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Original Article (Pages: 16498-16504)

HLADQ2 and HLADQ8 Alleles Are Associated with Celiac Disease in Children

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Abstract

Background: Celiac disease is the permanent intolerance to dietary gluten, the major protein component of wheat. The role of human leukocyte antigen (HLA) DQ2 heterodimer (DQA1*0501-DQB1*0201) in presenting gluten peptides to effectors T cells in celiac disease (CD) has been well documented. Epidemiological studies of the disease in Iran are not available. This study was aimed to investigate the frequency of HLADQ2 and HLADQ8 in children with celiac disease in Mashhad city.

Methods: This case-control study was conducted on 25 celiac patients and 25 matched healthy controls for HLA typing of DQ2/DQ8. CD diagnosis was reached in 25 subjects, according to the revised criteria of the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) and North American Society for Pediatric Gastroenterology, Hepatology and Nutrition criteria (NASPGHAN). Sensitivity, specificity, and negative and positive predictive values were calculated.

Results: Mean age was 134.06±30.48 months in case and control groups, with no statistical difference between the two groups. 48% of cases and controls were male, and 52 % were female. HLA-DQ2/8 was positive with 80% (CI 95%:64-95), sensitivity was 80% (CI 95%:58-92), specificity 48% (CI 95%:28-68), NPV 70.58% (CI 95%:44-88), PPV 61(CI 95%:42.2-76.5) and accuracy was 64%.

Conclusion: A positive association was found between HLA DQ2/8 and Iranian celiac disease. As negative and predictive values were high, HLA typing may be considered a beneficial test for diagnosis confirmation.

Key Words: Celiac disease, Children, HLA-DQ2 / 8, Genotyping, Gluten, Pediatric Gastroenterology, wheat.

* Please cite this article as: Jafari SA, Chaichi Z, Hammoud M, Kianifar H, Mir F. HLADQ2 and HLADQ8 Alleles Are Associated with Celiac Disease in Children. Int J Pediatr 2022; 10 (8):16498-16504. DOI: **10.22038/ijp. 2022.64904.4908**

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Received date: Apr.12,2022; Accepted date:Jun.14,2022

1- INTRODUCTION

Celiac disease is a gastrointestinal disease that damages the villi of the small intestine and interferes with the absorption of nutrients. People with celiac disease cannot tolerate a protein called gluten, which is found in wheat, barley, rye and possibly oats. By consuming gluten, the immune system responds by destroying the small intestine. Following damage to the villi of the intestine, the patient becomes malnourished regardless of the amount of food eaten. Recurrent abdominal pain, diarrhea, weight loss, unexplained anemia, fatigue, growth retardation, bloating, muscle contractions, and seizures are known symptoms of the disease and are associated with inadequate nutrient uptake (1, 2).

DQ2 (22.1%) and DQ7 (25%) are the most common HLA haplotype in Iran(3-6). Monsour et al., Looking for a simple experimental approach using tagging SNPs that predict the CD-associated HLA risk factors, described an HLA-tagging single nucleotide polymorphism (SNP) method for detecting the HLA risk alleles for CD. Using six SNPs, HLA genotyping for specific CD genotypes is also recommended to be performed in a high-throughput mode (7). Rostami Nejad et al. reported that 97% of CD cases and 58% of controls were carriers of HLADQ2 and/or HLA DQ8 heterodimers, either in the homozygous or heterozygous state(8).

Celiac disease is an inherited disease, and is passed down from generation to generation in the family. Sometimes the disease is activated for the first time following a diet after surgery, pregnancy, birth, viral infections and mental stress. The main component of the genetic predisposition of this disease is located in the HLA region on chromosome 6 (9, 10). The disease is significantly associated with HLA class II antigens, especially DQ2 and DQ8, and accounts for approximately 90% of the specific DQ2 alpha / beta heterodimer disease encoded by the DQA1 and DQB1 alleles (11, 12). However, HLA alleles explain only part of the genetic predisposition to celiac disease. In most European populations, the incidence of DQ2 is high, around 15 to 30%, but a small number of these people develop the disease (13).

Arthropathy in celiac disease is caused by immune damage to intestinal mucosal cells and tissue transglutaminase plays an important role in the immune response and is the most important autoantigen (14, 15). In addition to other functions, it can denature glutamine residues, and negatively charged deamination peptides are a high affinity for HLA-DQ2 and DQ8, which play a central role in the immune response in celiac disease (16).

Considering the role of genetics and environment in the development and prevalence of celiac disease in some families (17-19) and the association between some HLA antigens reported in previous studies and this disease (20, 21), it seems necessary to determine the type of HLA in celiac patients in Iran for identifying and treating these patients in early stages. Therefore, the present study was designed to compare the prevalence of HLA among children with celiac disease with healthy counterparts and specify the diagnostic value of HLA typing in the disease.

2- METHODS

2-1. Participants

In this case-control study, 25 children diagnosed with celiac based on serology and pathology tests and 25 age and sex-matched healthy kids were enrolled. A questionnaire including demographic and clinical information was completed for all participants.

CD was diagnosed on the basis of characteristic clinical symptoms, serological investigations and positive

result of small-bowel biopsy; healthy controls were tested on the basis of clinical symptoms and serological investigations, without small-bowel biopsy.

2-2. Genotyping

A 3 ml blood sample was collected in free-DNA EDTA tubes. DNA extraction was done using a BIOTECH DAN extraction kit and stored in -20 ◦C.

HLA typing was done using the PCR-SSP method and 24 primers for HLA-DRB1*04, HLA-DRB1*07, HLA-DRB1*03, HLA-DRB1*11, HLA-DQB1*0201-05, HLA-DQB1*0301-0314, HLA-DQA1*02-08, HLADQA1*03, HLADQA1*05 alleles. Electrophoresis of the PCR products was done on a 2% agarose gel.

2-3. Statistical analysis

Variables were described as number (frequency) and mean (SD), and comparisons between groups were performed using the chi-square or Fisher's exact tests and T-test for categorical and continuous variables, respectively. Data were analyzed using SPSS v16. A P-value less than 0.05 was considered significant.

3- RESULTS

The mean age of diagnosis was 6.76±1.37 months in celiac patients. The mean age of celiac and healthy groups was 135.52±29.25 and 132.60±32.19 months, respectively. Moreover, each group included 12 boys and 13 girls. There was no significant difference in age and sex between groups (p=0.074 for age and p=1.00 for sex).

Several symptoms were identified in patients including diarrhea (72%), constipation (4%), stomach ache (68%), vomit (72%), weight loss (96%), no weight gain (100%), and short stature (96%).

HLA typing indicated a significant difference in HLA-DQ2 between groups as it was observed in 18 (72%) celiac patients and 7 (28%) healthy subjects (p=0.004). HLA-DQ8 was also observed in 2 (8%) patients and 6 (24%) healthy controls (**Table 1**).

Table-1: The distribution of HLA-DQ2/8 between celiac and healthy subjects

HLA type	Celiac patients, n (%)	Healthy subjects, n (%)
HLA-DQ2	18 (72)	7 (28)
HLA-DQ8	2 (8)	6 (24)
Positive for HLA-DQ2/8	20 (80)	13 (52)
Negative for HLA-DQ2/8	5 (20)	12 (48)

HLA-DQ2/8 was positive for 80% 0f celiac patients and 52% of healthy people. Based on these observations, the sensitivity and specificity of the test were 80% and 48%, respectively. Positive predictive value (PPV) was 61% CI 95% (43.68-73.31), and negative predictive value (NPV) was 70.58 CI 95% (46.86-86.72). Furthermore, the accuracy of the test was 64%.

4- DISCUSSION

The overall prevalence of celiac disease based on serological and biopsy tests has been reported as 3% and 2%, respectively, in Iran which is similar or even higher than the worldwide reports (22). Identifying patients at early stages is critical to treatment procedures and the management of the disease. The pathogenesis of the disease is associated

with the HLA-DQ2 and HLA-DQ8 alleles. However, there is not enough data on the distribution of HLA alleles predisposing the risk of children with celiac. Therefore, this study was conducted to evaluate the prevalence of HLA-DQ2/8 in celiac children for the first time in Iran and the diagnostic value of HLA typing in the disease. Our findings indicated a significantly higher rate of HLA-DQ2 in celiac children than healthy controls while HLA-DQ8 presented lower frequency. HLA-DQ2/8 testing represented a sensitivity, specificity and accuracy of 80%, 48%, and 64%, respectively.

The present study revealed a frequency of 72% for HLA-DQ2 in Iranian celiac children. A previous study on 49 Syrian pediatric patients reported an almost similar distribution as 77.6% of patients represented HLA-DQ2, compared to eight healthy controls with no history of CD, cancer or autoimmune diseases and negative for CD serological screening (23). Another study conducted on Turkish celiac children indicated a frequency of 67% for HLA-DQ2 (24). The reported rate of HLA-DQ2 in Turkish patients was a little lower than that of the Iranian and Syrian subjects. The high incidence of HLA-DQ2 has been reported in the European population with a range of 70% to 100%. However, there is a report indicating a prevalence of 45.2% in Moroccan celiac patients. A part of discrepancies between studies may result from the different genotyping methods as well as diversity in the genetic background related to the ethnicity of the considered population. However, it is clearly obvious that HLA-DQ2 is a determining factor for the risk of celiac in children.

In the present study, children with celiac also represented a frequency of 8% for HLA-DQ8 that was lower than that in the healthy individuals. The prevalence of HLA-DQ8 has been reported to be in the range of 0.6% to 25% in different studies (25-27). Similar to our results, HLA-DQ8 was observed in 10.2% of Syrian children suffering from celiac (23). Conversely, the prevalence of HLA-DQ8 in Turkish children suffering from celiac was 25%, which is higher than that among the population of the present study (23, 24). Another study investigated HLA-DQ2/DQ8 frequency in adult patients with celiac disease, their first-degree relatives, and normal population in Turkey. It revealed a significant relationship between HLA-DQ2/DQ8 presences in all groups (28).

These findings indicated the variety of HLA allele's distributions in the word. Therefore, it is crucial to evaluate the genetic pool of different population and design studies to find the diagnostic genetic panels of the disease.

The rate of patients with a negative test of HLA-DQ2/8 was 20% in the present study. Similarly, a study on Turkish children reported a rate of 24% for negative HLA-DQ2/8 in patients (24). Furthermore, 32.5% of Italian celiac patients were negative for this region (29). Inconsistent with our findings, considering Libyan children with celiac indicated a negative rate of 3% for HLA-DQ2/8 (30). Moreover, there is some evidence indicating DQ2-DQ8-negative in a low number of celiac patients (25, 26, 31). 100% of Czechoslovakia and Spain Celiac patients indicated DQ2-DQ8 A (32, 33). France, England, Norway, Finland and Brazil had a negative rate ranging from zero to 6.5% (31).

However, some of these studies have been conducted on celiac without considering age and other comorbid diseases. Moreover, based on the prevalence of HLA-DQ2/8 in celiac subjects compared to the controls, a negative predictive value was calculated at 70.58%. However, this value was almost 100% in other studies confirming the main role of HLA-DQ typing in the diagnosis of celiac (34-36).

On the other hand, negative HLA-DQ2/8 indicated that in addition to the HLA-DQ, other loci may be involved in the pathogenesis of the inherited celiac disease.

Considering celiac symptoms, lack of weight gain as well as weight loss were observed almost in all cases of the present study. Growth retardation has been reported in previous studies as the main complaints of celiac patients (37, 38). Moreover, we found diarrhea in 72% of patients that have been previously reported in 50% of celiac children, ranging from 30 to 65% (37, 39).

Inconsistent with these results, a previous study reported it in 3.2% of patients (37).

It has been confirmed that the age of celiac cases influences symptoms and outcomes of the disease as in a study conducted by Rodrigo et al., diarrhea was observed in 65% of celiac children and 26% 0f adult cases (29, 40).

5- CONCLUSION

In the present pilot study, investigating the distribution of HLA-DQ2/8 genotypes, provided information on the genetic background of the Iranian pediatric patients with Celiac disease, for the first time. Moreover, the diagnostic value of HLA-DQ2/8 was considered. HLA-DQ2 was found to have a significantly higher rate in the children suffering from celiac than in the controls that represented the possible important role of HLA-typing to diagnose new patients at early ages and begin the management procedures in early stages. However, our results should be cautiously interpreted and further studies in different parts of Iran and various ethnic groups should be implemented to confirm these findings.

6- ETHICAL CONSIDERATIONS

The study was approved by the ethics committee of Mashhad University of Medical Sciences and a consent form was assigned by parents.

7- ACKNOWLEDGEMENT:

We appreciate the assistance of the Clinical Research Development Unit of Akbar Hospital in performing this research.

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