Celiac disease antibody levels reflect duodenal mucosal damage but not clinical symptoms

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Abstract

Objectives: This study aimed to investigate, in a real-world population, whether the histological and clinical phenotype differ at baseline and during follow-up in patients with high and low CD antibody titers.

Materials and methods: The study cohort consisted of 96 consecutive patients diagnosed to have CD during the years 2010–2018. The clinical parameters, symptoms and laboratory results were registered and histomorphometry was analyzed from the available duodenal biopsies taken during the primary and follow-up esophageal-gastric duodenoscopies. Patients having immunoglobulin A transglutaminase antibody (tTG-ab) levels above 70U/mL were classified as high titer patients.

Results: Measured by the villous-crypt ratio, the duodenal mucosa was more severely damaged in the high tTG-ab group than in the low tTG-group at baseline (n=70, 0.61 ± 0.63 vs. 1.02 ± 0.87, p=0.003) and during the follow-up when the patients were on gluten-free diet (n=27, 1.80 ± 0.72 vs. 2.35 ± 0.64, p=0.041). Interestingly, the high tTG-ab group members had fewer gastrointestinal symptoms at baseline than those in the low tTG-ab group (43% vs. 68%, p=0.013) but lower vitamin D levels (68 ± 34 nmol/L vs. 88 ± 29 nmol/L, p=0.034) and more often microcytosis (28% vs. 10%, p=0.040). During the follow-up, these differences were no longer detected.

Conclusions: At baseline, CD patients with high tTG-ab have more severe duodenum injury and signs of malabsorption but fewer symptoms. After gluten-free diet has been initiated, the mucosal healing in the high tTG-ab group is prolonged, but symptoms and signs of malabsorption recover equally in both groups.

Keywords

celiac disease, malabsorption, anemia, tissue transglutaminase antibody, histomorphometry, gastrointestinal symptoms

Introduction

Celiac disease (CD), characterized by duodenal damage and intraepithelial lymphocytosis, is an immune-mediated small intestine enteropathy triggered by gluten exposure. To date, gluten-free diet (GFD) remains the only treatment in CD. The global prevalence of CD is estimated to be around 1–2%, but the biopsy-confirmed prevalence is only half of that [1]. Traditionally, small bowel biopsies taken by esophageal-gastric duodenoscopy (EGD) have been required for the diagnosis of CD. However, high CD antibody titers have been found to be a highly sensitive and specific marker of small bowel duodenal injury and, thus, CD [2,3]. In addition, it has been found that the histological analysis of duodenal sections has several diagnostic pitfalls and different readers can even give different diagnoses on the same biopsy specimen, questioning the use of duodenal specimens as "gold standard" in CD diagnostics in routine practice [2,4].

In classical CD, patients have severe malabsorption with nutritional deficiencies [5]. However, after the introduction of modern CD antibody tests, CD patients are nowadays more often diagnosed as asymptomatic in at-risk groups or having non-classical extraintestinal manifestations. Despite the diverse clinical phenotype of the patients, all these patients have ongoing active CD predisposing to long-term complications, such as osteoporosis [6]. Moreover, especially in poorly treated or in the event of a rare GFD-refractory CD, the risk of lymphoproliferative malignancies is increased [7,8]. There are discrepant reports about the overall mortality among CD patients as compared to non-CD equals [8,9], but some evidence suggests that newly-diagnosed CD, delayed diagnosis, malabsorption symptoms and poor compliance with GFD may worsen the overall prognosis [10].

Accordingly, the option to make a non-invasive diagnosis of CD has been available in European pediatric guidelines since 2012, but the first national adult guidelines allowing this have only recently been released in Finland [11,12]. The updated Finnish national guidelines offer an option to diagnose CD in adult individuals with tTG-ab above 10 times the upper limit of normal (ULN; i.e.

>70 U/mL) and positive endomysium antibodies [3,11]. However, there are limited prospective data about the clinical and histological follow-up of high tTG-ab and low tTG-ab CD patients in a real-world adult population. Thus, the aim of the present study was to investigate, in a real-world setting, whether the histological and clinical characteristics differ at baseline or after GFD initiation in adult CD patients with low tTG-ab (ULN ≤ 70 U/mL) and high (ULN >70 U/mL).

Materials and Methods

Study cohort and clinical data

The study cohort consisted of 18- to 80-year-old consecutive individuals who were diagnosed with CD in Vaasa Central Hospital, Vaasa, Finland, between January 1st 2010 and September 30th 2018 (n=96, 95 of whom were Caucasians). The individuals were searched from the in-hospital registry (Exreport) using the search terms CD and EGD. The CD diagnoses were based on the demonstration of distal duodenal mucosal villous atrophy with crypt hyperplasia as evaluated by experienced local pathologists. The biopsy specimens were obtained with standard forceps via EGD. tTG-ab was analyzed by the ImmunoCap (FEIA) method. The normal range for tTG-Ab was <7 U/mL.

The data was gathered from medical records and consisted of basic patient information, endoscopic view according to the clinician's assessment, laboratory results and histopathological parameters. Moreover, the commitment to GFD was assessed as poor, good or excellent (or information not available) according to the medical records. The existence of gastrointestinal symptoms (gastrointestinal reflux related symptoms excluded) was assessed at baseline and during follow-up. Almost all baseline laboratory parameters (98%) were taken 0–180 days before primary EGD (the rest 2% of laboratory tests were taken 192-263 days before the primary EGD or in 5 cases, 21-71 days after the primary EGD and before the initiation of GFD) . Laboratory tests that were taken as close as possible to the follow-up EGD or about one to two years after the primary EGD were accepted as follow-up laboratory tests. However, laboratory tests taken during apparent acute illness were avoided. The time between the primary EGD and laboratory tests, secondary EGD or other baseline or follow-up data was documented. If the laboratory test results were available only in the referral without reporting the exact test day, the day the referral was written was considered the test day.

Histopathological analysis

In most cases, a minimum of three to four representative forceps biopsies were taken from the distal duodenum. The biopsy specimens were paraffin-embedded and standard 5-µm-thick sections were cut and stained with hematoxylin-eosin. Morphometric analyses were done according to our standard operating procedure [4]. Morphometric measurements were done in a blinded manner without knowledge of laboratory, demographic, or original histopathology results. Morphological measurements were performed only on specimens with the plane of sectioning perpendicular to the luminal surface. The small-intestinal mucosal villus height to crypt depth ratio (Vh:CrD) was evaluated from at least three separate villus-crypt units by measuring villi lengths (µm) and crypt depths (µm), and the primary outcome was given as the average of the ratios. IEL densities were counted under light microscopy in HE-stained sections. At least 300 epithelial cells were counted in a continuous length of the epithelium, and the results were expressed as the number of IELs per 100 epithelial cells. Vh:CrD >2 was considered normal [13].

Statistics

The statistical analysis was performed using IBM SPSS statistics 25 (Armonk**,** NY**:** IBM Corp, USA**).** Shapiro-Wilk test was used to identify the nature of distribution in continuous variables. If the data was not normally distributed and/or the sample size in both or either of the study arms was <20, Mann-Whittney U test was performed, and if the data was in normal distribution with sample size in both study arms ≥20, an Independent-Samples t-test was used to analyze the statistical significances of differences between the groups. ANCOVA method (analysis of co-variances) was performed when the effect of covariates needed to be controlled. The Chi-Square test was used in categorical variables. Furthermore, two-tailed Pearson correlation coefficient was used to analyze the correlation between two continuous variables. The p-value <0.05 was considered statistically significant.

Ethics

The study was approved by the organization (1/2019, Vaasa Central Hospital). The ethical committee of the Hospital District of Southwest Finland approved the study (111/1803/2018) and the National Supervisory Authority for Welfare and Health (Valvira) approved the re-analysis of the duodenal tissue specimens (V/10439/2019). Due to retrospective data collecting, the informed consent was waived.

Results

In total, there were 156 patients who met the inclusion entry terms. After exclusions (a prior CD diagnosis (n=34), age < 18 years (n=6), age > 80 years (n=4), not eventual CD diagnosis (n=4), failed search result (n=2), no measured tTG-ab (n=10)), the study cohort consisted of 96 subjects, of which 56 formed the low tTG-ab group and 40 the high tTG-ab group (Figure 1). Three patients (3%) had seronegative CD (tTG-ab < 7U/mL). In total, the 96 study participants underwent 140 EGDs with biopsies (i.e. 44 follow-up EGDs). Of the biopsies, 123 were available for histomorphological analysis but 15 of them (12%) were of too poor quality for histomorphological analysis. The suspicion of CD in the 96 study participants was based on GI symptoms in 55 patients (57%) and/or on extraintestinal manifestations, such as anemia and/or iron deficiency (n=23, 24%), eczema (n=9, 9%), general disease symptoms (e.g. weight loss, fever, malaise, lethargy, lymphadenopathy; n=20, 21%), elevated liver enzymes (n=6, 6%) or screening due to known CD case in the family (n=5, 5%) or due to associated condition (n=10, 10%). In total, the 55 patients with gastrointestinal symptoms had diarrhea (n=25), abdominal pain (n=13), flatulence (n=6), constipation (n=1) and/or dyspepsia (such as abdominal cramps/bloating/not specified) (n=29).

The baseline characteristics of both patient groups are depicted in Table 1. As the table shows, the individuals in the high tTG-ab group had fewer symptoms from the GI tract (p=0.013) but more often general disease symptoms (p=0.004) and microcytosis (p=0.040) than those in the low tTG-ab group. Additionally, the vitamin D level (p=0.034) was lower in the high tTG-ab individuals (p=0.034).

The adherence to GFD was similar in the groups when categorized as excellent (58% in the low tTG-ab group and 63% in the high tTG-ab group), good (11% and 15%, respectively), poor (11% and 0%, respectively) or data unavailable (20% and 23%, respectively) (p=0.186).

During follow-up, when on GFD, there were no longer any differences in the prevalence of GI tract symptoms between the low tTG-ab and the high tTG-ab groups (4/26 (15%) and 1/23 (4%),

respectively, p=0.203), microcytosis (88.4 ± 5.4 fL and 89.5 ± 3.8 fL, p=0.596, respectively) or vitamin D level (93 ± 53 nmol/L and 80 ± 37 nmol/L, p= 0.477, respectively) after excluding the individuals whose adherence to GFD was known to be poor (n=3 for all). The scattered follow-up data regarding general disease symptoms was considered too inconsistent to be analyzed.

Table 2A shows that the patients in the high tTG-ab group had more severely damaged duodenal mucosa in the biopsy specimens from the diagnostic EGD (p=0.002 for Vh, p=0.009 for CrD and p=0.003 for Vh:CrD) as compared to their counterparts with lower tTG-ab. Correspondingly, the Pearson correlation coefficient between tTG-ab and Vh (r -0.391, p<0.001), CrD (r 0.357, p=0.001) and Vh:CrD (r -0.325, p=0.003) were statistically significant. In contrast, there was no difference between the tTG-ab groups and IEL (p=0.686) or correlation between tTG-ab level and IEL (r 0.024, p=0.828). Additionally, the duodenal damage, as measured by Vh:CrD, did not differ between the patients with (0.90 ± 0.87) or without (0.77 ± 0.69) GI tract symptoms (p=0.785).

The histopathological results from the follow-up biopsies are presented in Table 2B after excluding the subjects whose adherence to GFD was known to be poor (n=1, in the low tTG-ab group; n=2 for IEL, both in the low tTG-ab group). The follow-up biopsies (n=27) were taken 529 days ± 160 days after the primary EGD (in the low tTg-ab 475 days ± 115 days and in the high tTg-ab group 573 days ± 180 days, p=0.067). As the table shows, Vh (p=0.093) and CrD (p=0.152) did not differ statistically significantly; however, Vh:CrD was statistically significantly different between the groups (p=0.041). After adjustment for the time interval between the primary and secondary EGDs, Vh:CrD remained significant (p=0.008). Vh:CrD was >2 in 10 (83%) subjects from the low tTG-ab group and in 8 (53%) patients from the high tTG-ab group (p=0.100) after exclusion of those with poor adherence to GFD (n=6). In the total study cohort, the results for baseline tTG-abs and histomorphological parameters did not correlate with the results obtained in the follow-up as Pearson's r was observed to be non-significant between the baseline tTG-ab and Vh (r -0.263,

p=0.185), CrD (r 0.194, p=0.333), Vh:CrD (-0.314, p=0.111) and IEL (-0.132, p=0.463) in the follow-up.

Discussion

Although one would logically assume that the symptoms of celiac disease and duodenal damage are linked, the literature is highly controversial on this matter. Some studies have not found any association between symptom severity and duodenal damage [14-16]. However, in a large patient cohort utilizing precise continuous Vh/CrD measurements of duodenal damage, a significant, albeit very small, correlation between gastrointestinal symptoms and architectural duodenal damage could be shown [17]. Hence, it seems that the logically assumed link between symptom severity and disease activity seems to be very low in CD patients and rarely seen in clinical practice. Interestingly, in the present study, those CD patients whose tTG-ab was elevated more than ten-fold above ULN had fewer GI symptoms than those with lower tTG-ab titer. Additionally, the patients with high tTG-ab had more severe duodenal damage at the time of diagnosis and, presumably because of it, more often signs of malabsorption, such as microcytosis, an undirect surrogate of iron deficiency, and low vitamin D level. Presumably, malabsorption may be one reason behind the greater prevalence of general disease symptoms as well.

CD has changed dramatically in phenotype in the last decades and we now find patients without symptoms, especially in at-risk groups, as well as those presenting with only extraintestinal manifestations [18,19]. Our findings could signal an even more problematic presentation of CD indicating that some patients do not evince symptoms despite severe duodenal damage. As GI symptoms are the feature of CD that is most well recognized by doctors, the diagnosis of CD could be delayed in these patients [20,21]. Moreover, these patients are hence at risk of long-term complications such as osteoporosis due to the diagnostic delay, but also because of the silent duodenal damage [22].For clinical practice, this prompts awareness for active case finding in patients with possible extraintestinal manifestations in at-risk groups, but it also raises the question of screening in general population to find these patients without GI symptoms, although mass screening for CD has not yet been shown beneficial or cost-effective [23].

The healing of the duodenal mucosa after GFD also seems to be a more time-consuming process. According to our study, the response of the duodenal mucosa to GFD approximately one-and-half years after diagnosis was not as comprehensive in the patients in the high tTG-ab group as in the patients in the low tTG-ab group. This was also reported by Pekki *et al.* as they showed that CD individuals with defectively recovered duodenal mucosa one year after the CD diagnosis had had higher tTG-ab at the baseline as compared to the CD individuals with a well-recovered duodenum [24]. Indeed, the duodenal mucosa does not always recover within 1 to 2 years despite full commitment to GFD [25,26]. Whether this happens in the long-term is controversial, as some reports are for [26] and some against [27] a full recovery. Importantly, of the known risk factors for refractory CD at least male gender, older age, severe symptoms or seronegativity at the diagnosis of CD [28] do not associate with the high tTG-ab group, as our study shows. However, Dotsenko *et al*. recently showed that despite years on a strict GFD the duodenal epithelium function is not the same in CD patients compared to those without CD, which can even lead to micronutrient deficiency [29]. Whether tTG-ab level associates with impaired epithelium function is not yet known. It is important, however, to find the CD patients early to avoid aggravated duodenal damage and to preserve the epithelium function, but whether routine follow-up EGDs are cost-effective may be put into question because they are often performed too early, and even when providing signs of excellent endoscopic and histological recovery, it may not be the whole truth, as shown by Dotsenko *et al*. [29]. Furthermore, the prevalence of refractory CD, the fear of which is often the conscious or subconscious reason for endoscopic follow-up, is only 0.3% among all CD patients [28]. Hence, the follow-up EGD could be opted out if a CD patient is symptom-free and relevant laboratory values are normal. However, if the GFD is not helping and the patient is still symptomatic or has signs of malabsorption in the follow-up despite adherence to GFD, EGD should be performed. Likewise, when there is suspicion of seronegative CD, the patient should undergo diagnostic and follow-up EGDs [11].

CD histology is still seen as a gold standard in CD diagnostics, even though CD histology is difficult due to the need for orientation, which is usually suboptimal in about 10% of cases in clinical practice [30]. This was apparent in our study, as up to 12% of these routine practice samples were inadequate for precise morphometric measurements, reflecting the poor quality of routine sampling. According to the Finnish guidelines, it is currently allowed to make a non-invasive CD diagnosis in adults with tTG-ab at least ten times the ULN combined with positive endomysium antibodies [11]. Previously, non-invasive diagnosis was reserved for children in European guidelines, but there are now increasing number of studies indicating that adults can also be safely diagnosed without biopsy [3, 31]. The shift towards the use of objective laboratory-based parameters such as CD antibodies seems justified in clinical practice. However, it must be remembered that small-bowel mucosa damage is linked to mortality and prognosis in CD, not antibody titers. Thus, small-bowel biopsy will retain its role in the future, especially in research and drug trials [32].

Our study has some limitations. The retrospectively gathered data inevitably implies some limitations in the availability and accuracy of the data. The possible inaccuracy, however, was controlled by registering, for instance, the adherence to GFD and the time interval between the primary EGD and other examinations, including blood specimens. Moreover, lacking or obviously inaccurate data was ignored. The detailed morphometric quantitative analysis of the biopsy specimens from the primary and follow-up EGDs and the real-world clinical data obtained from a secondary center can be seen as strengths of the present study, as current evidence with this information is scarce, particularly from an adult population.

In conclusion, the present study shows that patients with high antibody titers have more severe duodenal damage and signs of malabsorption but fewer gastrointestinal symptoms. After initiation of GFD, the duodenal mucosa recovers more slowly in patients with baseline tTG-ab >70 U/mL than in patients with tTG-ab ≤ 70U/mL, but there is no evidence that this is a sign of a refractory CD.

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Disclosure of interest

The authors report no conflict of interest.

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