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Original Article

Biochemistry Section

HLA-DR/DQ Genotypes in Kurd Patients with Rheumatoid Arthritis: Relation to Disease Activity

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ABSTRACT

Background: Specific alleles present at the HLA-DR/DQ loci seem to be associated with disease activity of rheumatoid arthritis.

Aim: In the present study, our aim was to investigate the distribution of HLA-DR/DQ alleles among Kurd patients with rheumatoid arthritis and to ascertain their relationship with disease activity.

Materials and Methods: Sixty five patients with rheumatoid arthritis (RA) and 65 apparently healthy subjects participated in the study. Diagnosis and disease activity were confirmed. Blood analyses, including those of laboratory markers of disease activity, were done. The 28 joint disease activity score (DAS-28) was calculated. HLA-DR/DQ typing was performed by polymerase chain reaction (PCR).The association between HLA-DR/DQ genotypes and disease activity was determined.

Results: The most frequent alleles which were identified in RA patients were HLA-DRBI*01(23.1%) and HLA-DQBI*6(34.6%), whereas in healthy subjects, they were HLA-DRBI*11(17.7%) and HLA-DQBI*03(35.4%). Patients with active disease had high frequencies of HLA-DQBI*6 (40.0%) as compared to those with moderate disease activity (16.7%); OR=3.33. Patients with severe RA had increased frequencies of HLA-DQBI*6 (56.3%) as compared to those with mild RA (10.0%); OR = 11.57.

Patients with positive rheumatoid factor (RF) and positive Anti-citrullinated peptide antibody (Anti-CCP), also had high frequencies of HLA-DQBI*06 (38.4% and 39.4%) as compared to frequencies of 11.1% and 15.4% which were seen in patients with negative rheumatoid factor and negative anti-CCP (OR= 4.98 and3.10) respectively.

Conclusion: HLA-DQBI*06 was found to be more common in Kurd patients and it was significantly associated with disease activity; this may indicate a high risk for developing a more progressive type of the disease.

Keywords: HLA genotypes, Rheumatoid arthritis, Disease activity

INTRODUCTION

The occurrence of rheumatoid arthritis is strongly associated with the expression of specific Human Leukocyte Antigen (HLA) class II alleles in several racial groups [1,2]. Depending upon geographical location, association of HLA-DR/DQ genotypes with RA varies from one population to another and genetic typing of patients with RA at presentation can be used to predict their outcomes [3]. Patients who carry certain HLA-DR/DQ alleles in the early stage of the disease may be characterized by an increased inflammatory reactivity that may indicate a high risk for developing a more progressive type of the disease [4]. However, only little is known about the HLA-DR/DQ polymorphism in our population, especially among those with rheumatoid arthritis. Thus, it is important to study frequencies of HLA-DR/DQ alleles in rheumatoid arthritis and to determine their association with disease activity, as this polymorphism might give a clue about the nature and prognosis of the disease.

MATERIALS AND METHODS

A random sampling procedure was used to select a representative sample of RA patients who were registered at Duhok Center for Rheumatic Disease and Medical Rehabilitation, between January 2011 and March 2012. During a period of three successive months, a total of 130 subjects who were living in different areas of Duhok governorate were enrolled in the study. Of these, 65 patients (56 females and 9 males) had been previously diagnosed with RA, who routinely attended the centre for follow-up and management. The remainder was 65 apparently healthy subjects with no history of rheumatic disease or autoimmune disease. They were 51 females and 14 males who were recruited from among medical staff of Azadi Teaching Hospital in Duhok. Participants were interviewed and informed about the nature of the study and then, verbal consents were obtained from each subject. The study protocol was approved by the ethical Committee of the General Directorate of Health in Duhok, Kurdistan Region, Iraq. The enrollment was done according to the revised 2010 criteria of American College of Rheumatology (ACR) and European League Against Rheumatism(EULAR) classification [5]. All patients underwent a routine medical history taking and physical examinations. Physical examinations were performed under the supervision of rheumatologist, in order to assess disease activity. According to the information which was obtained from the physical examinations, laboratory data, and imaging studies, the patients were divided into three groups: those with mild disease (that is; with less than six inflamed joints, no extra-articular disease, and no evidence of erosions or cartilage loss on plain radiographs), those with moderate disease (with inflamed joints score between 6 and 20, absence of extra-articular disease, evidence of inflammation on plain radiography), and those with severe disease (that is, with more than 20 inflamed joints, anaemia of chronic disease, bone erosions and loss of cartilage, extra-articular disease). Disease activity was assessed by using the Disease Activity Score-28(DAS-28) as: Low disease activity (DAS-28<3.2), moderate activity (DAS-28 3.2 to <5.1) and high activity (≥5.1). Visual analog scale (VAS) was used for assessment of disease severity. Blood analyses, including those done for rheumatoid factor, Anti-CCP and other markers of disease activity were done. Blood samples were collected after an overnight fast. EDTA blood samples were used for determination of haematological parameters by using Beckman Coulter. Rheumatoid factor, high sensitivity C-reactive protein and interlukin-1a and 6 were determined by doing enzyme immunoassays. HLA-DR/DQ typing was performed by doing polymerase chain reaction (PCR). Blood samples were taken from patients and they were stored in a frozen condition at -20^{0}C until DNA extraction. DNA was extracted

by using Proteinase K which was provided by Promega, USA. All genotyping was undertaken by using Morgan HLA SSP DRB/DQ Typing Kit .

Data were collected and analyzed by using SPSS, version 19.0 for Windows (SPSS, Chicago, USA). Quantitative data were analyzed by using independent sample t-test; qualitative data were analyzed by using Chi-square test. Frequencies, p values, and odds ratios were calculated.

RESULTS

The general characteristics of the study patients and healthy subjects have been described in [Table/Fig-1]. All 65 patients who were enrolled in the study had typical clinical and biological features of rheumatoid arthritis. Patients were predominantly women (86.2%). HLA-DR/DQ genotypes of RA patients and healthy controls have been shown in [Table/Fig-2]. The most frequent alleles which were identified in the RA patients were HLA-DRBI*01 and HLA-DQBI*06, whereas in healthy subjects they were HLA-DRBI*11 and HLA-DQBI*03. In both RA patients and healthy controls, the rare alleles which were identified were HLA-DRBI* 09 and HLA-DRBI*12. The distribution of the most frequent HLA-DR/DQ genotypes by severity of disease has been shown in [Table/Fig-3]. Patients with severe RA had increased frequencies of HLA-DQBI*6 as compared to mild RA patients. Patients with active disease (DAS-28 =>5.1) had higher frequencies of HLA-DQBI*6 as compared to those with moderate disease activity [Table/Fig-4]. Patients with positive RF and positive Anti-CCP also had high frequencies of HLA-DQBI*06 as compared to sero-negative groups [Table/Fig-5,6].

Characteristics	RA Patients (n=65)	Healthy subjects (n=65)	p-value
Age, years	42.5±8.8	42.0±9.1	0.67
Duration of disease, years	6.5±5.2	-	-
DAS-28	5.8±0.8	-	-
VAS	69.3±10.3	-	-
RF, IU/ml	127.1±118.3	3.8±1.7	0.003
Anti-CCP, IU/ml	26.7±19.7	4.9±1.9	0.001
Hs-CRP, mg/l	24.2±10.6	4.4±2.1	0.001
IL-1a, pg/ml	48.5±21.8	8.2±4.1	0.001
IL-6, pg/ml	88.4±34.9	18.9±6.4	0.001
ESR, mm/hr	48.1±16.6	6.2±2.2	0.008
Hb, g/dl	12.1±1.3	12.9±1.1	0.06
WBCx10/l	8.5±1.6	6.7±1.2	0.04

[Table/Fig-1]: Sample characteristics

HLA genotype	RA Patients		Healthy subjects		OR*
	No. of allele 130	F (%)	No. of alleles 130	F (%)	
HLA-DRBI*					
01	30	(23.1)	16	(12.3)	3.6
03	16	(12.3)	21	(16.2)	0.43
04	26	(20.0)	18	(13.9)	1.53
07	0	(0.0)	5	(3.9)	-
08	5	(3.9)	6	(4.6)	0.82
09	0	(0.0)	0	(0.0)	-
10	6	(4.6)	9	(6.9)	0.65
11	14	(10.8)	23	(17.7)	0.56
12	0	(0.0)	0	(0.0)	-
13	9	(6.9)	14	(10.8)	0.61
14	5	(3.9)	6	(4.6)	0.82
15	17	(13.1)	10	(7.7)	1.80
16	4	(3.1)	0	(0.0)	-
HLA-DQBI					
02	16	(12.3)	18	(13.9)	0.87
03	36	(27.7)	46	(35.4)	0.69
04	8	(6.2)	3	(2.3)	2.77
05	25	(19.2)	35	(26.9)	0.64
06	45	(34.6)	28	(21.5)	1.93

[Table/Fig-2]: Sample HLA-DR/DQ genotypes
F: frequency of alleles, * Patients odd ratio versus healthy subjects

HLA genotype	Mild		Moderate		OR*	Severe		OR**
	No. of alleles 10	F (%)	No. of alleles 56	F (%)		No. of alleles 64	F (%)	
HLA-DRBI*								
01	3	(30.0)	16	(28.6)	0.93	11	(17.2)	0.48
03	1	(10.0)	6	(10.7)	1.08	9	(14.1)	1.47
04	1	(10.0)	14	(25.0)	3.00	11	(17.2)	1.87
11	2	(20.0)	4	(7.1)	0.30	8	(12.5)	0.57
13	1	(10.0)	3	(5.4)	0.50	5	(8.9)	0.76
15	1	(10.0)	4	(7.1)	0.30	12	(18.8)	1.61
HLA-DQBI								
03	3	(30.0)	14	(25.0)	0.77	19	(29.7)	0.98
05	1	(10.0)	12	(21.4)	2.45	12	(18.8)	1.61
06	1	(10.0)	8	(12.5)	1.50	36	(56.3)	11.57

[Table/Fig-3]: Distribution of the most frequent HLA-DR/DQ genotypes by severity of disease in RA patients
*Moderate RA odds ratios versus mild RA
** Severe RA odds ratios versus mild RA

HLA genotype	DAS-28= 3.5-5.1		DAS-28=>5.1		OR*
	No. of alleles 30	F (%)	No. of alleles 100	F (%)	
HLA-DRBI*					
01	9	(30.0)	21	(21.0)	0.62
03	5	(16.7)	11	(11.0)	0.61
04	6	(20.0)	20	(20.0)	1.00
11	3	(10.0)	11	(11.0)	1.11
13	1	(3.3)	8	(8.0)	2.50
15	2	(6.7)	15	(15.0)	2.47
HLA-DQBI					
03	11	(36.7)	25	(25.0)	0.57
05	7	(23.3)	18	(18.0)	0.72
06	5	(16.7)	40	(40.0)	3.33

[Table/Fig-4]: Distribution of the most frequent HLA-DR/DQ genotypes by disease activity score (DAS-28) in RA patients
F: frequency of alleles; *Active RA odds ratios versus moderate RA

DISCUSSION

The present study is the first prospective investigation which was done on genotypes their correlation with disease activity and severity in Kurd population. The most striking finding of the present study was the appearance of higher frequencies of HLA-DQBI*06 allele in RA patients as compared to the data which was collected from available studies that were related directly to RA [6,7]. Moreover, HLA-DQBI*06 allele was much more frequently seen in our RA patients with severe and high disease activity, as well as in RF and Anti-CCP positive patients as compared to other genotypes.

In relation to disease activity, we observed that HLA-DQBI*06 was frequently seen in 40.0% of the active disease group (DAS-28>5.1). In contrast, other studies had failed to detect such an association in RA patients [8]. For example, a study which was done by Wakitani et al., [9] in Japan had demonstrated that HLA-DRBI*04 was

HLA genotype	Rheumatoid factor				OR
	< 24 IU/ml		≥24 IU/ml		
	No. of alleles 18	F (%)	No. of alleles 112	F (%)	
HLA-DRBI*					
01	1	(5.6)	29	(28.9)	5.90
03	2	(11.1)	14	(12.5)	1.14
04	1	(5.6)	25	(22.3)	4.80
11	0	(0.0)	14	(12.5)	-
13	1	(0.0)	8	(7.1)	1.30
15	1	(5.6)	16	(14.3)	2.83
HLA-DQBI					
03	1	(5.6)	35	(31.3)	7.70
05	4	(22.3)	21	(18.8)	0.80
06	2	(11.1)	43	(38.4)	4.98

[Table/Fig-5]: Distribution of the most frequent HLA-DR/DQ genotypes by RF in RA patients
F: frequency of alleles; *Positive RF odds ratios versus negative RF

HLA genotype	Anti-CCP				OR
	<15.1 IU/ml		≥15.1 IU/ml		
	No. of alleles 26	F (%)	No. of alleles 114	F (%)	
HLA-DRBI*					
01	2	(7.7)	28	(26.9)	3.90
03	2	(7.7)	14	(13.5)	1.68
04	1	(3.9)	25	(24.0)	7.02
11	4	(15.4)	10	(9.6)	0.52
13		(3.9)	8	(7.7)	1.88
15	1	(3.9)	16	(15.4)	4.08
HLA-DQBI					
03	3	(11.5)	33	(31.7)	3.12
05	3	(11.5)	22	(21.2)	1.87
06	4	(15.4)	41	(39.4)	3.10

[Table/Fig 6]: Distribution of the most frequent HLA-DR/DQ genotypes by Anti-CCP in RA patients
F: frequency of alleles; *Positive Anti-CCP odds ratios versus negative Anti-CCP

associated more strongly with a high disease activity subset. In a study done by Arouf et al., [6], it was reported that the presence of RF was more frequent in patients who carried the HLA-DRBI* 04 allele. However, in our study, the most frequent allele of HLA-genotypes which was identified in RA patients was HLA-DQBI*6 (34.6%), whereas in healthy controls, it was HLA-DRBI*03 (35.4%). Association between severity of disease and HLA-genotypes has been reported by several studies. Our results were in agreement with reports which indicated a greater role of genes which were related to HLA-DQBI alleles, which were related to severity of RA and development of extra-articular manifestation [10]. Other studies did not observe a significant role of HLA-DQBI alleles in the severe form of RA and they indicated that only HLA-DRBI*04 allele had a role in it [11]. This observation may signify that HLA-DQBI*06 allele was more prognostic than HLA-DRBI*04 allele, at least in our population. This discrepancy seen in our results as compared to those of other studies may be related to genetic and geographical variations [12]. Our results were in agreement with some reports on ethnic groups, especially on Pakistani population, that had a high frequency of HLA-DQBI*06 allele [13]. However, it would be desirable to augment the size of the sample to confirm this observation, which was a limitation of this study.

In the current study, we confirmed the association between HLA-DRBI*01 and DRBI*04 in RF positive patients. A similar association was found between HLA-DQBI*03 and DQBI*06. Indeed, the allele, DQBI*06 was highly represented in the RF positive patients. In contrast, other studies had reported that HLA-DRBI*04 allele was more frequent in RF positive patients [14].

Serum Anti-CCP seemed to play a pivotal role in the pathogenesis of RA, as it was highly specific [15]. It has been reported that high frequencies of HLA-DRBI*01, DRBI*04, DQBI*03 and DQBI*06 were associated with the presence of Anti-CCP antibodies [16]. The association between HLA-DR/DQ and Anti-CCP has been well established. About 40% of RA patients with anti-CCP positivity have HLA-DQBI*06 allele. This finding confirmed a link between RA and the most frequent HLA-DQ allele in our population.

CONCLUSION

In conclusion our study showed that Kurd RA patients had higher frequencies of HLA-DQBI*06 allele, which had predisposed them to active disease state. Doing genetic studies, especially on HLA-DQBI*O6 as a prognostic marker in patients with RA, may be of great importance.

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