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Frequency of HLA-DRB1 alleles in rheumatoid arthritis patients in Zahedan, southeast Iran

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BACKGROUND AND OBJECTIVES: Analysis of the role of different alleles of human leukocyte antigen (HLA) in rheumatoid arthritis (RA) patients is necessary in many populations and geographical areas. The aim of the present study was to investigate the frequency of HLA-DRB1 alleles in RA patients, comparing with that in control group in southeast Iran.

DESIGN AND SETTING: Case-control study of rheumatoid arthritis patients referred to rheumatology clinic at university hospital.

PATIENTS AND METHODS: The frequency of HLA-DRB1 alleles was determined in 79 RA patients and 93 healthy subjects in Zahedan, southeast Iran. HLA-DRB1 allele types were identified by polymerase chain reaction with sequence-specific primer (PCR-SSP).

RESULTS: The HLA-DRB1*10 allele showed a significantly higher frequency in patients with RA (OR=2.698, 95% CI=1.087-6.699, P=.045), while the frequency of DRB1*03 allele in these subjects was significantly lower than that in the control group (OR=0.447, 95% CI=0.2285-0.8729, P=.021). The frequencies of DRB1*01, DRB1*04, DRB1*07, DRB1*09, DRB1*11, DRB1*13, DRB1*14, DRB1*15, DRB1*16 were not significantly different between RA subjects and the control group.

CONCLUSION: The data suggest that the DRB1*10 allele is a risk factor and DRB1*03 is protective for RA in this population.

Rheumatoid arthritis (RA) is a chronic arthritic condition that can lead to deformities and disabilities. The exact pathogenesis is unknow, but both genetic and environmental factors play key roles in this disease process.[1] The prevalence of RA is about 1% of the population worldwide, and genetic factors have been estimated to account for 60% of the disease risk.[2] Major histocompatibility complex (MHC) genes account for about 50% of the genetic predisposition in most autoimmune diseases.[3]

Human leukocyte antigen (HLA) molecules play a central role in the immune response by presenting processed antigenic peptides to T cells. A specific sequence, present within the peptide-binding cleft of HLA class II molecules, has been implicated in genetic susceptibility to RA.

An association between RA and HLA-DRB1–shared epitope (SE), including DRB1*04 and DRB1*01 alleles, has been reported.[4] HLA molecules with specific shared epitopes (SEs) are considered to constitute about 30% to 40% of the genetic risk for RA.[5]

It has been reported that DRB1*0401 has a key role in predisposition to the most severe form of the disease, while some of the other DRB1 alleles may provide protection. However, the significant association of particular alleles with RA is not consistent in all human populations in different geographical areas or among different ethnic groups. Hence the analysis of different alleles of HLA in RA patients of many populations and geographical areas with regard to either having protective role or being a susceptibility factor is necessary. The aim of the present study was to determine the frequency of HLA-DRB1 alleles in RA patients in comparison with healthy subjects (control group) in Zahedan, southeast Iran.

PATIENTS AND METHODS

This pilot study included 79 patients (70 women and 9 men) with an average age of 44.5 years (range, 17-75 years) fulfilling the American College of Rheumatology (ACR) criteria for RA.[6] All the subjects were patients of the Rheumatology Clinic at Zahedan University of Medical Sciences. The control group consisted of 93 healthy individuals (65 women and 28 men) with a mean age of 45.4 years (range, 23 to 70 years) who were unrelated to the RA patients. The project was approved by the Ethical Committee of Zahedan University of Medical Sciences, and informed consent was obtained from all patients and healthy individuals. Blood samples were collected in Na-EDTA tubes from patients and healthy controls. DNA was extracted from blood according to the protocol of CTS-PCR-SSP kit (University of Heidelberg, Germany). The HLA-DRB1 alleles were determined in patients and control group by applying polymerase chain reaction with sequence-specific primer (PCR-SSP) using the above kit. The chi-square test was used in the statistical analysis.

RESULTS

The frequencies of HLA-DRB1 alleles in RA patients and normal individuals are summarized in **Table 1**. There were significant differences in the frequencies of particular alleles. The frequency of the HLA-DRB1*10 allele was significantly higher in RA patients (20.3%) than in normal subjects (8.6%) (OR=2.698, 95% CI=1.087-6.699, P=.045). The frequency of HLA-DRB1*16 was higher in RA patients (22.8%) than in normal subjects (11.8%), but the difference was not statistically significant (OR=2.20, 95% CI=0.9686-4.996, P=.067). The frequency of HLA-DRB1*03 in RA patients (22.8%) was significantly lower than that in normal subjects (40.2%) (OR=0.447, 95% CI=0.2285-0.8729, P=.021). The frequencies of HLA-DRB1*01, HLA-DRB1*04, HLA-DRB1*07, HLA-DRB1*09, HLA-DRB1*11, HLA-DRB1*13, HLA-DRB1*14, HLA-DRB1*15, DRB4* and DRB5* were not significantly different between RA patients and normal subjects.

DISCUSSION

Rheumatoid arthritis, a destructive autoimmune polyarthritis, is associated with an HLA-DRB1 shared epitope (SE), which contains a common amino acid sequence in positions 70-74.[4] The present study showed an association between some HLA-DRB1 alleles and RA susceptibility in our study population in southeast Iran. In particular, we found that DRB1*10 allele is contributory to susceptibility, while DRB1*03 is a

Table 1. The allele frequency of HLA-DRB in rheumatoid arthritis patients and normal subjects.

HLA-DRB alleles	Number of patients (%) (n= 79)	Number of normal subjects (%) (n= 93)	OR	95% CI	*P*
DRB1*01	26 (32.9)	30 (32.3)	1.030	0.52-2.05	.942
DRB1*03	18 (22.8)	37 (40.2)	0.45	0.22-0.92	.026*
DRB1*04	14 (17.7)	9 (9.7)	2.01	0.76-4.93	.187
DRB1*07	9 (11.4)	13 (13.9)	0.79	0.29-2.13	.781
DRB1*09	3 (3.8)	4 (4.3)	0.88	0.15-4.83	.825
DRB1*10	16 (20.3)	8 (8.6)	2.70	1.01-7.39	.048*
DRB1*11	12 (15.2)	12 (12.9)	1.21	0.47-3.11	.833
DRB1*13	7 (8.8)	9 (9.7)	0.91	0.29-2.83	.936
DRB1*14	4 (5.1)	7 (7.5)	0.66	0.15-2.62	.729
DRB1*15	14 (17.7)	25 (26.9)	0.59	0.26-1.30	.212
DRB1*16	18 (22.8)	11 (11.8)	2.20	0.90-5.41	.082
DRB3*	37 (46.8)	58 (62.4)	0.53	0.28-1.02	.591
DRB4*	25 (31.6)	29 (31.2)	1.02	0.51-2.05	.920
DRB5*	29 (36.7)	37 (39.8)	0.88	0.45-1.71	.798

OR: odds ratio, CI: confidence interval.

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protective allele. We also found a trend of positive association of RA with DRB1*16 and DRB1*04, although nonsignificant.

DRB1 alleles lacking SE were found by Larsen et al to be protective for RA.[7] Antigen presenting to the CD4+ T cells via HLA-DRB1 commences pathophysiological processes, for instance, activation of T lymphocytes, B lymphocytes, tissue macrophage and dendritic cells, that lead to RA.[8,9]

Association between HL-DRB1*01, HL-DRB1*04, HL-DRB1*10 and Italian RA patients has been reported.[10] Other studies demonstrated association between these alleles and RA; they have recently been reviewed in a number of meta-analyses or review articles.[11,12] Consistent with the above results, we found DRB1*10 to be a predisposing factor in the studied population; however, in contrast, DRB1*01 allele was found to be equally distributed between RA and control groups.

HLA-DRB1*04 allele has frequently been found to be associated with RA susceptibility;[4,13,14] and the HLA-DRB1*0404 sub-allele, strongly related with high titers of anti–cyclic citrullinated peptide (CCP) antibodies.[15] In contrast with these studies, no significant association was seen between DRB1*04 and RA in our subjects. However, a positive trend was observed with this allele in RA subjects compared to the control group, implying that a survey on DRB1*04 in larger-population samples of both RA patients and healthy people can assist in arriving at a more reliable conclusion on this commonly noticed allele. Further, analysis of the sub-alleles of DRB1*04 can shed more light on the role of this allele with regard to RA association.

A meta-analysis showed that HLA-DRB1 alleles (mainly HLA-DRB1*0404) are associated with RA in Latin Americans.[16] Another meta-analysis, in Asian-Mongoloids (Korean, Japanese, Chinese and Thai), showed that HLA-DRB1*0101, *0401, *0405, *0410 and *1001 are contributory to susceptibility, while HLA-DRB1* 0301, *0403, *0406, *0701, *1301 and *1405 are protective.[11] Like the results among Asian populations, the HLA-DRB1*03 was found in our study to play a protective role against RA. An exclusive association of DRB1*03 with anti–CCP-negative RA was also reported.[17] In conclusion, the results showed that DRB1*10 allele is a risk factor and DRB1*03 is among the protective factors for RA in our population.

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