ORIGINAL ARTICLE

Immunohistologic analysis of the duodenal bulb: a new method for celiac disease diagnosis in children

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Background and Aims: Anti-tissue transglutaminase antibodies (anti-tTG) have simplified celiac disease (CD) diagnosis. However, in atypical forms of CD, intestinal biopsy sampling is still required. This prospective study investigates whether histologic analysis of the duodenal bulb combined with intestinal IgA anti-tTG deposit immunoassay makes CD diagnosis possible in at-risk children with low concentrations of serum anti-tTG.

Methods: Histologic and intestinal IgA anti-tTG deposit immunoassays were used.

Results: Two hundred forty-five symptomatic children positive for serum anti-tTG (>7 U/mL) were enrolled and divided into 3 groups: extensive duodenal atrophy (n = 209), with IgA anti-tTG deposits throughout the duodenum and high serum anti-tTG concentrations (157 ± 178 U/mL); bulb duodenal atrophy (n = 22), with widespread IgA anti-tTG deposits in 9 and in the bulb alone in 13 and low serum anti-tTG concentrations (13.9 ± 8.7 U/mL); and normal duodenum (n = 14), with widespread IgA anti-tTG deposits in 8 and in the bulb alone in 6 and low serum anti-tTG concentrations (10.6 ± 6.2 U/mL). All patients in the first 2 groups were diagnosed with CD and 8 from the third group. All improved after 1 year of gluten-free diet. Bulb duodenal analysis led to a 12% (30/245) increase in CD diagnosis. No CD-related lesions were observed in the 30 control subjects.

Conclusions: In children at risk for CD, bulb duodenum biopsy sampling is essential to identify villous atrophy and detect IgA anti-tTG deposits even in absence of intestinal lesions. These mucosal autoantibodies could well represent a new standard for diagnosing CD. (Gastrointest Endosc 2018;■:1-6.)

Celiac disease (CD) is a gluten-dependent autoimmune disorder affecting genetically susceptible individuals carrying the human leucocyte antigen (HLA) DQ2 or DQ8 haplotypes.[1] The immune response is characterized by the production of specific IgA autoantibodies against the tissue transglutaminase enzyme (anti-tTG) in the intestinal mucosa.[2] These autoantibodies have greatly simplified the diagnosis of CD in daily clinical practice.[3]

Interestingly, high IgA anti-tTG serum concentrations indicate severe enteropathy not only in symptomatic patients but also in screening-detected patients with no symptoms of CD, suggesting that intestinal biopsy specimens may not be necessary for definitive CD diagnosis.[4] For this reason, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition has proposed and verified new diagnostic guidelines that advise against intestinal biopsy sampling in certain well-defined clinical situations.[5,6] However, pediatricians and gastroenterologists have to deal with several atypical forms of CD in which the histologic assessment of the intestinal biopsy specimen still plays a pivotal diagnostic role.[5] In general, biopsy specimens have been taken from the distal part of

Abbreviations: AEA, anti-endomysium antibodies; anti-tTG, anti-tissue transglutaminase antibodies; CD, celiac disease; GFD, gluten-free diet; HLA, human leucocyte antigen; IEL, intraepithelial lymphocyte.

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the duodenum and from the duodenal bulb to identify a patchy distribution of villous atrophy.[7] Taavela et al[8] recently showed that morphologic injuries are common in the bulb duodenum even in the absence of CD, thereby increasing the risk of false-positive diagnoses. In the same study the authors demonstrated that the presence of intestinal IgA anti-tTG deposits is effective for confirming that the bulb mucosal lesions are actually CD-related. However, this study involved symptomatic CD patients with high serum IgA anti-tTG concentrations (10 times the cut-off value) in which the intestinal biopsy sampling could have been avoided after the new European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines.[5]

The aim of this prospective study, based on a large number of children, was to assess whether both histologic analysis and intestinal IgA anti-tTG deposits of the duodenal bulb (in addition to that of the distal duodenum) would increase the diagnostic yield of CD, especially in suspected CD patients who tested positive for serum IgA anti-tTG with low concentrations. We also investigated whether serum concentrations of IgA anti-tTG can predict the extent of gluten-dependent immunohistologic changes in the bulb and distal duodenum. In selected cases with patchy distribution of villous atrophy and of intestinal IgA anti-tTG deposits, we investigated the extension of the gluten-dependent inflammation by using the high sensitive and specific phage display anti-tTG antibody assay.[9]

METHODS

Patients

Patients were prospectively recruited from the Institute for Maternal and Child Health–IRCCS "Burlo Garofolo" in Trieste (Italy) from July 2014 to March 2016. Consecutive children undergoing upper GI endoscopy for suspected CD, because of suggestive symptoms and serum IgA anti-tTG positivity, were enrolled and classified on the basis of the extent of mucosal atrophy in the bulb and distal duodenum. Children with other GI disorders (such as inflammatory bowel diseases) were asked to take part in the study as a control group. Written informed consent was obtained from the parents of the children enrolled, and the study was approved by the hospital's independent ethical committee (CE/V-131).

Small-bowel mucosal morphology

In each patient, 4 intestinal biopsy specimens were taken by endoscopy: 2 samples each from the bulb and distal duodenum. The specimens, correctly oriented on acetate cellulose filters, were stained with hematoxylin and eosin and were analyzed by light microscope. Immunohistochemical staining for T lymphocytes with anti-CD3 monoclonal antibodies (Dako, Glostrup, Denmark) was routinely performed. Intraepithelial lymphocyte (IEL) density was expressed as a percentage of the number of epithelial cells (number of IELs/100 epithelial cells), with a density value >25 considered as abnormal. The histologic analysis was based on Oberhuber's criteria[10] and the latest proposed classification.[11] An independent specialist (V.V.) evaluated the biopsy samples without prior knowledge of our subjects' clinical and laboratory data.

Intestinal IgA anti-tTG

Intestinal IgA anti-tTG was investigated in bulb and distal duodenum specimens by using a previously described immunoassay.[12,13] Briefly, unfixed frozen sections were incubated first with monoclonal mouse antibody against tTG (CUB7402; NeoMarkers Fremont, California, USA) followed by Alexa fluor 594-conjugated anti-mouse IgG secondary antibody (Thermo Fisher Scientific Waltham, Massachusetts, USA), and then with fluorescein isothiocyanate-labeled rabbit antibody against human IgA (Dako, Glostrup, Denmark). Multicolor analysis was performed using an Axioplan 2 fluorescence microscope (Carl Zeiss, Oberkochen, Germany) to localize IgA anti-tTG deposits, which appear as yellow spots.

Phage-display antibody libraries

Phage-display antibody assay was used to analyze intestinal biopsy specimens from randomly selected CD patients with patchy immunohistologic changes and from control subjects. Phage-antibody libraries were constructed, as previously described,[9] from the subjects' distal duodenum biopsy sample B lymphocytes to search for CD-specific mucosal anti-tTG antibodies, which are primarily composed of the *IGHV5-51* gene from the VH5 gene family. Selective IgA *IGVH5-51* genes were amplified from the cDNA and assembled into single-chain fragment-variable fragments by cloning into the phagemid vector pDAN5. Single-chain fragment-variable recognizing human tTG were selected by affinity chromatography. After 3 cycles of selection, 45 clones were screened for reactivity to human tTG protein by ELISA.

Serology tests and HLA typing

Serum IgA anti-tTG were measured using an ELISA assay (Eurospital, Trieste, Italy) following the manufacturer's instructions. Serum IgA anti-tTG values higher than 7 U/mL were considered positive. Serum IgA anti-endomysium antibodies (AEAs) were measured by an indirect immunofluorescence method using human umbilical cord as substrate, as previously described.[9] Whole blood was used to test for the presence of the susceptibility alleles for CD using polymerase chain reaction with allele-specific primers that identify HLA DQ2 and DQ8, using the Eu-Gene-Risk kit (Eurospital).

Statistical analysis

Data are reported as mean ± standard deviation for continuous variables and as proportion for categorical