ORIGINAL ARTICLE

HLA-DR alleles among Pakistani patients of coeliac disease

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Abstract

Objectives: To investigate whether certain DR alleles might also contribute to the genetic susceptibility among Coeliac disease patients in Pakistan.

Methods: The case-control study was conducted at the Military Hospital, Rawalpindi, from October 2011 to January 2012, and analysed 25 children diagnosed to have coeliac disease as per the criteria set by the European Society of Paediatric Gastroenterology and Nutrition, which included histopathological alterations in duodenal biopsies, clinical response to gluten withdrawal, and presence of anti-endomyseal antibodies. Patients were compared with a group of 150 healthy subjects. Dioxyribonucleic acid was extracted from peripheral blood collected in ethylenediaminetetraacetic acid.K3. Human leukocyte antigen DRB1 typing was carried out on allele level (DRB1*01 — DRB1*16) using sequence specific primers. Human leukocyte antigen type was determined by agarose gel electrophoresis and results were recorded. Phenotype frequency of various alleles among the patient group and the control group was calculated by direct counting, and significance of their association was determined by Fisher Exact Test.

Results: A total of 11 (44%) female paediatric coeliac patients in age range 1-9 (mean 7.2±4.8 years) and 14 (56%) male paediatric patients in the age range 6-14 (mean 8.6±5.1 years) were genotyped for HLA-DRB1 loci. A statistically significant positive association of the disease with HLA-DRB1*03 (n=23; 92% versus n=31; 21% in controls, p <0.01) was observed.

Conclusion: HLA-DRB1*03 is associated with increased risk of developing coeliac disease.

Keywords: Coeliac disease, Human leukocyte antigen. (JPMA 63: 1271; 2013)

Introduction

Coeliac disease (CD) is an autoimmune disorder of the small intestine that occurs in genetically predisposed people of all ages from middle infancy onward. It is caused by reaction to gliadin (gluten protein) found in grains like wheat, barley and rye.[1] Upon exposure to gliadin, the enzyme transglutaminase modifies the protein and the immune system cross reacts with the small bowel tissue, leading to truncation of villi.[2] This histological change is associated with a variety of symptoms related to malabsorption.

CD is a genetically-determined disease as suggested by a concordance rate of 75-80% in monozygotic twins and a high frequency of illness in first-degree relatives of patients.[3]

Studies from around the world using dioxyribonucleic acid (DNA) typing methods have proved that 95% of CD patients have a particular pair of HLA-DQ alleles with a combination of DQA1*0501 and DQB1*0201 alleles which are located in cis position in DR3 positive individuals and in trans position in DR7/DR5 positive individuals.[4-7] However, HLA-DR alleles involved vary when different ethnic groups are considered. Besides, strong association between CD and other autoimmune diseases which share the HLA-DR3-DQ2 haplotype has already been reported.[8] Thus, CD has one of the strongest HLA class II associations of any human illness.

CD is a fairly common gastrointestinal (GI) disorder with a prevalence of 1 in 200 in most populations, including western Caucasians.[9] In Iranian population, CD has been found in as many as 0.6% (1:166) of the population.[10] A retrospective study from north India has revealed a significant increase of 15.5 cases per year.[11] CD is considered nonexistent in people of African, Chinese or Japanese descent, in whom the prevalence of the HLA-DQ2 is negligible.[12]

CD has remained a virtually unexplored entity amongst Pakistanis and there are no reports on the molecular genotyping of HLA class II alleles in Pakistani patients. This is the first report on the molecular analysis of HLA polymorphism in children with CD in Pakistan.

Patients and Methods

The study investigated 25 unrelated children attending the paediatric clinic at Military Hospital Rawalpindi

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from October 2011 to January 2012, with suggestive clinical features of CD i-e., chronic diarrhoea, iron deficiency anaemia and failure to grow. All patients showed endoscopic duodenal biopsy changes suggestive of CD based on Marsh criteria and showed a good clinical response to gluten withdrawal for 4-6 weeks as per the diagnostic criteria laid down by the European Societey of Paediatric Gastroenterology and Nutrition (ESPGAN).[13] Patient sera were checked for various CD associated antibodies i-e., endomyseal antibodies, IgA-reticulin antibodies and IgA- gliadin antibodies. These patients had different socioeconomic backgrounds and belonged to the provinces of Punjab and Khyber Pakhtunkhwa with only two patients from Balochistan.

HLA data obtained from these patients was compared with a panel of 150 healthy unrelated individuals.

Peripheral blood was collected into tubes containing ethylenediaminetetraacetic acid (EDTA), and chromosomal DNA was extracted according to the manufacturer's instructions (Puregene DNA Purification Kit; Gentra Systems, Inc.).[14] Then 100ul DNA hydration solution was added to the extracted DNA to achieve the required concentration of 100ng/ul.

DNA was amplified using Sequence Specific Primers (SSPs) for HLA-DRB1*01 -*16. Amplified DNA was electrophoresed on 2% Agarose followed by Ethidium bromide staining for 30 min. HLA-DR alleles were determined by recording specific band patterns observed on the gel under ultraviolet (UV) illumination.

The phenotype frequency of various alleles among patients and controls was calculated by direct counting, and significance of their association was determined by Fisher exact test using OpenEpi info software. Level of significance was set at 0.05. Odds ratio (OR) and relative risk (RR) were calculated at 95% confidence intervals (CI).

Results

The median age of the patients at onset of their first symptoms was 2 years (range 1 to 8 years). However, the median age at diagnosis was 6 years (range 2 to14 years) (Table-1). Most of the patients were severely malnourished, stunted and anaemic. Marsh criteria stage 3 biopsy changes were observed in 16 (64%) patients whereas 9 (36%) patients had stage 4 charges in biopsy. All the 25 children were detected positive for anti-endomyseal, reticulin and gliadin antibodies in their serum samples which were taken before the commencement of gluten withdrawal.

Table-1: Demographic data of Pakistani paediatric CD patients (n=25).

	Parameter	Observation
1	Sex M:F	14:11
2	Median age at onset	2 years(range 1 to 8)
3	Median age at diagnosis	6 years(range 2 to14)
4	Chronic diarrhea (abdominal pain/distention)	23 (92%)
5	Stunting (<85% height for age)	18 (72%)
6	Wasting (<80% weight for age)	10 (40%)
7	Mean weight for height (before gluten withdrawal)	89% (±14%)
8	Mean weight for height (after gluten withdrawal)	96.4% (±10%)
9	Mean height for age (before gluten withdrawal)	83.4% (±7%)
10	Mean height for age (after gluten withdrawal)	90.3(±5%)
11	Anemia	
	◆ Mild (8-12 gm%)	◆ 17 (67%)
	◆ Moderate (5-8 gm%)	◆ 6 (24%)
	◆ Severe (<5 gm%)	◆ 2 (8%)
12	Small Intestine Histopathology (villous atrophy, cryptic hyperplasia, inflammatory cells in lamina propria)	Marsh criteria: Stage 3: 16 (64%) Stage 4: 9 (36%)

Table-2: Distribution of HLA-DR alleles in Pakistani Paediatric CD patients.

HLA-DR allele	Phenotype frequency		P Value	OR	RR
	CD (n=25) %	Control (n=150)%			
DRB1*15	3(12)	59(39)	0.01	0.2	0.2
DRB1*16	0	4(2)	0.888	0	0
DRB1*03	23(88)	31(21)	0.001	44	25
DRB1*04	1(4)	30(21)	0.079	0.155	0.182
DRB1*11	0	28(20)	0.032	0	0
DRB1*12	2(8)	23(16)	0.45	0.44	0.49
DRB1*13	1(4)	23(16)	0.195	0.21	0.24
DRB1*14	2(8)	21(14.7)	0.552	0.501	0.54
DRB1*07	5(20)	56(39.4)	0.101	0.38	0.43
DRB1*08	0	7(4)	0.55	0	0
DRB1*09	0	6(4.2)	0.64	0	0
DRB1*10	2(8)	27(19)	0.8	1.14	1.12

All patients mounted good clinical response to gluten withdrawal. Height and weight revealed considerable improvement and symptoms stood ameliorated.

Besides, 23 (92%) patients were positive for DRB1*03 compared to 31 (21%) in controls (p <0.001) (Table-2). Another important observation was statistically significant decrease in the frequency of DRB1*15 among patients compared with controls (n=33; 12% vs. n=58; 39%; p<0.001).

Of the 25 patients, 9 (36%) were DRB1*03 homozygous; 1 (4%) was DRB1*07 homozygous; 2 (8%) each were DRB1*03/*14, DRB1*03/*10 and, DRB1*03/*12

heterozygotes; while 3 (12%) each were DRB1*03/*04, DRB1*03/*07 and, DRB1*03/*15 heterozygotes.

Discussion

Clinical and histological features of CD in Pakistan indicate chronic diarrhoea, iron deficiency anaemia and stunting to be the most common presenting complaints in patients.

The results obtained on HLA association in Pakistani patients are in agreement with those reported in other ethnic groups, with high frequency of DR3+ haplotypes being reported in studies from India (94.2% vs. 22%),[11] Ireland (91% vs. 39%),[15] and Latin America (64.5% vs.15.1%).[16] DR15 was present in significantly low frequency in our CD patients, indicating a possible protective role of DR15 in the development of CD in our population.

Although HLA-DQ alleles were not determined directly, given the tight linkage of DQ2 to DR3 and DR7,[17-21] but it is likely that all CD patients express the DQ2 heterodimer, suggesting high prevalence of DR3-DQ2 haplotype in our CD patients. Similar findings were depicted by another local study, where majority of CD patients were found to carry HLA-DQ2 and HLA-DQ8 haplotypes.[22]

An analysis of extended haplotypes in CD in Asian Indians revealed A26-B8-DR3-DQ2[17] (haplotype 8.2) to be the most common haplotypes in contrast to the common Caucasoid ancestral haplotype A1-B8-DR3-DQ2 (haplotype 8.1). Such haplotype diversion suggests that genes lying outside DR3-DQ2 region may exert an additional influence on CD susceptibility beyond HLA-DQ association in CD patients.

Despite the strong association of HLA-DR3-DQ2 with CD, only a minor proportion of those having these alleles develop the disease. This raises the question of how healthy individuals bearing the typical DQ2 heterodimer remain protected. It is possible that HLA genes are not the only ones to be involved in the determination of disease as the concordance rate is about 30% in HLA identical siblings.[17] In some studies, the role of adjacent major histocompatibility (MHC) genes (such as tumour necrosis factor-alpha (TNF-α) -308, MHC class I chain-related genes (MIC), and others has also been highlighted.[23] Attempts to define polymorphism in T-cell receptors,[24] TAP-1(Antigen peptide transporter 1) alleles,[25] and Diabetes mellitus[26] alleles in CD have failed to demonstrate a significant association with any of these marker systems. Nonetheless, chromosome 2q region including Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) has been implicated as an additional marker of susceptibility in CD in Finnish families.[27] In addition, the MIC-A and MIC-B genes are interesting candidate susceptibility genes in CD, because the MIC molecules are ligands for TCR$\gamma\delta$ T cells. MIC-A*008 (5.1) allele is in strong linkage with HLA-B8 and may be of concern in coeliac patients.

Conclusion

Taken together, the data reaffirms the observation that CD has one of the strongest HLA class II associations of any human illness. Our findings highlight the fact that multiple DR3-DQ2 haplotypes are crucial in development of CD with DR3 conferring an odds ratio of 22 when compared with other DR alleles. At least one other gene (not limited to HLA) is required and this gene appears to have a recessive genetic expression. When the additional gene has been identified (perhaps by genome screening using microsatellite polymorphism), CD may take its place as a uniquely well-characterised HLA-associated immune-mediated disease with the specific enabling HLA molecule established, the important non-HLA gene(s) identified, and the triggering environmental factor well-characterised.

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