Accuracy of HLA-DQ genotyping in combination with IgA anti-tissue transglutaminase serology and a "scoring system" for the diagnosis of celiac disease in Turkish children

Murat Çakır[1], Maşallah Baran[2], Fahri Uçar[3], Ulaş Emre Akbulut[1], Neşe Kaklıkkaya[4], Şafak Ersöz[5]

[1]Division of Pediatric Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, Departments of [3]Medical Biology and Genetics, [4]Medical Microbiology, and [5]Pathology, Karadeniz Technical University, Trabzon, and [2]Department of Pediatric Gastroenterology, Hepatology and Nutrition, Tepecik Training and Research Hospital, Izmir, Turkey
E-mail: muratcak@hotmail.com

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The aim of the study was to analyze the accuracy of (i) HLA-DQ typing and anti-tissue transglutaminase antibodies immunoglobulin A (tTG-IgA) serology and (ii) a "simple scoring system" (SSS) for the diagnosis of celiac disease (CD). The study included 91 patients with positive tTG-IgA, who had been tested for HLA-DQ. Patients were divided into 3 groups: typical CD, atypical CD, and non-CD. The sensitivity, specificity, positive (PPV) and negative predictive value (NPV), positive (PLR) and negative likelihood ratio (NLR) and accuracy of the test combining genotyping and tTG-IgA positivity and the simple scoring system for the diagnosis of CD were evaluated. The combination of genotyping and strong tTG-IgA positivity had a sensitivity of 93.5%, specificity of 61.5%, PPV of 93.5%, NPV of 61.5%, PLR of 2.4, NLR of 0.1 and accuracy of 89% for "CD." SSS had a higher specificity (84.6%), higher PPV (97.3%), higher NPV (68.7%), higher PLR and higher accuracy (92.3%). The combination of genotyping and strong tTG-IgA positivity missed two patients with typical CD (4%) and three patients with atypical CD (10.7%). Two cases with malabsorptive symptoms (33.3%) and three patients without malabsorptive symptoms (42.8%) would have been misdiagnosed as CD if these tests were used. Intestinal biopsy is still mandatory for diagnosis of CD in Turkish children.

Key words: celiac disease, intestinal biopsy, HLA-DQ typing, tissue transglutaminase, type 1 diabetes.

Celiac disease (CD) is chronic autoimmune enteropathy induced by the ingestion of gluten-containing grains in genetically susceptible children. It is a T cell-mediated enteropathy with a strong HLA association, in which the immune response is directed mainly against deamidated cereal gluten peptides that have been modified by the enzyme transglutaminase 2. The deamidated gluten-HLA complex activates the T cells in the intestinal mucosa, which subsequently causes mucosal damage and atrophy. It was shown that HLA complex-associated immune response is specific to HLA-DQ2 and HLA-DQ8 heterodimers, especially in white populations[1]. Approximately 90-95% of CD patients had HLA DQ2 and/or DQ8 molecules, whereas their prevalence is approximately 30-35% in the healthy population[1-4].

In a recent population-based study, the prevalence of CD was found to be 0.47% in healthy Turkish schoolchildren[5]. In a multicenter study, the overall prevalence of CD was found to be 1%, with large variations between countries (2% in Finland, 1.2% in Italy, 0.9% in Northern Ireland and 0.3% in Germany)[6]. The prevalence of the disease seems to be on the rise in developed countries[7].

The clinical manifestations of CD vary greatly. The classical (typical) form is characterized by malabsorption syndrome, failure to thrive and abdominal distension, but in recent years the clinical picture of the disease has changed to include milder (atypical) forms with extra-digestive symptoms such as refractory iron deficiency anemia, short stature and osteopenia/osteoporosis. Sometimes it may be associated with other autoimmune diseases or genetic syndromes[7].

According to the ESPGHAN criteria in 1990, diagnosis of CD was based on positive serology combined with typical intestinal mucosal abnormality as defined by Marsh and Oberhuber. The anti-endomysial antibodies immunoglobulin A and anti-tissue transglutaminase antibodies immunoglobulin A (tTG-IgA) tests are widely available serological tests with high sensitivity and specificity[8]. But according to recent ESPGHAN guidelines for the diagnosis of CD, endoscopic biopsy can be omitted, especially in patients with typical CD and increased levels of tTG-IgA (>10 times above the upper limit) combined with positive HLA-DQ2 and/or DQ8 heterodimers that are diagnostic for CD[4].

It is true that there are some limitations of intestinal biopsy: it is an invasive procedure; lesions can be patchy; proper orientation of the specimen is not standardized; and assessment is subjective. Nonetheless, we held the view that intestinal biopsy was still mandatory for definite diagnosis, since the treatment includes a lifelong, strict gluten-free diet. Therefore, we aimed to analyze the accuracy of (i) HLA-DQ typing and tTG-IgA and (ii) a "simple scoring system" for the diagnosis of CD in Turkish children.

Material and Methods

The study included 91 patients with positive tTG-IgA serology, who had been tested for HLA-DQ genotyping. Patients were divided into three groups. Group 1 included patients with typical CD based on positive tTG-IgA and both malabsorptive symptoms and typical histological findings (n=50)[7]. Group 2 included patients with atypical CD based on positive tTG-IgA and typical histological findings, associated with extra- and atypical gastrointestinal symptoms such as constipation, vomiting and recurrent abdominal pain or autoimmune diseases/genetic syndromes, or who were diagnosed during screening (n=28)[7]. Group 3 included patients with positive tTG-IgA who did not have typical histological findings for CD (n=13).

Demographic and clinical findings of the patients are shown in Table I. Patients with IgA deficiency (n=2) and those for whom only EMA serology was performed during the diagnostic evaluation (n=10) were excluded from the study.

tTG-IgA antibodies were determined in the serum using an enzyme-linked immunosorbent assay technique (Euroimmun, Luebeck, Germany). Results were considered negative (<20 RU/ml), weak positive (>20-≤100 RU/ml), moderate positive (>100-≤200 RU/ml) and strong positive (>200 RU/ml).

Genomic DNA of patients in the study was isolated from 200 ml aliquots of peripheral venous blood samples using the BioRobot EZ1 magnetic bead-based workstation (Qiagen, Hilden, Germany). Genotyping of HLA-DQ2 and HLA-DQ8 alleles was performed for all subjects by the polymerase chain reaction and sequence-specific oligonucleotide probe (PCR-SSOP) hybridization method using Luminex technology (Gen-Probe LIFECODES, Stanford, CA). All studies were conducted in the HLA Tissue Typing Laboratory of Karadeniz Technical University. (The laboratory is accredited by the Turkish Ministry of Health and routinely undergoes quality control testing by the European Federation of Immunogenetics [EFI] and the United Kingdom's National External Quality Assessment Service [NEQAS].)

The sensitivity, specificity, positive (PPV) and negative predictive value (NPV), positive (PLR) and negative likelihood ratio (NLR) and accuracy of the test combining HLA-DQ2/DQ8 genotyping and tTG-IgA positivity and the "simple scoring system" (SSS) for the diagnosis of CD were evaluated.

The simple scoring system was developed by ESPGHAN, published in 2012 and based on symptoms, serum antibody testing, HLA-DQ genotyping and histology. A total score of >4 was accepted as CD.[4] In terms of serology, only tTG-IgA positivity was considered for purposes of the simple scoring system (EMA and anti-DGP could not studied in all patients).

All of the biopsies of the patients in the study group were reevaluated by the pathologist

according to the established criteria for the diagnosis of CD as defined elsewhere[9].

Results

Patients with CD

The HLA-DQ genotyping and tTG-IgA levels of the patients are shown in Table II. HLA-DQ2 and/or HLA-DQ8 positivity in patients with typical (Group 1) and atypical (Group 2) CD was 98% and 96.4%, respectively. Overall, HLA-DQ2 and/or HLA-DQ8 positivity in CD patients was 97.4% (76/78). Two patients were negative on both HLA-DQ2 and HLA-DQ8 genotyping. tTG-IgA was strongly positive in 98% and 92.8% of the patients in Groups 1 and 2, respectively. Overall, 96.1% of the patients had strongly positive tTG-IgA levels.

Overall, both HLA-DQ2 and/or HLA-DQ8 and strong tTG-IgA positivity in CD patients was 93.5% (95% CI: 88-98.9%).

The simple scoring system for the diagnosis of CD revealed scores of 6 ± 0.6 (range 4-7) and 5.2 ± 0.6 (range 3-6) for Groups 1 and 2, respectively. The overall score of patients with CD was 6 ± 0.9 points. Forty-nine (98%) of the 50 patients in Group 1 and 24 (85.7%) of the 28 patients in Group 2 had >4 points.

Non-CD patients

Three of 13 patients in the non-CD group had type 1 diabetes (2 were newly diagnosed). tTG-IgA levels were >200 RU/ml in two of the three, and 159 RU/ml in the other. Five endoscopies were performed on three patients; all of them revealed Marsh 0-1 lesions. At follow-up, tTG-IgA levels in three patients had returned to normal under a gluten-containing diet.

Three patients had chronic constipation that was refractory to medical and supportive treatment. tTG-IgA levels were weakly positive in all of them. Endoscopic biopsies revealed mild eosinophilia in one, nonspecific duodenitis in another and normal results in the remaining patient. One patient had normal tTG-IgA levels a year later at follow-up; the other patients are being followed.

Six patients had malabsorptive symptoms, including failure to thrive in all and chronic diarrhea in three cases. Two patients with weak positive tTG-IgA levels had a final diagnosis of eosinophilic enteropathy. One of these patients responded to a gluten-free diet, while the other received steroids. Both patients had normal tTG-IgA levels at follow-up. Duodenal biopsies were normal in the remaining four patients, in three of whom tTG-IgA levels were >200 RU/ml. At follow-up, tTG-IgA levels were normal in three of four patients. The other patient had tTG-IgA levels >200 RU/ml, and will undergo a second endoscopy.

One patient, with idiopathic short stature, had a tTG-IgA level that was weakly positive. Endoscopy revealed increased intraepithelial lymphocytes with normal villous architecture.

The HLA-DQ genotyping and serology of the non-CD patients are shown in Table II. Twelve (92.3%) of the 13 patients had positive HLA-DQ2 and/or HLA-DQ8 genotyping. tTG-IgA levels were strongly positive in 5 (38.4%) of 13 patients. Overall, the presence of both HLA-DQ2 and/or HLA-DQ8 and strong tTG-IgA positivity in non-CD patients was 38.4% (95% CI: 11.9-64.8%).

The simple scoring system gave an overall score of 3.8 ± 0.8 points for non-CD patients. Eleven (84.6%) of the 13 patients had ≤4 points.

Accuracy of HLA-DQ genotyping in combination with serology, and the simple scoring system for the diagnosis of CD

The sensitivity, specificity, PPV, NPV, PLR, NLR and accuracy of (i) HLA-DQ genotyping in combination with serology and (ii) the simple scoring system are shown in Table III. The combination of HLA-DQ2 and/or HLA-

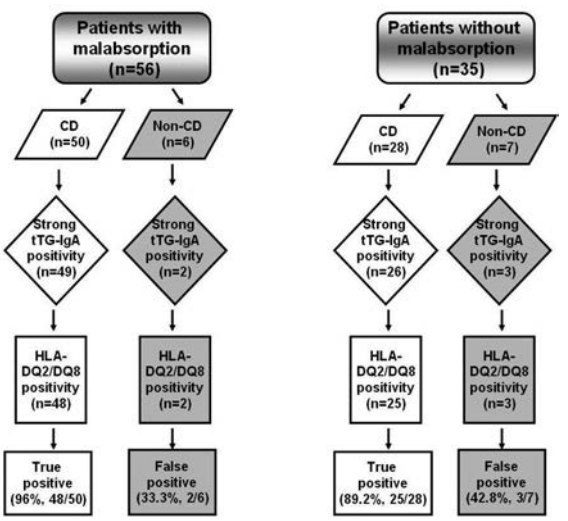

Fig. 1. Accuracy of both HLA-DQ typing and strong tTG-IgA positivity for the diagnosis of typical and atypical CD.

Table I. Demographic and Clinical Findings of Patients (n=91).

Parameters	CD patients		Overall	Non-CD patients
	Group 1 (n=50)	Group 2 (n=28)	(n=78)	Group 3 (n=13)
Age, years ± SD	7.3 ± 4.9	9.8 ± 3.6		7.5 ± 4.6
Gender, female/male, n	31/19	17/11	48/30	7/6
Primary symptoms, n (%)				
Malabsorptive symptoms	50 (100)	-	50 (64.1)	6 (46.1)
Short stature	-	13 (46.4)	13 (16.6)	1 (7.6)
Anemia	-	4 (14.2)	4 (5.1)	-
Hypoglycemia	-	1 (3.5)	1 (1.2)	-
Chronic constipation	-	1 (3.5)	1 (1.2)	3 (23)
Asymptomatic	-	9 (32.1)	9 (11.5)	3 (23)
Associated diseases				
Type 1 diabetes	2 (4)	8 (28.5)	10 (12.8)	3 (23)
Autoimmune thyroiditis	1 (2)	3 (10.7)	4 (5.1)	-
Liver involvement	-	1 (3.5)	1 (1.2)	-
Pityriasis	1 (2)	-	1 (1.2)	-
Hyperphosphatasia	1 (2)	-	1 (1.2)	-
Recurrent intestinal pseudo-obstruction	-	-	-	1 (7.6)
Arnold-Chiari malformation	-	-	-	1 (7.6)

Table II. HLA-DQ Genotyping and Serology of CD (n=78) and non-CD (n=13) Patients.

Parameters	CD patients			Non-CD patients
	Group 1 (n=50)	Group 2 (n=28)	Overall (n=78)	Group 3 (n=13)
HLA-DQ genotyping				
HLA-DQ2 and/or HLA-DQ8	49 (98)	27 (96.4)	76 (97.4)	12 (92.3)
HLA-DQ2/HLA-DQ2	22 (44)	6 (21.4)	28 (35.8)	5 (38.4)
HLA-DQ2/others	5 (10)	7 (25)	12 (15.3)	1 (7.6)
HLA-DQ8/HLA-DQ8	3 (6)	6 (21.4)	9 (11.5)	2 (15.3)
HLA-DQ8/others	4 (8)	1 (3.5)	5 (6.4)	2 (15.3)
HLA-DQ2/HLA-DQ8	15 (30)	7 (25)	22 (28.2)	2 (15.3)
Serology				
Weak tTG-IgA positivity	-	-	-	7 (58.8)
Moderate tTG-IgA positivity	1 (2)	2 (7.1)	3 (3.8)	1 (7.6)
Strong tTG-IgA positivity	49 (98)	26 (92.8)	75 (96.1)	5 (38.4)
Both HLA-DQ2 and/or HLA-DQ8 and strong tTG-IgA positivity	48 (96)	25 (89.2)	73 (93.5)	5 (38.4)

DQ8 and strong tTG-IgA positivity had a sensitivity of 96%, specificity of 61.5%, PPV of 90.5%, NPV of 80%, PLR of 2.4, NLR of 0.06 and accuracy of 88.8% for typical CD. For atypical CD, the respective numbers were 89.2%, 61.5%, 83.3%, 72.7%, 2.3, 0.17 and 80.4%. Sensitivity and specificity were 93.5% and 61.5% respectively for typical plus atypical CD, while PLR, NLR and accuracy were 2.4, 0.1 and 89%.

The simple scoring system had a sensitivity of 93.5%, specificity of 84.6%, PPV of 97.5%, NPV of 68.7%, PLR of 6, NLR of 0.07 and accuracy of 92.3%.

Table III. Sensitivity, Specificity, PPV, NPV, PLR, NLR and Accuracy of HLA-DQ Genotyping in Combination with Serology, and the Simple Scoring System.

	Sensitivity	Specificity	PPV	NPV	PLR	NLR	Accuracy
For typical CD (n=50 vs. 13)							
HLA-DQ2 and/or HLA-DQ8 & strong tTG-IgA positivity	96	61.5	90.5	80	2.4	0.06	88.8
Simple scoring system	98	84.6	96	91.6	6.3	0.02	95.2
For atypical CD (n=28 vs. 13)							
HLA-DQ2 and/or HLA-DQ8 & strong tTG-IgA positivity	89.2	61.5	83.3	72.7	2.3	0.17	80.4
Simple scoring system	85.7	84.6	92.3	73.3	5.5	0.16	85.3
For overall CD (n=78 vs. 13)							
HLA-DQ2 and/or HLA-DQ8 & strong tTG-IgA positivity	93.5	61.5	93.5	61.5	2.4	0.1	89
Simple scoring system	93.5	84.6	97.3	68.7	6	0.07	92.3

PPV: positive predictive value, NPV: negative predictive value,
PLR: positive likelihood ratio, NLR: negative likelihood ratio

Figure 1 shows the accuracy of both HLA-DQ typing and strong tTG-IgA positivity for the diagnosis of typical and atypical CD. The combination of HLA-DQ2 and/or HLA-DQ8 positivity and strong tTG-IgA positivity missed two patients with typical CD (4%) and three patients with atypical CD (10.7%). In addition, two patients with malabsorptive symptoms (33.3%) and three patients without malabsorptive symptoms (42.8%) would have been misdiagnosed with CD based on the combination of HLA-DQ2 and/or HLA-DQ8 positivity and strong tTG-IgA positivity.

Discussion

In this study, we found that the simple scoring system, which includes histological findings, had higher sensitivity and specificity than the combination of HLA-DQ2 and/or HLA-DQ8 positivity and strong tTG-IgA positivity for the diagnosis of CD in children.

In previous studies, the sensitivity and specificity of HLA-DQ2 and/or HLA-DQ8 positivity were found to be 96.2% and 54%, respectively[4]. Due to the high specificity of HLA-DQ typing for CD, it is recommended for use in the evaluation of the diagnosis in high-risk populations. A two-step strategy is recommended in high-risk patients, including patients with type 1 diabetes or Down syndrome, or first-degree relatives of celiac patients based on, first, selection of individuals with potential CD by HLA-DQ typing, followed by serology and biopsy in patients with the HLA-DQ genotype. Karinen et al.[10] investigated the usefulness of HLA-DQ genotyping for the identification of CD in first-degree relatives of celiac patients (n=382) and found that approximately 20% of these relatives did not carry any of the alleles and had negative serology for CD. On the other hand, 99.3% of the celiac patients among the first-degree relatives carried at least one of the alleles. Kurppa et al.[11] studied the prevalence of CD in the relatives of celiac patients (n=3031) and found that celiac-type human leukocyte antigens had good correlation with the antibodies. Csizmadia et al.[12] found that once-in-a-lifetime screening is not enough to detect CD in patients with Down syndrome. They proposed a cost-saving two-step strategy for screening, based on selection of individuals with potential CD by HLA-DQ typing and on longitudinal serologic CD screening in this selected group. The prevalence of CD is approximately 8-10% in children with type 1 diabetes, but increases to 30-35% in patients with both type 1 diabetes and positive HLA-DQ typing for CD. However, there have been no prospective studies about the usefulness of

HLA-DQ typing for predicting CD in patients with type 1 diabetes[13]. In our study, 97.4% of patients had celiac-type human leukocyte antigens: 98% of symptomatic patients and 96.4% of atypical patients. Using HLA-DQ typing, the diagnosis could have been missed in two of 78 patients (2.5%).

Serological analyses of patients with CD have been extensively studied, and anti-endomysial antibodies immunoglobulin A and tTG-IgA are presently recognized as the most effective tests, with high sensitivity and specificity, above 95%[14]. The PPV of strong tTG-IgA positivity (>200 RU/ml, or >10 times the upper normal limit) for CD was found to be close to 100% in symptomatic cases, and it was concluded that small-bowel biopsy was not necessary for these cases[15]. On the other hand, Freeman[16] reported that in approximately 20% of adult patients with strong tTG-IgA positivity, there was no association with CD. In the patient group in the present study, two of the 14 patients had normal mucosal findings, and one had unclassified sprue. False tTG-IgA positivity has been reported in patients with liver disease or small bowel inflammation, or during the course of infectious disease[17]. In recent reports, it has been shown that findings of normal intestinal mucosa with strong tTG-IgA positivity do not exclude subsequent CD. Kurppa et al.[18] showed mucosal deposition of transglutaminase 2 in the mucosal villi of such patients who had normal mucosal architecture, a normal villous height/crypt depth ratio and normal intraepithelial lymphocytes. They suggested that mucosal atrophy may develop in these patients if they continue gluten consumption. In some reports, it has been indicated that these patients may also show spontaneous seroconversion, and mucosal damage may be seen after a long period of time[19].

Three of 13 non-CD patients in our study had type 1 diabetes mellitus, and in two of the three the diabetes was newly diagnosed. Past studies, in both children and adults, have shown that CD occurs in patients with type 1 diabetes at a prevalence that varies from 4.4% to 11.1%, compared with 0.5-1% of the general population. The age at onset of type 1 diabetes is earlier in patients with both diseases than in those with type 1 diabetes alone. The risk of CD is negatively and independently associated with age at onset of diabetes, with a higher risk being seen in children aged <4 years than in those aged >9 years. In patients with type 1 diabetes, diabetes is usually diagnosed first, with CD preceding onset of diabetes in only 10-25% of patients; CD diagnosis in type 1 diabetes patients generally occurs during annual screenings. The prevalence of CD was found to be 6-8% among newly diagnosed type 1 diabetes patients[20, 21]. It has been shown that type 1 diabetes before the age of 20 years is characterized by strong HLA-defined genetic disease susceptibility, a more frequent autoantibody response to various cell antigens, a higher frequency of preceding infections and more severe metabolic decompensation at presentation than is the case in patients diagnosed in adulthood[22]. A higher frequency of autoantibodies at presentation may cause false tTG-IgA positivity in younger patients with type 1 diabetes. Waisbourd-Zinman et al.[23] showed in a recent study that high anti-tTG antibody levels in children diagnosed with type 1 diabetes spontaneously normalize in approximately one-third of cases. They suggest that in asymptomatic children with type 1 diabetes and mildly elevated tTG levels, physicians should consider serologic follow-up on a gluten-containing diet for at least 12 months, rather than immediate duodenal biopsy. We recommend careful monitoring with intestinal biopsy in patients with both a new diagnosis of type 1 diabetes and strong tTG-IgA positivity, since some of them could spontaneously seroconvert as other antibodies.

A high prevalence of CD has been reported in patients with chronic constipation in previous studies[24]. Screening for CD is recommended especially in the case of patients where laxative treatment is ineffective and there is failure to thrive. All of the patients in our study group had weakly elevated tTG-IgA levels. Alterations in intestinal permeability or prolonged exposure of intestinal enterocytes to gluten antigens due to decreased transit time may cause elevation in tTG-IgA levels in CD. Two of the patients in the non-CD group who had weakly elevated tTG-IgA levels had a final diagnosis of eosinophilic enteropathy. As in the case of chronic constipation, alterations in intestinal permeability may cause tTG-IgA elevations in these patients.

The limitations of our study are (i) the lack of transglutaminase 2 staining in the intestinal

mucosa of patients with elevated tTG-IgA and normal mucosal architecture, (ii) the small number of patients in the study group and (iii) the lack of HLA-DQ typing in a healthy population in order to compare the prevalence of HLA-DQ positivity in CD and healthy populations. Furthermore, it should be kept in mind that, since villous atrophy may develop in patients with positive genotyping and strong tTG-IgA positivity over time, long-term follow-up is mandatory for a more definitive conclusion.

In conclusion, intestinal biopsy is still mandatory for the diagnosis of CD in Turkish children, despite the fact that it is an invasive procedure, lesions can be patchy and assessment is subjective.

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