ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
February 2021; 20(1):67-75.
Doi: 10.18502/ijaai.v20i1.5413

Clinical Relevance of *HLA-DRB1* and *-DQB1* Alleles in Iranian Systemic Lupus Erythematosus Patients

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Received: 3 September 2020; Received in revised form: 12 November 2020; Accepted: 3 December 2020

ABSTRACT

Given the potential link between genetic risk factors and clinical features of systemic lupus erythematosus (SLE), this study aimed to explore the relationship between human leukocyte antigen (*HLA)-DRB1/DQB1* alleles and haplotypes and clinical sub-phenotypes of the disease in a group of Iranian SLE patients.

HLA-DRB1 and *HLA-DQB1* alleles were determined by PCR-SSP in 127 SLE patients and 153 ethnically-matched healthy controls. The relationships between various clinical manifestations and HLA alleles/haplotypes were analyzed in the patients.

We observed the positive associations of *DRB1*07* and *DRB1*07-DQB1*02* haplotypes with articular and pulmonary involvement (p=0.006 and p<0.001 respectively), *DRB1*03* and *DQB1*02* alleles, and *DRB1*03-DQB1*02* haplotypes with cutaneous (p=0.03, p=0.004 and p=0.02 respectively) and renal involvement, and *DRB1*13* as well as *DRB1*13-DQB1*06* haplotypes with renal involvement. Conversely, negative associations of DRB1*13 with cutaneous and gastrointestinal disorders (p=0.004 and p=0.02 respectively) and DRB1*01 with renal involvement (p=0.03) were found in our patients. Patients carrying susceptible *HLA-DRB1* alleles had a higher risk for expression of cutaneous involvement (p=0.03), anti-coagulant antibody development (p=0.01), and a lower risk for pulmonary disorders compared to patients' negatives for susceptible alleles (p=0.04).

Our findings on associations between HLA risk allele (DRB1*03) as well as non-risk alleles with particular clinical manifestations and between the potentially protective allele (DRB1*01) and protection against renal involvement indicate the important role of HLA class II genes in predisposing of specific serological and clinical features of SLE disease which could be implicative for therapeutic applications and better management of SLE patients.

Keywords: *HLA-DRB1* chains; *HLA-DQB1*; Systemic lupus erythematosus

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INTRODUCTION

The systemic lupus erythematosus (SLE) is one of the most complex systemic autoimmune diseases

characterized by heterogeneous clinical manifestations, multiple organ involvement, and different immunological abnormalities such as non-organ specific auto-antibodies development and alteration in serum levels of complement proteins.[1] Despite the etiological role of genetic components as well as environmental and hormonal factors in disease susceptibility, the exact etiology of SLE is still unknown.[2,3] The link between genetic predisposition and auto-antibodies profile as well as various clinical presentations in SLE patients have been reported differently in various ethnic groups.[4-6] In this context, the pathogenic role of human leukocyte antigen (HLA) genes as the most consistent and the strongest genetic risk factor has been shown clearly.

Although there is a consensus on the susceptibility role of some HLA alleles such as *HLA-DRB1*03, DRB1*15, DRB1*16,* and *HLA-A*01/B*08/DR3* haplotype in Caucasian SLE patients,[7] *DRB1*15* and *DRB1*09* in Asians[8-10] and *DRB1*03* as well as *DRB1*11* in Latin Americans,[11] the contribution of other non-HLA genes reflects the polygenic basis of SLE.[12,13] Elucidation of the exact mechanism of this relationship either with disease predisposition and/or protection or more importantly with clinical manifestations and serological finding is still under debate.[14] Several studies on different ethnic groups have shown the associations between HLA class II alleles and some clinical manifestations and auto-antibodies status but, the contradictory results of these investigations imply the more complex nature of SLE.[15-17] For instance, the link between *DRB1*03, DRB1*16* and *DQB1*05* alleles and renal disorders, DRB1*15, and cutaneous involvement and association of protective alleles for disease with protection against some of the clinical manifestations have been demonstrated in different populations.[6,17,18] Due to the lack of enough and consistent data in this regard, we attempted to further analyze the relationship between *HLA-DRB1* and *-DQB1* alleles and haplotypes and clinical as well as serological subphenotypes of the disease in a group of Iranian SLE patients.

PATIENTS AND METHODS

One hundred twenty-seven SLE patients (15 males and 112 females) with the mean age 37.98±10.86 that fulfilled the criteria for diagnosis and classification of disease based on American College of Rheumatology (ACR)[19] were enrolled in this cross-sectioned and case-control study.

The mean age at the onset of disease was 32.50±10.85 years and the mean disease duration was 5.48±5.13 years. Characterization and definition of the clinical manifestations were based on the ACR criteria and standard protocol.[19] Sixteen clinical features were recorded in our SLE patients with the majority of articular (90.6%), cutaneous (66.9%), hematological (47.2%), and renal (42.5%) involvement respectively. However, none of the SLE patients with articular manifestations and/or positive for Anti-CCP antibodies showed rheumatoid arthritis (RA) features based on ACR/EULAR 2010 rheumatoid arthritis classification criteria.[20]

Also, 153 ethnically matched healthy control subjects (96 males and 57 females) from the same geographic area were recruited as controls for determining the susceptible and protective HLA alleles for SLE disease.

All participants in this study signed an informed consent approved by our institutional medical ethics committee (IR.UMSHA.REC.1397.697), Hamadan University of Medical Sciences, and according to the recommendations of the Helsinki declaration.

HLA Class II Genotyping

Low-resolution typing of *HLA-DRB1* and *-DQB1* alleles was carried out by polymerase chain reaction sequence-specific primer (PCR-SSP) method for all study subjects. Briefly, after DNA extraction from whole blood samples by using the modified salting-out method,[21] PCR with sequence-specific primers was performed using a commercial *HLA-DR/DQ* typing kit (Olerup SSP DR-DQ SSP Combi Tray, Stockholm, Sweden) as per manufacturer's instructions. Finally, the determination of *HLA-DRB1* and *DQB1* alleles were executed by SCORE software provided by the same company. *HLA-DRB1-DQB1* haplotypes were assigned according to known linkage disequilibrium of *DRB1* and *DQB1* alleles and by using an expectation-maximization algorithm as implemented in the R statistical computing environment (R-project).

Serological assessments including C-reactive protein (CRP), antinuclear auto-antibodies (ANA), Anti-double stranded DNA (Anti-dsDNA), Anti-cardiolipin, Anti-SSA/Ro, Anti-SSB/La, complement, RF, and Anti-CCP were carried out; using commercial kits as described in our previous report (Submitted

data). Also, paraclinical variables measurements such as complete blood count (CBC), erythrocyte sedimentation rate (ESR), and urine analysis were performed for all samples.

Statistical Analysis

The frequencies of *HLA-DRB1* and *-DQB1* alleles and haplotypes were compared between patients and controls using the chi-square test with Yates' correction or Fisher's exact test as applicable. The risk contributed by haplotypes and genotypes were assessed by calculation of odds ratio (OR) with 95% confidence interval. Paired t-test was performed to analyze quantitative data. Also, we used the Benjamini–Hochberg method for multiple comparisons to control the false discovery rate (Benjamini and Hochberg, revised version 2010). All statistical analyses were performed by SPSS (version 22.0, Chicago, USA) and Epi Info (Version 7.1.3.10) software and p values<0.05 were considered as statistically significant.

RESULTS

Among 127 SLE patients, 78 cases had less than 5 years and 49 patients showed more than 5 years of disease duration. The mean age at onset of disease was 32.50±10.85 years and patients were categorized into three subgroups in terms of this variable; 8 cases showed disease onset before 18 years, 108 patients affected between 18 and 49 years and 11 cases had aged over 50 years at the time of disease onset. The most frequent clinical features in our patients were arthritis (90.6%), cutaneous involvement (66.9%), hematologic disorders (47.2%), and renal involvement (42.5%) respectively. Also, 94.5% of patients had ANA and 70.1% were positives for the anti-dsDNA antibody. Comparison of these patients' characteristics in terms of the presence or absence of HLA risk allele (DRB1*03 and DRB1*16) didn't show significant differences between the positive and negative patients for risk alleles. The only significant statistical difference was shown for the higher prevalence of cutaneous involvement in the patients carrying risk allele versus those patients negative for the risk allele (p=0.04, Table 1).

Comparisons of *HLA-DRB1/-DQB1* alleles and haplotypes frequencies between the patients and healthy controls have been described in detail previously (Submitted data). We found the *HLA-DRB1*03* and *DRB1*16* as risk alleles and *DRB1*01* as a possible protective allele for SLE disease. Also, *DRB1*03-DQB1*02* and *DRB1*16-DQB1*05* haplotypes showed a susceptibility role, whereas *DRB1*01-DQB1*05* haplotype showed a possible protective role for the disease. Furthermore, genotypes' analysis revealed that DRB1*03/XX and DRB1*16/XX (X stands for alleles other than DRB1*03 and *16) genotypes were significantly associated with a higher risk of disease (p=0.04 and p=0.01 respectively). While DRB1*01/*11 genotype showed a lower risk for SLE disease (p=0.01, Table 1). The only significantly associated genotype with some of the clinical manifestations was DRB1*03/*03 or *03/X that was associated with skin involvement (p=0.03), nephritis (p=0.04), and neurologic symptoms (p=0.005).

Likewise, analyzing the associations between HLA class II alleles and different auto-antibodies appearance in the patients showed the significant associations of DRB1*03 with auto-antibody development against SSA/Ro, SSB/La and coagulant factor, DRB1*04 with anti-β2gpI, anti-cardiolipin, RF and ACPA, DRB1*07 with anti-dsDNA, DRB1*11 with anti-SSA/Ro, SSB/La, DRB1*13 with anti-Sm and anti-β2gpI, DRB1*14 with anti-Smith, DRB1*15 with anti-SSA/Ro, anti-SSB/La, DQB1*02 with anti-SSB/La, DQB1*05 with anti-Sm and DQB1*06 with anti-Sm and anti-coagulant antibodies. Moreover, negative associations of DRB1*04 with anti-Sm and anti-SSA/Ro, DRB1*07 with anti-SSB/La, DRB1*11 with anti-Sm, and RF, DQB1*03 with anti-dsDNA and anti-Sm antibodies, and DQB1*05 with anti-coagulant antibodies were observed in our SLE patients (Submitted data).

Associations between *HLA-DRB1/-DQB1* Alleles and Haplotypes and Clinical Manifestations

We analyzed the associations between HLA class II alleles and haplotypes and all of 16 clinical patterns in our patients and only the significant relationships have been shown in Table 2.

We observed the positive associations of *DRB1*07* allele and *DRB1*07-DQB1*02* haplotypes with arthritis and pulmonary involvement (p=0.006 and p<0.001 respectively), *DRB1*03* and *DQB1*02* alleles, and *DRB1*03-DQB1*02* haplotypes with cutaneous (p=0.03, p=0.004 and P=0.02 respectively) and renal involvement (p=0.04 and p=0.01 respectively),

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Table 1. Demographics and patients characteristics based on the presence of risk human leukocyte antigen (HLA) alleles

Variables	Risk alleles		*p*
	Positive (n=54)	Negative (n=73)	
Age (year), (Mean±SD)	38.5±11.36	37.58±10.53	
Gender (Male/female)	5/49	10/63	0.62
Disease onset age (years)			
<18	2	6	
18-49	47	61	
≥50	5	6	0.66
Disease duration			
≤5 years (n=78)	38	40	
>5 years (n=49)	16	33	0.13
Decreased complement (Y/N)	21/27	33/40	0.59
CRP (+/-)	12/42	25/48	0.20
ESR			
≥30	21	29	
<30	33	44	1.00
Clinical manifestations			
Arthritis (+/-)	49/5	66/7	1.00
Dermatitis (+/-)	42/12	43/30	**0.04**
Nephritis (+/-)	26/28	28/45	0.35
Hematologic (+/-)	26/28	34/39	1.00
Minor symptoms (+/-)	14/40	29/44	0.44
Serological findings			
ANA (+/-)	49/5	71/2	0.23
Anti-dsDNA (+/-)	35/19	54/19	0.35

*Minor symptoms were pulmonary involvement (11%), ocular symptoms (7.9%), cardiovascular involvement (11%), gastrointestinal symptoms (1.6%), neurologic symptoms (7.1%), thrombotic symptoms (2.4%), and Antiphospholipid antibody syndrome (3.9%). ANA: Anti-nuclear antibody, Anti-dsDNA: Anti-double stranded DNA.

Table 2. Comparison of the frequencies of clinical manifestations in systemic lupus erythematosus (SLE) patients positive and negative for DRB1 and DQB1 alleles

Clinical manifestations (n)	HLA alleles	Positive (%)	Negative (%)	*p*	Risk Ratios
Arthritis (115)	DRB1*07	16 (72.7%)	99 (94.2%)	**0.006**	0.771 [0.594-1.000]
Cutaneous involvement (85)	DRB1*03	30 (81%)	55 (61.1%)	**0.038**	1.326 [1.057-1.664]
	DRB1*13	9 (40.9%)	76 (72.3%)	**0.004**	0.524 [0.300-0.915]
	DQB1*02	44 (81.4%)	41 (56.1%)	**0.004**	1.450 [1.142-1.842]
Renal involvement (54)	DRB1*01	0 (0%)	54 (44.6%)	**0.038**	-
	DRB1*03	21 (56.7%)	33 (36.6%)	**0.048**	1.547 [1.047-2.288]
	DRB1*13	14 (63.6%)	40 (38.0%)	**0.049**	1.600 [1.059-2.417]
Pulmonary disorders (14)	DRB1*07	9 (40.9%)	5 (4.7%)	**<0.001**	8.590 [3.186-23.165]
Cardiac disorders (14)	DRB1*04	6 (23.0%)	8 (7.9%)	**0.039**	2.913 [1.108-7.660]
Ocular involvement (10)	DRB1*10	3 (75.0%)	7 (5.6%)	**0.001**	13.178 [5.276-32.912]
	DQB1*05	7 (18.4%)	3 (3.3%)	**0.008**	5.464 [1.492-20.014]
Thrombotic disorders (3)	DRB1*08	3 (75.0%)	1 (0.8%)	**<0.001**	-
	DQB1*04	3 (75.0%)	1 (0.8%)	**<0.001**	-

*DRB1*10* and *DQB1*05* alleles and *DRB1*10-DQB1*05* haplotypes with ocular involvement (p=0.001, p=0.008, and p=0.001 respectively), *DRB1*08* and *DQB1*04* alleles and *DRB1*08-DQB1*04* haplotypes with thrombotic disorders (p<0.001), *DRB1*04* and *DRB1*04-DQB1*03* haplotypes with cardiovascular involvement (p=0.03 and p=0.02 respectively) and *DRB1*13* and *DRB1*13-DQB1*06* haplotypes with renal involvement (Table 2 and Table 3). Conversely, negative associations of *DRB1*13* alleles and *DRB1*13-DQB1*06* haplotypes with cutaneous and gastrointestinal disorders respectively (p=0.004 and p=0.02) as well as *DRB1*01* and *DRB1*01-DQB1*05* haplotypes with renal involvement (p=0.03) were found in our SLE patients (Table 2 and Table 3).

Moreover, a comparison of the frequencies of different clinical features between the patients' subgroups (positives and negatives for risk alleles) showed that patients carrying susceptible *HLA-DRB1* alleles (DRB1*03 and DRB1*16) had a higher risk for the expression of cutaneous involvement (p=0.03, Table 4), anti-coagulant antibody development (p=0.01, Table 5) and lower risk for pulmonary disorders compared to patients who were negatives for susceptible alleles (p=0.04, Tables 4).

Analyzing the auto-antibodies status in patients with and without risk alleles didn't show statistical differences except for anti-coagulant antibody (p=0.01, Table 5).

Finally, we found a significant link between arthritis symptoms and anti-β2gpI (p=0.04) and between the renal involvement and decreased levels of complement proteins (p=0.1, Table 6).

Table 3. Significantly associations of human leukocyte antigen (*HLA)-DRB1-DQB1* haplotypes with some of the clinical manifestations in systemic lupus erythematosus (SLE) patients

Symptoms	DRB1-DQB1 Haplotypes	No. of positive/negative patients (Positive rate %)	*p*	*Risk Ratio*
Arthritis	DRB1*07-DQB1*02	14/6 (70.0)	**0.004**	0.741 [0.554-0.991]
Cutaneous involvement	DRB1*03-DQB1*02	29/6 (82.8)	**0.021**	1.361 [1.089-1.700]
	DRB1*13-DQB1*06	9/13 (40.9)	**0.006**	0.565 [0.337-0.946]
Renal involvement	DRB1*01-DQB1*05	0/6 (0.0)	**0.038**	-
	DRB1*03-DQB1*02	21/14 (60.0)	**0.017**	1.672 [1.138-2.457]
	DRB1*13-DQB1*06	14/8 (63.6)	**0.034**	1.670 [1.120-2.489]
Pulmonary disorders	DRB1*07-DQB1*02	9/11 (45.0)	**<0.001**	9.630 [3.601-25.747]
Cardiac disorders	DRB1*04-DQB1*03	6/18 (25.0)	**0.026**	3.218 [1.231-8.413]
Ocular involvement	DRB1*10-DQB1*05	3/1 (75.0)	**0.001**	13.178 [5.276-32.912]
Digestive disorders	DRB1*13-DQB1*06	2/20 (9.0)	**0.029**	-
Thrombotic disorders	DRB1*08-DQB1*04	3/1 (75.0)	**<0.001**	-

Table 4. Frequencies of some of the clinical manifestations in systemic lupus erythematosus (SLE) patients positive and negative for human leukocyte antigen (HLA) risk alleles and haplotypes

Clinical manifestations (N)	Risk alleles				Risk haplotypes			
	Positive (%) N=54	Negative (%) N=73	*p*	Risk Ratios	Positive(%) N=52	Negative(%) N=75	*p*	Risk Ratios
Arthritis (115)	49(90.7%)	66 (90.4%)	*1.00*	1.02 [0.50-2.06]	47 (90.3%)	68 (90.6%)	*1.00*	0.98[0.48-1.98]
Cutaneous (85)	42 (77.7%)	43 (58.9%)	***0.03***	1.72 [1.02-2.92]	41(78.8%)	44 (58.6%)	***0.02***	1.84[1.05-3.20]
Hematologic (60)	26 (48.1%)	34 (46.5%)	*1.00*	1.03 [0.96-1.55]	26 (50.0%)	34 (45.3%)	*0.71*	1.11[0.73-1.69]
Renal (54)	26 (48.1%)	28 (38.3%)	*0.28*	1.25 [0.84-1.87]	26 (50.0%)	28 (37.3%)	*0.20*	1.35[0.89-2.04]
Pulmonary(14)	2 (3.7%)	12 (16.4%)	***0.04***	0.31 [0.08-1.13]	2 (3.8%)	12 (16.0%)	***0.04***	0.32[0.08-1.18]
Ocular (10)	4 (7.4%)	6 (8.2%)	*1.00*	0.93 [0.42-2.05]	4 (7.6%)	6 (8.0%)	*1.00*	0.97[0.44-2.14]
Cardiovascular (14)	6 (11.1%)	8 (10.9%)	*1.00*	1.00 [0.53-1.91]	6 (11.5%)	8 (10.6%)	*1.00*	1.05[0.55-2.00]

Table 5. Prevalence of different auto-antibodies in systemic lupus erythematosus (SLE) patients positive and negative for human leukocyte antigen (HLA) risk alleles

Auto-antibodies (No of positives)	Risk alleles		*p*	Risk ratio
	Positive (%) N=54	Negative (%) N=73		
ANA (120)	49 (90.7%)	71 (97.2%)	0.13	0.57 [0.34-0.95]
Anti-dsDNA (89)	34 (62.9%)	55 (75.3%)	0.32	0.72 [0.48-1.08]
Anti-Sm (23)	10 (18.5%)	13 (17.8%)	1.00	1.02 [0.61-1.72]
Anti-SSA/Ro (52)	26 (48.1%)	26 (35.6%)	0.20	1.33 [0.89-1.99]
Anti-SSB/La (34)	19 (35.1%)	15 (20.5%)	0.07	1.48 [0.99-2.20]
Anti-β2gp (38)	13 (24.0%)	25 (34.2%)	0.24	0.74 [0.45-1.21]
Anti-cardiolipin (31)	12 (22.2%)	19 (26.0%)	0.68	0.88 [0.53-1.45]
Anti-coagulant (26)	17 (31.4%)	9 (12.3%)	**0.01**	1.78 [1.22-2.60
RF (30)	15 (27.7%)	15 (20.5%)	0.40	1.24 [0.80-1.91]
Anti-CCP (12)	5 (9.2%)	7 (9.5%)	1.00	0.97 [0.48-1.97]

CRP: C-reactive protein, ESR: Erythrocyte Sedimentation Rate, ANA: Anti-nuclear antibody, Anti-dsDNA: Anti-double stranded DNA, Anti-sm: Anti-Smith, Anti-SSA/Ro: anti–Sjögren'ssyndrome-related antigen A auto-antibodies, Anti-SSB/La: anti–Sjögren'ssyndrome-related antigen B auto-antibodies, Anti-β2gp: anti-β2-glycoprotein, Anti-CCP: Anti–Cyclic Citrullinated Peptide Antibody, RF: Rheumatoid factor.

Table 6. Association between some of the serological findings and the major clinical manifestations in systemic lupus erythematosus (SLE) patients

Clinical manifestations (No of positives)	Antibodies	Positive (%)	Negative (%)	*p*	Risk Ratio
Arthritis (115)	Anti-B2gpI	31 (26.9%)	7 (6.0%)	**0.04**	0.864 [0.737-1.013]
	Anti-cardiolipin	25 (21.7%)	6 (5.2%)	0.07	0.860 [0.718-1.029]
	RF	30 (26.0%)	0 (0.0%)	0.06	-
Cutaneous involvement (85)	Anti-B2gp	21 (24.7%)	17 (20.0%)	0.09	0.768 [0.561-1.052]
	RF	16 (18.8%)	14 (16.4%)	0.07	0.749 [0.542-1.072]
Renal involvement (54)	Anti-dsDNA	42 (77.7%)	47 (87.0%)	0.12	1.494 [0.891-2.506]
	Anti-B2gpI	21 (38.8%)	17 (31.4%)	0.07	1.490 [1.005-2.209]
	Decreased complement	30 (55.5%)	24 (44.4%)	**0.01**	1.689 [1.126-2.534]

Anti-β2gpI: anti-β2-glycoprotein, RF: Rheumatoid factor, Anti-dsDNA: Anti-double stranded DNA.

DISCUSSION

Pathogenesis of SLE as a polygenic autoimmune disorder results from a complex genetic interaction that is influenced by several environmental factors.[18,22] Various clinical and immunological sub-phenotypes of SLE disease, as well as ethnic variations of the patients, have challenged the genetic studies in SLE.[6,17,18,22] Although compelling evidence implicates genetic susceptibility for SLE particularly based on HLA class II alleles (e.g. DRB1*03, DRB1*15, and DRB1*16) but, the associations of those predisposing/protective HLA alleles with clinical subsets and auto-antibody patterns in SLE patients might be completely different from the disease itself.

Our patients showed a similar pattern for disease predisposing *HLA-DR* alleles as reported in the previous studies on different ethnic groups. We observed that *DRB1*03* and *DRB1*16* alleles conferred disease risk and *DRB1*01* had a possible protective role for SLE disease. Our previous study on these patients revealed the significant associations between risk alleles and even non-risk alleles with different auto-antibodies such as the association of anti-SSA/Ro

and anti-SSB/La with *DRB1*03*, anti-B2gpI, anti-cardiolipin, RF, and anti-CCP with *DRB1*04*, anti-Sm and anti-dsDNA with *DRB1*07*, anti-Sm and anti-B2gpI with *DRB1*13*, and anti-Sm with *DRB1*16* alleles (submitted data).

Herein, we present the associations between HLA class II alleles and different clinical manifestations in the same SLE patients. We observed the positive associations of *DRB1*07* allele and *DRB1*07-DQB1*02* haplotypes with arthritis and pulmonary involvement, *DRB1*03* and *DQB1*02* alleles, and *DRB1*03-DQB1*02* haplotypes with cutaneous and renal involvement, *DRB1*10* and *DQB1*05* alleles and *DRB1*10-DQB1*05* haplotype with ocular involvement, *DRB1*08* and *DQB1*04* alleles and *DRB1*08-DQB1*04* haplotypes with thrombotic disorders, *DRB1*04* and *DRB1*04-DQB1*03* haplotypes with cardiovascular involvement and *DRB1*13* as well as *DRB1*13-DQB1*06* haplotype with renal involvement. Conversely, negative associations of *DRB1*13* alleles and *DRB1*13-DQB1*06* haplotypes with cutaneous and gastrointestinal disorders respectively as well as *DRB1*01* and *DRB1*01-DQB1*05* haplotypes with renal involvement were found in our SLE patients.

Remarkably, patients carrying susceptible *HLA-DRB1* alleles showed a higher risk for expression of cutaneous involvement, anti-coagulant antibody development, and a lower risk for pulmonary disorders compared to patients negative for susceptible alleles. Of note, some of the major clinical manifestations were correlated with auto-antibodies profile such as arthritis with anti-β2gpI and renal involvement with anti-dsDNA antibodies and decreased levels of complement proteins. Our results indicate that the contributions of multiple HLA alleles to certain clinical manifestations possibly through auto-antibodies development are higher than the contribution of HLA alleles to the disease itself.

These findings are in line with Galeazzi et al and Vasconcelos et al[18, 6] studies that showed a positive association between DRB1*03 and renal involvement. Moreover, we found a significant correlation between this risk allele and cutaneous involvement which is consistent with other studies that highlighted the link between DRB1*15 and cutaneous involvement.[17,23,24] Of note, we showed that the protective *HLA-DRB1*01* and *DQB1*05* alleles for the disease were also protective against renal involvement which is consistent with the above-mentioned study[18] that showed a similar protective effect for *DRB1*01* alleles against renal disorders. These findings are in agreement with the So-Young et al study on Koreans that reported the protective alleles for disease (DRB1*12:02 and *DRB1*11:01*) were also protective against several clinical manifestations including skin and renal involvements.[17]

Furthermore, they showed a positive link between *DRB1*03* and lung involvement, whereas we found this link for the DRB1*07 allele. Considering the renal involvement, we showed a predisposing role for *DRB1*13* and *DQB1*06* alleles as well as *DRB1*03*alleles. While Galeazzi et al demonstrated that the susceptibility *DRB1*16* and *DQB1*05* alleles were associated with a high risk of renal disorders.

In contrast to our results, Vasconcelos et al study [6] showed a protective role for *DRB1*13* as well as *DRB1*09* alleles against nephritis and disease itself as well. However, the absence of relationships between *HLA-DRB1* alleles and clinical sub-phenotypes in SLE patients was reported in two different ethnic groups, Egyptian with juvenile SLE[25] and Brazilian children with SLE.[26] These contradictory results deserve more investigations to find out the clear associations either positive or negative in this regard.

We also found significant associations between arthritis and anti-β2gpI and between renal involvement and anti-dsDNA antibodies regardless of the HLA genotype. Our previous report on auto-antibodies status of these patients clearly showed the predisposing role of risk alleles (DRB1*03 and DRB1*16) for anti-SSA/Ro, anti-SSB, and anti-coagulant antibodies development, and the current work emphasize the potential link between HLA alleles and clinical presentations which could be the consequence of auto-antibodies production. For instance, patients carrying the *DRB1*03* and *DQB1*02* alleles were prone to produce anti-SSA/Ro, anti-SSB, and anti-coagulant antibodies and to express renal and cutaneous disorders. DRB1*07 was associated with a high risk of having anti-Sm and anti-dsDNA along with a high risk to develop arthritis and pulmonary disorders. Also, a direct correlation between DRB1*13 and ant-Sm/anti-β2gpI production could be related to an increased risk of renal involvement in those patients carrying the *DRB1*13* alleles.

In a mechanistic view, Norris et al proposed a potential mechanism undergirding the link between

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HLA risk alleles, auto-antibodies, and skin involvement. They suggested that following UV exposure in patients carrying risk alleles, the increased synthesis and expression of Ro antigen has occurred on the surface of epidermal keratinocytes, and subsequently, circulating anti-Ro antibody binds to this overexpressed antigen on the surface of keratinocytes. Thereafter, the Fc domain of bound antibodies mediates cellular damage through antibody-dependent cell-mediated cytotoxicity (ADCC) phenomenon and ultimately, skin lesions will develop in these patients.[27]

Another finding was the negative association between *DRB1*04* allele and anti-SSA/anti-Sm antibodies which could be indicative of less incidence of cardiac involvement in SLE patients. On the other hand, patients carrying the *DRB1*04* alleles were positive for anti-β2gpI, anti-cardiolipin, rheumatoid factor, and anti-CCP antibodies which could be implicative for the predisposing role of DRB1*04 in the development of cardiovascular disorders. Given the possible role of antiphospholipid antibodies such as anti-cardiolipin and anti- β2gpI in cardiovascular diseases and elucidation of a direct link between DRB1*04 and anti-CL and anti-β2gpI, one can imagine the contribution of genetic factors (DR4) in the development of cardiac disorders.[28] Interestingly, patients with arthritis were positives for anti- β2gpI (p=0.04), anti-cardiolipin (p=0.07) and RF (p=0.06), and patients with renal involvement were positives for anti-dsDNA (p=0.05), anti- β2gpI (p=0.07) and they had decreased levels of complement proteins (p=0.01). Similar challenging results were found for DRB1*13 positive cases with anti-Sm and anti- β2gpI antibodies and low risk for cutaneous and gastrointestinal involvements.

Altogether, our findings further confirm the predisposing role of *HLA-DRB1* and *HLA-DQB1* alleles in auto-antibodies development and various clinical manifestations in SLE patients. Although, the elucidations of different HLA alleles in this regard in comparison to other ethnic groups of patients are reasonable because of ethnicity heterogeneity in the studied populations so far. Nevertheless, more studies are warranted to determine the exact nature of these relationships and their clinical relevance in SLE disease.

In conclusion, we observed significant associations between the HLA risk allele (*DRB1*03*) as well as non-risk alleles with particular clinical manifestations and between the potentially protective allele (*DRB1*01*) and protection against renal involvement. Moreover, these HLA and clinical features relevancies were more evident in conjunction with serological patterns. Despite recruiting a small number of patients (a limitation of the study), our results further confirm the important role of HLA *class II* genes in predisposing specific serological and clinical features of SLE disease which could be implicative for therapeutic applications and better management of SLE patients.

CONFLICT OF INTEREST

The authors have nothing to declare.

ACKNOWLEDGEMENTS

This work was supported financially by Vice-Chancellor for Research and Technology, Hamadan University of Medical Sciences (Grant No: 9711166853).

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