SEVERITY OF DUODENAL HISTOLOGY AND TISSUE TRANSGLUTAMINASE ANTIBODY LEVELS CORRELATE WELL IN ADULT CELIAC DISEASE IRRESPECTIVE OF CLINICAL FEATURES

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SEVERITY OF DUODENAL HISTOLOGY AND TISSUE TRANSGLUTAMINASE ANTIBODY LEVELS CORRELATE WELL IN ADULT CELIAC DISEASE IRRESPECTIVE OF CLINICAL FEATURES (Abstract): Celiac disease (CD) is a chronic immune-mediated enteropathy that occurs in genetically predisposed individuals. The clinical phenotypes range from classical gastrointestinal manifestations to only atypical signs, thus being a challenge to diagnose. **Aim:** To investigate the relationship between duodenal histology, specific antibody levels and clinical presentation in adult Romanian CD patients. **Material and methods:** Study design: retrospective retrieval of information prospectively entered into a structured database including 81 adult patients diagnosed with CD admitted with symptoms of abdominal disturbances (diarrhea, heartburn, nausea, vomiting, regurgitation, abdominal pain) to the Iasi Institute of Gastroenterology and Hepatology, “Sf. Spiridon” Emergency Clinical County Hospital between January 2012 and December 2016. The demographic, clinical, serological, and histological characteristics of individuals with CD were reviewed. **Results:** The female: male ratio was 3:1, 60 (71.1%) female patients, mean age 40.02±12.14 years. A total of 48.1% patients presented with gastrointestinal (GI) complaints and 51.9% presented mostly with non-GI manifestations, with a slightly more advanced age at symptom onset in the latter (38 yrs. vs. 47 yrs.). Marsh-Oberhuber classification was used to assess mucosal injury and Marsh 3c lesions were found in 25 (30.9%) cases. When assessing the serological parameters, IgA anti-tissue transglutaminase (IgA-tTG) antibody (61.45±76.458 u/mL vs.162.02±106.179 u/mL, p=0.001) and IgA anti-gliadin antibody (IgA-AGA) levels (61.83±69.41u/mL vs. 77.15±71.02 u/mL, p=0.001) correlated with intestinal villous atrophy (Marsh 3a and 3c) in CD patients by Spearman rank correlation. Of the symptoms, abdominal distention and diarrhea were associated with abnormal histology. Hemoglobin levels were determined and anemia was diagnosed in 61.7% of the patients with elevated IgA-tTG levels (r= -0.316; p=0.004), IgA-AGA (r= -0.301; p=0.006) and Marsh 3b-3c lesions (p=0.0048). Of the biological markers included in the statistical analysis, low iron levels (cut off 30 mg/dl), hypocholesterolemia and low protein levels were associated with Marsh 3b lesions (p=0.006) and elevated tTG-IgA levels (r= -0.384; p=0.001). **Conclusions:** IgA-tTG and AGA levels correlate with duodenal villous atrophy in adult CD patients. An IgA-tTG level >160 was nearly always associated with severe CD histopathology. GI and non-GI symptoms are not reliable predictors of CD. **Keywords:** CELIAC DISEASE, MARSH CLASSIFICATION, CLINICAL MANIFESTATIONS, ANTI-TISSUE TRANSGLUTAMINASE.
Celiac disease (CD) is a chronic immune-mediated enteropathy that occurs in genetically predisposed individuals (1, 2) and the most frequent food intolerance with an increasing prevalence over the last three decades (3). This has been partially prompted by globalization, which led to an increased consumption of gluten-containing food worldwide. The development of new sensitive and specific serological tests and their extended availability, as well as the best knowledge of the disease including a wide clinical spectrum have greatly facilitated CD diagnosis, being indirectly responsible for the rising prevalence (4). In Europe, a recently published large international, multicenter study investigating a wide population sample revealed an overall prevalence of 1% (5).

Unfortunately, no one test for CD has a perfect sensitivity or specificity. The clinical phenotypes range from classical gastrointestinal manifestations to only atypical signs, thus making the clinical diagnosis a challenge (6-8). As a rule, CD diagnosis can be made by serological tests, searching for ant tissue transglutaminase (tTG) and anti-endomysium (EMA) auto-antibodies, but confirmation of intestinal damage relies on small bowel biopsy and histological analysis, mainly in Europe (9). Nonetheless, the prevalence of seronegative CD is 6-22% of all diagnosed cases (10), the histological abnormalities associated with CD can be patchy (11,12), and a normal endoscopic appearance of the duodenum may occur in the presence of villous atrophy (13-16). The aim of the study was to investigate the relationship between duodenal histology, specific antibody levels and clinical presentation in adult CD Romanian patients.

**MATERIAL AND METHODS**

The study design included a retrospective retrieval of information prospectively entered into a structured database carried out at the Iasi Institute of Gastroenterology and Hepatology, which serves as a tertiary care referral center. The study was conducted between January 2012 and December 2016 and included 81 adult CD patients. In all CD cases, the diagnosis was established by a combination of positive IgA-tTG, IgA-AGA and biopsy results. Abdominal pain, diarrhea, constipation, bloating, nausea and vomiting were considered GI manifestations, and enclosed fatigue, joint pain, irritability, anorexia, anemia, aphthous stomatitis, short stature non-GI manifestations.

After obtaining a written informed consent, all subjects underwent upper endoscopy during which duodenal biopsies were obtained. The final Marsh score for each patient was graded according to the most affected site (highest Marsh score). The biopsies were evaluated by a single experienced pathologist blinded to all clinical data. The mucosal specimens were graded independently according to the Marsh-Oberhuber classification as follows: Marsh 0 denotes normal villi and crypt depth with no excess of intraepithelial lymphocytes (IEL), Marsh 1-2 denotes normal villi but intraepithelial lymphocytosis without (1) or with (2) hyperplastic crypts, and Marsh 3a-c denotes varying degrees of villous atrophy with hyperplastic crypts (17). Multiple biopsies (minimum of three) were taken during upper endoscopy from the bulb and distal duodenum. Immunohistochemical studies were performed using anti-CD3 antibody and an IELs count was noted (lymphocytosis >25/100 enterocytes).

Quantitative analysis was performed us-
Severity of duodenal histology and tissue transglutaminase antibody levels correlate well in adult celiac disease irrespective of clinical features

ing ELISA technique. IgA and IgG-tTG were reported according to the manufacturer’s cut off as UI/mL and classified as negative ≤8 IU/mL and positive ≥8 IU/mL. Serum samples containing more than 12 U/mL were considered positive for tTG and for AGA, respectively, as recommended by the manufacturer. Total IgA serum levels were measured. A serum IgA concentration below 0.07 g/dl was graded as IgA deficiency by an immunoturbidimetric assay. Detailed information regarding demographics, symptoms at presentation, duration, type of investigations performed, type of treatment including gluten-free diet were recorded.

Statistical analysis was performed using SPSS 18.0 (SPSS Chicago Inc., IL, USA). Continuous data were expressed as mean ± SD and categorical data as percentage. A P-value less than 0.05 was considered statistically significant. The study was approved by the ethical committee and all participants were informed about the study according to the study protocol and gave their written informed consent.

RESULTS

A total of 81 patients met the inclusion criteria of the study with a female to male ratio of 3:1, with a majority of 60 (71.1%) female patients, mean age 40.02±12.14 years (fig.1). The baseline characteristics of the participants are shown in Table I. Family history of CD was identified in 12 (14.8%) cases. The proportion of newly diagnosed CD patients was high: 68 (84%), 48 female patients (70.6%), mean age 40.09±12.67. The prevalence of associated diseases was low, 7 (8.6%), 3 (3.7%) cases being diagnosed with thyroid disease and 4 (4.9%) cases with chronic idiopathic hypertransaminasemia.

A total of 39 (48.1%) patients presented with GI complaints and 42 (51.9%) of patients presented mostly with non-GI manifestations, with a slightly more advanced age at symptom onset in in the latter (38 yrs. vs. 47 yrs.). Interestingly, GI symptoms were more common in patients with lower IgA-tTG <100U/mL
levels, 21 (26.9%), compared to patients with levels >100 U/mL, \( p=0.007 \). Marsh-Oberhuber classification was used to assess mucosal injury and varying degrees of villous atrophy were identified in 59 (72.8%) cases as follows: complete atrophy lesions (Marsh 3c) in 25 (30.9%) cases, Marsh 3a in 24 (29.6%), Marsh 3b in 10 (12.3%), and Marsh 1, 2 in 22 (27.1%) cases. The severity of duodenal lesions was not influenced by patients’ age (\( F=1.691; \text{df}=3; \ p=0.176 \)).

**TABLE 1**

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>n (%)</th>
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<tbody>
<tr>
<td>Age, median (range), yrs.</td>
<td>38(18-72)</td>
</tr>
<tr>
<td>Females, n, (%)</td>
<td>60 (71.1%)</td>
</tr>
<tr>
<td>Gastrointestinal symptoms, n, (%)</td>
<td>39(48.1%)</td>
</tr>
<tr>
<td>Extra intestinal symptoms, n, (%)</td>
<td>42(51.9%)</td>
</tr>
<tr>
<td>Family history of CD, n, (%)</td>
<td>12 (14.8%)</td>
</tr>
<tr>
<td>Newly diagnosed patients, n, (%)</td>
<td>68(84%)</td>
</tr>
<tr>
<td>Patients on a gluten free diet, n, (%)</td>
<td>10(12.3%)</td>
</tr>
<tr>
<td>CD associated comorbidity*, n, (%)</td>
<td>7(8.6%)</td>
</tr>
</tbody>
</table>

*Thyroid disease, dermatitis herpetiformis, ataxia, type 1 diabetes, autoimmune liver disease, unexplained hypertransaminasemia, selective IgA deficiency.

When assessing the serological parameters, IgA-tTG antibody levels (61.45±76.458 u/mL vs. 16.02±106.179 u/mL, \( p=0.001 \)) and IgA-AGA antibody levels (61.83±69.41u/mL vs. 77.15±71.02 u/mL, \( p=0.001 \)) correlated with intestinal villous atrophy (Marsh 3a and 3c) in CD patients by Spearman rank correlation (fig.2). Mild enteropathy (Marsh 1, 2) was identified in 22 (27.1%) cases with mean tTG levels of 32.44 ± 37.994 U/mL. Strongly positive antibody levels (IgA-tTG>160 units) were highly specific (>97%) for Marsh 3c lesions. Of the GI symptoms anemia, nausea and diarrhea were associated with abnormal histology (Marsh 3a-c lesions) when compared to mild duodenal lesions (Marsh 1, 2). The prevalence of non-GI manifestations was similar and was not influenced by the severity of mucosal lesions. Nevertheless, patients with villous atrophy presented more often with anemia compared with patients with mild enteropathy (\( p=0.048 \)).

Hemoglobin, ferritin, iron, vitamin B12, albumin, folate, calcium and total protein levels were measured. When assessing the hematological parameters, hemoglobin levels were determined and anemia was diagnosed in 61.7% patients with Marsh 3b and 3c histological lesions (\( p=0.0048 \)), elevated IgA-tTG levels (\( r=-0.316; \ p=0.004 \)) and IgA-AGA (\( r=-0.301; \ p=0.006 \)). Of the biological markers included in the statistical analysis, low iron levels (cut off 30 mg/dL), hypocholesterolemia and low protein levels were associated with Marsh 3b lesions (\( p=0.006 \)) and elevated IgA-tTG levels (\( r=-0.384; \ p=0.001 \)). The lowest mean iron levels were found among patients with subtotal and total villous atrophy, \( p=0.004 \).
Severity of duodenal histology and tissue transglutaminase antibody levels correlate well in adult celiac disease irrespective of clinical features.

Fig. 2. Correlation between IgA-tTG level and severity of mucosal damage according to Marsh-Oberhuber classification.

Fig. 3. Gender and age-group distribution of IgA-tTG level.
DISCUSSION

Gastrointestinal symptoms alone cannot accurately differentiate CD from other common gastrointestinal disorders (20-50% of patients with CD met the Rome criteria for irritable bowel syndrome) (18,19). Whilst current literature suggests a discriminatory role for a high-risk group (anemia, weight loss, diarrhea, dermatitis herpetiformis) our data suggest that no single clinical feature is a reliable predictor or risk stratifier for CD and the disease occurs irrespective of clinical presentation (20,21). According to our results, CD is more frequent among young female patients, with a shift in clinical pattern occurrence towards atypical presentation in older patients, which is associated with misdiagnosis and delayed diagnosis.

The results showed significant correlations between degrees of small-bowel mucosal morphologic damage in CD. Patients with IgA-anti-TG levels >160U/mL virtually always have CD, whereas the disease can be histologically absent in a significant number of patients with low serum tTG level. In our study, strongly positive antibody levels (IgA-anti-TG >160 units) were highly specific (>97%) for Marsh 3c lesions. We also noted that GI symptoms were more common in patients with lower IgA-anti-TG compared to patients with elevated levels. Alterations of laboratory parameters were particularly evident for the parameters expressing intestinal malabsorption, reasonably reflecting a more severe impairment of intestinal absorptive function in patients with villous atrophy.

The relationship between specific antibody level, degree of mucosal damage and clinical presentation in CD remains controversial. In the article by Taavela et al, the ratio of small-bowel villous height to crypt depth and results from serology tests correlate with the reported symptoms and quality of life of patients with CD. Patient-reported outcomes are therefore of value, in addition to histology findings, in assessing patients with CD (22). Mubarak et al. aimed to investigate whether CD patients with tTG levels ≥100 U/mL are different from patients with lower tTG levels. Patients with high tTG levels had lower average body weight-for-height standard deviation scores than did patients with lower tTG levels. In the low tTG group, GI symptoms were more common; specifically, abdominal pain and nausea were more frequent among patients with low tTG. In contrast, patients with solely extra intestinal manifestations were only present in the high tTG group (23). Dahlbom et al. demonstrated that high levels of IgA-tTG antibodies were associated with the grade of mucosal villous atrophy and a more severe clinical presentation. The combined measurement of IgA-tTG enables a noninvasive prediction of small intestinal villous atrophy with high accuracy, and may reduce the need for biopsy in patients with suspected CD (24).

Our study has some limitations. Our investigation is uncontrolled and retrospective but reflects current clinical routine practice. The inclusion of all adult CD patients over a 5 year study period, with the availability of a small number of cases forms nonetheless the basis for future prospective studies to create a national CD patient database to access reliable information responding to the specific needs of the Romanian society and provide knowledge and even prevention tools (25).

CONCLUSIONS

Our study confirms the chameleonic na-
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ture of CD. Adult Celiac disease is currently much more common than previously estimated and there is a need for well-designed epidemiological studies to increase detection rate. Celiac disease is confirmed to be more prevalent in female than in male patients (F:M = 3:1). IgA tTG levels correlate with duodenal villous atrophy in adult CD patients. An IgA-tGA level >160 was nearly always associated with CD histopathology. GI and non-GI symptoms are not reliable predictors of CD. No single clinical feature on its own is a reliable predictor or risk stratifier for CD.

REFERENCES


**NEWS**

**GIANT CELL MYOCARDITIS: A BRIEF REVIEW**

Giant cell myocarditis (GCM) was first described in 1905 and is characterized by a mixed myocardial infiltrate with multinucleated giant cells and cardiomyocyte necrosis. In larger autopsy series, the incidence of GCM has been reported to range from 0.007% to 0.051%. Given that autopsies are not routinely performed on an unselected population, and patients dying of GCM may not undergo autopsy, it is likely these figures underestimate the true incidence. In the United States in the 1990s, a group of major heart failure referral centers reportedly diagnosed GCM (via autopsy, endomyocardial biopsy, apical wedge resection, or cardiac explantation) an average of once every 21 months. Giant cell myocarditis is a rare, progressive inflammatory myocardial disease with high mortality, affecting a relatively young population. Although much progress has been made during the past decades in understanding the pathophysiology, there are still many deficiencies in our knowledge. Rapid diagnosis is critical, as the management differs from that of other myocardial processes containing giant cells, and early institution of combined cyclosporine-based immunosuppression can dramatically impact the disease course. Promising future directions include the development of serum biomarkers and utilization of gene expression profiling to potentially more rapidly diagnose and monitor GCM progress (Xu J and Brooks EG. Giant Cell Myocarditis: A Brief Review. Archives of Pathology & Laboratory Medicine 2016, 140 (12): 1429-1434).