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ORIGINAL ARTICLE

Crosslinks Between Human Leukocyte Antigen DRB1*01 and Human Leukocyte Antigen DRB1*13 Allelic Variants and Occurrence of Rheumatoid Arthritis in Patients From Federation of Bosnia and Herzegovina

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ABSTRACT

Objectives: This study aims to analyze human leukocyte antigen A (HLA-A), human leukocyte antigen B (HLA-B), human leukocyte antigen C (HLA-C), HLA-DRB1*, HLA-DRB3*, HLA-DRB4*, HLA-DRB5*, HLA-DQB1* loci expression in patients with rheumatoid arthritis (RA) in the Federation of Bosnia and Herzegovina.

Patients and methods: Deoxyribonucleic acid was isolated from peripheral blood of 48 RA patients (22 males, 26 females; mean age 36 years; range 2 to 63 years) and 104 healthy control individuals (52 males, 52 females; mean age 43 years; range 2 to 76 years). Deoxyribonucleic acid samples were analyzed using polymerase chain reaction-sequence-specific primers and sequence specific oligonucleotides methods.

Results: The most frequent allelic groups in RA patients were HLA-DRB1*01 (odds ratio=2.795; 95% confidence interval: 1.441-5.421; p=0.004) and HLA-DRB1*04 (odds ratio=2.573; 95% confidence interval: 1.214-5.453; p=0.023). Among RA patients, the most frequent genotype for the allelic group HLA-DRB1*, in the light of the common epitopes theory, was observed for DRB1*01/DRB1*13. This genotype indicates an increased incidence and relative risk (odds ratio=11.09).

Conclusion: The most common genotype in our RA patients was DRB1*01/DRB1*13, which showed increased frequency and a high relative risk. This genotype variant may be considered a predisposing factor for the development of RA.

Keywords: Allele groups; human leukocyte antigen genotypes; human leukocyte antigen system; rheumatoid arthritis.

Rheumatoid arthritis (RA) is a chronic, progressive inflammatory disease that affects mesenchymal tissue throughout the body and is one of the connective tissue diseases.[1] The prevalence of RA in the general population is about 1% (females about three times more frequently than males). Prevalence increases with age; however, sex differences are reduced in older age groups. RA is becoming more prevalent in all races.[2,3] The disease usually begins during the fourth and fifth decade of life and approximately 80% of all RA patients develop this disease between 35 and 50 years of age. Family studies indicate a genetic predisposition. Compared with the general population, individuals who have relatives suffering from RA have an increased risk of developing the disease.[4] Genetic factors associated with RA can be divided into those associated with the major histocompatibility (MHC) complex and those originating from other parts of the genome.[6] The role of genetic factors in the etiology of RA established a connection between the discovery of the disease with class II MHC complexes, namely the genetic variant human leukocyte antigen (HLA)-DRB1*04. Previous studies described genetic variants that are negatively associated with the development of RA, such as HLA-DRB1*07, HLA-DRB1*08

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and HLA-DRB*13, which are considered to play a protective role in preventing the occurrence of RA.[7] Patients often have circulating immunoglobulin M and immunoglobulin G reactive antibodies with a constant part of an immunoglobulin molecule. These antibodies, called rheumatoid factors, are used as a diagnostic indicator of RA. They are present in 60-90% of patients and in 5-10% of the healthy population. Collagen type II possesses one immunodominant T-lymphocyte epitope that binds to both variants of the predisposing HLA-DRB1* gene (HLA-DRB1*01 and HLA-DRB1*04). T-cells which recognize this epitope have a key role in the development of collagen-induced arthritis in transgenic mice expressing the HLA-DR1 and HLA-DR4 antigens.[8]

There are several hypotheses to explain the mechanics of this disease. The "shared epitope" hypothesis: allelic variation in the HLA-DRB1* gene reflects differences in the amino acid residues of HLA antigens' β-chain. Each of the HLA-DR molecules associated with RA has the same or very similar amino acid sequence in the third hypervariable region of the β-chain. The variants of HLA-DRB1 genes associated with RA, including HLA-DRB1*04, HLA-DRB1*01, HLA-DRB1*10, HLA-DRB1*14 and HLA-DRB1*09, encode the same amino acid sequence at positions 67-74 in the β-chain of HLA-DR molecules, except for the change of one amino acid for another basic (arginine → lysine) at position 71, encoded by HLA-DRB1*04. This same sequence of amino acid residues at positions 67-74 in the third hypervariable region of the β-chain, encoded by the mentioned variant of HLA-DRB1 gene, is called a "shared epitope".[9] According to one existing interpretation of the "shared epitope" hypotheses, specific peptides which bind to the peptide-binding groove of HLA alleles encoded with predisposing genes can lead to a loss of tolerance, leading to development of RA.[10] Disclosed are numerous possible causal agents including mycoplasma, Epstein-Barr virus, cytomegalovirus, rubella virus and parvovirus.[11] One possibility is the existence of persistent infection in the joint structures or retention of microbial products in synovial tissues. Alternatively, the immune response in connective joint components can be induced by microorganisms or a response to microorganisms which affect its integrity and detection of antigen peptides. In relation to this fact, previous researches determined reactivity to collagen type II and heat shock proteins.[12] There is a possibility that 'molecular mimicry' by infectious microorganisms leads to accumulation of cross-reactive determinants that are expressed in the joint structures of the host.[13] More recent studies are placing focus on the possible role of 'superantigens' originating from many microorganisms including staphylococci, streptococci and Mycoplasma arthritis.[14] Superantigens are proteins which have the ability to bind to HLA-DR molecules and specific Vβ segments of heterodimeric T-lymphocyte receptors and stimulate specific T-lymphocytes that express the Vβ product.[12] Other possible etiological mechanisms in RA include the breakdown of tolerance to the normal structure of one's own body, causing reactivity to self-antigens in the joint, such as collagen type II, or loss of immunoregulatory control mechanisms that result in polyclonal activation of T-lymphocytes.[12] Loss of tolerance may occur due to changes in the HLA molecule. In the HLA-B27, the cysteine sulfhydryl group in position 67 may be oxidized in some tissues, which leads to structural changes in the peptide binding cleft.[15] In this study, we aimed to analyze HLA-A, HLA-B, HLA-C, HLA-DRB1*, HLA-DRB3*, HLA-DRB4*, HLA-DRB5*, HLA-DQB1 loci expression in patients with RA in the Federation of Bosnia and Herzegovina.

PATIENTS AND METHODS

Total deoxyribonucleic acid was isolated from peripheral blood in volume of 10 mL (with ethylenediaminetetraacetic acid as anticoagulant) using the Ready deoxyribonucleic acid Spin Kit (Inno-Train, Diagnostik GmbH, Taunus, Hessen, Germany) of 48 RA patients (22 males, 26 females; mean age 36 years; range 2 to 63 years) and 104 healthy control individuals (52 males, 52 females; mean age 43 years; range 2 to 76 years) at Department for Molecular Imunnogenetics, Institute of Transfusion Medicine of FBiH, Sarajevo, Bosnia and Herzegovina between January 2012 to June 2014. We selected polymerase chain reaction-sequence specific primers for HLA genotyping, which was performed

using Polymerase Chain Reaction-Sequence Specific Oligonucleotides Microspheres Fluor analyzer (Luminex Corp., Austin, Texas, USA). Genotyping Polymerase Chain Reaction products were determined using ultraviolet Transilluminator MultiDoc-It (UVP Cambridge, UK) after their separation on agarose gel (1.5%) for horizontal electrophoresis and results were documented by Polaroid camera. We analyzed exon 2 and exon 3 of or MHC class I and exon 2 of gene for MHC class II.

The study protocol was approved by the Institute for Transfusion Medicine FBiH (No.03/05-37-875/012 from 18.12.2012) Ethics Committee. A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Statistical analysis of our results was carried using PowerMarker software version 3.25 (BRC, North Carolina, USA) and OpenEpi software version 2.3.1. (CreateSpace, CA, USA). Analysis of the allele group frequency within the gene locus was calculated by Fisher's exact test. Assessment of the strength of association between HLA gene variants and disease and significant differences were determined using 95% confidence interval.

RESULTS

Table 1 presents the values of the frequency of allelic groups in MHC class I (HLA-A*, HLA-B*, HLA-C* loci) in RA patients.

Human leukocyte antigen-A* gene locus is determined by 11 different allelic groups (A*01, A*02, A*03, A*11, A*23, A*24, A*26, A*31, A*32, A*33 and A*68). However, within this gene locus, HLA- A*25, A*29, A*30, A*34, A*36, A*43, A*66, A*69, A*74 and A*80 allelic variants were not detected. Estimated allele frequency was highest in HLA-A*02 allele group (0.292).

Within HLA-B* gene locus, presence of 17 different allelic groups of the confirmed 37 was revealed (HLA-B*07, B*08, B*13, B*14, B*15, B*18, B*27, B*35, B*37, B*38, B*39, B*40,

Table 1. Frequencies of allelic groups of major histocompatibility class I in rheumatoid arthritis patients

n	HLA-A*	2n=96	f_a	HLA-B*	2n=96	f_a	HLA-C*	2n=96	f_a
1.	A*01	10	0.104	B*07	7	0.073	C*01	3	0.031
2.	A*02	28	0.292	B*08	6	0.063	C*02	9	0.094
3.	A*03	12	0.125	B*13	2	0.021	C*03	8	0.083
4.	A*11	10	0.104	B*14	4	0.042	C*04	20	0.208
5.	A*23	1	0.010	B*15	4	0.042	C*05	2	0.021
6.	A*24	16	0.168	B*18	4	0.042	C*06	5	0.052
7.	A*25	-	-	B*27	6	0.063	C*07	22	0.229
8.	A*26	9	0.094	B*35	20	0.208	C*08	4	0.042
9.	A*30	-	-	B*37	2	0.021	C*12	15	0.156
10.	A*31	4	0.042	B*38	6	0.063	C*14	2	0.021
11.	A*32	3	0.031	B*39	7	0.073	C*15	1	0.010
12.	A*33	1	0.010	B*40	3	0.031	C*16	5	0.052
13.	A*68	2	0.021	B*41	-	-	C*17	-	-
14.	A*69	-	-	B*42	-	-	C*18	-	-
15.	A*29	-	-	B*44	5	0.052			
16.				B*45	-	-			
17.				B*46	-	-			
18.				B*47	-	-			
19.				B*48	-	-			
20.				B*49	3	0.031			
21.				B*50	1	0.010			
22.				B*51	14	0.146			
23.				B*52	-	-			
24.				B*53	-	-			
25.				B*54	-	-			
26.				B*55	2	0.021			
27.				B*56	-	-			
28.				B*57	-	-			
29.				B*58	-	-			

n: Presence of allelic groups in sample; HLA: Human leukocyte antigen; f_a: Frequency of allelic group.

Table 2. Frequencies of allelic groups of major histocompatibility class II in rheumatoid arthritis patients

n	HLA-DRB1*	2n=96	f_a	HLA-DRB3*,4*,5*	2n=96	f_a	HLA-DQB1*	2n=96	f_a
1.	DRB1*01	22	0.229	DRB3*	53	0.576	DQB1*02	12	0.125
2.	DRB1*03	9	0.094	DRB4*	21	0.228	DQB1*03	33	0.344
3.	DRB1*04	16	0.167	DRB5*	18	0.196	DQB1*04	-	-
4.	DRB1*07	3	0.031				DQB1*05	37	0.385
5.	DRB1*08	2	0.021				DQB1*06	14	0.146
6.	DRB1*09	-	-						
7.	DRB1*10	-	-						
8.	DRB1*11	11	0.115						
9.	DRB1*12	4	0.042						
10.	DRB1*13	9	0.094						
11.	DRB1*14	5	0.052						
12.	DRB1*15	9	0.094						
13.	DRB1*16	6	0.063						

n: Presence of allelic groups in sample; HLA: Human leukocyte antigen; f_a: Frequency of allelic group.

B*44, B*49, B*50, B*51 and B*55), while the highest frequency was observed for HLA-B*35 (0.208). Following allelic groups were not detected in HLA-B* gene locus: HLA-B*15, B*37, B*41, B*42, B*45, B*46, B*47, B*48, B*52, B*53, B*54, B*56, B*57, B*58, B*59, B*67, B*73, B*78, B*81, B*82, B*83 and B*95.

Human leukocyte antigen-C* gene locus was determined by the presence of 12 different allelic groups of 14 that was so far identified in this group (C*01, C*02, C*03, C*04, C 05, C*06, C*07, C*08, C*12, C*14, C*15, C*17). Within the HLA-C gene locus, the highest frequency among allelic groups had HLA-C*07 (0.229) allelic group.

Table 3. Frequencies of allelic groups of HLA-A*, HLA-B* and HLA-C* gene loci in control group

n	HLA-A*	2n=208	f_a	HLA-B*	2n=208	f_a	HLA-C*	2n=208	f_a
1.	A*01	30	0.144	B*07	7	0.033	C*01	14	0.067
2.	A*02	59	0.283	B*08	13	0.062	C*02	9	0.430
3.	A*03	23	0.110	B*13	6	0.028	C*03	13	0.062
4.	A*11	17	0.081	B*14	1	0.004	C*04	35	0.168
5.	A*23	9	0.043	B*15	6	0.028	C*05	6	0.028
6.	A*24	21	0.101	B*18	17	0.081	C*06	21	0.101
7.	A*25	5	0.024	B*27	5	0.024	C*07	59	0.283
8.	A*26	16	0.076	B*35	32	0.153	C*08	1	0.004
9.	A*30	1	0.004	B*37	2	0.008	C*12	34	0.163
10.	A*31	6	0.028	B*38	11	0.052	C*14	6	0.028
11.	A*32	8	0.038	B*39	14	0.067	C*15	7	0.033
12.	A*33	4	0.019	B*40	9	0.043	C*16	-	-
13.	A*68	9	0.043	B*41	7	0.033	C*17	3	0.014
14.	A*69	-	-	B*42	-	-	C*18	-	-
15.	A*29	-	-	B*44	15	0.072			
16.				B*45	1	0.004			
17.				B*46	-	-			
18.				B*47	2	0.008			
19.				B*48	-	-			
20.				B*49	12	0.057			
21.				B*50	3	0.014			
22.				B*51	26	0.125			
23.				B*52	3	0.014			
24.				B*53	-	-			
25.				B*54	-	-			
26.				B*55	5	0.024			
27.				B*56	2	0.009			
28.				B*57	6	0.028			
29.				B*58	3	0.014			

n: Presence of allelic groups in sample; HLA: Human leukocyte antigen; f_a: Frequency of allelic group.

Allelic groups HLA-C*17 and C*18 were not detected in the HLA-C gene locus.

Within HLA-DRB1* gene locus, 11 different allelic groups were determined (HLA-DRB1*01, DRB1*03, DRB1*04, DRB1*07, DRB1*08, DRB1*11, DRB1*12, DRB1*13, DRB1*14, DRB1*15 and DRB1*16) (Table 2). In this gene locus, following allelic groups were not detected: HLA-DRB1*09, DRB1*10 and DRB1*18. The highest frequencies had HLA-DRB1*01 (0.229) and HLA-DRB1*04 allelic groups (0.167). Allele frequencies in HLA-DRB3*,4*,5* gene loci were 0.576, 0.228 and 0.196, respectively.

Four different allelic groups (HLA-DQB1*02, DQB1*03, DQB1*05 and DQB1* 06) were detected in HLA-DQB1* gene locus and DQB1*05 (0.385) and DQB1*03 (0.344) gene loci had highest frequency. DQB1* 04 allele group was not established.

We analyzed the frequency of the variant gene loci (allele groups) of HLA-A*,B*,C* (MHC I class) as well as HLA-DRB1* DRB3*,4*,5* and DQB1* gene loci (MHC class II) in control group (Table 3). Each individual had a genotype consisting of two allelic groups so that the number of the considered allelic groups was 208 (104 genotypes).

The analysis of the HLA-A* gene locus showed that it is determined by 13 different allelic groups (HLA-A*01, A*02, A*03, A*11, A*23, A*24, A*25, A*26, A*30, A*31, A*32, A*33 and A*68), and the highest estimated frequency was for HLA-A*02 (0.283). HLA-A*29, A*34, A*36, A*43, A*66, A*69, A*74 and A*80 allelic groups were not identified.

Among 37 so far identified, the HLA-B* gene locus contains 24 different allelic groups (HLA-B*07, B*08, B*13, B*14, B*15, B*18, B*27, B*35, B*37, B*38, B*39, B*40, B*41, B*44, B*45, B*47, B*49, B*50, B*51, B*52, B*55, B*56, B*57 and B*58) and highest estimated frequency was for HLA-B*35 (0.153). HLA-B*42, B*46, B*48, B*53, B*54, B*59, B*67, B*73, B*78, B*81, B*82, B*83, B*95 allelic groups were not detected.

Human leukocyte antigen-C gene locus contains 12 different allelic groups (C*01, C*02, C*03, C*04, C*05, C*06, C*07, C*08, C*12, C*14, C*15 and C*17) and only two allelic groups were not detected. HLA-C*07 allele group had the highest frequency (0.283).

Table 4 presents HLA-DRB1* gene locus, the presence of 12 different allelic groups was determined (HLA-DRB1*01, DRB1*03, DRB1*04, DRB1*07, DRB1*08, DRB1*10, DRB1*11, DRB1*12, DRB1*13, DRB1*14, DRB1*15 and DRB1*16), and the highest frequency was estimated for HLA-DRB1*13 (0.149). HLA-DRB1*09 allele group was not detected. HLA-DRB3*, 4*, 5* gene loci had allelic frequencies 0.554, 0.163 and 0.282, respectively. Five different allelic groups (HLA-DQB1*02, DQB1*03, DQB1*04, DQB1*05 and DQB1*06) were present within HLA-DQB1* gene locus. HLA-DQB1*05 allele group had the highest frequency (0.278). This gene locus (HLA-DQB1*) had all allelic variants identified so far.

Exact *p* values using case-control test confirmed association ($p<0.05$) of specific genotypes and

Table 4. Frequencies of allelic groups of human leukocyte antigen-DRB1*, HLA-DRB3*, HLA-DRB4*, HLA-DRB5* and human leukocyte antigen-DQB1* gene locus in control group

n	HLA-DRB1*	2n=208	f_a	HLA-DRB*3,4,5	2n=202	f_a	HLA-DQB1*	2n=208	f_a
1.	DRB1*01	20	0.096	DRB3*	112	0.554	DQB1*02	37	0.177
2.	DRB1*03	26	0.125	DRB4*	33	0.163	DQB1*03	57	0.274
3.	DRB1*04	15	0.072	DRB5*	57	0.282	DQB1*04	5	0.024
4.	DRB1*07	18	0.065				DQB1*05	58	0.278
5.	DRB1*08	6	0.028				DQB1*06	51	0.245
6.	DRB1*09	-	-						
7.	DRB1*10	2	0.009						
8.	DRB1*11	26	0.125						
9.	DRB1*12	3	0.144						
10.	DRB1*13	31	0.149						
11.	DRB1*14	14	0.067						
12.	DRB1*15	24	0.115						
13.	DRB1*16	23	0.110						

n: Presence of allelic groups in sample; HLA: Human leukocyte antigen; f_a: Frequency of allelic group.

Table 5. Comparative analysis of allelic group distribution for HLA-DRB1* gene locus between rheumatoid arthritis and control groups

n	HLA-DRB1* (control)	2n	f_a	HLA-DRB1* (experiment)	2n	f_a	OR	95%Cl	*p*
1.	DRB1*01	20	0.096	DRB1*01	22	0.229	2.795	1.441-5.421	0.004*
2.	DRB1*04	15	0.072	DRB1*04	16	0.167	2.573	1.214-5.453	0.023*
3.	DRB1*03	26	0.125	DRB1*03	9	0.094	0.724	0.325- 1.611	0.557
4.	DRB1*07	18	0.065	DRB1*07	3	0.031	0.340	0.097- 1.185	0.115
5.	DRB1*13	31	0.149	DRB1*13	9	0.094	0.590	0.269-1.295	0.250
6.	DRB1*16	23	0.110	DRB1*16	6	0.063	0.536	0.210- 1.363	0.261

n: Presence of allelic group in sample; HLA: Human leukocyte antigen; f_a: Frequency of allelic group; OR: Odds ratio; CI: Confidence interval; * Statistically significant at 0.05 level.

genetic variants with the presence/absence of RA for HLA-DRB1* gene locus.

Table 5 presents the significant difference between RA patients and control patients for HLA-DRB1* genotype.

Significant higher frequency was estimated for HLA-DRB1*01 allele group in RA patients (odds ratio [OR]=2.795; 95% confidence interval=1.441 to 5.421; p=0.004), and HLA-DRB1*04 allele group in RA patients (OR=2.573; 95% confidence interval=1.214-5.453; p=0.023). Based on these results, HLA-DRB1*01 and HLA-DRB1*04 allelic groups can be considered as risky variant of the gene for the development of RA.

Control group had the higher frequency for allelic groups, but the results were not statistically significant: HLA-A*02 (p=0.564), HLA-B*18 (p=0.484), HLA-B*51 (p=0.527), HLA-C*12 (p=0.970) and DQB1*06 (p=0.350). Allelic groups HLA-B*41, B*45, B*47, B*52, B*56, B*57, B*58, C*17, DRB1*10 and DQB1*04 were detected in the control group, but in very low frequencies. HLA-C*16 allele group was detected only in RA patients. Frequency of HLA-DRB1*01/DRB1*13 genotype was significantly higher in RA patients (p=0.024).

This variant of genotype can be considered a predisposing factor for the development of RA. Its increased frequency was the result of the presence of allelic group HLA-DRB1*01, in which higher frequency in RA patients was established (Table 6). The frequency of other genotypes in the

Table 6. Comparative values of frequency for most common HLA-DRB1*01 and HLA-DRB1*04 gene loci between rheumatoid arthritis and control groups

Genotype	HLA-DRB1 HLA-DRB4* (control group)	n	f_a	HLA-DRB1* HLA-DRB4* (experimental group)	n	f_a	OR	95% Cl	*p*
1.	DRB1*01/DRB1*13	2	0.019	DRB1*01/DRB1*13	5	0.270	11.09	1.359-105.5	0.024
2.	DRB1*01/DRB1*15	1	0.009	DRB1*01/DRB1*15	3	0.062	6.86	0.695-67.8	0.186
3.	DRB1*01/DRB1*11	1	0.009	DRB1*01/DRB1*11	3	0.062	6.86	0.695-67.8	0.186
4.	DRB1*01/DRB1*16	3	0.028	DRB1*01/DRB1*16	3	0.062	2.24	0.436-11.5	0.565
5.	DRB1*01/DRB1*03	5	0.048	DRB1*01/DRB1*03	2	0.041	0.860	0.161-4.60	0.999
6.	DRB1*01/DRB1*14	1	0.009	DRB1*01/DRB1*14	2	0.041	4.47	0.396-50.6	0.469
7.	DRB1*01/DRB1*08	0	-	DRB1*01/DRB1*08	2	0.041	4.47	0.396-50.6	0.196
8.	DRB1*01/DRB1*12	0	-	DRB1*01/DRB1*12	1	0.020	-	-	0.631
9.	DRB1*01/DRB1*04	1	0.009	DRB1*01/DRB1*04	1	0.020	-	-	0.099
10.	DRB1*04/DRB1*15	5	0.048	DRB1*04/DRB1*15	4	0.083	1.8	0.461-7.02	0.606
11.	DRB1*04/DRB1*07	4	0.038	DRB1*04/DRB1*07	3	0.062	1.66	0.358-7.75	0.776
12.	DRB1*04/DRB1*11	2	0.019	DRB1*04/DRB1*11	2	0.041	2.21	0.30-16.23	0.751
13.	DRB1*04/DRB1*04	0	-	DRB1*04/DRB1*04	1	0.020	-	-	0.631
14.	DRB1*04/DRB1*12	0	-	DRB1*04/DRB1*12	1	0.020	-	-	0.631
15.	DRB1*04/DRB1*16	2	0.019	DRB1*04/DRB1*16	1	0.020	1.66	0.358-7.75	0.999
16.	DRB1*01/DRB1*01	2	0.019	DRB1*01/DRB1*01	0	-	1.08	0.09-12.2	0.933
17.	DRB1*01/DRB1*07	2	0.019	DRB1*01/DRB1*07	0	-	1.08	0.09-12.2	0.933
18.	DRB1*04/DRB1*13	1	0.009	DRB1*04/DRB1*13	0	-	-	-	0.999

HLA: Human leukocyte antigen; n: Presence of allelic group in sample; fa: Frequency of allelic group; OR: Odds ratio; CI: Confidence interval; * Statistically significant at 0.05 level.

HLA-DRB*1 and HLA-DRB*4 gene loci was not statistically significant.

In control group, HLA-DRB1*01/DRB1*08, DRB1*01/DRB1*12, DRB1*04/DRB1*04 and DRB1*04/DRB1*12 genotypes were not detected; however, these genotypes were present in RA patients in minimal gene frequency. HLA-DRB1*01/DRB1*01, DRB1*01/DRB1*07 and DRB1*04/DRB1*13 genotypes were not established for RA patients.

DISCUSSION

Recent research about crystallographic structure of the HLA-DRB1* gene further confirms that the specific epitopes are the main structural part of peptide binding clefts in the HLA-DR (A1, B1) heterodimer and that they play a crucial role in the formation of 'rheumatoid pockets'.[1] This theory of connection between RA and DRB1 gene with a common epitope includes approximately 80% of populations in the northern Europe, while this connection occurs in only 43% of Greek patients.[3] The results obtained for HLA class II polymorphisms in RA patients in Federation of Bosnia and Herzegovina (FB&H) point to the role of allelic groups DRB1*01 (OR=2.795) and DRB1*04 (relative risk=2.573) in susceptibility to this disease. As these allelic groups are very frequent in the population, the value of the relative risk is small (relative risk=2). Thus, patients with RA at locus DRB1* showed a marked increase in the frequency of allelic groups DRB1*03 and DRB1*11 and a reduced frequency of DRB1*16, which is not typical for RA patients in FB&H. Statistical analysis confirmed the increase in DRB1*03 and DRB1*11 expression in patients with RA and did not confirm a reduction of DRB1*16. Many Caucasian populations demonstrate an association of alleles DRB1*01 and DRB1*04 with RA, or different roles of individual subtypes in the predisposition to RA. Similar results were found in RA patients in our population and the allele group HLA-DRB1*01 (Frequency [F]=0.229; p=0.004) was most frequent, followed by the allelic group HLA-DRB1*04 (F=0.167; p=0.023). The population of Spain had an approximately equal representation of HLA-DRB1*04 (51.1%) and HLA-DRB1*01 (44.7%). In the Spanish population,[11] 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.[16] Similar results for DRB1*04 were found in studies conducted in Slovakia[17] and Hungary.[18] Research on the distribution of HLA-DRB1 locus conducted in Turkey[19] 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[20] 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. Studies conducted in Japan confirmed a positive correlation between HLA-DRB1*04 and development of RA.[21] HLA-DRB1*13 was found to be significantly reduced in RA patients in comparison with the control group in many studies. In the group of RA patients from Turkey, HLA-DRB1*13 was present at a reduced frequency (15.4%), in comparison to the control group (37.3%); OR=0.31; p<0.001.[19] A strong protective effect of HLA-DRB1*13 in preventing RA was found in the population of Finland.[16] Stark et al.[17] established the protective effect of HLA-DRB1*07 and HLA-DRB1*13. HLA-DRB1*13 in the FB&H control group had the highest frequency (0.149) in RA patients (0.094) but showed no statistical significance (p=0.250), so this cannot be considered a protective effect. In the locus DQB1*, we found increased frequencies of DQB1*03 (0.343) and DQB1*05 (0.385) variants that were in disequilibrium with DRB1*01 and DRB1*04. Analysis of genotypes with respect to the presence of the allele group DRB1* showed that the most common genotype in our patients was DRB1*01/DRB1*13, which showed an increased frequency and a high relative risk (OR=11.09), and the DRB1*04/DRB1*15 genotype, the increase of which in comparison to the control group was not statistically significant, p=0.606. This genotype variant may be considered a predisposing factor for the development of RA.

Increased frequency of this genotype resulted from a high frequency of HLA-DRB1*01 in RA patients. Frequencies of other genotypes in the HLA-DRB1* gene locus were not statistically significant ($p>0.05$). The limitation of our study is the small sample size.

In conclusion, this study suggests that in FB&H, RA is primarily associated with the locus HLA-DRB1* and allelic groups HLA-DRB1*01 and HLA-DRB1*04. Further studies with larger sample sizes are required to obtain a complete overview of the genetic background of this disease. This research should be considered as a pilot study in which we presented a small insight into the genetic structure of RA patients in the FB&H.

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