HLA-DQ2 and HLA-DQ8 Genotyping in a Sample of Iranian Celiac Patients and Their First-Degree Relatives

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Background:
Recent studies have shown a critical role for HLA-DQ2 and HLA-DQ8 in the pathogenesis of celiac disease. No study has been performed on the prevalence of these two HLA types in Iranian celiac patients.

Materials and Methods:
We enrolled 24 celiac patients and 37 first-degree relatives in whom the diagnosis of celiac was excluded by serologic tests. HLA typing for HLA-DQ2 (DQB1*02), HLA-DQ8 (DQB1*03), HLA-DQ B1*05 and HLA-DQ B1*06 was performed using polymerase chain (PCR) reaction.

Results:
Twenty two (91.7%) celiac patients and twenty seven (73%) controls were positive for the HLA-DQ2 and/or HLA-DQ8 heterodimers. There was no significant difference between the two groups (p=0.068). However, celiac patients were statistically more positive for homozygote HLA-DQ2, whereas non-celiac participants were more positive for homozygote HLA-DQ8 (p<0.05).

Conclusion:
The total prevalence of HLA-DQ2 and/or HLA-DQ8 alleles did not significantly differ between the two groups. Hence, first-degree relatives of celiac patients appear to be more susceptible for developing celiac disease. On the other hand, the higher prevalence of homozygote HLA-DQ2 in celiac patients shows its stronger role in disease pathogenesis. Further studies on larger populations are needed in Iran.

Keywords: Celiac disease; HLA-DQ2; HLA-DQ8; Iran; HLA typing; Disease risk

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INTRODUCTION
Celiac disease is a common food-induced enteropathy that affects approximately 1% of the world’s population(1). The disease is screened by serologic exams and the diagnosis is confirmed by a small intestinal biopsy(2,3). However, the sensitivity of serologic tests varies among different laboratories and never reaches 100%(2,4,5). Changes in a small intestinal biopsy are not specific for celiac disease; additionally, the biopsy may be taken from sites without histologic changes (false negative)(6).
Although the disease is triggered by ingestion of gluten, studies have shown that it occurs in genetically susceptible individuals(7). Recent studies have revealed a strong association of celiac disease with HLA class II genes, known as HLA-DQ2 and HLA-DQ8(8). HLA-DQ molecules present gluten peptides to antigen-specific CD4-positive T-lymphocytes, inducing inflammatory responses and ultimately causing the disease(9,10).

There are two types of HLA-DQ2, termed as HLA-DQ2.5 and HLA-DQ2.2. The earlier type has the strongest association and is coded by HLA-DQA1_0501 and HLA-DQB1_0201. HLA-DQ8 is coded by HLA-DQA1_301 and HLA-DQB1_0302(11-16). In patients with atypical histologic changes, HLA-genotyping can be useful since the absence of both HLA types can virtually exclude the diagnosis of celiac disease(17).

Although the prevalence of celiac disease has been investigated by different studies in Iran, no study has assessed HLA-genotyping in Iranian celiac patients. Therefore, we designed a study to investigate the prevalence of HLA-DQ2 and HLA-DQ8 molecules in a population of celiac patients in Zahedan, which is located in Southeast Iran.

MATERIALS AND METHODS
This descriptive study enrolled 24 patients with celiac disease who referred to a Gastroenterology Clinic in Zahedan during 2009-2011. The diagnosis of celiac disease was made according to a positive anti-tissue transglutaminase (anti-tTG) IgA plus villous atrophy on duodenal biopsy (modified Marsh classification: III)(18). The control group comprised 37 first-degree relatives in whom the diagnosis of celiac disease was ruled out by negative anti-tTG IgA and negative anti-gliadin IgG.

The exclusion criteria were history of any autoimmune disease and type I diabetes mellitus. All participants were informed about the study and written consents were obtained from both the patients and the control group. The protocol was approved by the Ethical Committee of Zahedan University of Medical Sciences.

In order to perform HLA-genotyping, 2ml of venous blood was drawn from all participants. DNA was extracted by a rapid genomic DNA extraction procedure and stored at -20°C. HLA-genotyping for all participants was performed using polymerase chain reaction (PCR) to determine the prevalence of HLA-DQ2 (DQB1*02) and HLA-DQ8 (DQB1*03). At the same time, HLA-DQB1*05 and HLA-DQB1*06 were also genotyped as the wild-type genotypes.

Data were analyzed using SPSS software (version 11.5) and the t-test was used as appropriate. p-values less than 0.05 were considered statistically significant.

RESULTS
All participants completed the study. According to the results, there were significantly more celiac patients (45.8%) positive for homozygote HLA-DQ2 than non-celiac participants (8.1%; p<0.05). Non-celiac participants were statistically more positive for homozygote HLA-DQ8 (13.6%) compared to celiac patients (0%; p<0.05).

However, the total prevalence of HLA-DQB1*02 and/or HLA-DQB1*03 alleles did not show a significant difference between the groups. Hence, 91.7% of celiac patients and 75.0% of non-celiac participants had at least one HLA-DQB1*02 or HLA-DQB1*03 allele (p<0.05; Table 1).

Table 1: HLA genotyping of celiac and non-celiac participants for HLA-DQ2 and HLA-DQ8.

<table>
<thead>
<tr>
<th>HLA typing</th>
<th>Celiac patients (%)</th>
<th>Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1<em>02/ B1</em>02</td>
<td>11 (45.8)</td>
<td>3 (8.1)</td>
</tr>
<tr>
<td>B1<em>03/ B1</em>03</td>
<td>0 (0)</td>
<td>5 (13.6)</td>
</tr>
<tr>
<td>B1<em>02/ B1</em>03</td>
<td>3 (12.5)</td>
<td>4 (10.8)</td>
</tr>
<tr>
<td>B1<em>02/ B1</em>05/06</td>
<td>4 (16.7)</td>
<td>11 (29.7)</td>
</tr>
<tr>
<td>B1<em>03/ B1</em>05/06</td>
<td>4 (16.7)</td>
<td>4 (10.8)</td>
</tr>
<tr>
<td>Others</td>
<td>2 (8.3)</td>
<td>10 (27)</td>
</tr>
</tbody>
</table>

DISCUSSION
According to our results, the total prevalence of HLA-DQ2 and/or HLA-DQ8 did not show any significant difference between those with celiac disease and their first-degree relatives. It was somewhat expectable, since the role of genetic susceptibility in the pathogenesis of celiac has been completely proven(4,19, 20).

The lack of a difference between these groups showed a higher risk for the development of celiac disease among first-degree relatives. Approximately 10% of first-degree relatives of celiac patients have been shown to suffer from this disease(4,21).

Megiorni et al. performed a large study in Italy to type HLA DR/DQ genes in 437 celiac patients and 834 first-degree relatives. According to the results 91% carried DQ2 and/or DQ8 heterodimers and...
approximately 1% were DQ2/DQ8/β2/α5 negative. The prevalence of high-risk HLA molecules among family members of celiac patients was 57% (sisters), 71% (brothers) and 58% (parents). However, 17.6% of sisters, 10.8% of brothers and 3.4% of parents were found to have celiac disease(22).

Most patients with celiac disease carry HLA-DQ2 and the remainder carry HLA-DQ8 or at least a heterodimer of HLA-DQ2 such as DQ B1*0201 or DQ A1*0501(15, 23). However, according to our results, 2 (8.3%) of the celiac patients did not carry either of the two alleles. This could be attributed to not assessing the other heterotypes of HLA-DQ2 or HLA-DQ8 (such as DQ A1*0501 or DQ A1*03).

According to the results of a large study performed on 1008 celiac patients in Europe, 6% of patients were homozygotes for HLA-DQ8 and did not carry any HLA-DQ2 alleles(15). According to our results, no patient was a homozygote for HLA-DQ8 and approximately 29% were heterozygotes for DQ B1*03 (a heterotype of HLA-DQ8). This might have been due to the small number of our patients or due to not assessing the other heterotypes of HLA-DQ8.

One of the main limitations of our study was the small number of our patients. This was mainly the result of conducting this study in a localized geographic area. The second limitation was the unavailability of laboratory facilities to assess other heterotypes of HLA-DQ2 and HLA-DQ8.

In conclusion, according to our results, the first-degree relatives of celiac patients are genetically more susceptible to celiac disease. Further studies on larger group of patients and their relatives that assess other heterotypes of HLA-DQ2 and HLA-DQ8 are needed in this geographic area. Additionally it seems better to choose a third group of non-celiac participants who are not relatives of the celiac patients as the control group.

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