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Presence of HLA-DQ2 and HLA-DQ8 /DR4 celiac disease predisposing alleles in tested group of patients in Bosnia and Herzegovina

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Abstract

Celiac disease (CD) is an autoimmune disease characterized by gluten intolerance. The main cause of this immune - mediated enteropathy is gluten, a protein mainly present in wheat, rye, barley and spelt. The predisposition to celiac disease is determined by HLA class II genes encoding MHC II heterodimer molecules, more specifically HLA-DQ2 and HLA-DQ8. Up to date the exact number of people suffering from celiac disease in Bosnia and Herzegovina is still not known because there is no public registry for this disease. The aim of this study was to evaluate the HLA-DQ2 and HLA-DQ8/DR4 presence in tested group of patients (n = 23) referred to Polyclinic Atrijum in Sarajevo in period from August 2022. to August 2023. HLA-DQ2 and DQ8/DR4 allele identification was performed using Real-time PCR technique. According to the obtained results a total of 26% (n=6) of tested patients were positive for HLA-DQ2 and HLA-DQ8/DR4 alleles. Two of the patients were positive for HLA-DQ2 alleles and four patients were positive for HLA-DQ8/DR4 alleles. To our knowledge this is the first study evaluating the presence of HLA-DQ2 and HLA-DQ8 genotypes in tested population in Bosnia and Herzegovina.

Introduction

Celiac disease (CD) is an autoimmune disorder characterized by gluten intolerance. Gliadin, a protein mainly present in wheat, rye, barley and spelt is a main trigger of this immune - mediated enteropathy (Caio et al. 2019). Reported prevalence of celiac disease is 0.5-1% in the general population. However, diagnosis for this disease is challenging due to the variety of symptoms that patients may have. Patients suffering from celiac disease most often report symptoms of diarrhea, nausea, vomiting, heartburn, and abdominal distension. A small number of patients are completely symptom-free, while there are also those who suffer from severe gastrointestinal symptoms, such as anemia and abdominal pains (Lebwohl and Rubio-Tapia, 2021). The exact number of people suffering from celiac disease in Bosnia and Herzegovina is still not known since there is no public registry for this disease. A recent epidemiological study conducted in 15 Mediterranean countries, estimates that in the next ten years, there will be more than five million newly diagnosed patients (Greco et al. 2011). It is important to note that due to the wide spectrum of symptoms, as many as 75%–90% of affected individuals remain undiagnosed (Fuchs et al. 2018). The pathologic process of celiac disease it is thought to be a combination of genetic, environmental, and immunological factors. The predisposition to celiac disease is determined by a large number of genes, whereby HLA class II genes encoding MHC II heterodimer molecules, more specifically HLA-DQ2 and HLA-DQ8, are considered a key factor in the development of this condition (Kupfer and Jabri, 2012). HLA molecules DQ2 and DQ8 are located in the short arm of chromosome number 6, more specifically in the region 6p21.3 and they react to gliadin as soon as it enters the small intestine. The primary susceptibility allele for Celiac disease is HLA-DQ2 which is composed of HLA-DQA1*05 and DQB1*02, rest of the cases is related to HLA-DQ8 that is composed of HLA-DQA1*03 DQB1*03:02 that is associated in haplotype with HLA-DR4 allele. More than 95% of individuals with celiac disease carry the HLA-DQ2 or HLA-DQ8 haplotype. The HLA-DQ2 antigen is present in approximately 90% of patients, while the HLA-DQ8 antigen is present in 5-10% of patients with celiac disease (Sciurti et al. 2018, Stanković et al. 2014). The aim of this study was to evaluate the HLA-DQ2 and HLA-DQ8/DR4 presence in tested group of patients referred to Polyclinic Atrijum in Sarajevo in period from August 2022. to October 2023.

Material and methods

Participants

A total of 23 patients (10 males and 13 females; mean age, 29 years) was referred to Atrijum Polyclinic (Sarajevo) in period from August 2022. to October 2023. for HLA-DQ2 and HLA-DQ8/DR4 genetic testing. To conduct this research written informed consent was obtained from all participants. If participant was a minor, parental consent was taken.

HLA-DQ2 and HLA-DQ8/DR4 genotyping

Peripheral blood was drawn on BD Vacutainer Blood Collection tubes K2 EDTA 5.4 mg (BD Biosciences, USA). A total of 200 µl of whole blood was used for DNA extraction. Genomic DNA was extracted using PureLink® Genomic DNA Kit (Invitrogen, Thermo Fisher Scientific, USA). A total of 3□ of purified isolated DNA is used for downstream analysis. Testing for HLA-DQ2 and DQ8/DR4 genotype was preformed using geneMAP™ Celiac (DQ2, DQ8, DR4) Detection Kit (Turkey). In the presence of target alleles, the real time amplification curves are generated by allele specific PCR primers then mutation genotyping is done by melting curve analysis using Mic PCR (Bio Molecular Systems, Australia). Each isolated DNA is tested with multiplex (endogenous control and HLA-DQ2,

HLA-DQ8 and HLA-DR4 specific primers) Real-time-PCR primer mixes.

Results and Discussion

In this study, twenty-three patients (10 males and 13 females) were genotyped for HLA-DQ2 and DQ8/DR4 genes. According to the obtained results a total of 26% (n=6) of tested patients were positive for HLA-DQ2 and HLA-DQ8/DR4 alleles (Table 1). Two of the patients were positive for HLA-DQ2 alleles and four patients were positive for HLA-DQ8/DR4 alleles. The presence of HLA-DQ8 genotype was twice more frequent than HLA-DQ2 (Figure 1).

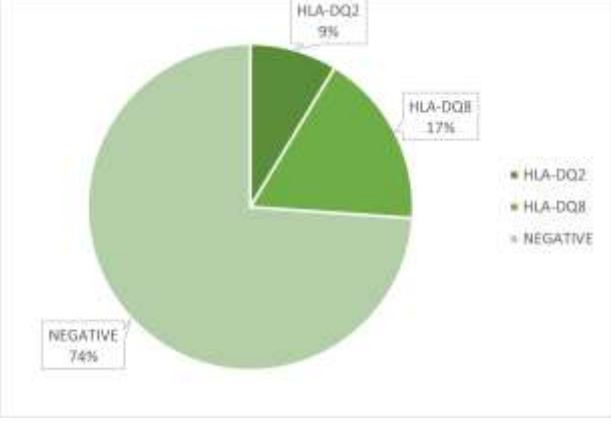

Figure 1. Chart representing HLA-DQ2 and HLA-DQ8/DR4 prevalence in tested patients (n=23)

According to research from 2022, the frequency of HLA-DQ2 and HLA-DQ8 among celiac disease patients was 93% and 4%, respectively. Among HLA-DQ2 positive, HLA-DQ2.5 and HLA-DQ2.2 were found in 92% and 8%, respectively (Alam et al. 2022). In another study, the presence of HLA DQ2 and DQ8 was identified in 98,4% of 74 celiac patients, of which 79,7% had only HLA DQ2; 8,1% had only HLA DQ8 and 10,8% had both antigens histocompatibility (Cecilio and Bonatto, 2015). Study including patients with celiac disease, diabetes type I and healthy controls showed that while patients with celiac like symptoms had DQ2 (60%) and DQ8 (8.5%) and all were positive for anti-tTG test, the patients with diabetes mellitus type 1 having celiac like symptoms possessed DQ2 (31.4%), DQ8 (25%), and DQ2/DQ8 (34%) , on the other hand control had only DQ2 (8.5%) and other alleles were negative (Siddiqui et al. 2021). Individuals positive for human leukocyte antigens (HLA) DQ2 and/or DQ8 have a greater chance for developing CD. However, it is important to emphasize that CD cannot be fully explained by this genetic predisposition alone. HLA genetic testing is not a typical diagnostic procedure, since a positive result indicates the presence of genetic predisposition for this disease, but not necessarily its development. Specifically, from 30% of the population who are carriers of HLA DQ2/DQ8 antigens, only 3% develop gluten intolerance (Hunt and van Heel, 2009). Therefore, a negative genetic test results for HLA DQ2/DQ8 are mainly used to exclude the diagnosis of CD.

Table 1. A total of 6 patients out of 23 were positive for HLA-DQ2 (n=2) and HLA-DQ8/DR4 alleles (n=4).

		***n* patients tested: 23**
ALLELS	***n***	**Fr (%)**
HLA-DQ2	2	9%
HLA-DQ8	4	17%

Conclusion

According to the obtained results a total of 26% of tested patients were positive for HLA-DQ2 and HLA-DQ8/DR4 alleles. The presence of HLA-DQ8 genotype was twice more frequent than HLA-DQ2. To our knowledge this is the first study evaluating the distribution of HLA-DQ genotypes in Bosnian and Herzegovinian population. However, to identify the role of HLA-DQ2 and HLA-DQ8/DR4 haplotypes in development of celiac disease in Bosnian and Herzegovinian population, a study with larger pool of clinically asymptomatic and symptomatic patients should be conducted.

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Conflict of interest

The authors state that there is no conflict of interest.

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