Association of HLA-DRB1 Gene Polymorphism in Rheumatoid Arthritis Patients in Babylon Province, Iraq

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Abstract

Background: The heritability of rheumatoid arthritis (RA) is approximately 40% to 65% for seropositive RA and 20% for seronegative RA. The risk of developing RA has been associated with human leukocyte antigen HLA-DRB1 alleles: HLA-DRB1*04, HLA-DRB1*01, and HLA-DRB1*010 in deferent ethnocentrism. These HLA-DRB1 alleles contain a stretch of conserved five amino acid sequence, the shared epitope (SE), in the third hypervariable region of their DRB1 chain, which has been associated with the risk of developing RA.

Aim of the study: to study the possible association of HLA-DRB1 gene polymorphism of Arabic ethnocentrism PA patients in Babylon Province, Iraq.

Patients and methods: The present case control study was conducted on sixty one patients (18 males and 43 females) of Arabic ethnocentrism RA patients admitted to Rheumatoid Unit in Merjan Teaching Medical City, Babylon Province, Iraq, as well as 127 apparently healthy control subjects (41 males, 86 females) as control group.

Results: The risk of HLAD-RB1*01 allele was assessed through calculation of odds ratio (OR) which was estimated to be 1.95 (95 % confidence interval of 1.03 to 3.70). The risk of HLAD-RB1*04 allele was assessed through calculation of odds ratio (OR) which was estimated to be 4.46 (95 % confidence interval of 2.32 to 8.55). The risk of HLAD-RB1*010 allele was assessed through calculation of odds ratio (OR) which was estimated to be 3.12 (95 % confidence interval of 1.39 -7.00).

Conclusion: The current study documented that RA is significantly associated with the locus HLA-DRB1* and allelic groups HLA-DRB1*01, HLA-DRB1*04 and HLA-DRB1*010 in Iraqi patients with RA.

Key words: *HLA-DRB1 Gene Polymorphism, Rheumatoid Arthritis, Babylon*

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by inflammatory arthritis and extra-articular involvement. RA with symptom duration of fewer than six months is defined as early, and when the symptoms have been present for more than six months, it is defined as established [1-3]. Twin studies have shown a concordance rate of 15% to 30% among monozygotic twins and 5% among dizygotic twins. The heritability of rheumatoid arthritis is approximately 40% to 65% for seropositive rheumatoid arthritis and 20% for seronegative rheumatoid arthritis. The risk of developing rheumatoid arthritis has been associated with HLA-DRB1 alleles: HLA-DRB1*04, HLA-DRB1*01, and HLA-DRB1*010. These HLA-DRB1 alleles contain a stretch of conserved five amino acid sequence, the shared epitope (SE), in the third hypervariable region of their DRB1 chain, which has been associated with the risk of developing RA [4-6].

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In several twin studies, the heritability of RA was estimated to be approximately 60%, pointing towards a substantial influence of genetic risk factors on the development of RA disease [7]. Recent genome-wide association studies (GWAS) have identified 101 single nucleotide polymorphism (SNPs) in total, showing the highest contribution of the *HLA-DRB1* gene to the development of RA [8-11]. *HLA-DRB1*-encoded proteins are components of human leukocyte antigen-DR (HLA-DR) molecules and together with HLA-DQ and HLA-DP, they represent the major determinants in the induction of adaptive immune responses. They are expressed, amongst others, by antigen-presenting cells (APCs) and are able to present peptides to $CD4^+$ T cells. In the 1970s, HLA-Dw4 was shown to be present in the majority of the RA patients which was confirmed by serological HLA-typing identifying HLA-DR4 and HLA-DR1 in association with RA [12]. Nowadays, the list of HLA alleles conferring increased risk for RA development is largely known albeit with altered nomenclature. The predisposing HLA-DR alleles were found to have a particular sequence in common, located in the beta chain (HLA-DRB1) at positions 70–74 [12]. This has later become known as the shared epitope sequence and as such, the HLA-DR alleles carrying this particular sequence were designated as 'Shared Epitope alleles'(SE-alleles). In 2005, it was discovered that the genetic contribution of the HLA locus did not apply to RA as such, but rather to ACPA-positive RA only [13]. These data are important as they indicate that ACPA-positive and ACPA-negative RA represent different disease entities with a different underlying pathophysiology. More recently, positions 11 and 13, which are also part of the peptide-binding groove, have been implicated in the association between HLA and RA [14]. However, as these positions are the most polymorphic in the HLA-region, these two positions most likely represent the best proxy for the predisposing HLA molecules explaining their association with RA in statistical terms. Because of significant controversy about HLA DRB1 gene polymorphism in association with RA and due to the rarity of Iraqi studies on people living in Babylon province with this regard, the current study was justified and conducted.

Patients and Method

The present case control study was conducted in Department of Biochemistry, College of Medicine, University of Babylon, and Rheumatoid Unit, Merjan Teaching Medical City, Hilla City, Babylon Province, Iraq. The duration of current study was extended from September 2018 to July 2019. Sample size was determined according to sample size equation. Sixty one patients (18 male and 43 female) with RA clinically diagnosis by specialist physician attended to out clinic of Merjan Teaching Medical city, Hilla City with mean age of (47.43 ±11.34 years), as well as 127 apparently healthy control subjects (41 males, 86 females) with mean age of (48.94 ±12.36 years). Disease severity score of RA patients was determined by use DAS-28. DNA was isolated from white blood cells (WBCs) and (HLAD-RB1) gene promoter polymorphism was determined by Polymerase chain reaction with sequence-specific primers (PCR-SSP).

Results

The genetic study in the current study involved the assessment of the frequency of HLA-DRB1 allelic frequency in patients with RA and contrasting the results to that of comparable healthy subjects serving as control group. These HLAD-RB1 alleles included HLA-DRB1*01, HLA-DRB1*04 and HLAD-RB1*10. HLA-DRB1*01 allele was identified in 26 (42.6 %) patients and in 35 (27.6 %) control subjects; the difference was statistically significant ($P = 0.039$); the allele frequency being higher in patients group. The risk of HLA-DRB1*01 allele was assessed through calculation of odds ratio (OR) which was estimated to be 1.95 (95 % confidence interval of 1.03 to 3.70). This indicates that persons with HLA-DRB1*01 allele are at approximately 2 times the chance to have RA that persons lacking this allele. The etiologic fraction (EF) of HLA-DRB 1*01 allele in association with RA was 0.21, as shown in Table 1.

Table 1: HLA-DRB1*01 allele frequency in patients and control groups

HLAD-RB1*01	Rheumatoid arthritis n = 61	Control n = 127	P ¥	OR	95 % CI	EF
Positive	26 (42.6 %)	35 (27.6 %)	0.039 S	1.95	1.03 - 3.70	0.21
Negative	35 (57.4%)	92 (72.4 %)				

n: number of cases; ¥: chi-square test; OR: odds ratio, CI: confidence interval, EF: etiologic fraction; S: significant at $P > 0.05$

HLA-DRB1*04 allele was identified in 36 (59.0 %) patients and in 31 (24.4 %) control subjects; the difference was statistically highly significant ($P < 0.001$); the allele frequency being higher in patients group. The risk of HLAD-RB1*04 allele was assessed through calculation of odds ratio (OR) which was estimated to be 4.46 (95 % confidence interval of 2.32 to 8.55). This indicates that persons with HLA-DRB1*04 allele are at approximately 4.5 times the chance to have rheumatoid arthritis that persons lacking this allele. The etiologic fraction (EF) of HLA-DRB1*04 allele in association with rheumatoid arthritis was 0.42, as shown in Table 2.

Table 2: HLA-DRB1*04 allele frequency in patients and control groups

HLADRB1*04	Rheumatoid arthritis n = 61	Control n = 127	P ¥	OR	95 % CI	EF
Positive	36 (59.0 %)	31 (24.4 %)	< 0.001 HS	4.46	2.32 - 8.55	0.42
Negative	25 (41.0 %)	95 (74.8 %)				

n: number of cases; ¥: chi-square test; OR: odds ratio, CI: confidence interval, EF: etiologic fraction; S: significant at $P > 0.05$

HLA-DRB1*010 allele was identified in 16 (26.2 %) patients and in 13 (10.2 %) control subjects; the difference was statistically highly significant ($P = 0.004$); the allele frequency being higher in patients group. The risk of HLA-DRB1*010 allele was assessed through calculation of odds ratio (OR) which was estimated to be 3.12 (95 % confidence interval of 1.39 -7.00). This indicates that persons with HLA-DRB1*04 allele are at approximately 4.5 times the chance to have rheumatoid arthritis that persons lacking this allele. The etiologic fraction (EF) of HLA-DRB1*010 allele in association with rheumatoid arthritis was 0.37, as shown in Table 3.

Table 3: HLA-DRB1*010 allele frequency in patients and control groups

HLADRB1*010	Rheumatoid arthritis n = 61	Control n = 127	P ¥	OR	95 % CI	EF
Positive	16 (26.2 %)	13 (10.2 %)	0.004 HS	3.12	1.39 -7.00	0.37
Negative	45 (73.8 %)	114 (89.8 %)				

n: number of cases; ¥: chi-square test; OR: odds ratio, CI: confidence interval, EF: etiologic fraction; S: significant at $P > 0.05$

Correlation of HLA-DRB1*01, HLA-DRB1*04 and HLA-DRB1*010 to disease characteristics in patients with rheumatoid arthritis were shown in Table 4. HLA-DRB1*01 was positively correlated to disease activity (r = 0.016); however, the correlation was insignificant (P = 0.902), Table 4. HLA-DRB1*01 was negatively correlated to ACCP (r = - 0.118); however, the correlation was insignificant (P = 0.365), Table 3.16. HLA-DRB1*01 was positively correlated to RF (r = 0.531); and the correlation was highly significant (P < 0.001), Table 4.

HLA-DRB1*04 was negatively correlated to disease activity (r = -0.098); however, the correlation was insignificant (P = 0.452), Table 4. HLA-DRB1*04 was negatively correlated to ACCP (r = - 0.189); however, the correlation was insignificant (P = 0.144), Table 4. HLA-DRB1*04 was positively correlated to RF (r = 0.112); however, the correlation was insignificant (P = 0.392), Table 4.

HLA-DRB1*010 was positively correlated to disease activity (r = 0.134); however, the correlation was insignificant (P = 0.303), Table 4. HLA-DRB1*010 was negatively correlated to ACCP (r = -0.054); however, the correlation was insignificant (P = 0.681), Table 4. HLA-DRB1*010 was positively correlated to RF (r = 0.014); however, the correlation was insignificant (P = 0.917), Table 4.

Table 4: Correlation of HLA-DRB1*01, HLA-DRB1*04 and HLA-DRB1*010 to disease characteristics in patients with rheumatoid arthritis

Characteristic	HLAD-RB1*01		HLADRB1*04		HLADRB1*010	
	r	P	r	P	r	P
DAS-28	0.016	0.902	-0.098	0.452	0.134	0.303
ACCP	-0.118	0.365	-0.189	0.144	-0.054	0.681
RF	0.531	<0.001 **	0.112	0.392	0.014	0.917

r: Spearman correlation coefficient; DAS: disease activity score; MDA: malondialdehyde; ACCP: anti-cyclic citrullinated antibody; RF: rheumatoid factor; **: highly significant at $P \leq 0.01$

Discussion

In the currents study, HLA-DRB1*01 allele was identified in 26 (42.6 %) patients and in 35 (27.6 %) control subjects; the difference was statistically significant (P = 0.039); the allele frequency being higher in patients group. The risk of HLAD-RB1*01 allele was assessed through calculation of odds ratio (OR) which was estimated to be 1.95 (95 % confidence interval of 1.03 to 3.70). This indicates that persons with HLA-DRB1*01 allele are at approximately 2 times the chance to have rheumatoid arthritis that persons lacking this allele. The etiologic fraction (EF) of HLAD-RB1*01 allele in association with rheumatoid arthritis was 0.21.

In the current study also, HLA-DRB1*04 allele was identified in 36 (59.0 %) patients and in 31 (24.4 %) control subjects; the difference was statistically highly significant (P < 0.001); the allele frequency being higher in patients group. The risk of HLA-DRB1*04 allele was assessed through calculation of odds ratio (OR) which was estimated to be 4.46 (95 % confidence interval of 2.32 to 8.55). This indicates that persons with HLA-DRB1*04 allele are at approximately 4.5 times the chance to have rheumatoid arthritis that persons lacking this allele. The etiologic fraction (EF) of HLA-DRB1*04 allele in association with rheumatoid arthritis was 0.42. An association between RA and HLA-DRB1–shared

epitope (SE), including DRB1*04 and DRB1*01 alleles, has been reported. HLA molecules with specific shared epitopes (SEs) are considered to constitute about 30% to 40% of the genetic risk for RA [15]. 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 [15]. In addition to a positive association between RA and the presence of HLA-DRB*01 (OR=3.5) and HLA-DRB1*04 (OR=4.0), there was also a positive association between RA and HLA-DRB1*10 (OR=4.4). In our patients, we have established the absence of the DRB1*10 allele group. In the population of Finland, the association between RA and presence of HLA-DRB1*04 was confirmed [19]. Similar results for DRB1*04 were found in studies conducted in Slovakia [20] and Hungary [21]. Research on the distribution of HLA-DRB1 locus conducted in Turkey [22] showed that HLA-DRB1*04 is present in high frequency (46.2%) in RA patients, in comparison to healthy subjects (20.9%); OR=3.24. Analysis of the population from the area of northern Italy [23] showed a weaker correlation between RA and HLA-DRB1*04 and an increased frequency of HLA-DRB1*01 in RA patients (24%), and in the control group, respectively (16%), accompanied by a relative risk of 1.5.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine, University of Babylon and all experiments were carried out in accordance with approved guidelines.

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