Huang WX, Hillert J. CTLA-4 gene expression is influenced by promoter and exon 1 polymorphisms. Genes Immun 2001; 2: 145–52.
19. Wang XB, Zhao X, Giscombe R, Lefvert AK. A CTLA-4 gene polymorphism at position -318 in the promoter region affects the expression of protein. Genes Immun 2002; 3: 233–4.
20. Muraki Y, Tsutsumi A, Takahashi R, Suzuki E, Hayashi T, Chino Y, et al. Polymorphisms of IL-1 beta gene in Japanese patients with Sjögren's syndrome and systemic lupus erythematosus. J Rheumatol. 2004; 31:720–5.
21. Wang B, Zhu JM, Fan YG, et al. The association of IL1alpha and IL1beta polymorphisms with susceptibility to systemic lupus erythematosus: a meta-analysis. Gene. 2013; 527:95–101.
22. Manchanda PK, Bid HK, Mittal RD. Ethnicity greatly influences the interleukin-1 gene cluster(IL-1b promoter, exon-5 and IL-1Ra) polymorphisms: a pilot study of a north Indian population. Asian Pac J Cancer Prev. 2005; 6:541–6.
23. Lind H, Haugen A, Zienolddiny S. Differential binding of proteins to the IL1B -31 T/C polymorphism in lung epithelial cells. Cytokine. 2007; 38:43–8.
24. Zienolddiny S, Ryberg D, Maggini V, Skaug V, Canzian F, Haugen A. Polymorphisms of the interleukin-1 β gene are associated with increased risk of non-small cell lung cancer. Int J Cancer. 2004; 109(3): 353-6.
25. Yencilek F, Yildirim A, Yilmaz SG, et al. Investigation of Interleukin-1β Polymorphisms in Prostate Cancer. Anticancer Res. 2015; 35(11):6057-61.
26. Hayashi F, Watanabe M, Nanba T, Inoue N, Akamizu T, Iwatani Y. Association of the −31C/T functional polymorphism in the interleukin-1β gene with the intractability of Graves' disease and the proportion of T helper type 17 cells. Clin Exp Immunol. 2009; 158(3):281-286.
27. Yang J, Yang X, Zou H, Chu Y, Li M. Recovery of the immune balance between Th17 and regulatory T cells as a treatment for systemic lupus erythematosus. Rheumatology 2011; 50(8):1366-72.
28. Ghanima AT, Elolemy GG, Ganeb SS, Elazem AA, Abdelgawad ER. Role of T helper 17 cells in the pathogenesis of systemic lupus erythematosus. Egypt J Immunol. 2012; 19(2):25-33.
29. Shah K, Lee WW, Lee SH, et al. Dysregulated balance of Th17 and Th1 cells in systemic lupus erythematosus. Arthritis Res Ther. 2010; 12(2):R53
30. Aml El Sayed Abodu, Haneya A.A. Ali, Omina A. El-Dydamoni and Hend M. Maghraby et al. Expression of T helper 17 cells retinoid acid related orphan receptor Gammat (ROR γt) mRNA in SLE. EJMM: 2018; 27(1):117-123.