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Human Leukocyte Antigen-DQ Genotyping in Pediatric Celiac Disease

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ABSTRACT

Purpose: The purpose of this study was to determine the pattern of human leukocyte antigen (HLA)-DQ genotype in children diagnosed with celiac disease (CD) (biopsy proven), and to compare this with a control group; and secondarily, to correlate HLA genotypes with clinical profiles of CD.
Methods: This cross-sectional comparative observational study included 26 controls and 52 patients diagnosed with CD who presented at Sir Padampat Mother and Child Health Institute, Jaipur, from May, 2017 to October, 2018. HLA DQ genotype was assessed for each patients and correlated with clinical profiles.
Results: HLA DQ2/DQ8 genotypes were significantly more common in CD (present in 100.0% cases) than in controls (23.1%) in Northern India (Rajasthan). When HLA DQ2.5 and DQ8 were present together, individuals had significantly more atypical presentations and severe findings on duodenal biopsy. Similarly, patients with the HLA DQ 2.5 genotype were also predisposed to more severe endoscopic findings, while HLA DQ2.2 predisposed them to less severe biopsy findings. HLA DQ8 was significantly associated with later age at diagnosis (>5 years) and shorter stature. The highest HLA DQ relative risk (RR) for CD development was associated with HLA DQ2.5 and DQ2.2 in combination, followed by HLA DQ2.5 and DQ8 in combination, while HLA DQx.5 and HLA DQ2.2 together had the lowest risk.
Conclusion: HLA DQ2/DQ8 genotypes are strongly associated with pediatric CD patients in northern India. These genotypes and their combinations may be associated with different clinical presentations of CD, and may help predict severity of CD.

Keywords: Human leukocyte antigen-DQ; Genotype; Celiac disease; Children

INTRODUCTION

Celiac disease (CD) is defined as an immune-mediated systemic disorder that is elicited by gluten and related prolamins in genetically susceptible individuals and characterized by varying combinations of gluten-dependent clinical manifestations, CD-specific antibodies, human leukocyte antigen (HLA)-DQ2 or HLA-DQ8 haplotypes, and enteropathy [1]. Worldwide, the disease affects approximately 1% of the general population, though the prevalence varies between countries [2].

HLA proteins are encoded by highly polymorphic HLA-DQ genes located at the HLA class II loci on the short arm of chromosome 6 (6p21.3) [3]. DQ2- or DQ8-restricted CD4 T cells' response to gluten is seen in CD. Studies from the Western world suggest that 30–35% of the general population express CD-associated HLA genotypes [4-6]. The prevalence of HLA-DQ2 in north Indian populations is around 16–31% while that of DQ8 is around 0–5% [7]. Over 90% of CD patients express the HLA-DQ2.5 heterodimer (DQA1*05 and DQB1*02). Most of the remaining cases are HLA DQ8 (DQA1*0301 and DQB1*0302)-positive [8]. The majority of DQ2.5/DQ8-negative celiac patients (about 5%) present DQ2.x molecules. Very rarely, CD patients can carry different DQ molecules, viz., DQX.5 and DQX.x [9,10].

A recent guideline by European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) 2012 proposed that it may be possible to avoid intestinal biopsies in children with characteristic symptoms of CD, transglutaminase (TGA IgA) levels >10×upper limit of normal (ULN, confirmed by positive endomysial antibodies [EMA] in a different blood sample), and who are HLA-DQ2/DQ8-positive [11]. In asymptomatic children at increased risk for CD, HLA-DQ2 and HLA-DQ8 testing is valuable because CD is unlikely if both haplotypes are negative. HLA testing is also useful for patients with an uncertain diagnosis of CD, such as those with negative CD-specific antibodies and mild infiltrative changes in proximal small intestinal biopsy specimens [1]. However, the ESPGHAN 2020 guidelines recommend that HLA DQ2 and DQ8 typing is not required in TGA-IgA-positive patients if they qualify for CD diagnosis with biopsy or have high serum TGA-IgA (≥10×ULN) and EMA-IgA positivity. HLA DQ2/DQ8 typing is useful in patients as risk of false negatives in serology [12].

The high negative predictive value (NPV) of HLA typing tests, however, indicates that the absence of HLA-DQ2/DQ8 may exclude the possibility of future development of CD with almost 100.0% certainty [6,13].

There are very few studies on HLA-DQ genotyping in India [11,14]; we could not find any study from India correlating HLA haplotypes with clinical profile of CD.

MATERIALS AND METHODS

This hospital-based cross-sectional comparative observational study was conducted in the Department of Pediatric Medicine, SPMCHI, SMS Medical College, Jaipur, and in the Advance Haematology and HLA Laboratory, SMS Medical College, Jaipur from May 2017 to October 2018. The study design was approved by the Institutional Ethics Committee of Sawai Man Singh Medical College (Approval No. 57773/18.12.2017), Jaipur.

The minimum sample size was determined by considering the 95% confidence intervals and 80% powers reported in previous studies [1]. Hence, for the present study, we planned to include 25 individuals in the study group and 25 in the control group; however, the final sample comprised 52 cases and 26 controls.

Children aged 1–18 years with CD-positive histopathology as per the modified ESPGHAN criteria (Marsh stages II and III); attending pediatric out patient department, a gastroenterology clinic, or admitted as in patient department in Sir Padampat Mother and Child Health Institute were prospectively enrolled after obtaining informed written consent

from their parents. Their history and physical examination results, including anthropometric data and histopathology of intestinal biopsies were analyzed. Healthy children (gender matched) with age-appropriate weight and height were selected randomly from general population, and those with no family history of CD and serology negative for CD were enrolled as controls, after obtaining informed written consent from their parents.

Both cases and controls were HLA-DQ genotyped. For the HLA-DQ study, 5–6 mL of peripheral blood from each patient was collected in EDTA vials, which underwent manual DNA extraction. The DNA was then quantified with the NanoDrop at a wavelength of 260/280 (<2 in 260; >2 in 280), and then kept on allele quoted trays. Subsequently, the samples underwent the polymerase chain reaction and electrophoresis, and a gel documentation system reported the alleles present using specialized software. Bag Health Care kit with four sets of alleles was used. Eleven alleles in total were reported by the Bag Health Care kit. The four sets of alleles used are as follows:
- DRB1*03-DQA1*05:01-DQB1*02:01
- DRB1*07-DQA1*02:01-DQB1*02:02
- DRB1*11-DQA1*05:05-DQB1*03:01
- DRB1*04-DQA1*03:01-DQB1*03:02

All data was compiled into a master chart using Microsoft Excel 2007 (Microsoft, Redmond, WA, USA); qualitative data was expressed as percentage and proportions, and quantitative data was expressed as mean and standard deviation. All scale variables were compared using a two-sample independent t-test. Fisher's exact test, a two-sample Z-test for proportions, and Pearson's Chi square test were used for qualitative data, and a two-sample independent t-test was used for quantitative data. The level of significance was kept 5% for all statistical analyses. A p-value less than 0.05 was considered statistically significant. All data were analyzed using PASW Statistics for Windows, Version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

This study included 52 CD-positive individuals, of which 32 were males (male:female ratio: 1.6:1). The mean age was 7.86 years. Pallor was most common finding at physical examination upon presentation (63.5%), followed by abdominal distension (61.5%), short stature (50.0%), diarrhea (44.2%), chronic abdomen pain (42.3%), failure to thrive (38.5%), constipation (21.2%), recurrent vomiting (9.6%), and delayed puberty (3.8%) (**Table 1**).

Table 1. Clinical presentation in Celiac disease in children

Clinical presentation	Male (n=32)	Female (n=20)	Total (n=52)
Abdominal distension	21 (65.6)	11 (55.0)	32 (61.5)
Diarrhoea	11 (34.4)	12 (60.0)	23 (44.2)
Failure to thrive	15 (46.9)	5 (25.0)	20 (38.5)
Pallor	21 (65.6)	12 (60.0)	33 (63.5)
Short stature	17 (53.1)	9 (45.0)	26 (50.0)
Chronic abdominal pain	15 (46.9)	7 (35.0)	22 (42.3)
Constipation	6 (18.8)	5 (25.0)	11 (21.1)
Recurrent vomiting	4 (12.5)	1 (5.0)	5 (9.6)
Delayed puberty	1 (3.1)	1 (5.0)	2 (3.8)

Values are presented as number (%).

Table 2. HLA distribution among cases (n=52) and controls (n=26)

HLA genotype	HLA* distribution		
	Cases (n=52)	Controls (n=26)	Total (n=78)
HLA-DRB1*03-DQA1*05:01-DQB1*02:01 (DQ2.5)	29 (55.8)	3 (11.5)	32 (41.0)
HLA-DRB1*07-DQA1*02:01-DQB1*02:02 (DQ2.2)	2 (3.8)	3 (11.5)	5 (6.4)
HLA-DRB1*11-DQA1*05:05-DQB1*03:01 (DQx.5)	0 (0.0)	0 (0.0)	0 (0.0)
HLA-DRB1*04-DQA1*03:01-DQB1*03:02 (DQ8)	4 (7.7)	0 (0.0)	4 (5.1)
HLA-DRB1*03-DQA1*05:01-DQB1*02:01+HLA-DRB1*07-DQA1*02:01-DQB1*02:02 (DQ2.5+DQ2.2)	8 (15.4)	0 (0.0)	8 (10.3)
HLA-DRB1*03-DQA1*05:01-DQB1*02:01+HLA-DRB1*11-DQA1*05:05-DQB1*03:01 (DQ2.5+DQx.5)	2 (3.8)	0 (0.0)	2 (2.6)
HLA-DRB1*03-DQA1*05:01-DQB1*02:01+HLA-DRB1*04-DQA1*03:01-DQB1*03:02 (DQ2.5+DQ8)	7 (13.5)	0 (0.0)	7 (9.0)
Total	52 (100.0)	6 (23.1)	58 (74.4)
Pearson chi-square	Value	df	p-value
	60.366	6	0.000

Values are presented as number (%).
HLA: human leukocyte antigen.

Regarding clinical presentation of CD, 40 patients (76.9%) presented with typical disease and the remaining 12 (23.1%) presented with atypical symptoms. Thus, typical CD presentations outnumbered atypical presentations. Abdominal distension was the most common typical symptom, while pallor was the most common atypical manifestation, followed by short stature.

One hundred percent of cases were positive for the HLA-DQ2/DQ8 genotype, in contrast to only 23.1% of controls; this difference was statistically significant (p=0.000). Among CD-positive cases, HLA-DQ2.5 (55.8%) was most common, followed by HLA-DQ2.5 and DQ2.2 in combination (15.4%), HLA-DQ2.5 and DQ8 in combination (13.5%), HLA-DQ8 (7.7%), HLA-DQ2.2 (3.8%), HLA-DQ2.5 and DQx.5 in combination (3.8%). HLA-DQx.5 alone was not present in any of the participants. The controls carried only HLA-DQ2.5 (11.5%) and HLA-DQ2.2 (11.5%), with equal proportions (**Table 2**). HLA-DQ2 alone or in combination was carried by 92.3% of CD-positive cases, while HLA-DQ8 was carried by 7.7% of cases. HLA-DQ8 alone or in combination was present in 21.1% of cases.

The highest HLA-DQ relative risk (RR) for CD development was found in patients with HLA-DQ2.5 and DQ2.2 in combination (RR=8.660), followed by HLA-DQ2.5 and DQ8 in combination (RR=7.642), HLA-DQ2.5 (RR=4.833), HLA-DQ8 (RR=4.585), and HLA-DQ2.5 and DQx.5 in combination (RR=2.547). The minimum RR was associated with HLA-DQx.5 (RR=0.509) and HLA DQ2.2 (RR=0.333) (**Table 3**). The HLA-DQ genotype testing sensitivity was 100.0% and the specificity was 76.9%. Moreover, the positive predictive value of the test was 89.7%, while the NPV was 100.0%.

HLA-DQ2.5 and DQ8 genotype pattern in combination was significantly associated with atypical presentation (p=0.041). The HLA-DQ8 genotype was significantly associated with late age (>5 years) at diagnosis (p=0.034) and short stature (<−2standard deviation [SD]) (p=0.031). HLA-DQ2.5 and DQ8 in combination was significantly associated with normal

Table 3. HLA genotypes and HLA relative risk

Risk category	HLA genotypes	Relative risk
High	HLA-DQ2.5 and DQ2.2	8.660
	HLA-DQ2.5 and DQ8	7.642
Intermediate	HLA-DQ2.5	4.833
	HLA-DQ8	4.585
	HLA-DQ2.5 and DQx.5	2.547
Low	HLA-DQx.5	0.509
	HLA-DQ2.2	0.333

HLA: human leukocyte antigen.

weight for age/underweight (*p*=0.003), where normal weight is defined as +2SD to –2SD, underweight as –2SD to –3SD, and severe underweight as <–3SD. HLA DQ2.5 and DQ2.2 in combination had a higher proportion of patients with moderate anemia than mild/no anemia (23.1% vs. 12.82%), but the difference was not statistically significant (*p*=0.396). (Hemoglobin levels as per WHO standards: 6–59 months: mild (10–10.9), moderate (7–9.9), severe (<7); 5–11 years: mild (11–11.4), moderate (8–10.9), severe (<8); 12–14 years: mild (11–11.9), moderate (8–10.9), severe (<8); >15 years female (non-pregnant): mild (11–11.9), moderate (8–109.9), severe (<8); >15 years male: mild (11–12.9), moderate (8–10.9), severe (<8)). The HLA-DQ2.5 genotype was more frequently carried by cases with typical presentation than those with atypical presentation (62.5% vs. 33.3%), but this difference was not statistically significant (*p*=0.062). HLA-DQ2.5 alone and HLA-DQ2.5 and DQ8 in combination were significantly associated with Marsh stage IIIB/C (*p*=0.049 and 0.004, respectively) i.e., more severe duodenal findings, while HLA-DQ2.2 was significantly (*p*=0.034) associated with less severe Marsh stage II/IIIA.

DISCUSSION

In our study, the HLA-DQ2/DQ8 genotype was carried by 52 celiac disease (CD)-positive cases (100.0%) while it was present only in 26 controls (23.1%) (*p*=0.000). These findings are similar to those of several previous studies [11,15,16]. Ramakrishna et al. [17] estimated the prevalence of CD-associated HLA-DQ2/DQ8 in three populations in India: Northern (38.1%), North-Eastern (31.4%) and Southern India (36.4%). Across various studies, HLA-DQ2/DQ8 serotypes have been reported in 13–30% of different populations in India [14]. The allele prevalence of HLA-DQB1*02 in northern India ranged from 16–31%, while that of HLA-DQB1*0302 ranged from 0–5% which is in accordance with our results [7]. The significant differences in the frequency of HLA-DQ2 and HLA-DQ8 alleles in Indian (Rajasthan) patients compared with the controls demonstrates the importance of these alleles in CD development, and supports the possibility of using HLA-DQ typing in confirming CD diagnosis. We found that the HLA-DQ genotype patterns of CD-positive individuals and controls are similar between the European population and that of the northern India [6,9,10,18].

In our study, HLA-DQ2.5, HLA-DQ2.2, and HLA-DQ8 alone or in combination were found in 88.5%, 19.2%, and 21.1% of patients, respectively, while HLA-DQ2.5 and DQ2.2 and HLA-DQ2.5 and DQ8 in combination were present in 15.4% and 13.5% patients, respectively, similar to the findings of Rostami-Nejad et al. [19] and Murad et al. [16]

Khosravi et al. [20] observed that DQ2 and DQ8 were positive in 80% and 49% of CD patients, and 36% and 13% of control group participants, respectively. Likewise, we found that DQ2 and DQ8 were carried by 92.3% and 21.1% patients. However, the positivity rates for DQ2 and DQ8 in control group (23.1% and 0.0%, respectively) in our study were comparatively lower than those reported by Khosravi et al. [20]. Murad et al. [16] also found results similar to our study. Moreover, HLA-DQ2.5, HLA-DQ8, HLA-DQ2.5 and DQ2.2 in combination and HLA-DQ2.5 and DQ8 in combination showed a very strong significant association with CT-positive patients compared with controls in our study (*p*=0.000; 0.037; 0.002, and 0.004, respectively).

Murad et al. [16] found that the highest HLA-DQB RR for CD development was seen in patients carrying the DQ2.5/DQ8 genotype (1/10), while, patients carrying the DQ2.5/

DQ2.5 or DQ2.5/DQ2.2 genotypes had relative risks of about 1/12.5 and 1/20, respectively However, in our study the highest HLA-DQ RR for CD development was found in patients carrying HLA-DQ2.5 and DQ2.2 in combination (RR=8.660), followed by HLA-DQ2.5 and DQ8 (RR=7.642), while the minimum RR was associated with HLA-DQx.5 (RR=0.509) and HLA-DQ2.2 (RR=0.333). Romanos et al. [21] reported a higher risk of CD in individuals homozygous for the HLA-DQ2.5 or HLA-DQ2.5/DQ2.2 genotypes, compared with those homozygous for HLA-DQ2.2 or heterozygous for HLA-DQ2.5 or DQ2.2.

When analyzing the relationship between HLA genotype pattern and clinical presentation of CD, we found a significant association of HLA DQ2.5 and DQ8 in combination with atypical presentation; HLA-DQ2.5 and DQ8 in combination were also associated with normal/ underweight patients, while severe underweight and HLA-DQ8 were associated with later age at diagnosis of CD (>5 years) and short stature. Congia et al. [22] suggested that a combination of the DQA1*0501 and DQB 1*0201 genes may predispose a person to earlier onset and more severe manifestations of CD. Zubillaga et al. [23] concluded that DQ2 homozygosity was significantly associated with female sex, earlier age at diagnosis, and shorter delay between onset of symptoms and diagnosis. Due to the lack of quantitative analysis of alleles in our study, the association of HLA-DQ with age and sex could not be established.

In a study by Murad et al. [16], the frequencies of CD patients with Marsh I and Marsh II-carrying DQ2 alleles were 9.5% and 23.8% respectively, while it reached 4.8% in CD patients carrying DQ8. On the other hand, Marsh III score was more than five-fold higher in CD patients carrying DQ2 (52.4%) compared to CD patients carrying DQ8 (9.5%), indicating a high positive association of the DQ2 allele with Marsh III in CD patients. However, in our study, 98.1% cases had Marsh III stage on duodenal biopsy. Also, HLA-DQ2.5 and HLA-DQ2.5 and DQ8 in combination were significantly (p=0.049; and 0.004, respectively) associated with Marsh stage IIIB/C (i.e., more severe duodenal mucosal atrophy), while HLA-DQ2.2 was significantly associated (p=0.034) with lower severity Marsh stages (II/ IIIA). Rostami-Nejad et al. [19] reported that patients with Marsh I or II histology carry low HLA-risk alleles, such as HLA-DQ8, although no statistically significant correlations were detected. Despite an intensive literature search, we could not find any published studies correlating HLA-DQ genotypes with clinical features of CD. Few researchers have correlated HLA-DQ genotype with age of onset and gender in CD patients [22,23].

The primary limitation of this study was the low number of patients and controls in the study population. Moreover, histology was not assessed in the controls for ethical reasons. In addition, we could only perform qualitative assessment of HLA-DQ genotype patterns, and could not compare the effects of gene dosage quantitatively. As the size of the control group in our study was relatively small, our results relating to HLA-DQ2/DQ8 typing in the healthy population cannot be extrapolated to the whole population of a given geographical area. Further studies in this area are required in future to determine the exact proportion of individuals in the healthy general population carrying these genotypes.

The data presented in this study will facilitate the first steps towards defining the genetic patterns HLA in the Indian population with CD, and provide information that can be used in family screening, which will aid risk profiling of children, so that those requiring close follow-up and monitoring can be identified. This test can be used for screening of family members of CD patients, as well as individuals and members of families with other immune-related disorders, such as diabetes mellitus and autoimmune thyroiditis, as they have high

risk of developing CD [21]. As HLA-DQ2/8 are present in all cases of CD but only 23.1% of controls, HLA-DQ2/8 typing may be help rectify the diagnosis when a gluten-free diet is started before performing small-bowel biopsies in cases of suspected CD. Moreover, negative HLA test result may have positive psychological impact since the individual feel reassured that they have a very low CD risk. In addition, for some patients with positive serology but findings of Marsh stage 0 or I, HLA typing may be useful for the diagnosis of CD, as histology may produce a false negative in rare cases.

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