

Ethnic distribution of HLA-DQ2 and HLA-DQ8 alleles in celiac disease with serological confirmation: A cross-sectional study from a tertiary care center in Rawalpindi

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ABSTRACT

Objective: To determine the ethnic distribution of HLA-DQ2 and HLA-DQ8 alleles among Pakistani patients with serologically confirmed celiac disease (CD).

Material and Methods: This cross-sectional study was conducted at the Department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from 1st September 2021 to 30th September 2022. A total of 100 serologically confirmed CD patients were enrolled through consecutive non-probability sampling. Anti-tissue transglutaminase IgA was tested by ELISA (Diametra, Italy), while total serum IgA was measured to exclude IgA deficiency. Genomic DNA was extracted from EDTA blood using the Puregene® Blood Core Kit B (QIAGEN, Germany), and HLA typing was performed by sequence-specific primer PCR. Data were analysed using SPSS v26.0.

Results: HLA-DQ2 was detected in 92 (92.0%) and HLA-DQ8 in 7 (7.0%) of 100 patients; only 1 patient (1.0%), from the Pashtun group, was negative for both. HLA-DQ2 prevalence was 100% in Kashmiri (n=13) and Gilgit-Baltistani (n=9) patients, 94.9% (37/39) in Punjabis, 90.0% (9/10) in Sindhis, 83.3% (20/24) in Pashtuns, and 80.0% (4/5) in Balochis. HLA-DQ8 was most prevalent in Balochis (20.0%, 1/5) and Pashtuns (12.5%, 3/24). HLA-DQ2 was the dominant allele across all ethnic groups.

Conclusion: HLA-DQ2 is the dominant celiac-associated allele across all major Pakistani ethnic groups, with universal prevalence in Kashmiri and Gilgit-Baltistani populations. HLA-DQ8 is most frequent in Balochi and Pashtun subgroups. HLA genotyping supports diagnostic evaluation across ethnically diverse Pakistani communities.

Keywords: Celiac disease, Ethnicity, Genetic susceptibility, HLA-DQ2 Antigen, HLA-DQ8 Antigen.

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INTRODUCTION

Celiac disease (CD) is a chronic immune-mediated intestinal disorder triggered by the ingestion of gluten in genetically susceptible individuals. A recent systematic review and meta-analysis found that the pooled global prevalence of CD was 1.4%, with a prevalence of 0.6% in Asia [1]. However, data from South Asia remain limited and often non-stratified by ethnicity.

Human leucocyte antigens (HLA) DQ2 and DQ8 are central to antigen presentation of gluten-derived peptides to T lymphocytes, initiating the immune cascade in CD [2]. Western studies have repeatedly

confirmed the strong association of HLA-DQ2 and DQ8 haplotypes with CD, with nearly complete disease concordance in high-risk genotypes [3]. However, differences in allele frequencies, penetrance, and haplotype composition across populations suggest that extrapolation of these findings to all ethnic groups may be inappropriate [4]. Non-European populations, including those in the Middle East, North Africa, and South Asia, demonstrate unique haplotype distributions and disease associations. The absence of both HLA-DQ2 and HLA-DQ8 alleles virtually excludes CD, establishing HLA genotyping as a valuable negative predictive tool [5].

In a recent Pakistani study, Alam *et al.* (2022) reported the frequency of HLA-DQ2 and HLA-DQ8 among serologically positive CD cases was 93% and 4%, respectively, underscoring the strong genetic linkage of HLA class II alleles with disease susceptibility in the local population [6]. In Pakistan, increased awareness of celiac disease and improved serological tests, including anti-tTG IgA and endomysial antibody assays, have increased the

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frequency of diagnosis. Despite this, data on HLA allele distribution across Pakistan's genetically diverse ethnic groups remain sparse, limiting accurate assessment of genetic risk and targeted screening programmes.

The rationale for this study was to delineate the ethnic distribution of HLA-DQ2 and HLA-DQ8 alleles in serologically confirmed CD patients presenting to a tertiary care centre in Rawalpindi, Pakistan. By providing population-specific allele frequency data, this study aims to fill a critical knowledge gap and support contextualisation of CD risk across different Pakistani ethnic backgrounds.

MATERIAL AND METHODS

This observational, cross-sectional study was conducted at the Department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi over a 24-month period from 1st September 2021 to 30th September 2022. Ethical approval was obtained from the Institutional Review Board of AFIP (FC/IMM17-27/IRB/19/297 dated 31st August 2021). Written informed consent was obtained from all participants or their legal guardians.

A total of 100 serologically confirmed patients with suspected CD were enrolled using non-probability consecutive sampling. Inclusion criteria comprised individuals of either gender, aged between 2 and 50 years, with elevated anti-tTG IgA levels ≥ 10 times the upper limit of normal (ULN) according to ESPGHAN diagnostic guidelines [7]. Patients with selective IgA deficiency or incomplete records were excluded. Based on a reported HLA-DQ2/DQ8 combined prevalence of 94% in Pakistani CD patients [8], with a 95% confidence level and 5% margin of error, the estimated minimum sample size was 87 patients. Although one hundred patients were enrolled through consecutive sampling within the defined study period in order to increase the generalizability of the results. Ethnicity was self-reported and categorised into six groups: Punjabi, Pashtun, Kashmiri, Gilgit-Baltistani, Balochi, and Sindhi.

Anti-tTG IgA antibodies were detected using ELISA (Diametra, Italy). Total serum IgA was measured by radial immunodiffusion to exclude selective IgA deficiency.

Peripheral blood was collected into EDTA vacutainers. Genomic DNA was extracted using the Puregene® Blood Core Kit B (QIAGEN, Hilden, Germany) and standardised to a working

concentration of 100 ng/ μ l. Sequence-specific primer (SSP) PCR was performed on a GeneAMP® PCR System 9700 (Applied Biosystems, USA) with the following cycling conditions: initial denaturation at 95°C for 5 minutes; annealing at 58°C for 1 minute; final extension at 72°C for 10 minutes. PCR products were electrophoresed on a 2% agarose gel stained with ethidium bromide and visualised under UV light. Allele identification was based on specific band patterns. Internal kit controls validated each run. Primer sequences are detailed in Table-I.

Data were analysed using SPSS v26.0 (IBM Corp., Armonk, NY). Continuous variables were expressed as mean $\pm$ SD; categorical variables as frequencies and percentages. The chi-square test was applied for between-group comparisons of allele frequencies. Exact *p*-values and 95% confidence intervals are reported; *p* < 0.05 was considered statistically significant.

RESULTS

The study included One hundred serologically confirmed CD patients of which 62 (62%) were female and 38 (38%) were male, with a mean age of 25.05 ± 11.04 years. The Punjabi group was the largest (n=39), followed by Pashtuns (n=24), Kashmiris (n=13), Sindhis (n=10), Gilgit-Baltistanis (n=9), and Balochis (n=5).

Among Punjabis, 37 (94.9%) were HLA-DQ2 positive and 2 (5.1%) were HLA-DQ8 positive; none were negative for both alleles, Figure-I. Among the 24 Pashtun patients, 20 (83.3%) were HLA-DQ2 positive, 3 (12.5%) were HLA-DQ8 positive, and 1 (4.2%) was negative for both alleles. All 13 Kashmiri patients were HLA-DQ2 positive (100%); none expressed HLA-DQ8. All 9 patients from Gilgit-Baltistan were HLA-DQ2 positive (100%) with no HLA-DQ8 expression. Among the 5 Balochi patients, 4 (80.0%) carried HLA-DQ2 and 1 (20.0%) carried HLA-DQ8, with no dual-negative cases. Of the 10 Sindhi patients, 9 (90.0%) were HLA-DQ2 positive and 1 (10.0%) was HLA-DQ8 positive, Table-II.

Overall, HLA-DQ2 was the predominant allele across all ethnicities, detected in 92 of 100 (92.0%) patients. HLA-DQ8 was identified in 7 of 100 (7.0%) patients. Only a single patient (1.0%), from the Pashtun ethnic group, was negative for both HLA-DQ2 and HLA-DQ8. These results are summarised in Table-II.

Table-I: Primer Specifications for sequence-specific PCR amplification of HLA-DQ alleles.

HLA Type	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Product	Amplified Allele
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			Size	
HLA-DQ2.2	GTGCGTCTTGAGCAGAAG (B-5'07)	GCAAGGTCGTGCGGAGCT (B-3'07)	205 bp	DQB1*0201
HLA-DQ2.5	ACGGTCCCTCTGGCCAGTA (A-5'02)	AGTTGGAGCGTTTAATCAGAC (A-3'05)	186 bp	DQA1*0501
HLA-DQ8	TTCATCGTCAGCTGACCAC (A-5'06)	CAAATTGCGGGTCAAATCTTCT (A-3'03)	183 bp	DQA1*0302

bp = base pairs; SSP = sequence-specific primers.

Table-II: Ethnic Distribution of HLA-DQ2 and HLA-DQ8 Alleles in Serologically Confirmed Celiac Disease Patients (n=100).

Ethnicity	No. of Patients (n)	HLA-DQ2 Positive n (%)	HLA-DQ8 Positive n (%)	HLA-DQ2 & DQ8 Negative n (%)	Row Total
Punjabi	39	37 (94.9%)	2 (5.1%)	0 (0.0%)	39
Pashtun	24	20 (83.3%)	3 (12.5%)	1 (4.2%)	24
Kashmiri	13	13 (100.0%)	0 (0.0%)	0 (0.0%)	13
Gilgit-Baltistani	9	9 (100.0%)	0 (0.0%)	0 (0.0%)	9
Balochi	5	4 (80.0%)	1 (20.0%)	0 (0.0%)	5
Sindhi	10	9 (90.0%)	1 (10.0%)	0 (0.0%)	10
TOTAL	100	92 (92.0%)	7 (7.0%)	1 (1.0%)	100

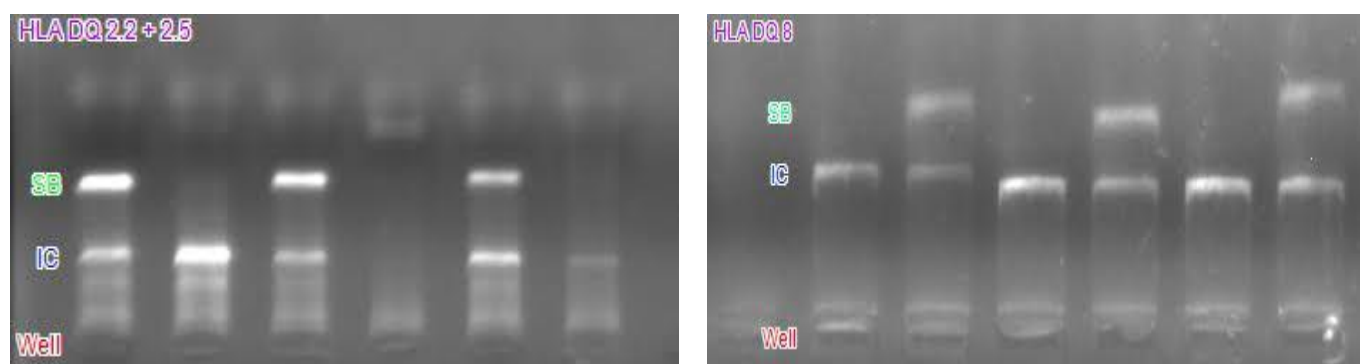


Figure-I: Allelic bands for HLA-DQ2 are visualized (left), and HLA-DQ8 allele bands are demonstrated (right).

DISCUSSION

This study provides one of the first structured analyses of the ethnic distribution of HLA-DQ2 and HLA-DQ8 alleles in Pakistani patients with serologically confirmed CD. Our findings demonstrate considerable heterogeneity in allele frequencies across major ethnic subgroups, highlighting the complex immunogenetic landscape of CD in the region.

The aggregate positivity for either HLA-DQ2 or HLA-DQ8 was 99% (99/100) in this cohort, with HLA-DQ2 identified in 92 (92.0%) and HLA-DQ8 in 7 (7.0%) patients. These figures are consistent with, and at the upper end of, frequencies reported in non-European populations, where DQ2 and/or DQ8 positivity ranges from 75–90% in Iranian and Turkish CD cohorts [10], and are comparable to European data where over 90% of cases carry HLA-DQ2 [3]. HLA-DQ2 was universal (100%) in Kashmiri and Gilgit-Baltistani patients and highest in Punjabi (94.9%) and Sindhi (90.0%) groups. HLA-DQ8 was most prevalent among Balochis (20.0%) and Pashtuns (12.5%), followed by Sindhis (10.0%) and Punjabis (5.1%). These findings echo regional genetic diversity

patterns, an Iranian study similarly demonstrated higher DQ8 frequencies in non-dominant ethnic minorities [15], and Latin American cohorts have shown elevated DQ8 rates in specific indigenous populations [16], consonant with our Balochi and Pashtun findings.

Most patients with CD carry HLA-DQ2.5, encoded by DQA1*05:01 and DQB1*02:01; HLA-DQ8 (DQA1*03:01/DQB1*03:02) accounts for the majority of remaining cases. Studying these haplotypes across Pakistani ethnic groups facilitates early disease recognition, family screening, and preventive guidance for genetically high-risk individuals [3].

Only one patient (1.0%), from the Pashtun ethnic group, was negative for both HLA-DQ2 and HLA-DQ8. This is substantially lower than the 6.8–24% dual-negative rates reported in admixed or non-European populations from northeastern Brazil and Turkey [11], suggesting a high degree of classical haplotype association in the present cohort. The involvement of alternate susceptibility haplotypes such as HLA-DQ2.2 or HLA-DQ7, associated with CD in ethnically diverse cohorts [12], cannot be

excluded in this single patient and warrants investigation in larger studies.

Previously, the prevalence of CD in non-Hispanic Caucasian populations was well characterised [13]; however, recent analysis of US NHANES data has revealed that up to 10% of CD patients are non-Caucasian [14], reflecting increasing recognition of CD in non-European groups. A prior Iranian study demonstrated that ethnicity significantly influences HLA-DQ subtype distribution, with higher DQ2 in Fars and higher DQ8 in Turkic populations [15] a pattern echoed in our data, where DQ8 was more prevalent in Balochi and Pashtun subgroups than in Kashmiri and Gilgit-Baltistani groups. Studies from Latin American and Amerindian groups have similarly demonstrated elevated DQ8 rates, attributed to ancestral genetic admixture patterns [16].

The primary diagnostic role of HLA genotyping in CD is exclusionary i.e. the absence of both DQ2 and DQ8 renders CD highly unlikely [17]. HLA typing is especially useful in cases where serology and biopsy are inconclusive despite strong clinical suspicion [18]. In this study, only one patient (1.0%) with confirmed positive serology lacked both alleles, reinforcing the high negative predictive value of DQ2/DQ8 typing in this Pakistani cohort. Broader genetic testing or extended HLA profiling may nonetheless be warranted in populations with diverse genetic backgrounds [19, 20].

Ethnic background must be considered when interpreting HLA typing results. The present data support inclusion of HLA genotyping not only for individual diagnosis but as part of community-based screening and family risk assessment programmes in Pakistan [21].

Strengths of this study include ethnic stratification of a Pakistani clinical cohort, use of validated SSP-PCR genotyping, and restriction to serologically confirmed cases. Limitations include the absence of biopsy-proven histological confirmation, small subgroup sizes particularly for Balochi patients, absence of healthy ethnicity-matched controls, single-centre design, and a sample that fell below the calculated minimum of 126 patients. Larger, multicentre studies incorporating duodenal histology and extended HLA profiling are needed to define true allele frequency differences and explore non-classical HLA variants in South Asian populations.

CONCLUSION

This study demonstrates clear ethnic variability in HLA-DQ2 and HLA-DQ8 expression among Pakistani

patients with serologically confirmed celiac disease. HLA-DQ2 was universally present in Kashmiri and Gilgit-Baltistani patients, and highly prevalent in Punjabi and Sindhi groups. HLA-DQ8 was most frequent in Balochi and Pashtun subgroups, followed by Sindhi. Only one patient from the Pashtun group, was negative for both alleles. These findings highlight the importance of considering ethnic background in genetic risk assessment, and demonstrate that HLA genotyping is a reliable diagnostic and family-screening tool for celiac disease across Pakistan's ethnically diverse population.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Mustajab Alam: Drafting the manuscript, acquisition, analysis, and interpretation of data. Critical review, literature search. Statistical review, critical review and discussion, final approval, responsible for every aspect of the research

Muhammad Hussain: Conception, data collection, formal Analysis, investigation, and resources, final approval, responsible for every aspect of the research

Muhammad Zain Ul Abdeen Javed: Drafting, designing the study content, review, writing, and editing, final approval, responsible for every aspect of the research

Fatima Sharif: Result analysis, review, final approval, responsible for every aspect of the research

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