POSITIVE AND NEGATIVE PREDICTIVE VALUE OF SEROLOGICAL TESTS IN CELIAC DISEASE CHILDREN BASED ON HISTOLOGICAL FINDINGS

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Abstract

The authors tried to establish the positive predictive value and negative predictive value of serological tests used in celiac disease screening on target population. Positive diagnosis of gluten intolerance was established assessing the histological villous alteration using Marsh classification (1992) modified by Oberhuber (1997). In order to optimize the serologic diagnosis during celiac disease screening among risk population, maximum specificity and sensitivity are obtained by using combination of antiendomisium and antitransglutaminase antibody assessment.

Key words: celiac disease, children, antiendomisium antibody, antitransglutaminase antibody

Introduction

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. Recent, a non-invasive diagnosis algorithm of serological tests for celiac disease developed.

Objectives

We intended to establish sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of serologic tests used for celiac screening. The diagnosis was based on histological intestinal injury, using Marsh classification.

Material and methods

70 consecutive patients (medium 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 2004 until March 2007 – group A. All patients enrolled in group A underwent intestinal biopsy using upper digestive endoscopy (patients aged more than 4 years), or Watson capsule (patients aged less than 4 years).

During the same period, a lot of 62 consecutive, randomized patients – lot B of study, (medium 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 group B a sample of intestinal biopsy was taken during upper digestive endoscopy.

All biopsy sample were blindly classified, using Marsh criteria (1992) modified by Oberhuber (1997): type I infiltrative, type II hyperplastic (infiltrative lympho-plasmocitic lesions in villous corion, associated by glandular crypt enlargement) and type III destructive (including partial, subtotal villous atrophy – type IIIa, IIIb and total villous atrophy – IIIc). (1)

At the time of admission in this study, a serum sample was taken for total serum immunoglobulin A level and also for IgA and IgG antigliadin antibody (AGA), IgA anti-endomisium antibody (EMA) and IgA anti – human tissue transglutaminase antibody (anti hu-tTG). 2 patients from lot B with selective IgA deficiency were excluded from this study.

For IgA EMA detection we used immunofluorescence technique using smooth muscle of monkey esophagus (ImmuGlo^{TM}Anti-Endomysial Antibody (EMA) Test Kit – provided by „IMMCO DIAGNOSTICS").

Detection of anti tTG antibodies was performed using ImmuLisa^{TM} anti-hu tTG ELISA. Test kits were provided also by „IMMCO DIAGNOSTICS".

Results and disscusions

14 patients from 70 enrroled in group A of study (20%) and 1 patient from 60 remained in group B after IgA deficiency subjects exclusion (1,67%) presented villous lesions corresponding to Marh type II, IIIa, IIIb and IIIc. The 115 remained patients with normal intestinal morphology were considered the control group. All 15 subjects with histologically confirmed celiac disease tested positive for IgA EMA and 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 patients from 115 (5%) tested positive for IgA anti hu-tTG antibody. 15 control subjects (13%) tested positive for IgA AGA and 30 control subjects (26 %) tested positive for IgG AGA.

Assessing these data, we calculated EMA and tTG sensitivity as 100% and IgA, IgG AGA 66%. The specificity was 100% for EMA, 95% for tTG, 74% for IgG AGA and 87% for IgA AGA. The negative predictive value was 100% for EMA and tTG, 94% for IgG AGA and 95% for IgA AGA. The positive predictive value was 100 % for EMA, 71% for tTG (p = 0, 03% vs. EMA), 25 % for IgG AGA and 66% for IgA AGA. Most of the control subjects who false

positive for were anti - hu tTG antibody had Crohn's disease or chronic liver disease.

Recent studies described a high number of asimptomatic (latent, silent) or atypical form of celiac disease. In many cases, this conditon is indicated by intestinal morphology alteration observed after upper digestive endoscopy performed for diyspeptic syndrom, or to evaluate an irritable bowel syndrome non-responsive to classic therapy . It is estimated that 5 % of patients with irritable bowel sindrome coressponding to Rome II criteria have celiac disease.(2), (3)

Typical form of disease presents classic clinic manifestation, positive serology and characteristic intestinal alteration.

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 s.a), positive serology and characteristic intestinal alteration.

Silent form of disease associates positive serology and intestinal villous injury in non-symptomatic patients.

Latent form of disease is characterized by positive serology without bowel morphology alteration in non-symptomatic patients.

Studies regarding atypical, silent or latent form of celiac disease have generated a great interest for methods of serologic screening in gluten enteropathy diagnosis. Using different serologic tests since 1997 permitted a better selection of cases for intestinal biopsy in celiac patients. (4)

Anti-reticulin antibodies used previously for gluten intolerance diagnosis proved to have low sensitivity and specificity, so these antibodies are excluded from diagnosis protocols.

IgA and IgG antibodies (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, s.a. 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. (5)

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 s.a. (6)

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. (7)

As many studied concluded, enzyme linked immunosorbent assay based on human tissuse transglutaminase outperforms the guinea pig based tissue transglutaminase assay (8), so we used in this study human antigen for tTG antibody.

Interpretation of serologic test in celiac disease must consider IgA selective deficiency source of fals negative results for IgA EMA and tTG. Also ESPGHAN criteria for celiac disease do not recommend serologic tests in patients aged less than 2 years, due to high frequency of false negative results. (9)

In 2007 at Barcelona, during ESPGHAN (European Society of Pediatric Gastroenterology, Hepatology and Nutrition) Symposium, a researchers group leaded by S. 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 acurracy is able to diagnose celiac disease without performing intestinal biopsy and associates EMA, anti-actine antibodies, tTG and seric level of protein zonulin. (10)

Conclusions

Although anti - tTG antibody evaluated in our study showed an optimum sensitivity, their low specificity determined positive predictive values wich were significantly lower than those of EMA assay.

In accordance with others studies, the positive predictive values of Ig A and IgG AGA were to 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 - EMA and tTG antibodies assessment.

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

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

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