

Association of tissue transglutaminase IgA antibodies with HLA DQ2/DQ8 haplotypes in clinically suspected cases of celiac disease in Pakistani population

Sara Waqar Khan, Sana Nawaz, Muhammad Aftab Hassan

Armed Forces Institute of Pathology (National University of Medical Sciences), Rawalpindi Pakistan

ABSTRACT

Objective: To determine the presence of anti TTG IgA in clinically suspected Celiac disease (CD) patients and associate their results with HLA-DQ 2/ DQ 8 haplotypes.

Material and Methods: This analytical cross-sectional study was conducted at Department of Immunology, Armed Forces Institute of Pathology, NUMS, Rawalpindi, from September 2024 to February 2025. A total of 277 clinically suspected Celiac disease cases were tested for anti-TTG IgA antibodies by ELISA technique and HLA-DQ 2/ DQ 8 haplotypes by sequence specific PCR. Associations were analyzed using chi-square tests and logistic regression.

Results: Out of 277 suspected celiac disease patients, 53 (19.1%) were anti-TTG IgA positive; among them, 43 (81%) had HLA-DQ2/DQ8. HLA-DQ2.5 was the most common genotype (48%), while overall HLA-DQ2 (and subtypes) was present in 59.6% and HLA-DQ8 in 2.16%. Anti-TTG IgA showed a significant association with HLA-DQ2/DQ8 ($p=0.001$). HLA-DQ2.5 was a significant predictor (OR=3.98, 95% CI: 1.88–8.44; $p<0.001$), whereas HLA-DQ2.2 and HLA-DQ8 were not significant.

Conclusion: HLA-DQ 2.5 is a strong genetic marker for celiac disease in Pakistan, significantly associated with anti-TTG IgA positivity. The limited predictive value of HLA-DQ 8 and HLA-DQ 2.2 highlights the need for population-specific diagnostic strategies. Combining serological and genetic testing enhances diagnostic accuracy, enabling early detection and improved disease management.

Keywords: Anti-TTG IgA, Celiac disease, HLA-DQ 2, HLA-DQ 8

This article can be cited as: Khan SW, Nawaz S, Hassan MA. Association of tissue transglutaminase IgA antibodies with HLA DQ2/DQ8 haplotypes in clinically suspected cases of celiac disease in Pakistani population. Pak J Pathol. 2026; 37(1): 1-5. DOI: <https://doi.org/10.55629/pakipathol.v37i1.908>

INTRODUCTION

Celiac disease (CD) is an autoimmune disorder characterized by an aberrant immune response against the small intestine following the ingestion of gluten, a protein present in wheat, barley, and rye. CD develops in genetically predisposed individuals, especially those with HLA-DQ 2 or HLA-DQ 8 genes. As a result, this autoimmune response damages the intestinal lining, leading to villous atrophy, crypt hyperplasia and increased intraepithelial lymphocytes. Over time, this intestinal damage leads to nutrient malabsorption and patient develops symptoms due to nutritional deficiencies [1].

Serological markers, especially anti-TTG IgA, are commonly used for diagnosing CD due to their high sensitivity and specificity [2,3]. However, in individuals with IgA deficiency, alternative markers

like TTG-IgG and DGP-IgG are considered [4]. More than 90% of individuals with CD carry the HLA-DQ 2 or HLA-DQ 8 haplotypes, and their absence serves as a strong indicator that further diagnostic testing is unnecessary [5]. Among these, HLA-DQ 2 is the most prevalent, present in 98.6% of CD cases worldwide, providing a high negative predictive value [6]. However, while these genetic markers are closely linked to CD, they are not exclusive to the disease. Around 40% of the general population carries HLA-DQ 2 or HLA-DQ 8 without developing CD. This percentage is even higher among those with a family history of the condition, particularly first-degree relatives of affected individuals [6]. This indicates that although HLA-DQ 2/DQ 8 are necessary for the development of CD, they are not sufficient on their own, emphasizing the role of additional genetic, environmental, and immune-related factors [7].

Once considered a condition primarily affecting Europeans, CD is now recognized as a global health issue, with an increasing prevalence in diverse regions such as South Asia and the Middle East [6]. In Islamabad, Pakistan, 11.7% of suspected CD patients tested positive for anti-TTG IgA [8] emphasizing the need for region-specific diagnostic approaches. A comprehensive strategy combining

Correspondence: Dr. Muhammad Aftab Hassan, Registrar Immunology, Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi Pakistan

Email: mahassan445@gmail.com

Receiving Date: 17 Apr 2025

Revision Date: 21 Jan 2026

Acceptance Date: 27 Feb 2025



Copyright © 2025. Muhammad Aftab Hassan, et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License, which permits unrestricted use, distribution & reproduction in any medium provided that original work is cited properly.

serology, genetics, and histology is crucial, especially in uncertain cases [9].

This study aims to assess the presence of anti-TTG IgA in suspected CD patients and associates their results with HLA-DQ 2/DQ 8 haplotypes. By evaluating the combined diagnostic efficacy of serological and genetic testing, this research seeks to enhance the accuracy of CD diagnosis and improve patient management. The findings will contribute to the development of refined diagnostic protocols suited to the Pakistani population, facilitating early detection and improved clinical outcomes

MATERIAL AND METHODS

This cross-sectional study was conducted at the Department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, over a six-month period from September 2024 to February 2025. Approval was taken from Institutional review board (READ-IRB/24/3559) prior to initiation of this research. The sample size was determined using the WHO formula, considering a 95% confidence level, a prevalence rate of 11.7% for celiac disease [8]. The sample size was calculated to be 159, however for better generalizability of results 277 samples were included in this study and non-probability consecutive sampling technique was used.

Patients of all ages and both genders who were screened for both anti-TTG IgA and HLA DQ 2/ DQ8 testing were included. The age of participants ranged from 1 to 80 years, with a median age of 10 years in males and 9 years in females. Patients with incomplete clinical or laboratory data or tested for only one of the parameters, immunocompromised individuals, and those who had been on a strict gluten-free diet before testing were excluded.

The anti-TTG IgA antibody test was conducted using Enzyme-Linked Immunosorbent Assay (ELISA) on serum samples. HLA-DQ 2/ DQ 8 genotyping was performed using Polymerase Chain Reaction (PCR - SSP) technique.

For data analysis, the Statistical Package for Social Sciences (SPSS) v27 software was used. Quantitative variables such as age were expressed as mean and standard deviation (SD) if normally distributed, while the median was calculated for skewed data. Qualitative variables like gender, anti-TTG IgA, and HLA DQ 2/DQ 8 were presented as frequencies and percentages. Normality was assessed using the Shapiro-Wilk test. The association between anti-TTG IgA and HLA DQ 2/DQ 8 genotyping was evaluated using the Chi-

square test. Logistic regression analysis was performed to evaluate the predictors of anti-TTG IgA positivity and their association with HLA DQ 2/DQ 8 haplotypes. A p -value of ≤ 0.05 was considered statistically significant.

RESULTS

A total of 277 patients with suspected celiac disease were included in the study, out of which 155 were males (56%) and 122 (44%) females. The median age was 10 years for males and 9 years for females. Among the participants, 53 (19.1%) tested positive for anti-TTG IgA, while 224 (80.9%) were negative. A strong genetic association was observed, as 81% ($n=43$) of the anti-TTG IgA positive cases had a positive HLA-DQ2/DQ8 status. In contrast, 128 (57%) of the anti-TTG IgA negative cases also carried the HLA-DQ2/DQ8 marker. Overall, 171(61.7%) of participants carried the HLA-DQ2/DQ8 marker, while 106 (38.3%) tested negative for it as shown in Table-I. Pearson Chi square test analysis, summarized in Table-I, reveals a significant association between HLA-DQ2/DQ8 genotyping with anti-TTG IgA positivity ($p = 0.001$). Moreover, individuals who carried the HLA-DQ2/ DQ8 genotype were found to have 3.22 times higher odds of being anti-tTG IgA positive compared to genotype-negative individuals (OR = 3.22, 95% CI: 1.54–6.74), indicating a meaningful link between genetic susceptibility and serological markers of celiac disease.

The distribution of HLA DQ subtypes along with anti tTG IgA serological status is presented in Table-II. While prevalence of HLA DQ subtypes is highlighted in Figure-I. The majority of participants carried HLA-DQ2 165 (59.6%), which includes HLA-DQ2.5 133 (48%), HLA-DQ2.2 (7.6%)21, and HLA-DQ2.5/DQ2.2 11(3.97%) out of 277 cases. In contrast, HLA-DQ8 was infrequent, present in only 6(2.16%) out of 277 cases. A significant proportion,106(38.3%), were categorized as HLA-DQ2/DQ8 negative, lacking detectable DQ subtypes. This distribution showed the predominance of HLA-DQ2 markers and their potential clinical relevance within the studied population.

To investigate the relationship between specific HLA genotypes and anti-tTG IgA positivity, a logistic regression analysis was performed, as presented in Table-III. The model demonstrated a strong ability to explain the data ($p < 0.001$), and the intercept was statistically significant (Estimate = -2.2618, $p < 0.001$). Logistic regression analysis revealed that HLA genotype significantly predicted anti-tTG IgA positivity

($p < 0.001$). The intercept estimate (-2.26) corresponds to a baseline probability of approximately 9% for seropositivity in the reference genotype group. Among the genotypes analyzed, HLA-DQ2.5 showed a significant association with anti-tTG IgA positivity. Individuals carrying this genotype were almost four times more likely to test positive for anti-tTG IgA (OR = 3.98, 95% CI: 1.88–8.44), suggesting a strong predictive value for this genetic marker in identifying celiac-related autoimmunity. However, other subtypes HLA-DQ2.2 and the combined DQ2.5/DQ2.2 did not show statistically significant associations with anti-tTG IgA

positivity, despite having odds ratios above 1 (OR = 1.01 and OR = 2.13, respectively; $p > 0.05$), indicating weaker or uncertain links.

HLA-DQ 8 ($p=0.988$) showed minimal predictive power and weaker or nonsignificant associations. This result is likely due to very small number of participants carrying this genotype, which limits its reliability in this analysis ($p = 0.988$). Overall, these findings highlight that HLA-DQ 2.5 is the most relevant genetic marker associated with anti-tTG IgA positivity in the studied population, reinforcing its role in identifying individuals at higher risk for celiac disease.

Table-I: HLA-DQ2/DQ8 status with Anti-TTG IgA.

		HLA-DQ2/DQ8		Total	p-value
		Positive	Negative		
Anti-TTG IgA	Positive	43	10	53	0.001*
	Negative	128	96	224	
Total		171	106	277	

Note: * $p < 0.001$ considered statistically significant

Table-II: Distribution of HLA DQ subtypes.

		Anti-tTG IgA		Total
		Positive	Negative	
HLA-DQ Type	Negative	10	96	106 (38.3%)
	HLA-DQ2.5	39	94	133 (48.0%)
	HLA-DQ2.5/DQ2.2	2	9	11 (3.97%)
	HLA-DQ2.2	2	19	21 (7.6%)
	HLA-DQ8	0	6	6 (2.16%)
	Total	53	224	277

Table-III: Logistic regression analysis.

Predictor	Estimate	P-value	Odds ratio	95% Confidence Interval	
				Lower	Upper
HLA-DQ2.5	1.3820	<0.001	3.983	1.880	8.438
HLA-DQ2.5/DQ2.2	0.7577	0.372	2.133	0.404	11.274
HLA-DQ2.2	0.0105	0.990	1.011	0.205	4.985
HLA-DQ8	-14.3043	0.988	<0.001	0.000	**∞

**The symbol ∞ (infinity) denotes that the upper confidence interval could not be reliably estimated due to the small number of observations in the HLA-DQ8 category, resulting in instability of the regression estimate.

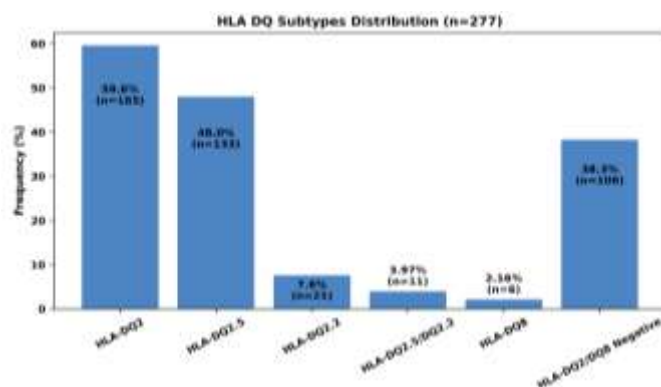


Figure-I: HLA DQ subtypes distribution.

DISCUSSION

Celiac disease is a complex autoimmune disorder, with its diagnosis primarily based on serological markers such as anti-TTG IgA and genetic predisposition linked to HLA-DQ2/ DQ8 haplotypes. This study aimed to assess the relationship between

anti-TTG IgA and HLA-DQ2/ DQ8 haplotypes in clinically suspected CD cases within the Pakistani population. By comparing the prevalence and association of these markers with regional and international studies, the study provides deeper insights into their diagnostic significance.

In this study, 19.1% of participants tested positive for anti-TTG IgA, reinforcing its role as a key serological marker for diagnosing CD. A study from Islamabad, Pakistan, reported a slightly lower prevalence of 11.7% [8]. Similarly, a cross-sectional study by Haseeb *et al.* found that 7.1% of the tested population was positive for TTG-IgA antibodies [10]. As nutritional deficiencies secondary to malabsorption and iron deficiency anemia are common in CD patients, similar findings were reported by Khan *et al.* that 5.4% of iron deficiency anemia patients tested positive for anti-TTG IgA [11].

Aforementioned findings suggest variations based on patient demographics and clinical settings. However, all studies emphasize the importance of anti-TTG IgA testing in suspected CD cases.

In this study, 61.7% of participants carried HLA-DQ2/DQ8 haplotypes, with HLA-DQ 2.5 being the most prevalent (48%). Similar findings were reported by Choung *et al.* where HLA-DQ 2 or HLA-DQ 8 was detected in 55% of suspected CD cases [12]. A study by Leja *et al.* found that among serologically positive individuals, 41.86% also carried the DQ2.5/DQ8 alleles [13]. In a study conducted by Dehghani *et al.* it was reported that 72.6% of pediatric CD patients were positive for HLA-DQ2/DQ8 [14]. Likewise, a study conducted in Denmark by Lund *et al.* analyzed blood samples from 9,754 symptomatic patients, revealing that 62% tested positive for HLA-DQ2/ DQ8 [15].

In this study, HLA-DQ8 was detected in only 2.2% of participants. Similarly, findings were reported in a study by Alam *et al.* in which HLA-DQ8 prevalence was 4% among confirmed CD patients in Pakistan [6], suggesting that HLA-DQ8 is less common in the Pakistani population. This variation may be influenced by environmental, geographic, and genetic factors. In contrast, global studies indicate that 5–10% of CD patients carry HLA-DQ8 [16], highlighting regional differences in genetic predisposition. The low prevalence observed in this study suggests a limited role of HLA-DQ8 in CD diagnosis within Pakistani population.

A significant association was observed between anti-TTG IgA positivity with HLA-DQ2/DQ8 genotyping ($\chi^2 = 10.441$, $p = 0.001$), with 81% of seropositive cases carrying these markers. HLA-DQ2.5 emerged as the strongest predictor, increasing the likelihood of seropositivity nearly fourfold (OR = 3.983, $p < 0.001$). Similarly, Mills *et al.* found a strong association between HLA-DQ 2.5 homozygosity and positive anti-TTG IgA serology ($p < 0.0001$) [4]. Choung *et al.* also reported a high odds ratio (OR = 96.9) for TTG-IgA positivity in individuals, homozygous for HLA-DQ 2.5, reinforcing its predictive significance [12]. A study conducted by Shi *et al.* found that individuals with HLA-DQ 2.5 had the highest risk, with 1 in 16 developing CD. Additionally, individuals who were heterozygous for DQB102:02 and DQB102:01 had a high genetic risk, as they carried two different alleles of DQB102, a combination linked to HLA-DQ2.2. While HLA-DQ 2.2 poses a slightly lower risk than HLA-DQ2.5, it remains an important genetic factor in CD development in this population [17].

In this study, HLA-DQ 2.2 (OR = 1.011, $p = 0.990$) and HLA-DQ8 (OR = 6.13, $p = 0.988$) showed minimal predictive value and lacked significant associations. Similarly, a study by Choung *et al.* found that individuals carrying a single HLA-DQ 2.2 haplotype did not show an association with TTG-IgA positivity, suggesting that HLA-DQ 2.2 may not be a key genetic contributor to CD development [12]. In this study, HLA-DQ 8 also showed weak predictive value with no significant link to anti-TTG IgA positivity. However, given the well-established global association of HLA-DQ8 with CD, it remains an important factor in diagnostic approaches, particularly in cases where individuals carry both HLA-DQ2.5 and HLA-DQ8.

Approximately 40% of the general population carries HLA-DQ2/ DQ8 without developing CD, but prevalence increases among first-degree relatives [6]. A study by Cecilio *et al.* reported that 89.6% of CD patients carried these markers compared to 55.4% in the general population [18].

Limitations of this study include its single-center, cross-sectional design, which may limit the external validity of the findings and preclude the establishment of causal relationship.

To summarize, this study confirms a strong association between anti-TTG IgA seropositivity and HLA-DQ 2/DQ 8 haplotypes in suspected CD cases in Pakistan, reinforcing the diagnostic value of anti-TTG IgA and the predominant role of HLA-DQ 2.5 in genetic susceptibility. The low incidence of HLA-DQ 8 indicates that regional genetic differences influence CD risk. While HLA-DQ 2.2 and HLA-DQ 8 had little predictive value in this sample, their established global importance suggests population-specific differences.

CONCLUSION

This study establishes a significant association between anti-tissue transglutaminase (anti-tTG) IgA positivity and HLA-DQ2/DQ8 haplotypes in clinically suspected celiac disease cases within the Pakistani population. HLA-DQ2.5 demonstrated a strong correlation with anti-tTG IgA positivity, indicating its major genetic susceptibility role, whereas HLA-DQ8 and HLA-DQ2.2 showed comparatively limited predictive value. These findings support the combined use of serological markers and HLA genotyping to enhance diagnostic precision and risk stratification of celiac disease in Pakistani population.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Sara Waqar Khan: Conception and study design, drafting of work, data acquisition and analysis, critical revision and final approval, accountable for all aspects of publication.

Sana Nawaz: Drafting of work, data acquisition and data analysis, critical revision and final approval, accountable for all aspects of publication.

Muhammad Aftab Hassan: Drafting of work, data analysis and final approval, accountable for all aspects of publication.

REFERENCES

- Alaa Jawad Kadhum. Evaluation of HLA-DQ2 and DQ8 haplotypes in patients with celiac disease correlation with circulating anti-tissue transglutaminase and anti-gliadin antibodies. *Biology*. 2021. DOI: <https://doi.org/10.51699/ajbns.v2i2.603>
- Clouzeau-Girard H, Rebouissoux L, Taupin J, Le Bail B, Kalach N, Michaud L, *et al*. HLA-DQ Genotyping combined with serological markers for the diagnosis of celiac disease: Is intestinal biopsy still mandatory? *J Pediatr Gastroenterol Nutr*. 2011; 52(6): 729–33. DOI: <https://doi.org/10.1097/MPG.0b013e31820a724d>
- Sghiri R, Hassine H Ben, Almogren A, Shakoor Z, Alswayyed M. Diagnostic performances of celiac disease serological tests among Saudi patients. *Saudi J Gastroenterol*. 2023; 29(1): 31–8. DOI: https://doi.org/10.4103/sjg.sjg_280_22
- Mills JR, Katzman BM, Ettore M, Murray JA, Gandhi MJ, Snyder MR. 72: Exploring Associations between genotype and serology in celiac disease. *Am J Clin Pathol*. 2015; 143 (suppl_1): A042–A042. DOI: <https://doi.org/10.1093/ajcp/143.suppl1.043>
- Siddiqui K, Uqaili AA, Rafiq M, Bhutto MA. Human leukocyte antigen (HLA)-DQ2 and -DQ8 haplotypes in celiac, celiac with type 1 diabetic, and celiac suspected pediatric cases. *Medicine*. 2021; 100(11): e24954. DOI: <https://doi.org/10.1097/MD.00000000000024954>
- Alam M, Tipu HN, Ahmad D, Hussain M, Khalid U Bin, Ijaz Y. HLA-DQ2 and HLA-DQ8 alleles in celiac disease patients and healthy controls. *J Coll Physicians Surg Pak*. 2022; 32(2): 157–60. DOI: <https://doi.org/10.29271/jcsp.2022.02.157>
- Schyum AC, Rumessen JJ. Serological testing for celiac disease in adults. *United European Gastroenterol J*. 2013; 1 (5): 319–25. DOI: <https://doi.org/10.1177/2050640613502788>
- Ahmed Kiani R, Ahmed I, Khan Yousafzai J, Mehmood W, Ahmed Khan S, Ahmad W, *et al*. Celiac disease in different age groups and gender in Pakistan. *JRMC*. 2018; 22. Available from: <https://www.journalrmc.com/index.php/JRMC/article/view/926>
- Raiteri A, Granito A, Giamperoli A, Catenaro T, Negrini G, Tovoli F. Current guidelines for the management of celiac disease: A systematic review with comparative analysis. *World J Gastroenterol*. 2022; 28(1): 154–75. DOI: <https://doi.org/10.3748/wjg.v28.i1.154>
- Haseeb A, Khattak AA, Hayat A, Rehman M, Jan F, Ahmed B, *et al*. Incidence and prevalence of celiac disease in Hazara Division, KP Pakistan by anti-tissue transglutaminase antibody as diagnostic tool. *Pak J Med Health Sci*. 2022; 16(12): 32–5. DOI: <https://doi.org/10.53350/pjmhs2022161232>
- Khan MA, Jan H, Khan MO, Hanif M, Hassan T, Khurshid L. Prevalence of celiac disease in adult patients with iron deficiency anemia. *Pak J Med Health Sci*. 2022; 16(5): 1006–8. DOI: <https://doi.org/10.53350/pjmhs221651006>
- Choung RS, Mills JR, Snyder MR, Murray JA, Gandhi MJ. Celiac disease risk stratification based on HLA-DQ heterodimer (HLA-DQA1 ~ DQB1) typing in a large cohort of adults with suspected celiac disease. *Hum Immunol*. 2020; 81(2): 59–64. DOI: <https://doi.org/10.1016/j.humimm.2020.01.006>
- Leja M, Shums Z, Nikitina-Zake L, Gavars M, Kikuste I, Milo J, *et al*. Prevalence estimation of celiac disease in the general adult population of Latvia using serology and HLA genotyping. *United European Gastroenterol J*. 2015; 3(2): 190–9. DOI: <https://doi.org/10.1177/2050640615569379>
- Dehghani SM, Dara N, Gharesifar B, Shahramian I, Dalili F, Salarzadei M. Prevalence of HLA DQ 2, 8 in children with celiac disease. *Hum Antibodies*. 2021; 29: 123–8. DOI: <https://doi.org/10.3233/hab-200437>
- Lund F, Pedersen MF, Kristiansen S. Estimation of the celiac disease prevalence in Denmark and the diagnostic value of HLA-DQ2/ DQ8. *Scand J Clin Lab Invest*. 2020; 80(8): 667–71. DOI: <https://doi.org/10.1080/00365513.2020.1829698>
- Sahin Y, Mermer S. Frequency of celiac disease and distribution of HLA-DQ2/DQ8 haplotypes among siblings of children with celiac disease. *World J Clin Pediatr*. 2022; 11(4): 351–9. DOI: <https://doi.org/10.5409/wjcp.v11.i4.351>
- Shi T, Liu W, Li T, Liu H, Hui W, Lin Q, *et al*. HLA-DQ genotype distribution and risk evaluation of celiac disease in Northwest China. *Scand J Gastroenterol*. 2023; 58(5): 471–6. DOI: <https://doi.org/10.1080/00365521.2022.2147801>
- Cecilio LA, Bonatto MW. The prevalence of HLA DQ2 and DQ8 in patients with celiac disease, in family and in general population. *Arq Bras Cir Dig*. 2015; 28(3): 183–5. DOI: <https://doi.org/10.1590/S0102-67202015000300009>