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Research Article

HLA Typing for Celiac Disease in Entity of Bosnia and Hercegovina-Republic of Srpska

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ABSTRACT

HLA genotyping proved itself as a useful tool for avoiding additional invasive diagnostic testing for autoimmune diseases. In order to determine the justification and need for these analyses in our population, the association was explored of HLA-*DQB1** locus with celiac disease (CD) in the entity in Bosnia and Herzegovina – Republic of Srpska, which has been already found in other populations. As diagnostic support for autoimmune disease, *HLA-DQ2* and *HLA-DQ8* typing were performed on 90 patients. Tests were carried out with Inno-Train's molecular Fluo gene detection system based on *PCR-SSP*, with the aim to investigate the prevalence of *HLA-DQ2* and *HLA-DQ8* in celiac disease. Out of 24 patients with confirmed celiac disease, 87.5% of patients had *HLA-DQ2*; while 12.5% had *HLA-DQ8*. With these results, we demonstrated the full justification and need for diagnostic of CD in future work. Given that the tests for HLA class II also contained the *HLA-DR* locus, the prevalence of allelic groups of both loci was determined for the first time in our entity.

Keywords: HLA genotyping; HLA-DQ; Celiac disease; Alleles

INTRODUCTION

Celiac disease (CD) is a chronic inflammatory disease in the small intestine, for which the effective treatment represents a strict lifelong gluten-free diet. In the Caucasian population, the prevalence of CD equals approximately 1% [1-3], however, with the awareness of many subclinical cases, the prevalence is considered to be higher. Many genes involved in the pathogenesis have been identified and a crucial role is known to be played by the Human Leukocyte Antigen (HLA) system. HLA super-locus contains hundreds of genes with different immunological functions and it is characterized by a high gene density, variability and extensive linkage disequilibrium. Certain combinations of alleles are passed to the offspring more often than usually expected, and this phenomenon is marked as a "haplotype" [4-6]. *HLA-DQA1* and *HLA-DQB1* genes are the main determinants for genetic susceptibility, encoding *HLA-DQ2* and *HLA-DQ8* molecules, which have been carried by almost all patients presenting the disease [7].

Numerous studies have demonstrated that roughly 90%-95% of CD patients' express *HLA-DQ2* protein, while nearly the rest of the patients' express *DQ8* protein [8]. The strong association of CD with HLA class II alleles is in accordance with a central role for CD4 T cells in disease pathogenesis. Gluten-specific CD4 T cells can be isolated from the mucosa of CD patients but not from healthy individuals, and these cells are restricted by *HLA-DQ2* or *DQ8* molecules [9]. Although not all *HLA-DQ2* and *HLA-DQ8* carriers eventually develop the disease, it is very useful to determine the existence of genetic susceptibility prior to painful and lengthy procedures, especially in pediatric patients, allowing streamlining of the diagnostic algorithm for a faster and safer way to the final diagnosis.

Considering all the above mentioned, the present study aimed to establish the frequency of *HLA-DQB1* alleles in patients diagnosed with CD in the population of the Republic of Srpska and to determine the usefulness of the corresponding genotyping procedures in diagnostic protocols.

MATERIALS AND METHODS

HLA genotyping

Genomic DNA was extracted from peripheral blood samples using the Ready-DNA Spin-Kit (Inno Train, Molecular Biology Grade,

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Germany). Inno-Train's molecular Fluo gene detection system based on *PCR-SSP* (Sequence Specific Priming) was used as a detection method for Human Leukocyte Antigens (HLA). Detection was performed by an endpoint method in the fluorescence reader Inno-Train Fluo Vista. Endpoint method detects the emissions of the various fluoro chromes in the Fluo Vista before and after the PCR is completed and calculates the difference using the Fluo gene analysis software, evaluated as positive or negative end result.

For *HLA-DQ2* and *HLA-DQ8* typing, we used HLA-Fluo gene *DRDQ* test. It detects *HLA-DQB1*, *HLA-DRB1*, *DRB3*, DRB4, and DRB5 alleles with at least low resolution. In the allelic group of *HLA-DRB1**03 and *HLA-DQB1**03 it is separated primers for high resolution of *HLA-DRB1**03:01 (DR17) and *HLA-DRB1**03:02 (DR18), as well *HLA-DQB1**03:01 (DQ7), *HLA-DR*QB1*03:02 (*DQ8*) and *HLA-DQB1**03:03 (*DQ9*), and it is possible to determine serological equivalents, in software based on the EMBL EBI HLA dictionary with the WHO assigned settings.

Patients and control group

In total 90 patients with clinical request and suspected celiac disease has been tested, 52 of them were pediatric. The diagnosis of CD was based on the criteria of The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition from 1989. All the relevant calculations were performed after the positive CD diagnosis, which was confirmed in 24 admitted patients. The remaining 66 individuals, with confirmed anemia or some other gastrointestinal disorder, were regarded as the control group.

The study was approved by the Ethics Committee of the University Clinical Centre of RS, and the Institute for Transfusion Medicine RS in Banjaluka. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Firstly, *HLA-DRB1* and *HLA-DQB1* allele groups were presented by counting them through a tabular presentation (Table 1). In the same way, results were presented for patients with confirmed CD (Table 2), and control group (Table 3). Responsible alleles *HLA-DQB1**02 and *HLA-DQB1**03:02 were disequilibrium fused to *HLA-DRB1* and a graphical representation of the prevalence of combinations in our population was made. The number of persons who had an allele in relation to the total number of respondents-phenotype frequency, number of individual alleles of a certain type-allelic frequency, and haplotype frequencies were estimated by direct counting.

To determine statistically significant differences of the examined alleles between the groups, a chi-square test was used, while Fisher's exact test was used if any of the values in 2 × 2 tables were less than 5. A P-value <0.05 was considered statistically significant.

RESULTS

HLA-DRB1 and *HLA-DQB1* loci were determined for all suspected CD patients. Among the investigated 90 patients, 34 had *HLA-DRB1**11 in their phenotype, 28 had *HLA-DRB1**03, 24 had *HLA-DRB1**07, while 23 had *HLA-DRB1**01. For seven patients homozygous allelic condition was detected. Frequencies of all the *HLA-DRB1* alleles are shown in Table 1.

Regarding the *HLA-DQB1* locus, the allelic group HLA-DQB*02 was detected in 45 patients, nine of which appeared homozygous. *HLA-DQB1**03:02 was detected in 13 patients, with one homozygous case. According to the participation in the phenotype, the group of alleles *HLA-DQB1**03:01 had the largest number in the test group – 48 of them all. The frequency of all groups of *HLA-DQB1* alleles is also shown in Table 1.

In the *HLA-DRB1* locus, out of the 24 patients diagnosed with CD, the *HLA-DRB1**03 allele group was detected in 15 subjects, while alleles *HLA-DRB1**07 and *HLA-DRB1**11 were present in nine patients each. In terms of participation in the phenotype, the group of *HLA-DRB1**03 alleles also had the highest frequency, followed by *HLA-DRB1**07, and *HLA-DRB1**11. Frequencies of all *HLA-DRB1* allelic groups are shown in Table 2.

Table 1: Frequencies of allelic groups for *HLA-DRB1* and DQB1 loci in all tested groups (N=90).

HLA loci	Allelic group	n	Allele frequency	n homozygous	Frequencies in phenotype (%)
HLA-DRB1	DRB1*01	23	0.1278	0	25.56
	DRB1*03	30	0.1667	2	31.11
	DRB1*04	17	0.0944	1	17.78
	DRB1*07	26	0.1444	2	26.67
	DRB1*08	2	0.0111	0	2.22
	DRB1*10	2	0.0111	0	2.22
	DRB1*11	35	0.1944	1	37.78
	DRB1*12	4	0.0222	0	4.44
	DRB1*13	16	0.0889	1	16.67
	DRB1*14	2	0.0111	0	2.22
	DRB1*15	13	0.0722	0	14.44
	DRB1*16	10	0.0556	0	11.11

HLA-DQB1	DQB1*02	54	0.3	9	50
	DQB1*03:01	48	0.2667	2	51.11
	DQB1*03:02	14	0.0778	1	14.44
	DQB1*03:03	4	0.0222	0	4.44
	DQB1*04	1	0.0056	0	1.11
	DQB1*05	33	0.1833	3	33.33
	DQB1*06	26	0.1444	2	26.67

Note: Phenotype frequency-the number of persons who have an allele in relation to the total no.

of respondents (N=90). Allelic frequency – the number of individual alleles of a certain type N=90 × 2=180). N homozygous – the number of persons who have a homozygous allelic group.

Table 2: Frequencies of allelic groups for *HLA-DRB1* and DQB1 loci in the group of patients with diagnosed celiac disease (N=24).

HLA loci	Allelic group	n	Allele frequency	n homozygous	Frequencies in phenotype (%)
HLA-DRB1	DRB1*01	3	0.0625	0	12.50
	DRB1*03	17	0.3542	1	66.67
	DRB1*04	6	0.1250	1	20.83
	DRB1*07	9	0.1875	0	37.50
	DRB1*11	9	0.1875	0	37.50
	DRB1*13	3	0.0625	0	12.50
	DRB1*16	1	0.0208	0	4.17
HLA-DQB1	DQB1*02	26	0.5417	5	87.50
	DQB1*03:01	10	0.2083	0	41.67
	DQB1*03:02	6	0.1250	1	20.83
	DQB1*05	4	0.0833	0	16.67
	DQB1*06	2	0.0417	0	8.33

Regarding the *HLA-DQB1* locus, the group of *HLA-DQB1**02 alleles was represented by 21 patients, followed by *HLA-DQB1**03:01 with nine patients, and *HLA-DQB1**03:02 with four subjects. According to the participation in the phenotype, the allelic *HLA-DQB1**02 group is characterized with the five homozygous, while *HLA-DQB1**03:02 is represented with only one homozygous.

After linking groups of alleles by disequilibrium, in the group of patients with confirmed CD, a total of three haplotypes with responsible allelic groups were detected. The most commonly detected combination was the *HLA-DRB1**03:01-*DQB1**02, occurring in 16 patients, followed by *HLA-DRB1**07-*DQB1**02, occurring in five patients, and finally *HLA-DRB1**04-*DQB1**03:02, occurring in the remaining three patients. The presence of the *HLA-DRB3* locus chain was also detected in 22 CD patients, *HLA-DRB4* in 12, and *HLA-DR*B5 in just one of them.

Regarding the *HLA-DR* loci, in the control group (comprising 66 patients) 26 patients had *HLA-DR*B*11, 20 of them had *HLA-DRB1**01, 17 had *HLA-DRB1**07, and 13 had *HLA-DRB1**03, just as the DRB1*15 allelic group. Also, in the control group, HLA-*DQB1**03:01 was detected in 38 patients, *HLA-DQB1**05 in 29, *HLA-DQB1**02 in 28, while *HLA-DQB1**06 allele was found in 24 patients (Table 3).

In the control group, 25 patients were the haplotype *HLA-DRB1**11-*DQB1**03:01, 20 of them with *HLA-DRB1**01-*DQB1**05, 15 with *HLA-DRB1**15-*DQB1**06, 14 with *HLA-DRB1**07-*DQB1**02, 13 with *HLA-DRB1**03:01-*DQB1**02, and 11 with *HLA-DRB1**13-*DQB1**06. Haplotype *HLA-DRB1**16-*DQB1**05 was present in seven subjects and *HLA-DRB1**04-*DQB1**03:02 in six of them. The remaining haplotypes were present in only one or two subjects. These were left out from further consideration.

When comparing the investigated CD and control groups, statistically significant differences were obtained for the *HLA-DRB1**03 and *HLA-DQB1**06 allele frequencies, with a P-value of 0.02 in both cases, and also for *HLA-DQB1**02, with the P-value of 0.008. Other common allelic groups in both loci had no statistical difference.

Table 3: Frequencies of allelic groups for *HLA-DRB1* and *DQB1* loci in the control group (N=66).

HLA loci	Allelic group	n	Allele frequency	n homozygous	Frequencies in phenotype (%)
HLA-DRB1	DRB1*01	20	0.1515	0	30.30
	DRB1*03	13	0.0948	1	19.70
	DRB1*04	11	0.0833	0	16.67
	DRB1*07	17	0.1288	2	25.76
	DRB1*08	2	0.0151	0	3.03
	DRB1*10	2	0.0151	0	3.03
	DRB1*11	26	0.1970	1	37.88
	DRB1*12	4	0.0303	0	6.06
	DRB1*13	13	0.0948	1	18.18
	DRB1*14	2	0.0151	0	3.03
	DRB1*15	13	0.0948	0	19.70
	DRB1*16	9	0.0606	0	13.64
HLA-DQB1	DQB1*02	28	0.2121	4	36.36
	DQB1*03:01	38	0.2879	2	54.44
	DQB1*03:02	8	0.0606	0	12.12
	DQB1*03:03	4	0.0303	0	6.06
	DQB1*04	1	0.0075	0	1.51
	DQB1*05	29	0.2197	3	39.39
	DQB1*06	24	0.1818	2	33.33

DISCUSSION

Almost 95% of CD-affected patients express *HLA-DQ2* and the rest of them are usually carriers of the *HLA-DQ8*, which was once again confirmed in this study, where 87.5% of *HLA-DQ2* positive cases and 12.5% of *HLA-DQ8* positive cases were reported in the group of patients suffering from CD [10]. Among the *HLA-DQ8* positive cases, one of them was homozygous. The other two had a *DRB3* chain. This result is in concordance with the data available for several European countries indicating that: 87% of French, 84% of Italian, and 88% of the UK CD patients carry *HLA-DQ2* locus. In the neighboring countries, a prevalence of 94.5% was reported in Serbia, 93.7% in Croatia, while 88.8% was reported in Slovenia [11,12].

Given the high frequency of DQ2.5 cis in the linkage of disequilibrium with the DRB1-03:01 allele, and the fact that it was first associated with a risk of developing CD [13], the frequency of this haplotype was investigated in the present study, where 66% of positive subjects in the population of CD patients were reported. 20.81% of positive cases were found in the linkage with the DRB1*07 allelic group and all the patients carrying DQ2 also have had a *DRB3* allele. Accordingly, in a serology study in Northern Serbia (Vojvodina), DR3 and DQ2 were reported as the most frequent HLA protein variants among CD patients [14]. Among the remaining subjects (12.5%) in our study, *DQB1**03:02 alleles were predominantly found, which is slightly higher than in the region. In Croatia, a prevalence of 4.8% was reported, in Slovenia 8.8%, and in Serbia only 2.7%. Finally, this study provides the first results of HLA-DR-DQ polymorphism occurrence in the population without diagnosed CD in Republic of Srpska.

The highest allelic frequency in the *HLA-DRB1* locus was found for allelic groups DRB1*11 (19.7%), followed by *HLA-DRB1**01 (15.15%), *HLA-DRB1**07 (12.9%), *HLA-DRB1**03, *HLA-DRB1**13 and *HLA-DRB1**15, that occur with the same prevalence (9.5%). Regarding the participation in the phenotype, exactly the same order of allelic group frequencies was obtained: *HLA-DRB1**11 (37.9%), *HLA-DRB1**01 (30.3%), *HLA-DR*B1*07 (25.8%) and *HLA-DRB1**03 and *HLA-DRB1**13 and DRB*15 which occur with the same frequency (19.7%).

In HLA-DQ loci, the most frequent allelic group was *HLA-DQB1**03:01 (28.8%), followed by *HLA-DQB1**05 (22%), HLA-DQB1*02 (21.2%), and *DQB1**06 (18.2%). Frequency obtained in phenotype shows the highest prevalence of HLA-*DQB1**03:01 (54.5%), followed by HLA-*DQB1**05 (39.4%), HLA-*DQB1**02 (36.3%), and *HLA-DQB1**06 (33.3%).

The most commonly occurring haplotype in the control group was *HLA-DRB1**11-*DQB1**03:01, found in 25 subjects (20.49%). *HLA-DRB1**01-*DQB1**05 was found in 14.75% of subjects, *HLA-DRB1**07-*DQB1**02 in 11.47%, *HLA-DRB1**15-*DQB1**06 in 10.65%, while *HLA-DRB1**03:01-*DQB1**02 haplotype was found in 9.38% of subjects. The results obtained in this study are very similar to the ones reported in a study from Serbia, where on a larger sample of respondents, an almost identical order of the

most common haplotypes was reported. The only exception was the *HLA-DRB1**15-*DQB1**06, instead of which in the Serbian population occurs *HLA-DRB1**16-*DQB1**05. A study in Northern Macedonia also reported similar results [15], same as a study in Croatia – Gorski kotar [16], where the most occurring haplotype was the same as in the present study, with a different order in percentages.

CONCLUSION

The present study provides the first results for the association of CD and HLA-*DQB1**02 and HLA-*DQB1**03:02 alleles in the Republic of Srpska, indicating that all the tested CD patients were the carriers of one of the responsible alleles, which represents an excellent lead for diagnostic procedures and is in concordance with the existing data available for other populations of Caucasians. The results of the haplotype allow us to see the similarities and differences with the surrounding populations in region.

AUTHORSHIP CONTRIBUTION

Milosavi M – Concept, Data Collection and Procesing, Analysis, Interpretation, Literature Search, Writing Manuscript

Andri Z– Supervision, Interpretation, Literature Search, Writing Manuscript

Guzijan G – Resources, Materials, Literature Search

Nedeljkovi BB– Supervision, Interpretation, Literature Search, Critical Review

DISCLOSURE OF CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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