HLA DQ2/DQ8 TYPING IN THE DIAGNOSIS OF CELIAC DISEASE IN SYMPTOMATIC CHILDREN

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Abstract

Background: A strong association between celiac disease (CD) and human leukocyte antigens (HLA) -DQ2 and HLA-DQ8 molecules was demonstrated and therefore DQ typing is nowadays used in CD diagnosis. We aimed to evaluate the usefulness of HLA DQ2/DQ8 typing in CD diagnosis of symptomatic patients with positive anti-tissue transglutaminase antibodies (anti-tTGA) and histologic findings compatible with CD.

Methods: We performed a retrospective study including patients investigated for CD who underwent HLA DQ2/DQ8 typing and intestinal biopsy. We selected patients that presented with CD suggestive symptoms, positive anti-tTGA and specific histologic findings. We extracted from the medical records: age, clinical symptoms, anti-tTGA titers and histologic findings (according to Marsh-Oberhuber classification). Regarding anti-tTGA titers, three groups were defined: anti-tTGA < 3-fold the upper limit of normal range, anti-tTGA between 3-fold and 10-fold the upper limit of normal range and anti-tTGA > 10-fold the upper limit of normal range. According to the clinical response to gluten free diet (GFD), we defined two groups: responsive (disappearance of symptoms or significant improvement under GFD) and non-responsive (mild improvement or persistent symptoms).

Results: Thirty seven patients were found to be HLA DQ2/DQ8 positive (29 children had mildly/moderately increased anti-tTGA; 8 cases had anti-tTGA>10-fold the cutoff) and 8 patients were HLA DQ2/DQ8 negative. We compared two groups: patients HLA DQ2/DQ8 negative with anti-tTGA mildly/moderately elevated values and patients HLA DQ2/DQ8 positive with anti-tTGA mildly/moderately elevated values. Mildly elevated or borderline anti-tTGA were found in 100% HLA DQ2/DQ8 negative patients vs. 37.9 % HLA DQ2/DQ8 positive patients. All HLA DQ2/DQ8 negative patients had mild/moderate intestinal lesions, while in HLA DQ2 and/or DQ8 positive patients mild/moderate enteropathy was the histologic finding in 62.1% of cases.

Conclusions: Patients with high anti-tTGA were HLA DQ2 and/or DQ8 positive and intestinal biopsy could have been avoided. HLA DQ2/DQ8 typing was useful in the evaluation of patients with mildly increased anti-tTGA, especially at young age, with mild/moderate histologic lesions and unresponsive to GFD. These data show that HLA testing plays an important role in the diagnostic algorithm of CD.

Key words: Celiac disease, HLA DQ2/DQ8 typing, children

Introduction

The involvement of genetic factors in celiac disease (CD) was observed many years ago, but is still under evaluation. The association of certain human leukocyte antigens (HLA) and CD was discovered by Falchuk et al. in 1972 [**Error! Reference source not found.**]. A decade later a strong association between CD and HLA-DQ2 and HLA-DQ8 molecules was demonstrated [**Error! Reference source not found.-Error! Reference source not found.**]. Forty loci have been identified to be involved in the etiopathogeny of the disease. All disease loci have been characterized as low-penetrance, with the exception of the high-risk genotypes in the HLA-DQA1 and HLA-DQB1 genes [6].

In consequence, DQ genotyping is used in CD diagnosis. The majority of celiac patients carry DQ2.5 heterodimers (DQA1*05 and DQB1*02) and a small percentage express HLA-DQ8 (DQA1*03 DQB1*0302). About 5% of celiac patients carry DQ2.x heterodimers (DQA1*05 and DQB1*02) [**Error! Reference source not found.**,8].

CD appears very rarely in patients negative for these DQ predisposing markers. So testing for their presence has high negative predictive value for the diagnosis. HLA DQ2/DQ8 typing is recommended for patients with uncertain diagnosis.

In the last two decades, the clinical picture of celiac disease became broader and new, improved diagnosis techniques were developed. Therefore, revision of the second ESPGHAN guideline was considered necessary.

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The present ESPGHAN guideline recommends HLA DQ2/DQ8 typing for asymptomatic patients from risk groups, for symptomatic patients with uncertain diagnosis: positive anti-tissue transglutaminase antibodies (anti-tTGA), but mild enteropathy on histologic examination or negative anti-tTG and mild infiltrative changes in small-bowel specimens and also for children with high anti-tTG titers in order to avoid intestinal biopsy [9].

Aim

The aim of the study was to evaluate the usefulness of gene testing for HLA DQ2 and DQ8 haplotypes in CD diagnosis of symptomatic children with positive anti-tTG antibodies and histologic findings compatible with CD.

Material and method

We performed a retrospective study including patients investigated for CD in the Pediatric Gastroenterology Department of ”Grigore Alexandrescu” Emergency Children’s Hospital, Bucharest, patients who underwent HLA DQ2/DQ8 typing.

We reviewed the medical records of all patients that underwent HLA DQ2/DQ8 typing and intestinal biopsy. We selected patients that presented with CD suggestive symptoms and signs, positive anti-tTGA and specific histologic findings.

Exclusion criteria were: asymptomatic patients from risk groups (first degree relatives with CD or patients with CD-associated diseases), patients with negative specific antibodies or patients that were only tested with anti-gliadin antibodies (the latter were patients diagnosed years back when other serological markers were not available) and patients with normal intestinal biopsy.

In selected patients, we extracted from the medical records: age, clinical symptoms and signs, anti-tTGA titers and histologic findings (according to Marsh-Oberhuber classification) [10]. Regarding anti-tTGA titers, three groups were defined: anti-tTGA < 3-fold the upper limit of normal range, anti-tTGA between 3-fold and 10-fold the upper limit of normal range and anti-tTGA > 10-fold the upper limit of normal range.

The clinical response to gluten free diet (GFD) was evaluated in patients that did not underwent HLA DQ2/DQ8 typing at the moment of initial diagnosis and were already under exclusion diet when tested. According to the clinical response, we defined two groups: responsive (disappearance of symptoms or significant improvement under GFD) and non-responsive (mild improvement or persistent symptoms).

Typing methodology was polymerase chain reaction with sequence-specific primers (PCR-SSP); we used the commercial kits ``HISTO TYPE Celiac Disease`` (BAG Health Care GmbH - Germany). The kit contains 23 amplification primers that identify DQB1*02:01 to *02:05 alleles; DQB1*03:01 to *03:17 and DQA1 *02:01; *03:01;*05:01 to *05:09 alleles.

Statistical analysis was performed using SPSS. The characteristics of HLA DQ2/DQ8 positive patients were compared to those of DQ2/DQ8 negative patients. Statistical significance was calculated using Fisher exact test. Values of $p < 0.05$ were considered significant.

The study was approved by the Ethics Committee of the ”Grigore Alexandrescu” Emergency Children’s Hospital.

Results

174 children investigated for CD underwent HLA DQ2/DQ8 typing. After we applied the exclusion criteria, we analyzed the medical records of 77 symptomatic patients that also had intestinal biopsy performed.

Patients with normal findings on intestinal biopsy, patients with negative celiac serologies or patients that were only tested for anti-gliadin antibodies were all excluded, so 45 patients fulfilled the inclusion criteria. The mean age was 34 ± 29.5 months (p=0.03).

Thirty seven patients were found to be HLA DQ2/DQ8 positive and 8 patients HLA DQ2/DQ8 negative.

Out of HLA DQ2/DQ8 positive cases, 8 patients had anti-tTGA > 10-fold the cutoff values. Mean age was 41.6 ± 40.2 months. 7 out of 8 children had gastrointestinal symptoms. Histological findings corresponded to Marsh 3 in all patients, 5 out of 8 children exhibiting severe intestinal damage (Marsh 3b or 3c).

We compared two groups: patients HLA DQ2/DQ8 negative with anti-tTGA positive, mildly to moderately elevated values (mean age: 19.5 ± 6.3 months) and patients HLA DQ2/DQ8 positive with anti-tTGA positive, mildly to moderately elevated values (mean age: 41.6 ± 40.2 months). Gastrointestinal manifestations were reported for 75% of patients without HLA DQ2 or DQ8 vs 68.9% of patients with HLA DQ2 and/or DQ8 (p=1). Mildly elevated or borderline anti-tTGA (< 3-fold the cutoff) were found in 100% HLA DQ2/DQ8 negative patients vs. in only 37.9 % HLA DQ2/DQ8 positive patients (p=0.003). All HLA DQ2/DQ8 negative patients had mild to moderate intestinal lesions (Marsh 2 - 2 cases and Marsh 3a – 6 cases), while in HLA DQ2 and/or DQ8 positive patients mild to moderate enteropathy was the histologic finding in just 62.1% of cases (p=0.04) and Marsh 3b and 3c in 37.9% cases. Poor clinical response to GFD exhibited 5 out of 7 HLA DQ2/DQ8 negative patients (71.4%) and 16 out of 22 HLA DQ2/DQ8 positive patients (27.3%) (p=0.07). These were patients that were already under GFD and therefore, for them, an evaluation of the clinical response could be made.

The results are presented in table 1.

Among patients with anti-tTGA > 10-fold the cutoff, we found: one patient DQ2 and DQ8 positive, one patient DQ2 positive with a double dose of *DQB1*02*, one patient DQ8 positive and 5 patients DQ2 positive, with a single dose of *DQB1*0)*. Among patients with mildly to moderately elevated anti-tTG, the distribution was as follows: one patient DQ2 and DQ8 positive, 4 patients DQ2 positive, with a double dose of *DQB1*02*, 6 patients DQ8 positive and 18 patients DQ2 positive with a single dose of *DQB1*02*. P=0.47.

Table 1: Characteristics of symptomatic patients with mild/moderate positive anti-tTGA.

	HLA DQ2/DQ8 positive	HLA DQ2/DQ8 negative	p
Age (mean age, SD) months	41.6 ± 40.2	19.5 ± 6.3	0.03
Typical symptoms (number, %)	20 (68.9)	6 (75)	1
Anti-tTGA < 3-fold (number, %)	11 (37.9)	8 (100)	0.003
Histology – Marsh 1, 2, 3a (number, %)	18 (62.1)	8 (100)	0.07
Not responsive to GDF (number, %)	6/22 (27.3)	5/7 (71.4)	0.07

Discussions

The study aimed to evaluate in a retrospective manner the usefulness of HLA genotyping in CD diagnosis in symptomatic patients, based on the experience of a single centre of pediatric gastroenterology.

All patients with anti-tTGA > 10-fold the cutoff had positive HLA DQ2 and/or DQ8. The majority had the classical form of the disease; two of them presented as celiac crisis, situation rarely encountered in clinical practice nowadays. In all patients, villous atrophy was the histologic finding. In all these patients with high level of anti-tTGA, the confirmation of genetic risk through HLA DQ2/DQ8 typing might have been sufficient for the diagnosis; the biopsy could have been avoided, according to recent studies [**Error! Reference source not found.-Error! Reference source not found.**].

Among patients with mildly or moderately increased anti-tTGA, we found a relatively high percentage (27.5%) of negative HLA DQ2/DQ8 cases, for which CD was practically excluded. The potential of a bias due to the fact that the study conducted was retrospective must be taken into consideration. Not all symptomatic patients investigated for CD underwent HLA testing, the indication being decided by the Pediatric Gastroenterology specialist.

All HLA DQ2/DQ8 negative patients had mildly increased or borderline anti-tTGA titers. Although anti-tTGA testing is now the most commonly used and recommended diagnosis test, some authors discuss it's limits [16]. Anti- tTGA may have false positive values related to the technique used or a high rate of false positives in antibody testing may be related to pathological situations such as inflammatory bowel disease [**Error! Reference source not found.**].

All HLA DQ2/DQ8 negative patients had mild to moderate enteropathy on biopsied specimens; two patients had Marsh 2 lesions and is obvious that HLA DQ testing is useful for exclusion of CD in patients with minor mucosal changes [**Error! Reference source not found.**,**Error! Reference source not found.**]. Still, 6 patients had Marsh 3a lesions.

Partial villous atrophy is currently encountered in small children related to different pathologies. In our study the cases of non-celiac enteropathy were young age children (mean age 19.5 months, ranging from 11 to 26 months). Two patients were diagnosed with cow's milk protein allergy. One case had Giardia lamblia infestation, chronic mucosal inflammation in children being sometimes related to this parasite. Considering the relatively high incidence of this infection in our country setting, Giardia may possibly be the etiology of the villous atrophy in some other patients diagnosed with CD [20]. Some authors consider that in these cases the villous atrophy is not associated with intraepithelial lymphocytes, specific to the histologic aspects of CD, and this could be considered a differentiation criteria. Other authors found a large number of intraepithelial lymphocytes related to Giardia infection [21, 22], so the diagnosis may be sometimes difficult [23,24].

Among children for which CD was infirmed, a higher number of patients had a poor response to GFD compared to the CD confirmed patients. It is considered that subjective response to gluten free diet has poor predictive value for coeliac disease, but the persistent or unsignificant improvement of symptoms makes the initial diagnosis uncertain.

The large number of cases for which CD was infirmed may be explained through the existence of false positive anti-tTG antibodies due to technical difficulties. Errors in the interpretation of biopsy specimens may appear; false-positive results concerning small bowel biopsies may occur due to poorly oriented biopsies or inter-observer variation in interpretation [**Error! Reference source not found.**,**Error! Reference source not found.**].

Due to it's high negative predictive value HLA DQ2/DQ8 typing allowed the diagnosis of CD to be excluded in a number of patients that were initially diagnosed with CD according to the ESPGHAN guideline (suggestive symptoms, anti-tTGA positive, histologic findings compatible with CD). Nevertheless these patients were of young age, had mildly to moderately increased anti-tTGA values and histologic lesions at most Marsh 3a and the majority didn't respond to GFD.

Conclusions

All symptomatic patients with high values of anti-tTGA were HLA DQ2 and/or DQ8 positive; in these CD confirmation through intestinal biopsy could have been

avoided. HLA DQ2/DQ8 typing also proved useful in the evaluation of patients with mildly increased anti-tTGA, especially at a young age, with mild to moderate histologic lesions and poor response to GFD. These data show that HLA testing plays an important role in the diagnostic algorithm of CD.

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