ORIGINAL ARTICLE

CONTENTS

Association of program cell death ligand-1 (rs4143815 G>C) with some clinical symptoms and oral ulcer types in systemic lupus erythematosus

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ABSTRACT

Aim: The study aims to detect Association of Program Cell Death Ligand-1(PD-L1) rs4143815 G>C with some Clinical Symptoms and Oral Ulcer Types in Systemic Lupus Erythematosus (SLE).

Materials and Methods: A case control design was suggested in the study enrolled about 60 SLE patients and 20 healthy individuals as a control group, PD-L1 variation implemented using allele specific PCR.

Results: The findings showed significant changes in age ($p<0.011$), sex ($p<0.000$), and non-sig in BMI ($p<0.654$). in SLE group about 23.3% of patients have a family history, 93.7% suffered from fever, 51.66% have a Butterfly-shaped rash, 73.3% suffered from oral ulcer, single oral ulcer was observed 65.90% and multiple ulcer observed 34.09%. Oral mouth was more frequent (55%) while pharynx ulcer was low frequent (3.3%). The rs4143815 G>C showed three genotypes (GC, CC and GG), Significant association of CC with SLE patients (OR 67.8874, $p <0.003$), A non-sig association was observed in GG with SLE (OR 5.8571, $p <0.395$), significant association of C allele with SLE group (OR 1.3529, $p< 0.0008$). The PD-L1 rs4143815 (G>C) genotyping distribution according to some clinical symptoms showed a non-significant association with all symptoms. The PD-L1 rs4143815 (G>C) genotyping distribution according to oral ulcer sites showed a non-significant association also.

Conclusions: The present results concluded closed correlation between rs4143815 G>C and SLE patients, but no association was observed with some clinical symptoms and oral ulcer types.

KEY WORDS: Program Cell Death Ligand-1, (rs4143815 G>C), Clinical Symptoms, Oral Ulcer Types, SLE

Wiad Lek. 2025;78(1):136-142. doi: 10.36740/WLek/197185 DOI

INTRODUCTION

One of the most diseases which increased in the last years in Iraq is SLE, this disease is an autoimmune disorders induced by a complex etiology, the interplay between genetic and environmental factors lead to clinically heterogeneous presentation [1]. The Immune regulatory molecules like programmed death receptor (PD-1) and ligands (PD-L1, PD-L2) are contributed in SLE development [2]. The Antibodies Targeting PD-1 receptors that block its stimulation is used to treat some cancers, Perhaps lead to the development of immune-associated inverse events that clinically appeared with symptoms same of autoimmune disorders such as SLE [1,3]. The PD-1 axis complexities are presented by the PD-1and PD-L1 expression of on lymphoid and myeloid subsets on the microenvironment of non- immune and immune cells. The role of PD-1 and its ligands in the immune response is well documented, it has been found that produced negative signals from them lead to attenuate and terminate immune response [4]. in an antigen presenting cell, the most interaction explained contributed the CD4 T cell PD-1 with both ligands 1 or 2, the Ligation of these receptors stimulates immunoreceptor tyrosine-based inhibitory motif (ITIM) activation in the PD-1 cytoplasmic tail that prevent stimulation sequences contained in the immunological synapse [5], moreover, PD-1 is detected on B cells and myeloid cells [6-8], while the ligands are detected on some cells such as lymphocytes, neutrophils tumors, epithelial, endothelial and stromal cells [9-14]. PD-L1 binds CD80 via antigen presenting cell and linked to CD4 T to reduce cell activation [4, 10]. Therefore PD-1 or PD-L1 signal regulates the adaptive immune response, the cell signals Dysregulated in SLE may discriminate

mechanisms contributed in the controlling of PD-1 response. Oral ulcer in SLE is one of the 4 criteria for the SLE diagnosis, many types of mouth ulcer were elucidated, ranging between common aphthous and injury, it may be a side impacts of SLE medications or rheumatoid arthritis [15-16]. Several ulcers are erupt on the hard palate and painless. In spite of rare, discoid lupus may develop in a child and appeared with ulcers on the soft palate, tongue and on the lining of the lips and cheeks (buccal mucosa), SLE often presents oral alteration in early stages of development [17]. The present study was aims to Association of program cell death ligand-1 with some clinical symptoms and oral ulcer types of SLE.

AIM

The study aims to detect Association of Program Cell Death Ligand-1(PD-L1) rs4143815 G>C with some Clinical Symptoms and Oral Ulcer Types in Systemic Lupus Erythematosus (SLE).

MATERIALS AND METHODS

STUDY SUBJECTS

A case control study was conducted to identify program cell death ligand-1 (PD-L1) rs4143815 G>C Association with some clinical symptoms and oral ulcer types of SLE A 60 SLE patients were enrolled in the present study which attended the chronic disease clinic of the Marjan hospital city; all cases were diagnosed by prof. Dr. Ali Al-kazaz /college of medicine, university of Babylon, and 20 healthy individuals were enrolled as a control group.

SAMPLE COLLECTION

Blood and data sample were collected from study subjects according to ethical approval of environment and health ministry in Iraq, and written consents from each contributor. Blood sample were collected then transferred to DNA Lab, College of Science, University of Babylon, for DNA extraction and (PD-L1) rs4143815 G>C genotyping identification, using the following primers:

FO: CTGTGACAGGGAGAAAGGATACTTCTG;
RO: AGCAAGTTTAGTTTGGCGACAAAATTGT;
FI: TTTGCCTCCACTCAATGCCTCAATATC;
RI: AACACTGAGACTCTCAGTCATGCAGAATAC [18] to produce Allele G=176 bp Allele C=203 bp, Control band = 322 bp, via 30 cycles 95, 58, and 72^{o}C for 30, 40 and 40 sec respectively, with final extension 72 ^{o}C for 10 min.

DATA VISUALIZATION AND ANALYSIS

The PCR products were visualized by electrophoresis pattern (1% agaros, 0.5 TBE, 100 V for 1 hour), 100-1000bp DNA ladder, data represented as mean ±SE for age and BMI, percentage% for genotyping, statistical analysis was used independent t test, and odd ratio (CI%) at p value < 0.05.

RESULTS

The present work was elucidated to evaluate association of PD-L1 (rs4143815 G>C) genotyping with some clinical symptoms and oral ulcer types in SLE patients, the study characteristics distribution exhibited significant differences between SLE and control group in age ($p<0.011$) and sex that showed the female percentage was higher than male significantly ($p<0.000$), non-sig in BMI differences between both groups ($p>0.654$). About 23.3% of patients have a family history, 93.7% suffered from fever, 51.66% have a Butterfly-shaped rash, 73.3% suffered from oral ulcer, single oral ulcer was observed in 65.90% and multiple ulcer observed in 34.09%, Table 1.

The oral ulcers were classified according to the ulcer sites, five types were detected (mouth, lips, tongue, gum and pharynx) oral mouth was more frequent 55% while pharynx ulcer was low frequent 3.3%, Fig. 1.

The DNA was extracted from whole blood and PD-L1 (rs4143815 G>C) was detected using allele specific PCR, the result was clarified in electrophoresis pattern Fig. 2, three genotypes were found (GC, CC and GG). A Significant association of CC with SLE patients (OR 67.8874, $p<0.003$), a non-sig association was observed in GG with SLE (OR 5.8571, $p>0.395$), significant association of C allele with SLE group (OR 1.3529, $p<0.0008$), Table 2.

The PD-L1 rs4143815 (G>C) genotyping distribution according to some clinical symptoms were elucidated in SLE patients, non-significant association were observed in all symptoms, Table 3.

The PD-L1 rs4143815 (G>C) genotyping distribution according to oral ulcer sites showed non-significant association also, GC more frequent in all types ($p>0.725$) (Table 4).

DISCUSSION

The current output proved significant role of PD-L1 rs4143815 G>C in SLE patients but non association with clinical symptoms, the SLE prevalence is increased in last years in Iraq, particularly among women which have higher percentage than men in present study, this agree with a study in United States, found the SLE incidence and prevalence were reached to 5.5 and 73 per

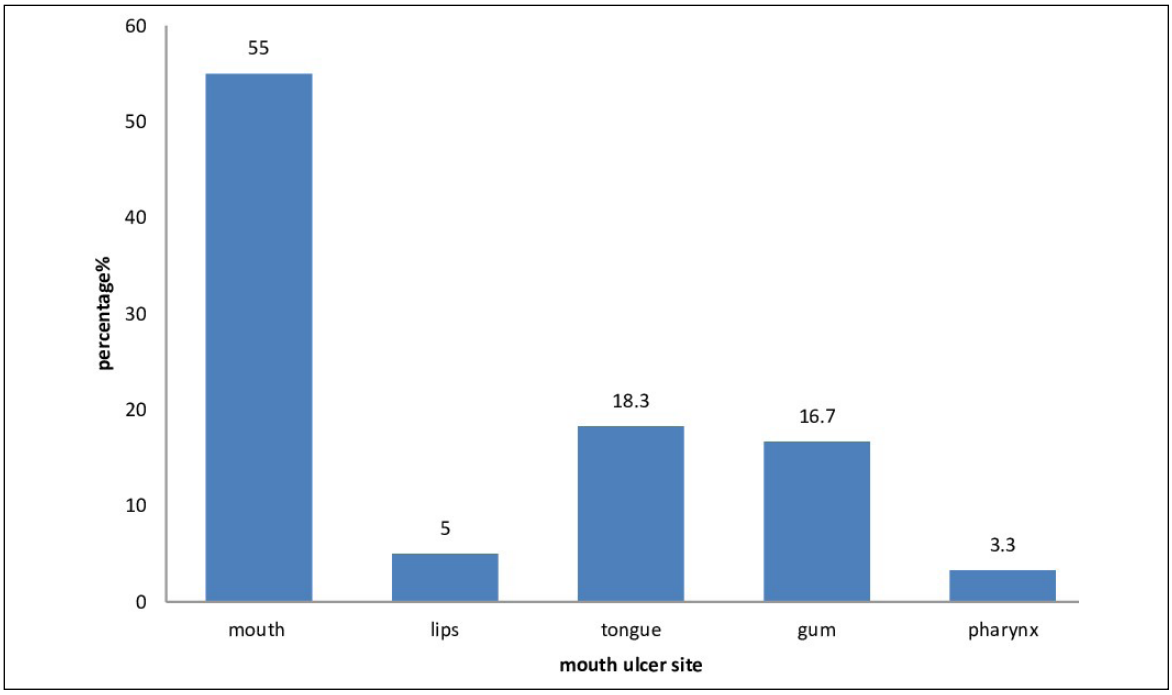

Fig. 1. The percentage of oral ulcer types of SLE patients.

Table 1. Characteristics distribution and some clinical symptoms of SLE group

Study variables	SLE group	Control group	p
Age (year)	35.80±1.453	28.97±1.43	0.011
BMI(kg\m2)	28.00±0.968	27.30±0.86	0.654
Duration (year)	7.00±0.841	-	-
Sex Male female	 5% 95%	 57.89% 42.10%	0.000
Married Yes no	 75% 25%	 47.36% 52.63%	0.000
Family history Yes no	 23 % 77%	-	
Fever Yes no	 93.7% 6.7%	-	
Butterfly-shaped rash Yes no	 52% 48%	-	
Oral ulcer Yes no	 73.3% 26.7%	-	
Number of oral ulcer Single Multiple	 65.90% 34.09%	-	

100,000 people respectively [19]. SLE predominantly impacts women of child-bearing age, with high percentage in African ancestry individuals [19-20]. The role of the PD1 and PDL-1in SLE has complex mechanisms, Regarding to PD-L1 that significant association in the present study, the PD-1^{+}Tfh cells number is increased with severity and progressing of SLE which regulated by PD-L1 ligation on B cell [21-23]. That may be prevented tyrosine phosphorylation of effector molecules as well as SYK, SHP-2, beneficially stopped signaling of B cell

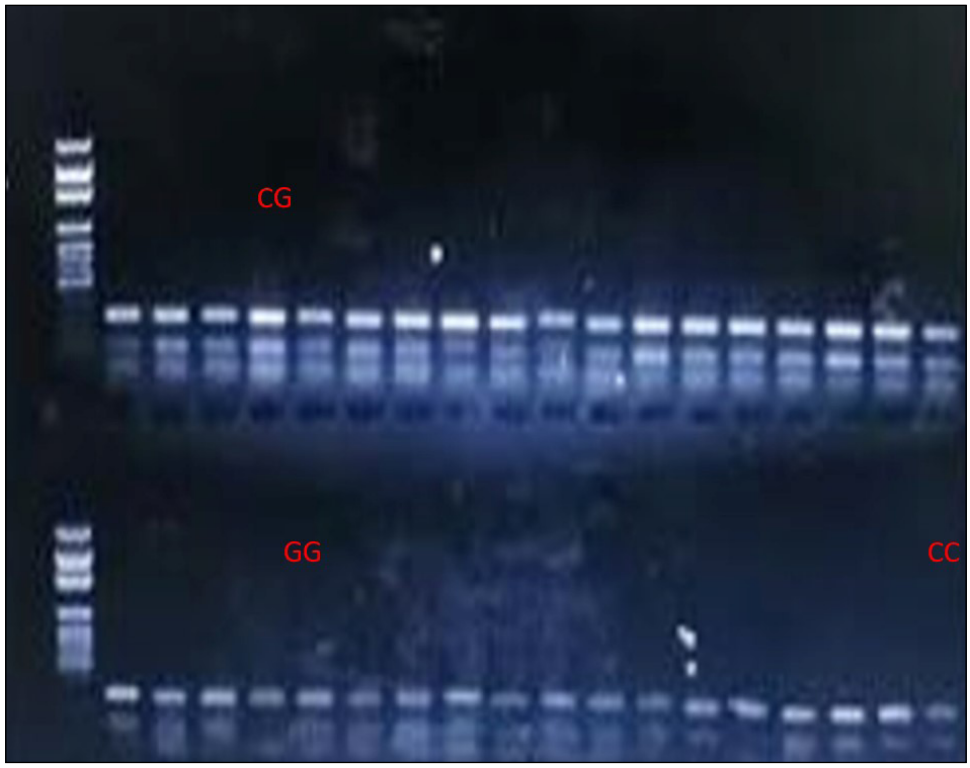

Fig. 2. Electrophoresis patterns of PD-L1 rs4143815 (G>C) genotyping in study groups (CC, GC and GG), DNA ladder 100 bp.

Table 2. PD-L1 rs4143815 (G>C) genotyping and allele frequency distribution in study groups (p<0.05)

rs4143815 (G>C) genotyping	SLE patients	Control group	Odd ratio	p
GC	(75%)	(100%)	67.8874 4.0677 to 1133.0016	0.0033*
CC	(20%)	0	5.8571 0.0990 to 346.6356	0.3959
GG	(5%)	0		
C	0.575	0.5	1.3529 1.1342 to 1.6138	0.0008*
G	0.425	0.5		

Table 3. The PD-L1 rs4143815 (G>C) genotyping distribution according to some clinical symptoms (p less than 0.05)

SEL symptoms	GC(%)	CC(%)	GG(%)	X^2	p
Fever					
Yes	42(70)	11(18.33)	3(5)	0.267	0.874
no	3(5)	1(1.66)	0		
Butterfly-shaped rash					
Yes	22(36.66)	8(13.33)	1(1.66)		
no	23(38.33)	4(6.66)	2(3.33)	1.624	0.444
Sex					
Male	3(5)	0	0	1.052	0.5908
female	42(70)	12(20)	3(5)		
Family history					
Yes	9(15)	4(6.66)	1(1.66)	1.118	0.5718
no	36(60)	8(13.33)	2(3.33)		
Oral ulcer					
Yes	31(51.66)	9(15)	3(5)	1.422	0.491
no	14(23.33)	3(5)	0		
Number of oral ulcer					
Single	24(40)	5(8.33)	2(3.33)	0.8008	0.670
Multiple	21(35)	7(11.66)	1(1.66)		

Table 4. PD-L1 rs4143815 (G>C) genotyping distribution according to oral ulcer sites

Oral ulcer sites	GC(%)	CC(%)	GG(%)	X^2	p
Mouth	22(44)	8(16)	3(6)	3.6363	0.7257
lips	3(6)	0	0		
tongue	8(16)	1(2)	2(4)		
Pharynx	2(4)	1(2)	0		

receptor [6], The B cells expansion in SLE, perhaps supposed that PD-1 in B cell is not beneficially produced or ligated in SLE in spite of elevation in transcript levels [24-25], The significant PD-1 production lacking on SLE patient CD8 T cells is detected depletion in regulatory cell signals for this cell type [26]. The macrophage in cancer, the expression of PD-1 is a negative association with their phagocytic potency, while the macrophages in SLE also produced PD-1 as a biomarker of reduction capability to clear apoptotic cells [7]. The PD-L1 gene (ID: 29,126) encoded type 1 transmembrane protein 40 kDa, which carried on at the chromosome 9p24.2, the 3'-UTR of mRNA is most vital regions in post-transcriptional gene expression regulation concerning to microRNAs targeting, that mediates repression of gene translational. The PD-L1 3'-UTR Structural variations, as well as duplications, deletions and translocations, result to PD-L1 production disturbing in numerous disease like tumors and autoimmune disease [27-29]. The rs4143815 G>C is a common SNP in PD-L1 gene which binding site of miR-570 and causes up-regulation production of PD-L1 by attenuated the miRNA mediated mRNA degeneration [30]. Oral ulcers in SLE have been reported in almost studies, in present study the high percentage of oral ulcer was reported but there was no association with PD-L1 in addition to types of oral ulcer, Zakeri et al., [31] found an oral ulcer in 61.4% of the study cases, and the most reported type was oral aphthous ulcers, erosion, hyperkeratosis and pigmentation, that observed on the hard palate, soft palate and the lower lip vermilion. However, in spite of the importance of the oral manifestations perception in the SLE prognosis, there are still not enough reports about them.

CONCLUSIONS

The present results concluded closed correlation between rs4143815 G>C and SLE patients, but no association was observed with some clinical symptoms and oral ulcer types. Further investigation should be implemented to evaluate the role of PDL-1 in the SLE symptoms.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 27.01.2024
ACCEPTED: 10.12.2024