IMPORTANCE OF DUODENAL BIOPSY DURING ROUTINE UPPER GASTROINTESTINAL ENDOSCOPY FOR DIAGNOSIS OF CELIAC DISEASE

SUMMARY: Celiac disease (CD) is a worldwide prevalent immune disorder caused by the ingestion of gluten containing grains in genetically susceptible individuals. To describe the value of duodenal biopsy during routine upper gastrointestinal endoscopy in an adult Romanian population for the diagnosis of celiac disease in clinical practice. A total of 150 outpatients were included in the study, all patients were interviewed for full medical history and the indication of upper gastrointestinal endoscopy with duodenal biopsy was established for gastrointestinal manifestations typical or atypical for celiac disease or/and extra-intestinal manifestations. Patients with celiac disease were followed serologically, clinically and endoscopically at one year after gluten free diet. Twelve patients (8%) had biopsy proven lesions of the CD spectrum (9 women and 3 men, mean age at diagnosis 47±10.50 years, median age 49 years, age range 24 – 60 years). According to the Marsh histopathological classification 66% were with villous atrophy and 33% with infiltrative-hyperplasic lesions. Celiac disease was more frequently diagnosed in females (75%) and in 4 and 5 decades of life. Only 2 (16.66%) patients had typical clinical features for CD. The most frequent clinical manifestations of the disease were diarrhea (41.66%), asthenia (50%), iron deficiency anemia (50%) and weight loss (50%). Following the results obtained at one year of gluten free diet the final incidence of the disease in the study group was 6%. Data from the current study confirm that celiac disease has a high incidence among adult patients in Romania. Duodenal biopsy during the routine gastrointestinal endoscopy should be included into clinical practice as a diagnostic method of celiac disease in high risk symptomatic patients and those with anemia and/or chronic diarrhea.

Key Words: celiac disease; upper gastrointestinal endoscopy; duodenal biopsy; symptomatic patients

IMPORTANȚA BIOPSIEI DUODENALE PRELEVATE ÎN TIMPUL ENDOSCOPIEI DIGESTIVE SUPERIOARE DE RUTINĂ ÎN DIAGNOSTICUL BOLII CELIACE

Boala celiacă (BC) este o boală cronică autoimună cu prevalență în creștere la nivel mondial, cauzată de ingestia de cereale care conțin gluten la pacienții cu susceptibilitate genetică. Scopul studiului a fost de a descrie valoarea în practica clinică a biopsiei duodenale preluate în timpul endoscopiei digestive superioare de rutină, în diagnosticul bolii celiacă la populația adultă din România. Au fost inclusi în studiu un număr de 150 de pacienți care au fost evaluați anamnestic și clinic, iar indicația de endoscopie digestivă superioară a fost stabilită în prezența manifestărilor clinice gastrointestinale tipice sau atipice ale bolii celiacă și/sau a manifestărilor extra-intestinale. Pacienții cu boală celiacă diagnosticată au fost urmați serologic, clinic și endoscopic la un an de la excluderea glutinei din alimentație. Doi prezece pacienți (8%) au prezentat leziuni histopatologice care s-au încadrat în spectrul bolii celiacă (9 femei și 3 bărbați, cu vârsta medie de 47±10.50 ani, mediana vârstei de 49 de ani cu limite între 24 și 60 ani). Conform clasiﬁcării histopatologice Marsh 66% au prezentat modificări de tip atrofie vilozitară și 33% modificări de tip infiltrativ-hiperplazice. Boala celiacă a fost mai frecvent diagnosticată la femei (75%) în decât al 4 și al 5 de viață. Doi (16.66%) pacienți au prezentat tabloul clinic clasă al bolii celiacă. Cei mai frecvente manifestări clinice întâlnite au fost diareea (41.66%), astenia (50%), anemia feriprivă (50%) și scăderea ponderală (50%). Urmărind rezultatele obținute la un an de dietă fără gluten incidența finală a bolii a fost de 6%. Datele obținute în studiul de față confirmă faptul că boala celiacă are o incidență crescută în rândul pacienților adulți din România. Biopsia duodenală preluate în timpul endoscopiei digestive superioare de rutină necesită a fi inclusă în practica clinică fiind o metodă utilă de diagnostic a bolii celiacă la pacienții cu risc crescut simptomatici, cu sau fără anemie feriprivă și sindrom diareic cronic.

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INTRODUCTION

Celiac disease (CD) is an immune-mediated enteropathy triggered in genetically susceptible individuals by the ingestion of gluten-containing grains (wheat, barley and rye). It is characterized by intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy, and by the classic malabsorption syndrome (diarrhea, steatorrhea and weight loss) or by minor unrelated symptoms such as iron deficiency anemia, osteopenic bone disease, amenorrhea and infertility (1). Serological screening in healthy volunteers around the world has estimated the prevalence at 0.5-1%. A recent meta-analysis indicated that the ratio of known to undiagnosed cases of celiac disease was 1:7, this suggests a failure in diagnosis of this disease (2, 3, 4).

Even though we currently possess sensitive and specific serological methods, the duodenal biopsy continues to be the “gold standard” for the diagnosis of CD (5). Knowledge of the diverse forms of presentation of CD, together with a high index of clinical suspicion and an improvement in the accessibility to endoscopy units have made it possible, in some geographical areas, that the reported prevalence of CD when taking a routine duodenal biopsy ranges from 1.0% to 5.2% (6).

Endoscopic duodenal biopsy is a diagnostic tool in the management of patients with chronic diarrhea, weight loss or symptoms that suggest malabsorption and ferropenic anemia, these type of CD is known as the classic (typical) form (7, 8).

The disease may not always be recognized because of the insidious nature of its presentation. Patients can also have the silent or atypical form of disease. These patients may present with non-specific abdominal pain, oesophageal reflux, osteoporosis and cryptogenic hypertransaminasemia (4, 5, 9).

However, the usefulness of duodenal biopsy during upper gastrointestinal endoscopy for diagnosis of CD is less well known in daily clinical practice (10).

In Eastern Europe and in Romania there is few data of the prevalence of CD in general population or in high risk population. In Romania in 2002 one study was made in selected population referred for endoscopy, according to this study the prevalence of CD was 2.22% (11).

Aims. The present study was designed to evaluate the incidence of CD among the adult out-patients biopsied during upper endoscopy with typical and atypical symptoms that can suggest the CD.

PATIENTS AND METHODS

One hundred and fifty out-patients (94 women and 56 men, mean age 43.93±13.13 years, median age 45 years, age range 17 – 66 years) were prospectively enrolled in the study during the period between January 2007 and December 2008. The study was explained to the patients and written consent was obtained. The most frequent indications for upper gastrointestinal endoscopy were gastrointestinal symptoms such as diarrhea, nausea and vomiting, abdominal pain, abdominal distension, flatulence, constipation, and asthenia. Anemia and hypertransaminasemia, as well as abnormalities in bone mineral density (BMD), were also recorded.

There is no typical classification for endoscopic lesions in CD, we used a system of grading of duodenal damage seen on endoscopy enounced by Tursi et al (12) in “normal” endoscopic appearance, “mild” endoscopic alterations (granular mucosa in the second part of the duodenum), “moderate” endoscopic alterations (scalloping of duodenal folds, reduction of duodenal folds), and “severe” endoscopic alterations (“mosaic” pattern of the mucosa in the second part of the duodenum and loss of duodenal folds). Biopsy samples (to detect villous atrophy) were also obtained from patients with only endoscopic appearances suspicious for celiac disease.

Duodenal biopsy and diagnosis criteria for CD

Four endoscopic biopsies from the 2nd-3rd portions of the duodenum were processed by using hematoxylin/eosin staining, and were evaluated by an expert gastrointestinal pathologist. Histopathological findings were staged according to the Marsh criteria (13), revised by Oberhuber (14) in: ‘infiltrative’ lesions with intraepithelial lymphocytosis (Marsh 1, cut-off value at 25 intraepithelial lymphocytes / 100); ‘infiltrative / hyperplastic’ lesions (Marsh 2); and partial (a), subtotal (b) and total (c) villous atrophy’ as Marsh 3. Other frequent causes of lymphocytic enteritis, such as parasites, NSAID ingestion, Crohn’s disease and autoimmune diseases and Helicobacter pylori (HP) infection were investigated by means of the urease test, were ruled out (15).

Patients with histopathological findings were screened for total anti-tissue transglutaminase (IgA and IgG) antibodies were determined by enzyme-linked immunosorbent assay (ELISA) with human recombinant t-TG as antigen, using a commercial kit (Test AESKULISA,
CeliCheck). Results were considered positive when the t-TGA levels were greater than 24 U/mL.

The final diagnosis of CD was considered when some degree of histological abnormality was found and a good response to a gluten free diet was achieved at least after one year of follow-up.

**Gluten free diet** (GFD) was recommended to all patients with histological abnormality. Clinical, histological, and serological assessments were carried out in all patients who adhered to a GFD at least 1 year after starting the diet.

A patient was considered to have achieved a complete clinical response when all symptoms disappeared and a partial clinical response was defined as a significant improvement but not normalization of well-being.

A complete histological response was defined as a decrease from Marsh 3 to Marsh 1 or Marsh 0, and in Marsh 1 cases, normalization in the IEL count or a reduction of at least 50% from the basal biopsy. A partial histological response was considered as an improvement in the degree of atrophy. Patients with Marsh 1 were particularly encouraged to undergo serological and histological retesting during follow-up.

**Statistical analysis.**

The Statistical Package for Social Science (SPSS), version 16. Simple statistics such as frequency, mean and standard deviation were used and chi-square, t-test and Mann-Whitney U test were used for comparison. The results were considered to have a statistical significance when the P values were <0.05.

**RESULTS**

Twelve patients of the 150 subjects included in the study were diagnosed with celiac disease (9 women and 3 men, mean age at diagnosis 47±10.50 years, median age 49 years, age range 24 – 60 years).

The most common gastrointestinal pathology diagnosed in the study group were gastrroduodenitis, peptic ulcer with or without Helicobacter Pylori infection in 38.66% of cases, functional dyspepsia in 16.66% of cases, irritable bowel syndrome in 16% of cases, parasitosis in 12% of cases and less frequent Crohn disease in 2% of cases, collagenous colitis in 2% of cases and other diseases (sarcoidosis, gastric lymphoma, giant duodenal polyp, blind loop syndrome) in 6.66% of cases.

Biopsy proven lesions of the CD spectrum had twelve (8%) patients which disclosed the following histological findings: three were Marsh 1, one Marsh 2 and eight patients with Marsh 3 (six 3a, one 3b and one 3c) lesions. Four (33.33%) cases had Helicobacter pylori infection (two Marsh 3 and two Marsh 1)

The patients with biopsy proven CD were tested for t-TGA, all had positive antibodies. The values of t-TGA increase with severity of the mucosal lesions.

The endoscopic markers of duodenal mucosa were present at 6 (50%) patients with histological findings. The severity of the endoscopic lesions were correlated with villous atrophy (Marsh 3 lesions), the patients with ‘infiltrative/hyperplasic’ lesions (Marsh 1 and 2) were without any endoscopic markers of CD.

All the patients were also assessed for the presence of gastrointestinal and extra-intestinal symptoms. Typical clinical features of CD including diarrhea, anemia and weight loss were present in 2 (16.66%) cases. 5 (41.66%) patients were with chronic diarrhea accompanied by the abdominal discomfort, nausea or vomiting and recurrent abdominal pain without any clinical manifestation of malabsorption syndrome. Atypical gastrointestinal symptoms and signs were present in 3 (25%) patients with CD.

Iron deficiency anemia was present in 5 (41.66%) cases, osteopenia in 1 (8.33%) case, hypocalcaemia and hypomagnesaemia have had 2 (16.66%) patients. Extra-intestinal symptoms associated with gastrointestinal manifestations were present: 6 (50%) patients were with asthenia and 5 (41.55%) patients with weight loss. In two cases the indications for duodenal biopsy were iron deficiency anemia and weight loss. Also in one case the hypertransaminasemia and in another case anti-nuclear antibodies were found (Clinical and immune-histological characteristics of celiac patients are shown in Table 1)

A progressive increase in severity of most symptoms from Marsh 1 to Marsh 3 was observed in almost all cases. Two patients with Marsh 1 were asymptomatic or with mild dyspeptic syndrome and another two patients with Marsh 1 and respectively Marsh 2 were with diarrhea and abdominal discomfort.

There were no differences between the patients with CD and control group (patients without CD) in terms of gastrointestinal complains like: abdominal pain, abdominal discomfort, chronic constipation, recurrent nausea and vomiting. Iron deficiency anemia was found significantly frequent in the patients with CD than in control group (p<0.05, Fisher test).

**Follow up after 1 year of gluten free diet**

One patient with Marsh 1 and Helicobacter Pylori infection and mild dyspeptic-like symptoms was not under GFD, the Helicobacter Pylori eradication was done

Follow up after 1 year of gluten free diet
and the patients returns after one year, the serology and biopsy were negative.

GFD was indicated to all patients with villous atrophy (Marsh 3) and to symptomatic patients with “infiltrative-hyperplasic” lesions (Marsh 1 and 2), one of them refused the diet and the follow-up after one year, after all 9 (90%) patients were under GFD.

A complete histological response as a decrease in severity of mucosal lesions from Marsh 3 to Marsh 1 or Marsh 0 was observed in 7/9 cases, all cases were asymptomatic after the first year of follow up. There was a statistically significant decrease in the values of t-TGA after one year of follow-up (p=0.004, Mann-Whitney test)

A partial histological clinical and serological response was observed in two cases: one from Marsh 3c to Marsh 3b and another one from Marsh 3a to Marsh 2 in which the GFD was intermittently maintained.

One year after GFD, adherence to diet was 70% (7 from 10 patients). Following the results obtained at one year CD was diagnosed in 9 patients, the final incidence of the disease in the study group was 6%.

DISCUSSIONS

Endoscopic evaluation with duodenal biopsy at high-risk symptomatic patients is the “gold standard” in the diagnosis of celiac disease. Screening studies indicates that the prevalence of CD is increasing and can reach up to 5% - 12% when the duodenal biopsy was performed during the routine upper gastrointestinal endoscopy (3).

In this study group were included 150 symptomatic subjects with classical symptoms of CD or with atypical gastrointestinal and extra-intestinal sings who can be considered at-risk patients for CD.

We observed a very high incidence of CD at the patients with clinical signs of alarm, namely in patients who have classic clinical features of disease (diarrhea, anemia and weight loss) incidence was 22.22%; in those with iron deficiency anemia and diarrhea incidence of CD was 18.18%; in those with isolated diarrhea incidence of CD was 10.29%; in those with isolated anemia incidence of CD was 20.69% and at the patients with endoscopic changes of duodenal mucosa the incidence was 17.14%. The results obtained fall in the reported data in literature that demonstrates the high incidence of CD in symptomatic patients, considered as patients at high risk of disease (3).

In the present study the incidence of biopsy proven CD was 8%. CD was diagnosed most common in the 4 and 5 decades and more frequently in females than in males (75% females vs 25% males) as has been observed in other similar studies (10).

In terms of clinical features classical clinical picture was present in 16.66% of patients. The most frequent clinical manifestations of the disease were diarrhea (41.66%), asthenia (50%), iron deficiency anemia (50%) and weight loss (50%) Atypical gastrointestinal manifestations such as abdominal discomfort, constipation, recurrent abdominal pain and dyspepsia were present in 25% of patients. Endoscopic changes of duodenal mucosa were present in 50% of patients with type atrophy changes.

Using clinical manifestations like diarrhea and/or anemia, weight loss and endoscopic changes as screening criteria lead to increasing rate of diagnosis of celiac disease

Almost 70% of patients with CD adhered to the follow-up program and to the GFD. Increased adherence to the diet can be explained on account of symptomatic

### Table 1 Clinical and immune-histological characteristics of celiac patients

<table>
<thead>
<tr>
<th>Gender</th>
<th>Female</th>
<th>9 (75%)</th>
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<tbody>
<tr>
<td>Male</td>
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<td>3 (25%)</td>
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<td>Age (years)</td>
<td>49 (24 – 60)</td>
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<tr>
<td>Clinical characteristics</td>
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<tr>
<td>Classic form of CD</td>
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<tr>
<td>Diarrhea</td>
<td>5 (41.66%)</td>
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<tr>
<td>Atypical digestive symptoms</td>
<td>3 (25%)</td>
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<tr>
<td>Asymptomatic</td>
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<tr>
<td>Extra-intestinal manifestations</td>
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</tr>
<tr>
<td>Asthenia</td>
<td>6 (50%)</td>
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<tr>
<td>Weight loss</td>
<td>5 (41.55)</td>
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<tr>
<td>Iron deficiency anemia</td>
<td>6 (50%)</td>
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<tr>
<td>Hypocalcaemia, hypomagnesaemia</td>
<td>2 (16.66%)</td>
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<tr>
<td>Osteoporosis</td>
<td>1 (8.33%)</td>
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<tr>
<td>Abnormal liver functions tests</td>
<td>1 (8.33%)</td>
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<tr>
<td>t-TGA</td>
<td>12 (100%)</td>
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<tr>
<td>Histology (Marsh type)</td>
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<tr>
<td>Marsh 1</td>
<td>3 (25%)</td>
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<tr>
<td>Marsh 2</td>
<td>1 (8.33%)</td>
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<td>Marsh 3</td>
<td>8 (66.66%)</td>
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status of patients. The reported degree of GFD adherence has been shown to vary greatly in different studies ranging from 10% to 90% and probably highly dependent on the patient-doctor relationship and confidence (16).

CONCLUSIONS

Duodenal biopsy performed during routine upper endoscopy should be incorporated in clinical practice in digestive endoscopic services. Clinicians should consider CD as possible cause of unexplained anemia, diarrhea, weight loss, asthenia but also at the patients with atypical gastrointestinal symptoms which are unresponsive to usual treatment.

REFERENCES