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**Children with celiac disease and high tTGA are genetically and phenotypically different**

**Mubarak A *et al*.** Celiac disease and high tTGA levels

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**Abstract**

**AIM**: To investigate whether celiac disease (CD) patients with tissue-transglutaminase antibody (tTGA) ≥ 100 U/mL are different from patients with lower tTGA levels.

**METHODS:** Biopsy-proven (Marsh III) pediatric CD patients (*n* = 116) were prospectively included between March 2009 and October 2012. The biopsies were evaluated by a single pathologist who was blinded to all of the patients’ clinical data. The patients were distributed into 2 groups according to their tTGA level, which was measured using enzyme-linked immunoassay: tTGA ≥ 100 U/mL and Ttga < 100 U/mL. The patients’characteristics, symptoms, human leukocyte antigen (HLA) genotype and degree of histological involvement were compared between the 2 groups.

**RESULTS**: A total of 34 (29.3%) children had tTGA values < 100 U/mL and 82 (70.7%) tTGA levels of ≥ 100 U/mL. Patients with high tTGA levels had lower average body weight-for-height standard deviation scores (SDS) than did patients with tTGA < 100 U/mL (-0.20 ± 1.19 SDS *vs* 0.23 ± 1.03 SDS, *P* = 0.025). In the low tTGA group, gastrointestinal symptoms were more common (97.1% *vs* 75.6%, *P* = 0.006). More specifically, abdominal pain (76.5% *vs* 51.2%; *P* = 0.012) and nausea (17.6% *vs* 3.7%, *P* = 0.018) were more frequent amongpatients with low tTGA. In contrast, patients with solely extraintestinal manifestations were only present in the high tTGA group (18.3%, *P* = 0.005). These patients more commonly presented with aphthous stomatitis (15.9% *vs* 0.0%, *P* = 0.010) and anemia (32.9% *vs* 11.8%, *P* = 0.019). In addition, when evaluating the number of CD-associated HLA-DQ heterodimers (HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8), patients with low tTGA levels more commonly had only 1 disease-associated heterodimer (61.8% *vs* 31.7%, *P* = 0.005), while patients with high tTGA more commonly had multiple heterodimers. Finally, patients with tTGA ≥ 100 U/mL more often had a Marsh IIIc lesion (73.2% *vs* 20.6%, *P* = < 0.001) while in patients with low tTGA patchy lesions were more common (42.4% *vs* 6.8%, *P* = < 0.001).

**CONCLUSION:** Patients with tTGA ≥ 100 U/mL show several signs of more advanced disease. They also carry a larger number of CD associated HLA-DQ heterodimers.

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**Key words:** Celiac disease; Serology; Anti-tissue transglutaminase antibodies; Human leukocyte antigen; Phenotype

**Core tip:** We prospectively investigated the differences between celiac disease (CD) (Marsh III) patients with tissue-transglutaminase antibody (tTGA) levels ≥ 100 U/mL and patients with lower tTGA levels. We found that patients with high tTGA more often carried multiple CD-associated heterodimers compared with patients with tTGA < 100 U/mL. In addition, high-tTGA patients have more advanced mucosal lesions that are also less patchy. Phenotypically, high-tTGA patients have a lower body weight and more often present with extraintestinal symptoms compared with patients with lower levels of tTGA, who more often have intestinal symptoms. These results provide further evidence that patients with tTGA ≥ 100 U/mL are truly a distinct group with more advanced disease.

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**INTRODUCTION**

Celiac disease (CD) is a highly prevalent disorder with a strong genetic component. The disease has a complex and variable clinical presentation: some patients display symptoms ranging from severe malabsorption to vague intestinal or extraintestinal manifestations, while others have no symptoms at all[1-3]. The disease is caused by inappropriate immune responses to gluten, a storage protein in wheat and the related grain species barley and rye[4]. The immune reaction mainly affects the small intestine, where it typically causes lymphocyte invasion in the epithelium, hyperplasia of the crypts and various grades of villous atrophy[5,6]. These histological lesions can be patchily distributed throughout the small intestine and can even occasionally be localized exclusively in the duodenal bulb[7,8]. Serologically, signs of inflammation are also evidencedby the presence of disease-associated antibodies, including endomysium antibodies (EMA) and tissue transglutaminase antibodies (tTGA)[4,9,10].

Until recently, these serological and histological manifestations were used in combination to detect CD, with histological evaluation being essential for establishing the diagnosis in all cases[9,11]. However, given the excellent sensitivity and specificity of serology, the new ESPGHAN guidelines now indicate that a biopsy can be omitted in symptomatic children with tTGA levels ≥ 100 U/mL (> 10 times the upper limit) and positive EMA, provided the patient also carries a disease-associated human leukocyte antigen (HLA) type and responds well to the diet[12]. In contrast, in patients with a tTGA < 100 U/mL, a biopsy is always necessary because a significant proportion of patients with these levels do not have CD.

It is unclear why patients with a tTGA ≥ 100 U/mL virtually always have CD. These high levels could be a sign of advanced disease. Patients with high serum tTGA may also have a different genetic risk profile. Because HLA genes makethe greatest genetic contribution, the aim of this study was to assess whether patients with a tTGA ≥ 100 U/mL have a different HLAdistribution compared withpatients with lower tTGA levels[13]. We also investigated whether more advanced small intestinal histological lesionswerepresent in patients with a tTGA ≥ 100 U/mL. In addition, as it remains to be resolved whether patients with tTGA levels ≥ 100 U/mL are phenotypically distinct from those with a tTGA < 100 U/mL, we set out to detect differences in clinical presentation between both groups.

**MATERIALS AND METHODS**

***Study population***

Pediatric patients who had a histologically confirmed diagnosis of CD between March 2009 and October 2012 in the Wilhelmina Children’s Hospital in Utrecht, The Netherlands, were prospectively included in the study. Patients were referred to us because of CD-associated symptoms or because they belonged to a group at risk for CD. Biopsies were collected from patients with abnormal serology. Biopsies were also collected from patients with negative serology but a strong clinical suspicion of the disease. Patients with immune globulin A (IgA) deficiency (*n* = 3) were excluded from the study. The clinical symptoms at presentation were collected from the medical records. The study was performed according to the guidelines of the local medical ethics board.

***Histological evaluation***

Biopsies were obtained using upper endoscopy. On average, 3.09 biopsies (range 1 to 5, SD 0.75) were obtained from the distal duodenum, and 2.41 (range 0 to 5, SD 1.03) were obtained from the duodenal bulb. The biopsies were evaluated by a single experienced pathologist who was blinded to all of the patients’ clinical data and whousedthe Marsh classification, as modified by Oberhuber[5,6]. The duodenal bulb and the distal duodenum were scored separately, but the final Marsh score for each patient was graded according to the most affected site (highest Marsh score). Only Marsh III lesions (*i.e.*, those characterized by an increased number of intraepithelial lymphocytes, crypt hyperplasia and villous atrophy) were considered diagnostic for CD. Patients with other histological findings were not included. Marsh III lesions were further classified according to the degree of villous atrophy: Marsh IIIa (partial villous atrophy), Marsh IIIb (subtotal villous atrophy) and Marsh IIIc (total villous atrophy).

***Serological assessment***

Serum IgA tTGA levels were measured using the ELiA Celikey IgA kit (Phadia AB, Uppsala, Sweden). Serum samples containing an antibody titerof more than 10 U/mL were considered positive, as recommended by the manufacturer. IgA EMA levels were detected *via* indirect immunofluorescence using sections of distal monkey esophagus mounted on glass slides (IMMCO Diagnostics Inc., Buffalo NY). Total IgA was measured in all patients, and a serum IgA concentration below 0.07 g/L was regarded as IgA deficiency.

***HLA-typing***

Genomic DNA was isolated from ethylenediaminetetraacetic acid-anticoagulated blood with a standardized DNAzol-based technique. The HLA-DQA1 and HLA-DQB1 alleles were typed using the sequence-specific oligonucleotide primed polymerase chain reaction technique with the Luminex-based OneLambda LABType SSO Class II DQA1/DQB1 typing kit, following the recommendations of the manufacturer (One Lambda Inc., Canoga Park, CA, United States). Samples were analyzed on a LABScanTM 100 System (Luminex, Austin TX, United States), and data were interpreted using the HLA-Fusion 2.0 Software package (OneLambda).

HLA-DQ2.5 (DQA1\*05:01, -DQB1\*02:01 or DQA1\*05:05, -DQB1\*02:02), HLA-DQ2.2 DQA1\*02:01, -DQB1\*02:02) and HLA-DQ8 (DQA1\*03:01, -DQB1\*03:02 or DQA1\*03:02, -DQB1\*03:02) were considered CD-associated HLA-types. The patients were scored for the number of CD-associated heterodimers that they couldform with their HLA-genotypes. For example, a patient who is homozygous for HLA-DQ2.5 (or HLA-DQ2.2 or HLA-DQ8) can form 4 different heterodimers that are associated with CD. The same is true of patients who are compound heterozygous for HLA-DQ2.5 and HLA-DQ2.2, because these patients can also make 4 different CD-associated heterodimers: HLA-DQA1\*05:01, -DQB1\*02:01; HLA-DQA1\*02:01, -DQB1\*02:02; HLA-DQA1\*05:01, -DQB1\*02:02 and HLA-DQA1\*02:01, -DQB1\*02:01 (the latter 2 of which are molecularly indistinguishable from the first 2). Patients who are heterozygous for HLA-DQ2.5 and HLA-DQ8 or HLA-DQ2.2 and HLA-DQ8 can only form 2 CD-associated heterodimers. Finally, patients with only 1 CD-associated HLAgenotype can only generate 1 CD-associated heterodimer.

***Statistical analysis***

The patients were divided in 2 groups: thosewith tTGA ≥ 100 U/mL and thosewith tTGA < 100 U/mL. Subsequently, the differences between the 2 groups in terms of gender, average age at diagnosis, average height and weight, the presence of a CD-associated disease, the presence of a first-degree relative with CD, symptoms, HLAtype, Marsh classification and histological differences between the duodenal bulb and the more distal duodenum were calculated using SPSS Version 20.0.

To test for statistical significance, the *χ*2 or Fisher exact test wasused for nominal variables. For continuous variables, the independent t-test or the Mann-Whitney *U*-test were used. A *P*-value < 0.05 was considered statistically significant.

**RESULTS**

***Patient characteristics***

A total of 116 patients met the study’s inclusion criteria. Of those, 34 (29.3%) patients had tTGA values <100 U/mL and 82 (70.7%) had a serum tTGA of at least 100 U/mL. Within the low tTGA group, 2 patients, a 10-month-old girl and a 2-year-old boy, had a tTGA level <10 U/mL and negative EMA, which is not an uncommon finding in very young children[11,14-18]. All of the remaining patients had positive EMA levels.

Of the total study population, 32 (27.6%) were male and 84 (72.4%) female, with no difference in gender distribution between the high and lowtTGA groups (Table 1). The mean age of the included patients at diagnosis was 6.5 years, ranging from 0.9 to 17.7 years. The average age at diagnosis was slightly higher (7.4 years) in the low tTGA group compared withthe high tTGA group (6.1 years), but this was statistically not significant. The patients in the high tTGA group were slightly shorter (-0.83 standard deviation score, SDS) compared withthe low tTGA group (-0.60 SDS), but this difference was not significant. In contrast, the average body weight-for-height was significantly lower (-0.20 SDS) in the high tTGA group compared with patients in the low tTGA group, who had an average weight of 0.23 SDS (*P* = 0.025).

Regarding comorbidity, 5 (4.3%) patients had Down syndrome, and 1 (0.86%) of those also had hypothyroidism. Another 4 (3.4%) patients had diabetes mellitus Type I, 1 (0.86%) patient had juvenile rheumatoid arthritis and 1 (0.86%) patient had Graves disease. Remarkably, all but 1 of the patients with comorbidity had tTGA ≥ 100 U/mL; however, this finding was not statistically significant. Finally, 9 (26.5%) patients in the low tTGA group had a first-degree relative with CD, compared with 14 (17.1%) patients in the high tTGA group; again, this difference was not statistically significant.

***Symptoms***

Only 5 (4.3%) patients were asymptomatic, 4 of which had a tTGA ≥ 100 U/mL and 1 of which had a tTGA < 100 U/mL (Table 2). The other 111 (95.7%) patients had various gastrointestinal and extraintestinal symptoms. Interestingly, gastrointestinal symptoms were significantly (*P* = 0.006) more common in the low tTGA group (*n* = 33; 97.1%) compared with the high tTGA group, in which 75.6% (*n* = 62) of the patients suffered from a gastrointestinal symptom. However, although patients with symptoms restricted to the gastrointestinal tract (without any extraintestinal manifestations) were also more common in the low tTGA group (23.5% *vs* 9.8%, respectively), this difference was not statistically significant (*P* = 0.074). In terms of specific gastrointestinal complaints, abdominal pain and nausea were significantly more common in the low tTGA group. Indeed, 76.5% (*n* = 26) of the patients in the low tTGA group had abdominal pain, compared with 51.2% (*n*  = 42) in the high tTGA group (*P* = 0.012). Similarly, in the low tTGA group, 17.6% (*n* = 6) of the patients suffered from nausea, compared with3.7% (*n* = 3) in the high tTGA group (*P* = 0.018). Moreover, there was a statistically non-significant trend (*P* = 0.096) towards more constipation in the low tTGA group (*n* = 14; 41.2%) compared with the high tTGA group (*n* = 21; 25.6%). In contrast, diarrhea was more common in the high tTGA group (*n* = 27; 32.9%) compared with the low tTGA group (*n* = 8; 23.5%), but the difference was not significant (*P* = 0.316). Similarly, a comparable trend (*P* = 0.277) was seen for vomiting, which occurred more often in the high tTGA group (11.0% *vs* 2.9%). Finally, the presence of bloating was comparable in both groups with more than 1/3 of the patients suffering from this symptom.

Extraintestinal symptoms occurred in 25 (73.5%) of the patients with low tTGA compared with 70 (85.4%) patients in the high tTGA group, but this difference was not statistically significant (*P* = 0.132). However, patients with solely extraintestinal symptoms (*i.e.*, without gastrointestinal symptoms) were only present in the high tTGA group (*n* = 15; 18.3%), a finding that was statistically significant (*P* = 0.005). Similarly, aphthous stomatitis only occurred in patients with high tTGA (*n* = 13; 15.9%). This was statistically significant, with a *P*-value of 0.010. Likewise, anemia was significantly (*P* = 0.019) more common in the high tTGA group: 27 (32.9%) of the patients with high tTGA had anemia, compared with 4 (11.8%) patients with low tTGA. There was also a trend towards more increased appetite (7.3% *vs* 2.9%), joint pain (11.0% *vs* 5.9%) and low weight (8.5% *vs* 5.9%) in the high tTGA group, but these differences were not statistically significant (*P*-value > 0.05). Tooth enamel defects were more common in the low tTGA group (5.9% *vs* 3.7%), but this was also not statistically significant (*P* = 0.629). Finally, the presence of fatigue, irritability, anorexia and short stature was comparable in both groups.

***HLA-types***

All of the patients carried at least one of the CD-associated HLAtypes. In the high tTGA group, the patients more often carried multiple CD-associated heterodimers (*P* = 0.005; Table 3). Illustratively, in the low tTGA group, more than half of the patients (*n* = 21; 61.8%) had only one CD-associated heterodimer, compared with 26 (31.7%) in the high tTGA group. Two patients (5.9%) with low tTGA had 2 CD-associated heterodimers, compared with 20 (24.4%) patients in the high tTGA group. Finally, 36 (43.9%) patients in the high tTGA group had 4 CD-associated heterodimers, compared with 11 (32.4%) patients with low tTGA.

***Histology***

In the low tTGA group, 5 (14.7%) patients had a Marsh IIIa lesion, 22 (64.7%) had a Marsh IIIb lesion, and only 7 (20.6%) had a Marsh IIIc lesion (Table 3). This was significantly different from the high tTGA group (*P*-value < 0.001). Illustratively, only 4 (4.9%) patients in the high tTGA group had a Marsh IIIa lesion; 18 (22.0%) had a Marsh IIIb lesion, and the largest proportion of the patients in the high tTGA group (*n* = 60; 73.2%) had flat mucosa (Marsh IIIc).

In 106 patients, both duodenal bulb and distal duodenum biopsies were taken. To assess the presence of patchy lesions, the Marsh classification in both locations was compared. A patchy lesion was defined as the absence of villous atrophy in either the duodenal bulb or the distal duodenum. In 7 (6.6%) patients, a Marsh III lesion was only found in the duodenal bulb, while the distal duodenum was spared. In 12 (11.3%) patients, the distal duodenum was the only affected site. Interestingly, a discrepancy between the diagnosis in the distal duodenum *vs* the duodenal bulb was more common in patients with low tTGA than in patients with high tTGA (42.4% *vs* 6.8%, *P*-value < 0.001). In addition, patchy lesions were more common in patients with Marsh IIIa (in 5 of 9 patients, 55.6%) than in patients with Marsh IIIb (in 13 of 35 patients, 37.1%) or IIIc lesions (in 1 of 62 patients; 1.6%, *P*-value < 0.001).

**DISCUSSION**

CD is defined as a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals[19].

Patients with tTGA levels ≥ 100 U/mL (> 10 times the upper limit) virtually always have CD, whereas the disease can be histologically absentin a significant number of patients with a lower serum tTGA level. In the present study, we show in a pediatric population that patients with a tTGA level ≥ 100 U/mL also have a different HLA-pattern and a more severe histological lesion and seem to be phenotypically different, with more extraintestinal symptoms and a lower body weight.

Patients with high tTGA levels are more likely to have 2 and 4 CD-associated heterodimers compared with patients with lower tTGA levels, who more often only have 1 CD-associated heterodimer (Table 3). This seems pathophysiologically logical. In CD, HLA-molecules on antigen-presenting cells in the lamina propria present gluten peptides to CD4+ T-cells, which in turn further activate the immune system, including B-cells[20-22]. Thus, increased cell-surface expression of CD-associated heterodimers will lead to more antigen presentation and therefore more T- and B-cell stimulation, which will eventually generate a stronger antibody response.However, because not all patients with multiple heterodimers had a tTGA ≥ 100 U/mL, and some patients with a single HLA-heterodimer also had tTGA levels ≥ 100 U/mL, other factors, such as non-HLA genes or environmental factors, are likely to contribute to the tTGA-level response. This finding is in line with a previous study showing a correlation between antibody level and HLAdose; patients homozygous for HLA-DQB1\*02 had significantly higher tTGA levels compared with patients with a single dose of HLA-DQB1\*02 and to patients not carrying any HLA-DQB1\*02[23]. In the current study, a comparable HLA-DQB1\*02 correlation was found, but the difference was not significant (*P* = 0.101; data not shown).

The current study also provided evidence that patients with high tTGA levels have more advanced mucosal lesions compared with CD patients with lower tTGA levels. First, patients with tTGA levels ≥ 100 U/mL had a more severe grade of villous atrophy, in line with previous studies showing an increasing tTGA titer with increasing villous atrophy[24,25]. However, we also showed that patchy lesions, defined as the absence of villous atrophy in either the duodenal bulb or the distal duodenum, were more common in patients with low tTGA than in patients with high tTGA, suggesting that in patients with high tTGA, the total area of mucosa involved is larger. In addition, patients with a lesser degree of villous atrophy, which is more common in the low tTGA group, also had a higher chance of patchy lesions, providing more evidence that the disease in these patients is truly less advanced.

Interestingly, we also found significant differences in clinical presentation between patients with high tTGA and those with levels < 100 U/mL. The group with high tTGA levels had lower body weight and more extraintestinal complaints than did patients with low tTGA (Table 2). This suggests that patients with high tTGA levels have more advanced or generalized disease. Other studies investigating the relationship between antibody levels and symptoms are rare. Dahlbom and colleagues found that children with an onset of CD in early childhood and/or severe malabsorption had higher tTGA levels than did patients with a late childhood onset of disease and/or moderate symptoms, and also when compared with patients presenting in adulthood[24]. Taavela *et al*[26] also showed that the serum levels of antibodies associated with CD correlated with gastrointestinal symptoms. None of these two studies specifically investigated the differences in intestinal and extraintestinal symptoms, so their results cannot be directly compared with our study. However, in both studies, a relationship between antibody levels and symptom severity was observed, once again suggesting that patients with a high tTGA have more advanced disease.

Finally, we showed that patients in the low tTGA group more often have a positive family history for CD (26.5% *vs* 17.1%), although this difference was not statistically significant. This difference could have resulted because patients with a positive family history are detected earlier than those without a positive history, before a very high tTGA level is reached. Conversely, patients with comorbidity were found more frequently (although statistically not significant) in the high tTGA group (12.2% *vs* 2.9%), which might be due to a more advanced disease progression in this group.

Our combined data confirm, in a pediatric population, the hypothesis that patients with tTGA ≥ 100 U/mL have more advanced disease, given the more severe histological involvement and the increased incidence of extraintestinal manifestations and lower body weight. Pathophysiologically, these patients also express more CD-associated HLA-heterodimers on their cells. These findings should also be investigated in adults.

**COMMENTS**

***Background***

Genetically predisposed symptomatic children with positive endomysium antibodies (EMA) and tissue-transglutaminase antibody (tTGA) levels ≥ 100 U/mL virtually always have the classical histological triad of an increased number of intraepithelial lymphocytes, crypt hyperplasia and villous atrophy. These features are diagnostic for celiac disease (CD); therefore, in children with these high tTGA values, recent ESPGHAN guidelines have suggested that a biopsy is unnecessary to confirm the disease. Incontrast, in patients with lower tTGA levels, a biopsy is still mandatory for histological confirmation because a significant number of these patients appear not to have CD.

***Research frontiers***

It is unknown whether CD patients with high tTGA are phenotypically and genotypically different from CD patients with low tTGA.

***Innovations and breakthroughs***

Authors prospectively investigated the differences between CD (Marsh III) patients with tTGA levels ≥ 100 U/mL and patients with lower levels. They found that patients with tTGA ≥ 100 U/mL more often carry multiple CD-associated heterodimers compared with patients with lower levels. In addition, these patients have more advanced mucosal lesions that are also less patchy. Phenotypically, they have a lower body weight and more often present with extraintestinal symptoms compared with patients with lower tTGA levels, who more often have intestinal symptoms.

***Applications***

The findings of the current study provide further evidence that patients with high tTGA values are truly a distinct group with more advanced disease. These results therefore support the new European Society for Paediatric Gastroenterology, Hepatology and Nutrition criteria.

***Terminology***

tTGA: these antibodies are directed against the enzyme tissue-transglutaminase, which is the auto-antigen in CD. This enzyme plays a key role in eliciting the immune response against gluten. EMA: the endomysium is the intercellular matrix that lies between the smooth muscle cells of the muscularis mucosae throughout the gastrointestinal tract. It is rich in the enzyme tissuetransglutaminase. Antibodies directed against the endomysium are actually directed against tissue-transglutaminase. Human leukocyte antigen (HLA)-DQ2/8: gluten-derived peptides, especially after enzymatic modification by the enzyme tissue-transglutaminase, show a very high affinity forHLA-DQ2/8. In contrast, gluten peptides barely show affinity to other HLA-DQ types. Therefore, having more of these CD-associated heterodimers will result in a stronger T- and B-cell response, while an absence of HLA-DQ2/8 excludes the presence of CD.

***Peer review***

The paper by Mubarak *et al* investigated celiac children with high tTGA titres *vs* low titres. Main findings are a genetic diversity, extra-intestinal pathologies, lower height/weight ratio in high titre group. The paper is interesting and well written.

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**Table 1 Characteristics of patients *n* (%)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Patient characteristic** | **tTGA < 100 U/mL (*n*  = 34)** | **tTGA ≥ 100 U/mL (*n*  = 82)** | ***P*-value** |
| Gender (M) | 8 (23.5) | 24 (29.3) | 0.529 |
| Average age (yr) | 7.40 ± 4.06 | 6.10 ± 3.82 | 0.114 |
| Average height in SDS | -0.60 ± 1.15 | -0.83 ± 1.22 | 0.331 |
| Average weight for height in SDS | 0.23 ± 1.03 | -0.20 ± 1.19 | 0.025 |
| CD associated comorbidity | 1 (2.9) | 10 (12.2) | 0.171 |
| First degree relative with CD | 9 (26.5) | 14 (17.1) | 0.248 |

tTGA: Anti-tissue transglutaminase antibodies; SDS: Standard deviation scores; CD: Celiac disease.

**Table 2 Symptoms in** **celiac disease patients *n* (%)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **tTGA < 100 U/mL (*n*  = 34)** | **tTGA ≥ 100 U/mL (*n*  = 82)** | ***P*-value** |
| Symptoms | | | |
| Asymptomatic | 1 (2.9) | 4 (4.9) | 1.000 |
| Gastrointestinal symptoms | | | |
| Any gastrointestinal symptom | 33 (97.1) | 62 (75.6) | **0.006** |
| Only gastrointestinal symptoms | 8 (23.5) | 8 (9.8) | 0.074 |
| Abdominal pain | 26 (76.5) | 42 (51.2) | **0.012** |
| Diarrhea | 8 (23.5) | 27 (32.9) | 0.316 |
| Constipation | 14 (41.2) | 21 (25.6) | 0.096 |
| Bloating | 12 (35.3) | 31 (37.8) | 0.799 |
| Nausea | 6 (17.6) | 3 (3.7) | **0.018** |
| Vomiting | 1 (2.9) | 9 (11.0) | 0.277 |
| Extraintestinal symptoms | | | |
| Any extraintestinal symptom | 25 (73.5) | 70 (85.4) | 0.132 |
| Only extraintestinal symptoms | 0 (0.0) | 15 (18.3) | **0.005** |
| Fatigue | 16 (47.1) | 35 (42.7) | 0.666 |
| Irritability | 9 (26.5) | 25 (30.5) | 0.665 |
| Anorexia | 13 (38.2) | 32 (39.0) | 0.937 |
| Increased appetite | 1 (2.9) | 6 (7.3) | 0.672 |
| Joint pain | 2 (5.9) | 9 (11.0) | 0.504 |
| Tooth enamel defects | 2 (5.9) | 3 (3.7) | 0.629 |
| Aphthous stomatitis | 0 (0.0) | 13 (15.9) | **0.010** |
| Anaemia | 4 (11.8) | 27 (32.9) | **0.019** |
| Short stature (height < -2 SDS) | 4 (11.8) | 11 (13.4) | 1.000 |
| Low weight (< -2 SDS) | 2 (5.9) | 7 (8.5) | 1.000 |

tTGA: Anti-tissue transglutaminase antibodies; CD: Celiac disease; SDS: Standard deviation scores.

**Table 3 Human leukocyte antigen distribution,** **Marsh classification in celiac disease patients *n* (%)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **tTGA < 100 U/mL**  **(*n*  = 34)** | **tTGA ≥ 100 U/mL (*n*  = 82)** | ***P*-value** |
| HLA-score  1 heterodimer  2 heterodimers  4 heterodimers | 21 (61.8)  2 (5.9)  11 (32.4) | 26 (31.7)  20 (24.4)  36 (43.9) | **0.005** |
| Marsh classification |  |  | < 0.001 |
| Marsh IIIa | 5 (14.7) | 4 (4.9) |  |
| Marsh IIIb | 22 (64.7) | 18 (22.0) |  |
| Marsh IIIc | 7 (20.6) | 60 (73.2) |  |
|  | *n* = 331 | *n* = 731 | < 0.001 |
| Patchy lesions2 | 14 (42.4) | 5 (6.8) |  |

1Only 106 patients out of the total study population also underwent duodenal bulb biopsies; 2Discrepancy in the diagnosis based on histology in the duodenal bulb *vs* in the distal duodenum. HLA: Human leukocyte antigen; tTGA: Anti-tissue transglutaminase antibodies; CD: Celiac disease.