HLA-DQ2 and *HLA-DQ8* Alleles in Celiac Disease Patients and Healthy Controls

Mustajab Alam[1], Hamid Nawaz Tipu[1], Dawood Ahmad[1], Muhammad Hussain[1], Usama Bin Khalid[2] and Yumna Ijaz[1]

[1]*Department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan*
[2]*Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan*

ABSTRACT

Objective: To compare *HLA-DQ2* and *HLA-DQ8* alleles between celiac disease patients and healthy control group.
Study Design: Observational cross-sectional study.
Place and Duration of Study: Department of Immunology, Armed Forces Institute of Pathology (AFIP), from April to December 2018.
Methodology: Subjects were included: 100 celiac disease patients selected by non-probability consecutive sampling, and 100 healthy subjects. After collecting peripheral blood in EDTA tubes, chromosomal DNA was extracted and amplified, using sequence specific primers. Post-amplification electrophoresis was performed on two per cent agarose gel, followed by ethidium bromide staining; and specific band patterns were recorded under ultraviolet illumination to determine the HLA-DQ alleles. The subtypes of *HLA-DQ2*, *i.e. HLA-DQ2.5* and *HLA-DQ2.2* were also assessed. Frequency, percentage, mean and SD were calculated. Post-stratification Chi-square test was applied.
Results: The mean age of celiac disease group and healthy subjects was 14.79 ± 5.32 years and 14.71 ± 5.21 years, respectively. 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. Statistically significant difference ($p < 0.05$) was observed between the celiac disease patients and healthy group. There was no significant difference observed among different age groups and gender ($p > 0.05$).
Conclusion: *HLA-DQ2* detection reliably diagnoses celiac disease among all age groups and either gender. It can be used as an effective marker for early diagnosis of celiac disease instead of invasive procedures such as intestinal biopsy. The diagnosis can be pin--pointed by presence of *HLA-DQ2.5.*

Key Words: *Celiac disease, HLA-DQ2, HLA-DQ8, HLA-DQ2.5, HLA-DQ2.2.*

How to cite this article: Alam M, Tipu HN, Ahmad D, Hussain M, Khalid UB. *HLA-DQ2* and *HLA-DQ8* Alleles in Celiac Disease Patients and Healthy Controls. *J Coll Physicians Surg Pak* 2022; **32(02)**:157-160.

INTRODUCTION

Celiac disease (CD) is a chronic autoimmune intestinal condition that is caused by immune-mediated enteropathy, triggered by ingested prolamins, commonly called gluten, found in wheat, barley and rye. CD is one of the most common genetic diseases arising from environmental factors (gluten) as well as genetic factors (HLA and non-HLA genes).[1] Initially thought to affect white Europeans exclusively, CD is now considered to be distributed globally.[2,3] In the population of European origin, CD is one of the most common life-long disorders, affecting approximately 1% of the general population.

Correspondence to: Dr. Mustajab Alam, Department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan
E-mail: mustajab.alamm1@gmail.com
Received: August 04, 2021; Revised: November 23, 2021; Accepted: December 22, 2021
DOI: https://doi.org/10.29271/jcpsp.2022.02.157

In North Africa, Middle-East and South-East Asia, the incidence of CD is rising. The enormous prevalence of CD in some American communities (5.6-11.1%) is possibly due to their strong genetic predisposition and sudden dietary changes over the last few centuries.[4] The prevalence of *HLA-DQ2* and *HLA-DQ8* in patients of celiac disease found in the literature is mostly from developed countries.[5]

Gastrointestinal symptoms (chronic diarrhea, abdominal pain, distension, bloating, weight loss, vomiting and constipation) confirmed by a small bowel biopsy (SBB) which shows villous atrophy, crypt hyperplasia and an increase in intra-epithelial lymphocytes and normalisation of these findings in response to a gluten-free diet are the classical features of CD. Previously, CD was predominantly considered to be a childhood disease. However, the clinical symptoms have become subtler with the delay in the introduction of wheat into infant diet; and diagnosis is now usually made in older children and adults. The capricious clinical picture of CD is due to the fact that genetic and immunological bases both influence the clinical presentation of the disease, age of commencement, degree of mucosal damage, dietary behaviours and gender.[6,7]

Table I: The specifications of sequence specific primers (SSP) used in PCR reaction.

HLA	5' Primer	Sequence	3' Primer	Sequence	Size of PCR Product	Amplified HLA-DQ alleles
HLADQ2.2	B-5'07	*GTGCGTCTTGTGAGCAGAAG*	B-3'07	*GCAAGGTCGTGCGGAGCT*	205bp	*DQB1*0201*
HLADQ2.5	A-5'02	*ACGGTCCCTCTGGCCAGTA*	A-3'05	*AGTTGGAGCGTTTAATCAGAC*	186bp	*DQA1*0501*
HLADQ8	A-5'06	*TTCACTCGTCAGCTGACCAC*	A-3'03	*CAAATTGCGGGTCAAATCTTCT*	183bp	*DQA1*0302*

Table II: Stratification of *HLADQ2* among disease group and healthy subjects with respect to age and gender.

Age (years)	*HLADQ2* in disease group		p-value (*HLA-DQ2* positive cases)	Age (years)	*HLA-DQ2* in healthy group		p-value (*HLA-DQ2* positive cases)
	Present	Absent			Present	Absent	
1-18 (n=79)	73 (92.4%)	6 (7.6%)	0.651	1-18 (n=77)	12 (15.58%)	65 (84.42%)	0.071
>18 (n=21)	20 (95.2%)	1(4.8%)		>18 (n=23)	8 (34.79%)	15 (65.21%)	
Total	93(93%)	7(7%)		Total (100)	20 (20 %)	80 (80 %)	
Gender			p-value	Gender			p-value
Male (n=50)	44(88%)	6(12%)	0.050	Male (n=50)	11 (22%)	39(78%)	0.617
Female (n=50)	49(98%)	1(2%)		Female (n=50)	9(18%)	41 (82%)	
Total	93(93%)	7(7%)		Total	20 (20%)	80 (80%)	

Table III: Stratification of *HLA-DQ8* among diseased and healthy subjects with respect to age and gender.

Age (years)	*HLADQ8* disease group		p-value (*HLA-DQ8* positive cases)	Age (years)	*HLA-DQ8* healthy group		p-value (*HLA-DQ8* positive cases)
	Present	Absent			Present	Absent	
1-18 (n=79)	4(5.1%)	75 (94.9%)	0.841	1-18 (n=77)	2 (2.59%)	75 (97.41%)	0.435
>18 (n=21)	0(0%)	21(100%)		>18 (n=23)	0 (0%)	23 (100%)	
Total 100	4(4%)	96(96%)		Total 100	2 (2%)	98 (98%)	
Gender			0.307	Gender			1.000
Male (n=50)	3(6%)	47(94%)		Male (n=50)	1 (2%)	49 (98%)	
Female (n=50)	1(2%)	49(98%)		Female (n=50)	1 (2%)	49 (98%)	
Total	4(4%)	96(96%)		Total	2 (2%)	98 (98%)	

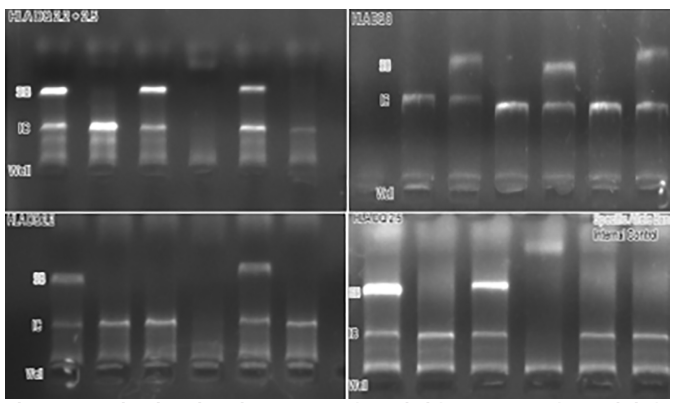

Figure 1: The bands of *HLA-DQ2* (top left) *HLA-DQ8* (top right), *HLA-DQ2.2* (bottom left) and *HLA-DQ2.5* (bottom right) as seen under UV illumination. SB = Specific allele band, IC = Internal control.

Typing HL-ADQ is now known as a valuable method to diagnose difficult cases of celiac disease or as an alternative to intrusive biopsy.[8] The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends analysis of HLA-DQ2 and HLA-DQ8 for diagnosis of CD. In Pakistan, while the association of HLADRB1 with celiac disease was previously determined, the data is scanty on the frequency of HLA-DQ in CD patients and their association thereof. In addition, their effectiveness is limited by trivial number of patients and the use of less responsive methods, like anti-gliadin and anti-reticulin antibodies. Hence, evaluating the prevalence of HLA-DQ2 and HLA-DQ8 in celiac patients in this population would help to determine its usefulness in the diagnosis of difficult cases and avoid the need for invasive duodenal biopsy.

The aim of this study was to find the association of *HLA-DQ2* and *HLA-DQ8* with celiac disease in symptomatic patients and the subtypes of *HLA-DQ2*, *i.e*. *HLA-DQ2.5* and *HLA-DQ2.2* in *HLA-DQ2* positive individuals.

METHODOLOGY

Approval was taken from the Institutional Ethical Committee to perform this research. This cross-sectional study was conducted at the Department of Immunology, Armed Forces Institute of Pathology (AFIP), from April to December 2018. Non-probability consecutive sampling technique was employed for sampling. Informed consent was obtained from the study participants. The data were obtained on a pre-designed questionnaire, including information related to their demographic characteristics and laboratory findings. The information was kept in a password-protected database and strict confidentiality was preserved.

Sample size was calculated by WHO calculator at 95% confidence interval, 5% margin of error, and disease prevalence at 1%.[9] Two hundred subjects were included; 100 celiac disease patients were selected on the basis of age greater than or equal to one year, belonging to either gender, with clinical features suggestive of celiac disease and anti-TTG (IgA) antibody levels ten times the upper reference limit. The patients having type 1 diabetes mellitus, including those having family history of type 1 DM, the patients having anti-TTG (IgA) levels less than ten times the upper limit, and known patients of IgA deficiency were excluded from the study. One hundred age and gender matched healthy subjects were selected from bone marrow and renal transplant donors.

The blood samples were collected by venipuncture from peripheral veins in EDTA containing tubes. Chromosomal DNA was extracted according to the manufacturer's directions, (Puregene® Blood Core Kit B; QIAGEN). The concentration of DNA was adjusted to 100 ng/µl. Using sequence specific primers (SSPs) for *HLA-DQ2* and *HLA-DQ8*, DNA was amplified on GeneAMP® PCR system 9700. The conditions used for PCR were: initial denaturation at 95°C for 5 minutes, followed by annealing at 58°C for one minute and extension at 72°C for 10 minutes. The sequence specific primers (SSP) are described in Table I. Internal controls (IC) available with the commercial kits were run with each test during the process of PCR and electrophoresis for validation of results.

Electrophoresis was done on 2% agarose gel for DNA, followed by staining with ethidium bromide for 30 minutes. The alleles of *HLA-DQ* were ascertained by documenting specific band patterns seen on the gel under ultraviolet radiance.

Statistical package for social sciences (SPSS) version 23.0 was used to measure quantitative and qualitative variables. For categorical variables, *DQ2* and *DQ8* frequency and percentages were calculated. Mean ± SD were calculated for age. Stratification was done for age and gender for controlling the effect changers. Chi-square test was used to see any significant difference between the groups. Statistically significant level was set at $p \leq 0.05$.

RESULTS

Celiac disease group had mean age of 14.79 ± 5.32 years, while healthy subjects had mean age of 14.71± 5.21 years. The occurrence of *HLA-DQ2* was higher among the celiac disease (93%) patients as compared to healthy patients (20%) ($p < 0.05$). The frequency of *HLA-DQ8*, although greater in celiac disease patients (4%) as compared to the healthy subjects (2%), but was found statistically not significant ($p = 0.683$). This suggests that *HLA-DQ2* had a significant association with the disease in contrast to *HLA-DQ8*.

Among the *HLA-DQ2* positive patients (n=93), the frequency of *HLA-DQ2.5* (*HLA-DQA1*05/HLA-DQB1*02*) and *HLA-DQ2.2* (*HLA-DQA1*02/HLA-DQB1*02*) was assessed and found 86 (92.4%) and 7 (7.6%), respectively. The photograph of the bands of *HLA-DQ2, HLA-DQ8*, *HLA-DQ2.5* and *HLA-DQ2.2* as seen under UV illumination are shown in Figure 1.

Chi-square test applied to the data after stratification showed that the confounding factors, like age and gender, had no significant effect ($p > 0.05$) on *HLA-DQ2* and *HLA-DQ8* in celiac disease group. Similarly, no significant difference was observed in healthy group. HLA *HLA-DQ2* detection reliably diagnoses celiac disease among all age groups, gender and ethnicities Tables II and III.

DISCUSSION

Celiac disease has a strong genetic background and diversity in its phenotype (depending on the point at which the illness is discovered); thus the observer needs a high degree of skepticism. In the pathogenesis of the disease, the factors involved are: gluten ingestion; shifts in the confluences of the intestinal mucosa (gliadin breaching the barrier and triggering the inflammatory process), and the involvement of a hereditary factor administered by a particular HLA.

HLA-DQ2 exists in 98.6% of CD patients in the world population and has a high negative predictive value.[3] In addition, *HLA-DQ2* and/or *HLA-DQ8* are present in approximately 40 percent of the general population who do not have CD, and this proportion rises in the individuals who have first-degree relatives with celiac disease.

When the patient is on a gluten-containing diet, positive serological tests and SBB are necessary for the diagnosis of CD.[10,11] Therefore, in settings with poor histopathological diagnostic ability, serology based and especially genetic tests would be much-prized to develop more precise diagnosis of CD.[12,13] As a matter of fact, a recent multi-centric study organised in Spain established that small intestinal biopsies could have been prevented without any chance of misdiagnosis in a large fraction of symptomatic patients.[14] Recent revisions of the CD diagnostic criteria have, therefore, adopted the greater involvement of *HLA-DQ* genotyping because of its high negative predictive value, in addition to antibodies, to diagnosis of CD.[15,16]

In this study, *HLA-DQ2* was seen in 93 percent celiac disease patients and *HLA-DQ8* was seen in 4 per cent celiac disease patients. The explanation for the higher pervasiveness of these antigens in this area compared to other places in the literature may include the genetic heterogeneity of the population, environmental and cultural factors. Further subtyping revealed that *HLA-DQ2.5* was more strongly associated with celiac disease, *i.e.* 92.4 % of *HLA-DQ2* positive patients had *HLA-DQ2.5*.

These findings are similar to those recorded in European populations, where the *HLA-DQ2* frequency is greater than ninety percent and the *HLA-DQ8* frequency is between five to ten percent.[17,18]

This work on Pakistani population provides a basis for association of HLA specifically *HLA-DQ2* and *HLA-DQ8* with celiac

disease. The subtypes of *HLA-DQ2 i.e*. *HLA-DQ2.5* having high association with the disease reveals its prevalence in local population. Further multicentric studies with larger sample size can be employed based on this data to make local guidelines for diagnostic testing of celiac disease.

CONCLUSION

HLA-DQ2 can be used as an effective marker for early diagnosis of celiac disease instead of invasive procedures such as intestinal biopsy. The lack of genotyping of *HLA-DQ2* in patient workup may lead to misdiagnosis of celiac disease, particularly *HLA-DQ2.5*. This can prevent invasive biopsy procedure and its associated complications.

ETHICAL APPROVAL:
This study was approved by Ethical Review Committee, Armed Forces Institute of Pathology (AFIP), Rawalpindi; and ethical approval was obtained prior to the initiation of research work.

PATIENTS' CONSENT:
Informed consents were taken from all the participants about the study; and detailed history was recorded.

CONFLICT OF INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
MA: Concept, study design, data collection, data analysis, interpretation and discussion.
HNT: Study design, data analysis and interpretation.
DA: Critical review.
MH: Data collection.
UBK: Statistical analysis.
YI: Data collection, and literature review.

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