INTRODUCTION

Celiac disease is an immune-mediated enteropathy caused by a permanent sensitivity to gluten in genetically susceptible individuals (DQ2 and/or DQ8 HLA haplotype). It occurs in symptomatic subjects with gastrointestinal and non-gastrointestinal symptoms, and in some asymptomatic individuals, including subjects affected by: type 1 diabetes, Turner syndrome, William’s syndrome, immunoglobulin A (IgA) deficiency and first degree relatives of individuals with celiac disease.

ABSTRACT

Introduction: Nowadays, the gold standard of celiac disease diagnosis is intestinal biopsy showing characteristic villous lesions. Objectives: Assessing sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of serological tests used for celiac disease screening. The diagnosis was based on histological intestinal injury, using Marsh classification. Material and methods: 70 consecutive patients presenting high suspicion of celiac disease were enrolled in the study during April 2005 - October 2006 (lot A). All patients from lot A underwent intestinal biopsy. During the same period, a lot of 62 consecutive, randomized patients - lot B - underwent upper digestive endoscopy for different causes non-related to gluten intolerance. For each patient from group B a sample of intestinal biopsy was also taken. We assessed for each patient immunoglobulin A (IgA) total serum level, IgA and IgG antigliadin antibodies (AGA), IgA anti-endomisium antibodies (EMA) and IgA anti-human tissue transglutaminase antibodies (anti hu-TTG). Results: Sn for EMA and ITG was 100%, while for IgA and IgG AGA was 86%. Sp was 100% for EMA, 95% for ITG, 74% for IgG AGA and 87% for IgA AGA. NPV was 100% for EMA and ITG, 94% for IgG AGA and 95% for IgA AGA. PPV was 100% for EMA, 71% for ITG, 25% for IgG AGA and 66% for IgA AGA. Conclusions: In order to optimize the serological diagnosis of celiac disease, screening tests among risk population must associate a combination with maximum specificity and sensitivity - EMA and ITG antibodies assessment.

Key Words: celiac disease, villous atrophy, anti-endomisium antibodies, anti-transglutaminase antibodies
In clinical practice, serological tests for celiac disease are useful in identifying patients who require intestinal biopsy findings to diagnose this condition. EMA and tTG tests are both highly sensitive and highly specific, with values for both parameters exceeding 96% in most studies. No identifiable differences between adults and children are noted with respect to these tests.¹

Nowadays, the gold standard of celiac disease diagnosis is represented by intestinal biopsy showing characteristic villous lesions. The biopsy sample can be taken by using Watson capsule or during upper digestive endoscopy.

In 2007, a non-invasive diagnosis algorithm of serological tests for celiac disease was developed by a team of Baltimore, USA researchers, associating assessment of IgA EMA, anti-actine antibodies, IgA anti hu-tTG and seric level of protein zonulin.

Serological screening of the general population will identify most cases of previously unrecognized celiac disease, but mass screening for celiac disease is not currently recommended, as the potential cost/benefits of such a strategy have not been determined. An active case-finding strategy targeting both symptomatic and asymptomatic individuals who are at risk for celiac disease is currently considered a more cost-effective approach to diagnosis.²

In Romania, the true prevalence of gluten enteropathy in children is not known. There is in course of development The Celiac Disease National Register by the members of Celiac Disease Working Group inside the Romanian Society of Pediatric Gastroenterology, Hepatology and Nutrition. In 2004, a group of researchers from IOMC Pediatric Clinic Bucharest, performed the most important screening study in Romanian schoolchildren for detection of atypical forms of gluten enteropathy. The prevalence of the disease in the lot of study was 2.17%.³

Recent epidemiological surveys in Europe and in the United States of America showed the prevalence of celiac disease in the general population is between 0.5 and 1%. The prevalence of childhood celiac disease has been reported to be between 1:285 and 1:77 in Sweden, 1:99 and 1:67 in Finland, and 1:230 and 1:106 in Italian schoolchildren. Population-based estimates of the incidence of small bowel biopsy-confirmed celiac disease in adults vary from 2-13/100000 per year.⁴

The prevalence of the celiac disease in Romanian adults is 2.22%.⁵ These results have to be interpreted with caution because many patients diagnosed as adults have had 20-40 years of untreated celiac disease, thus hardly represent truly incident new disease. Many cases remain undiagnosed, usually because they have atypical symptoms and because there is a lack of awareness of celiac disease by doctors. In addition to having chronic symptoms that might otherwise respond to a gluten-free diet, undiagnosed patients are exposed to the risk of long-term complications of celiac disease, such as anemia, infertility, osteoporosis, or cancer, especially intestinal lymphoma.

**OBJECTIVES**

We intended to establish sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of serological tests used for celiac disease screening - IgA and IgG antigliadin antibodies (AGA), IgA anti-endomisium antibodies (EMA) and IgA anti – human tissue transglutaminase antibodies (anti hu-tTG). The diagnosis was based on histological intestinal injury, using Marsh classification.

**MATERIAL AND METHODS**

70 consecutive patients (mean age 6.5 years, sex ratio G/B 48/22) presenting high suspicion of celiac disease (chronic diarrhea, small stature, weight loss, recurrent abdominal pain, or anemia resistant to oral martial therapy) were enrolled in this study during April 2005 until October 2008 – group A. All patients enrolled in group A underwent intestinal biopsy using upper digestive endoscopy (patients aged more than 6 years), or Watson capsule (patients aged less than 6 years).

![Figure 1. Clinical signs of high suspicion for celiac disease in group A.](image)

During the same period, a lot of 62 consecutive, randomized patients – group B of study, (mean age 9 years, sex ratio G/B 38/24) underwent upper digestive
endoscopy for different causes, non-related to gluten intolerance: recurrent vomiting, dyspepsia, gastritis, gastric or duodenal ulcer, hematemesis, cirrhosis with esophageal varices, alternating bowel habits). For each patient from lot B a sample of intestinal biopsy was taken during upper digestive endoscopy.

All biopsy samples were blindly classified, using Marsh criteria (1992) modified by Oberhuber (1997): type I infiltrative (infiltrative lympho-plasmocytic lesions in villous corion), type II hyperplastic (infiltrative lympho-plasmocytic lesions in villous corion, associated by glandular crypt enlargement) and type III destructive (including partial and subtotal villous atrophy – type IIIa, IIIb respectively and total villous atrophy – type IIIc).

IgA selective deficiency was considered an exclusion criteria.

At the time of admission in this study, a serum sample was taken for IgA total serum level and also for IgA and IgG AGA, IgA EMA and IgA anti hu-tTG. Two patients from group B with selective IgA deficiency were excluded from the study.

IgA and IgG AGA detection was made with ELISA technique, using Immulisa™ anti-gliadin antibodies IgA and ImmuLisa™ anti-gliadin antibodies IgG test kits from Immco Diagnostics. For IgA EMA detection we used indirect immunofluorescence technique using smooth muscle of monkey esophagus (ImmuGlo™ Anti-Endomysial Antibody Test Kits – provided by Immco Diagnostics). Detection of IgA anti tTG antibodies in this study was performed using ImmuLisa™ anti-hu tTG ELISA. Test kits were provided also by Immco Diagnostics.

Sn, Sp, PPV and NPV of serological tests were computed according to statistical formulas described below. Data processing was made by using SPSS12 application, using chi square test in order to compare the PPV for IgA EMA and IgA anti hu-tTG.

RESULTS AND DISSCUSSIONS

14 patients (20%) from 70 enrolled in group A of study and 1 patient (1.67%) from 60 remained in group B after exclusion of IgA deficiency subjects, presented villous lesions corresponding to Marsh type II, IIIa, IIIb and IIIc. Considering villous alterations as gold standard for diagnosis, we obtained a total of 15 celiac disease patients, according to intestinal histology.

Distribution of villous alterations was classified as follows: from a total of 15 celiac patients, 6 patients presented type II Marsh lesions (40%), 3 patients presented type IIIa Marsh lesions (20%), 4 patients presented type IIIb Marsh lesions (27%) and 2 patients presented type IIIc Marsh lesions (13%).

The group of 15 celiac disease patients histologically confirmed with this diagnosis presented an average age of 4.5 years and sex ratio G/B = 10/5 (67% girls and 32% boys).

The 115 remaining patients with normal intestinal morphology were considered the control group.
All 15 subjects with histologically confirmed celiac disease tested positive for IgA EMA and IgA anti hu-tTG, while both IgA and IgG AGA were positive only in 10 of 15 patients (66.7%).

None of the patients from control group had positive IgA EMA, but 6 subjects (5%) from 115 tested false positive for IgA anti hu-tTG antibodies. 15 control subjects (13%) tested false positive for IgA AGA and 30 control subjects (26%) tested false positive for IgG AGA.

From a total of six false positive control subject for IgA anti hu-tTG antibodies, 3 had viral B chronic hepatitis, 1 had viral C chronic hepatitis and 2 had Crohn's disease. From the 15 control subjects who tested false positive for IgA AGA, 10 had Giardia Lambilia infection, 2 had Rotavirus gastroenteritis and 4 had protein milk intolerance. From the 30 control subjects who tested false positive for IgG AGA, 10 had Giardia Lambilia infection, 2 had soy protein allergy, 3 had Crohn's disease, 3 had protein milk intolerance and 11 had post viral gastroenteritis disaccharides secondary intolerance.

Assessing these data, we calculated IgA EMA and IgA anti hu-tTG sensitivity as 100%. For IgA and IgG AGA antibodies, sensitivity was 66%. The specificity was 100% for EMA, 95% for anti hu-tTG, 74% for IgG AGA and 87% for IgA AGA. NPV was 100% for EMA and anti hu-tTG, 94% for IgG AGA and 95% for IgA AGA. PPV was 100% for EMA, 71% for anti hu-tTG (p = 0.03 versus EMA), 25% for IgG AGA and 66% for IgA AGA.

Recent studies described a high number of asimptomatic (latent, silent) or atypical form of celiac disease. In many cases, this condition is indicated by intestinal morphology alteration observed after upper digestive endoscopy performed for a dyspeptic syndrome, or to evaluate an irritable bowel syndrome non-responsive to classic therapy. It is estimated that 5% of patients with irritable bowel syndrome corresponding to Rome II criteria have celiac disease. Typical form of disease presents classic clinical manifestations (chronic diarrhea, failure to thrive), positive serology and characteristic intestinal alterations. Atypical form of disease presents different and/or minimal clinic manifestation (dermatitis herpetiformis, dental enamel hypoplasia of permanent teeth, osteopenia/osteoporosis, short stature, delayed puberty, etc.), positive serology and characteristic intestinal alterations. Silent form of disease associates positive serology and intestinal villous injury in non-symptomatic patients.

**Table 1. Definition of statistical parameters used in the present study.**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sn</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
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<tbody>
<tr>
<td>IgA AGA</td>
<td>66%</td>
<td>87%</td>
<td>66%</td>
<td>95%</td>
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<tr>
<td>IgG AGA</td>
<td>66%</td>
<td>74%</td>
<td>25%</td>
<td>94%</td>
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<tr>
<td>IgA EMA</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>IgA anti hu-tTG</td>
<td>100%</td>
<td>95%</td>
<td>71%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Table 2. Statistical parameters for celiac disease serological test.**

<table>
<thead>
<tr>
<th>Disease present (D+)</th>
<th>Disease absent (D-)</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Positive test (T+)</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Negative test (T-)</td>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td>Total</td>
<td>a+c</td>
<td>b+d</td>
</tr>
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</table>

a = real positive subjects (RP); b = false positive subjects (FP); c = false negative subjects (FN); d = real negative subjects (RN); Sn = a/a+c = RP/ total D+; Sp = d/b+d = RN/ total D-; PPV = a/a+b = RP/ total T+; NPV d/c+d = RN/ total T-.
Studies regarding atypical or silent form of celiac disease have generated a great interest for methods of serologic screening in gluten enteropathy diagnosis. Using different serological tests since 1997 permitted a better selection of cases for intestinal biopsy in celiac patients. Anti-reticulin antibodies used previously for gluten intolerance diagnosis proved to have low sensitivity and specificity, so these antibodies are excluded from diagnosis protocols.10

IgA and IgG AGA are quantitative assessed using ELISA technique. There is a great number of false positive patients for AGA, mostly of them presenting milk protein intolerance, parasitic enteritis – Giardia Lamblia, etc. Lately, specialized researchers developed a new serologic test for IgA and IgG AGA, based on deaminate gliadine peptides, with high accuracy. This new assay has a higher sensibility and sensitivity compared to conventional IgA and IgG AGA assay.11

EMA are detected on the smooth muscle of monkey esophagus or human umbilical tissue using indirect immunofluorescence. EMA decrease slowly after gluten exclusion and have a rapid increase tendency after gluten challenge. It is known that indirect immunofluorescence technique is operator dependent and there are different sources of error: number of function hours of fluorescence source, lens quality, microscope diaphragm opening.12

Since 1998, IgA and IgG tTG have been detected using ELISA technique. Recent, researchers developed a rapid diagnosis test for tTG, dot blot assay with similar sensibility and specificity as ELISA.13

As many studies concluded, enzyme linked immunosorbent assay based on human tissue transglutaminase outperforms the guinea pig based tissue transglutaminase assay, so in this study we used human antigen for tTG antibodies.14

Interpretation of serological test in celiac disease must consider IgA selective deficiency source of false negative results for IgA EMA and tTG. Also, European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) does not recommends serological tests in patients suspected of celiac disease aged less than 2 years, due to high frequency of false negative results.15 For these reasons, all patients included in present study were aged more than 2 years old and IgA selective deficiency was considered exclusion criteria, in order to be able to perform a valid assessment of serological tests accuracy based on histological findings.

In 2007 at Barcelona, during the 40th ESPGHAN Annual Meeting, a researchers group led by Sonia Niveloni from Mucosal Biology Research Center and Center for Celiac Research, Maryland University, Baltimore, USA, have comunicated their results regarding a new serologic diagnosis algorithm for celiac disease with positive and negative predictive value of 100%. This protocol with high accuracy is able to diagnose celiac disease without performing intestinal biopsy and associates IgA EMA, anti-actine antibodies, IgA anti hu-tTG and seric level of protein zonulin.

Celiac disease is a complex genetic disorder with multiple contributing genes. Linkage studies have identified several genomic regions that probably contain celiac disease susceptibility genes. The most important genetic factors identified are HLA-DQ2 and HLA-DQ8, which are necessary but not sufficient to predispose to gluten enteropathy. The associations found in non-HLA genome wide linkage and association studies are much weaker. This might be because a large number of non-HLA genes contribute to the pathogenesis of celiac disease. Hence, the contribution of a single predisposing non-HLA gene might be quite modest. Practically, all celiac disease patients carry HLA-DQ2 or HLA-DQ8, while the absence of these molecules has a negative predictive value for celiac disease close to 100%. Genetic risk profiles for celiac disease would be helpful in clinical practice for predicting disease susceptibility and progression.16

A recent study analyzing both patients with celiac disease and control patients from the United Kingdom, Ireland, and the Netherlands has found associations between celiac disease and 8 susceptibility variants outside the human leukocytic antigen region. Only the IL2–IL21 region had been previously linked to celiac disease, and 7 of the 8 regions contain genes involved in the immune response. Candidate genes (SH2B3 and CCR3) in risk regions identified in that study, as well as HLA-DQ and IL2–IL21, suggest that mechanisms are shared between celiac disease and type 1 diabetes. Another candidate gene (IL18RAP) is associated with both celiac disease and Crohn’s disease.17

The main role of HLA typing lies in its high negative predictive value to exclude celiac disease. The disease can be virtually excluded in nonbiopsy-proven white individuals on a gluten-free diet who are non-HLA-DQ2 and non-HLA-DQ8. HLA typing can be useful to help exclude the possibility of the future development of celiac disease in patients at high risk and can provide additional information if the clinical picture is unclear.

First-degree relatives of celiac disease patients should be HLA typed and if celiac disease cannot
be excluded (HLA-DQ2 or HLA-DQ8 positive), serologic tests might be performed in asymptomatic patients with a frequency of approximately every 5-10 years when patients still have growing potential (<20 years). In asymptomatic HLA-DQ2/DQ8 positive first-degree relatives aged over 20 years old, one single screening at the age of 50 years might be indicated, as complications of celiac disease can develop. When a first-degree relative is symptomatic, a low threshold for biopsy is indicated.

In a recent study, Dr. Hadithi and colleagues assessed the value of specific serologic tests, HLA-DQ typing, or both, in diagnosing celiac disease among 463 patients referred for small-bowel biopsy. Positive results of tTG or EMA testing alone had 81% sensitivity, 99.1% specificity and 99.1% negative predictive value in making a diagnosis of celiac disease. Positive results of all four serologic tests (tTG, EMA, IgG AGA, and IgA AGA) gave 100% specificity, positive predictive value, and posttest probability, but the sensitivity was only 19%. Testing positive for either HLA-DQ2 or HLA-DQ8 maximized sensitivity (100%) and negative predictive value (100%).

Dr. Shadi Rashtak and Dr. Joseph A. Murray from the Mayo Clinic College of Medicine, Rochester, Minnesota stated in a recent editorial: “Instead of doing both tests routinely, we suggest that clinicians use serologic testing or HLA typing (but not both), depending on the clinical situation”. “It makes logical sense to use HLA typing - a high-sensitivity rule-out test - when there is a high suspicion for celiac disease and to use serologic testing - a high-specificity rule-in test - when the suspicion is low.”

In 2008, at Iguassu Falls, Brazil, during the Third Word Congress of Pediatric Gastroenterology, Dr. Mongi Ben Hariz from Tunisia presented the results of his study regarding HLA DRB1, DQB1 and DQA1 polymorphism in Tunisian children with typical form of celiac disease, in comparison with those from mass screening (atypical and silent forms of disease). He confirmed in this study the high frequency of DQ2 haplotype in celiac disease patients and he identified a new protective haplotype - DRB1*13-DQA1*0102-DQB1*06. He also concluded that HLA polymorphism seems to have no impact on clinical form of disease.

In the coming years, identifying other target genes and understanding the pathways they influence, will lead to a better understanding of celiac disease pathogenesis. Ultimately, we might be able to define genetic risk profiles for more precise diagnoses and for predicting disease progression and they may lead to novel therapies.

CONCLUSIONS

In accordance with others studies, the positive predictive values of IgA and IgG AGA were too low to warrant submitting a patient to intestinal biopsy for suspected celiac disease only performing AGA serology.

In order to optimize the serologic diagnosis of celiac disease, screening tests among risk population must associate a combination with maximum specificity and sensitivity - IgA EMA and anti hu-tTG antibodies assessment.

Low values of IgA and IgG AGA sensitivity and specificity compared to those of EMA and tTG, can reduce or even exclude these tests from celiac disease serological screening protocol.

In order to develop a non-invasive diagnosis algorithm for gluten enteropathy, further studies on different age groups are needed regarding deaminate gliadine peptides antibodies, anti-actine antibodies, or zonulin. Until these tests will be available and accessible in any laboratory, intestinal biopsy remains the gold standard for celiac disease diagnosis.

REFERENCES