CLINICAL, MORPHOLOGICAL AND IMMUNOLOGICAL CORRELATIONS IN CELIAC DISEASE CHILDREN DIAGNOSED BY SCREENING

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REZUMAT

Obiective: Efectuarea unui studiu screening asupra grupului populaţional pediatric ce asociază factori de risc pentru boala celiacă (BC) şi corelarea formelor clinice cu nivelul seric al anticorpilor IgA anti transglutaminază tisulară (IgA tTG) şi cu severitatea leziunilor intestinale. Pacienţii şi metode: Cercetarea s-a desfăşurat în perioada ianuarie 2008 – decembrie 2008. Studiul a inclus un grup de pacienţi diagnosticaţi cu enteropatie glutenică, format din două subloturi. Primul a fost format din 10 pacienţi depistaţi cu BC prin screening serologic şi confirmare histologică. Al doilea sublot s-a constituit din 14 copii cunoscuţi cu BC şi monitorizaţi în clinica noastră. Testele serologice au inclus anticorpi IgA antiendomisiali (EMA) şi IgA tTG. În caz de pozitivare a cel puţin unuia dintre teste, s-a practicat biopsia de mucoasă intestinală, cu interpretarea histologică conform criteriilor Marsh. Rezultate: Din cei 24 de bolnavi cu celiaciu înrolaţi în studiu, 2 au prezentat forme silenţiase de boală, 13 au asociat forme atipice şi doar 9 au prezentat forma clasică a bolii. S-a stabilit o corelare semnificativă între titrul anticorpilor IgA tTG şi severitatea leziunilor intestinale. S-a stabilit de asemenea o corelaţie doar între forma de boală severă şi subsetul Marsh IIIc. Concluzii: Frecvenţa mare – 4,09% a BC depistate în urma screening-ului într-un grup selecţionat, concordă cu datele publicate recent, fiind mult mai mare decât prevalenţa bolii în populaţia generală. Polimorfismul simptomatologic al bolii celiace, precum şi lipsa de concordanţă între manifestările clinice şi tipul leziunilor intestinale, reţin în continuare biopsia intestinală ca procedură obligatorie pentru confirmarea enteropatiei glutenice. Cuvinte cheie: boala celiacă, anticorpi anti transglutaminază tisulară, biopsie intestinală, screening

ABSTRACT

Objectives: To perform a screening study on a pediatric group that associates certain risk factors for celiac disease (CD) and to correlate the clinical forms of disease with the serum level of IgA anti tissue transglutaminase antibodies (IgA tTG) and with the severity of intestinal injury. Patients and methods: The research was performed between January 2008 and December 2008. The study included a group of patients diagnosed with CD, divided in two subgroups. The first subgroup included 10 patients diagnosed with CD during the serological screening and confirmed by histological examination. The second subgroup included 14 known celiac children monitored in our clinic. The serological tests used for CD screening included IgA anti endomysial antibodies (EMA) and IgA tTG. In case of positive result for at least one of these tests, the intestinal biopsy was performed. The histological interpretation was done according to Marsh criteria. Results: From the total of 24 patients diagnosed with CD enrolled in this study, 2 children presented silent forms of disease, 13 of them associated atypical forms and only 9 of them presented the classical form of disease. It was established a significant correlation between IgA tTG antibodies serum level and the severity of intestinal injury. Furthermore, a correlation has been established between the severe form of disease and the subgroup Marsh IIIc. Conclusions: The high frequency (4.09%) of CD diagnosed after screening in a selected group, corresponds with the recent published dates, being higher than the prevalence of the disease in the general population. The polymorphism of CD presenting forms, as well as the lack of concordance between clinical symptoms and the type of intestinal injury, make the intestinal biopsy the gold standard for confirmation in case of clinical suspicion of gluten intolerance. Key Words: celiac disease, anti tissue transglutaminase antibodies, intestinal biopsy, screening

INTRODUCTION

Gluten intolerance is classically defined by the associations of three features: malabsorption, atrophy of intestinal mucosa, clinical and morphological response to gluten free diet. The actual tendency is to substitute this concept with the notion of gluten-sensitive enteropathy (celiac disease – CD), defined as an extreme immune response of intestinal mucosa to gluten proteins, that appears in genetically predisposed
subjects (HLA DQ2, DQ8) and can associate a diversity of histological anomalies, from a mild increase of intraepithelial lymphocytes to total villous atrophy.

It was established that the profile of anti-gliadin, anti-reticulin, EMA and tTG antibodies does not have absolute specificity and sensibility in children with gluten intolerance. For these reasons, the biopsy of intestinal mucosa remains the gold standard for the confirmation of the diagnosis. In the future, it will be necessary to establish a new definition for gluten intolerance, non-restricted by intestinal histological aspect. That definition will be probably based on genetic susceptibility and new immunological markers for CD.

Studies regarding atypical or silent form of CD have generated a great interest for methods of serological screening in gluten enteropathy. The development of different serological tests permitted a better selection of cases for intestinal biopsy in celiac patients. In clinical practice, serological tests for CD are useful in identifying patients who require intestinal biopsy in order to diagnose this condition. Anti-endomysium antibodies (EMA) and anti-tissue transglutaminase antibodies (tTG) assessment are both highly sensitive and highly specific tests, with values for both parameters exceeding 96% in most studies.

Prevalence of CD in the United States varies in different situations as follows: in average healthy people: 1 in 133, in people with related symptoms: 1 in 56, in people with first-degree relatives (parent, child, and sibling) who have CD: 1 in 22, in people with second-degree relatives (aunt, uncle, cousin) with CD: 1 in 39. The estimated prevalence of CD for African, Hispanic and Asian Americans is 1 in 236.

Recent epidemiological surveys in Europe have shown that the prevalence of celiac disease in the general population varies between 0.5 - 1%. The prevalence of childhood CD has been reported to be between 1:285 - 1:77 in Sweden, 1:99 - 1:67 in Finland, and 1:230 - 1:106 in Italian schoolchildren. Population-based studies estimated that the incidence of small bowel biopsy-confirmed CD in adults ranges between 2 and 13/100 000 per year.

In Romania, the true prevalence of gluten enteropathy in children is not known. The Celiac Disease National Register is under development 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 Shelter Institute for Mother and Child in 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 study group was 2.17%.

The incidence of the CD in the general population is appreciated as 0.7-2%, but the population mass screening is not accepted as being cost/efficient, although in present there are implemented several screening tests for disorders which are less frequent than CD - neonatal screening test for phenylketonuria, congenital mixedem. An explanation could be the onset of these two mentioned disorders in the neonatal period, while in celiac disease the precise moment of screening could not be established due to variable onset of the disease after gluten exposure.

In a 1999 study, Ventura et al. found that children diagnosed with CD between 2 and 4 years of age, presented in proportion of 10.5% a risk of developing an autoimmune disorder. Early diagnosis of celiac disease thus is important, as it might prevent complications and awareness is the key.

A recent study in North America shows that an active case-finding strategy in the primary care setting is an effective means to improve the diagnosis rate of CD. The serological screening in all subjects belonging to known risk groups increased the diagnosis rates more than 40 folds. In USA, in atypical cases of CD, the average length of time needed for diagnosis setting is four years; this type of delay dramatically increases an individual's risk of developing autoimmune disorders, neurological problems, osteoporosis and even cancer.

**OBJECTIVES**

The aim of this study was to perform a screening study on a pediatric group that associates certain risk factors for CD and to correlate the clinical forms of disease with IgA tTG serum level and with the severity of intestinal injury.

**PATIENTS AND METHODS**

The study developed between January 2008 and December 2008 at 1st Pediatric Clinic of Emergency Children Hospital „Louis Turcanu” Timisoara, on a group of 244 consecutive subjects aged from 6 months to 18 years. The criteria of inclusion in the study were: presence of least one of the following risk factors for CD: chronic diarrhea, anemia (Hb<11g/dl) resistant to oral martial therapy, growth deficiency (weight, height under the 10th percentile for age and sex), presence of autoimmune disease (type 1 diabetes mellitus, thyroiditis), chromosomal disturbances (Down, Turner, Williams syndrome), certified viral infections in patients medical history (adenovirus, rotavirus, herpes virus) or first-degree relatives of CD.

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diagnosed patients. Several subjects met two or more risk factors in the same time.

The study included one principal lot of CD patients, divided in two subgroups. The first subgroup included children diagnosed with CD after performing serological screening followed by histological confirmation. The second subgroup consisted in known patients diagnosed with CD in the precedent years and monitored in the Gastroenterology Division of First Pediatric Clinic, Emergency Children Hospital „Louis Turcanu” Timisoara.

The criteria of selection in the first subgroup are described below:

1. Subjects with suspicion of CD, associating risk factors;
2. Age between 6 months and 18 years;
3. Participants at the serological screening and further confirmed as having CD;
4. Informed consent of the legal tutors.

The criteria of selection in the second subgroup are described below:

1. Known patients with CD, addressed and monitored in First Pediatric Clinic of Emergency Children Hospital „Louis Turcanu” Timisoara between January 2008 and December 2008;
2. Age between 6 months and 18 years;
3. Informed consent of the legal tutors.

The criteria of exclusion:

1. Patients with IgA selective deficiency;
2. Lack of informed consent of the legal tutors for the participation in the study.

The inclusion in this research of two subgroups was dictated by the necessity of having a final significant number of patients. Due to the low value of CD prevalence comparing with others disorders, the annual number of newly diagnosed patients after screening was too low to perform statistical correlations. Thus, the final study lot associated to the first subgroup of patients diagnosed by screening, a second subgroup. This included 14 known celiac patients, that came for monitoring in our clinic during the period of study development (January 2008 - December 2008).

The screening was performed using the following serological tests: assessment of total IgA serum level, followed by IgA EMA and IgA tTG detection. In case of positivity of at least one of these two auto-antibodies, the intestinal biopsy was performed, with histological interpretation of biopsy sample. For patients aged less than 6 years old, we used Watson capsule and those older than 6 years underwent intestinal biopsy using upper digestive endoscopy.

In order to establish the diagnosis in patients aged less than two years old, in selected cases, the intestinal mucosa biopsy has been taken even in case of negative serological results, according to European Society of Pediatric Gastroenterology, Hematology and Nutrition (ESPGHAN) recommendations.

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

For IgA EMA detection we have used indirect immunofluorescence technique on smooth muscle of monkey esophagus - ImmuGlo™ Anti-Endomysial Antibody Test Kit, provided by Immco Diagnostics. Detection of IgA anti tTG antibodies in this study was performed using ImmuLisa™ anti-hu tTG ELISA. Test kits were also provided by Immco Diagnostics.

The statistic method used in this study was chi square test, SPSS12 application, in order to correlate the clinical forms of the disease with IgA tTG antibodies serum level and the severity of the intestinal villous alterations.

RESULTS AND DISCUSSIONS

In 2008, there were 244 subjects associating risk factors for CD submitted to the serological screening. The distribution of including criteria was as described below, some of them being added to the same patient: 134 cases with chronic diarrhea, 21 autoimmune disorders (15 cases of diabetes mellitus, 2 cases of thyroid dysfunction, one case of hypophysis dysfunction, one cases of systemic lupus erythematous, 2 cases of herpetiform dermatitis), 38 cases with iron deficiency anemia, 18 cases with rotavirus/adenovirus or herpes virus infection in their medical history, 5 first-degree relatives cases of known patients with CD (brothers), 5 cases with genetic disorders (Down syndrome, Turner syndrome), 11 cases with height deficiency and 104 cases with weight deficiency.

From the total of 244 children with associated risk factors for CD, 11 were identified as having at least one positive serological test. (Fig. 1) One of these refused the intestinal mucosa biopsy, but the positive auto-antibodies tests (IgA EMA and tTG), along with the classical digestive symptoms, allowed us to subscribe him as CD patient.

The histological evaluation was performed to the other 10 patients, but the diagnosis was confirmed only in 9 cases. One child aged 2 years and 2 months presented negative IgA EMA, positive IgA tTG auto-
antibodies and normal histological aspect of the intestinal mucosa (Marsh 0 type). This allowed us to exclude the CD diagnosis in this case and to consider IgA tTG as false positive result. Although, further clinical and serological observation for this case is needed and also haplotyping is mandatory in order to establish the risk of developing CD in the future.

Figure 1. The composition of the first subgroup after screening.

The calculated prevalence of CD in the study group that associated risk factors was 4.09%. (Fig. 2)

Figure 2. The percent of cases with confirmed CD after serological screening.

The number of known patients with CD from the second subgroup monitored in our clinic (who took part in this study between January 2008 and December 2008) was 14. From these, 3 of them refused the intestinal biopsy at the beginning. The diagnosis of CD was sustained by clinical and biological features along with the positive serological tests that became negative after 6 months of gluten free diet.

The characteristics of the study group composed of 24 CD patients are presented below.

The mean age of patients with CD enrolled in the study group (including the ones who refused the biopsy) was 6.5 years, from which 7 cases (29%) were aged less than 2 years, 11 (46%) were aged between 2 and 7 years, and 6 cases (25%) were older than 7 years.

The sex ratio in the study group was 14 girls/10 boys. (Fig. 3) Higher prevalence of CD in females was also described by other studies.

Figure 3. Sex distribution of patients with CD from the study group

From 24 celiac patients, in 4 cases, the intestinal biopsy was refused by parents as discussed (one case from the first subgroup refused the biopsy after screening and 3 children from the second subgroup were diagnosed in the previous years with CD due to typical symptoms and positive serology, but their parents refused from the beginning the biopsy). In the rest of 20 patients who accepted intestinal biopsy, the histological villous alterations were analyzed. Distribution of the intestinal injuries according to modified Marsh criteria was: 1 patient with Marsh I type lesion, 3 patients with Marsh II type lesions, 9 patients with Marsh IIIa lesions, one patient with Marsh IIIb lesion and 3 cases with total villous atrophy type Marsh IIIc. (Fig. 4)

Figure 4. Distribution of intestinal villous alterations in patients with CD who accepted the intestinal biopsy

IgA tTG serum level was quantitative assessed, been classified according to “cut-off” manufacture values as: negative for values less than 20 EU/ml, uncertain (borderline) for values between 20-25 EU/ml and positive for values over 25 EU/ml, classified further as moderate titer for values between 25-50 EU/ml and high titer for values over 50 EU/ml.

In this study, IgA EMA antibodies were detected on the smooth muscle of monkey esophagus using indirect immunofluorescence, as described in “Patients and methods” paragraph. EMA decrease slowly after gluten exclusion and have a rapid increase tendency after gluten challenge, so these antibodies are good screening markers, but they are not indicated for
monitoring the patients’ compliance to gluten free diet, when the serological parameter that should be followed is IgA tTG. EMA assessment is a qualitative method. The results can be positive, negative or in debatable cases, uncertain. 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, etc.10

From 10 patients confirmed in 2008 after screening with CD, only 4 (40%) presented the classic clinical symptoms, the rest of them associated atypical or silent forms of disease. This fact highlights the importance of serological screening in at-risk groups, in order to diagnose all forms of the disease. The oligo-symptomatic forms remained often under-diagnosed in the past, when only the typical symptoms (chronic diarrhea, abdominal distension and failure to thrive) were considered relevant in order to perform serological tests.

Analyzing all 24 patients enrolled in this study (subgroup I and II together), 2 cases (8%) presented silent form of the disease, 13 cases (54%) associated atypical form of CD and only 9 patients (38%) presented the classical form of disease. (Fig. 5)

Figure 5. Distribution of CD clinical forms of all 24 patients enrolled in the study.

Typical form of disease includes classical symptoms (chronic diarrhea, malnutrition, abdominal distension), positive serology and suggestive histological lesions. The atypical form associates atypical symptoms (chronic constipation, anemia resistant to iron therapy, dermatitis, dental enamel dystrophy, isolated height deficiency), along with positive serology and suggestive histological lesions. The silent form includes asymptomatic children diagnosed by screening with positive serology and intestinal injury at the biopsy. Latent or potential form of celiac disease describes a condition similar with the silent form, but without villous injury associated, presenting only positive serology. In these cases, mucosal alteration can develop in time.11

Confounding latent/potential form of gluten enteropathy with non CD subjects that associate false positive serological tests is an important diagnosis error. In such cases, due to its high negative predictive value, the haplotype analysis is mandatory and can make the difference: no association of DQ2/DQ8 HLA can exclude celiac disease.12

According to ESPGHAN recommendations, due to the high rate of negative serological tests in patients aged less than 2 years old, it isn’t mandatory to perform serological tests for CD diagnosis in this group of age. The serological criteria for diagnosis are debatable. In patients younger than 2 years old, with risk factors associated and negative serology results, the intestinal biopsy is further required for CD diagnosis. In this category of age, the diagnosis algorithm includes the protocol of three biopsies. The review ESPGHAN criteria reduced the number of additional biopsies after diagnosis to one, performed after gluten challenge.13

Among the first subgroup of 10 patients newly diagnosed with CD in 2008, 3 cases were aged less than 2 years and 2 of them had at least one uncertain or negative serological test. The intestinal histological changes sustained the diagnosis in all three cases. The distribution of the intestinal injuries in patients younger than 2 years old, included 2 cases with Marsh IIIc and one case with Marsh I type lesion.

According to clinical and biological changes, all cases of CD from this study were classified in three grades of severity:

1. Mild forms of CD included silent or atypical forms, without biological changes of hematological spectrum, carbohydrates, proteins or lipids level.
2. Moderate forms included atypical or classical forms associating chronic diarrhea with isolated biological changes.
3. Severe forms included classical forms of CD with obvious signs of malabsorption and maldigestion as well as severe biological changes (anemia, hypoglycemia, hypoproteinemia, hypocalcemia, etc.)

We have analyzed the existence of direct correlation between these CD forms and villous injury degree, and also the correlation between villous injury degree and IgA tTG serum level. For the second subgroup of known celiac patients monitored in our clinic, the initial IgA tTG serum level found at the moment of diagnosis, before starting the gluten free diet, was taken into consideration.

The statistic method used was chi square test, SPSS12 application. The item “clinical form of CD” is an ordinal variable with three possible values: 1
The symptomatic polymorphism of CD, as well as the lack of concordance between the clinical symptoms and the intestinal lesions degree, confers to the intestinal biopsy the gold standard value for diagnosis when there is the clinical suspicion of gluten intolerance.

The predictive value of serology in patients with villous alterations classified as Marsh I, II, IIIa and IIIb is unsatisfactory. With all these limits, the use of the serological tests decreased the necessity of multiple biopsies for diagnosis confirmation and for assessment of CD evolution in children.

The high frequency of CD cases (4.09%) diagnosed in 2008 by active serological screening in a selected group that associates risk factors, is in agreement with recent published data, being much higher than the prevalence of the disease in the general population.

According to the description of the celiac iceberg, the atypical and asymptomatic forms of CD were found in a percentage of 60% (6 from 10 patients)
during the screening performed in 2008. The socio-economic impact of early detection of the disease in pediatric patients with associated risk factors is major if it is taken into consideration the number of hidden cases that remain undetected and can generate severe complications and increase further the burden on financial and human resources.

REFERENCES