Celiac Disease without Villous Atrophy in Children: A Prospective Study

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Objective To establish whether children who are endomysial antibody (EmA) positive and have normal small-bowel mucosal villous morphology are truly gluten-sensitive and may benefit from early treatment with a gluten-free diet.

Study design Children who were EmA positive with normal small-bowel mucosal villi were compared with children who were seropositive with villous atrophy by using several markers of untreated celiac disease. Thereafter, children with normal villous structure either continued on a normal diet or were placed on a gluten-free diet and re-investigated after 1 year. Seventeen children who were seronegative served as control subjects for baseline investigations.

Results Normal villous morphology was noted in 17 children who were EmA positive, and villous atrophy was noted in 42 children who were EmA positive. These children were comparable in all measured variables regardless of the degree of enteropathy, but differed significantly from the seronegative control subjects. During the dietary intervention, in children who were EmA positive with normal villi, the disease was exacerbated in children who continued gluten consumption, whereas in all children who started the gluten-free diet, both the gastrointestinal symptoms and abnormal antibodies disappeared.

Conclusions The study provided evidence that children who are EmA positive have a celiac-type disorder and benefit from early treatment despite normal mucosal structure, indicating that the diagnostic criteria for celiac disease should be re-evaluated. *(J Pediatr 2010;157:373-80)*.

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In recent years celiac, disease has been recognized as one of the most common chronic disorders in childhood, with an estimated prevalence of 1% in Western populations.[1,2] The current diagnostic criteria for the disease are based on the demonstration of small-bowel mucosal villous atrophy with crypt hyperplasia.[3,4] During the last few decades, sensitive tests for endomysial (EmA) and transglutaminase 2 antibodies (TG2-ab) have been used increasingly to select patients for endoscopic studies, and as a result, children with positive celiac antibodies but normal small-bowel mucosal villous structure are frequently seen.[5-9] In such situations, the antibodies are often considered false-positive. However, the mucosal damage develops gradually from mild inflammatory changes to overt villous atrophy.[10] Some studies have suggested that positive celiac antibodies might be specific markers of early developing celiac disease.[6,7,9,11-13] The children who are seropositive may also have symptoms such as diarrhea,[6,7] abdominal pain,[9,13] and failure to grow[6,9] while still having normal villous structure. We have recently shown that adults who are EmA positive have a celiac-type disorder and evinced a positive response to a gluten-free diet irrespective of the small-bowel mucosal morphology.[14] The early diagnosis of children would seem to be equally relevant, because many complications of celiac disease, for example decreased bone mineral density,[15,16] short stature,[17] and dental enamel defects,[18] may remain permanent if untreated. However, prospective trials in children who are EmA positive and have normal villi are lacking, and it is unclear whether early treatment would be beneficial in children.

In this prospective study, we compared children who are EmA positive and have normal small-bowel mucosal villous structure to similar subjects who are seropositive and have villous atrophy and control subjects by using several markers of untreated celiac disease. Subsequently, the children with positive EmA and normal villi either continued on a gluten-containing diet or started an experimental gluten-free diet and were re-examined after a follow-up period. The main objectives were to ascertain whether children who are EmA positive

EmA	Endomysial antibodies
HLA	Human leukocyte antigen
IEL	Intraepithelial lymphocyte
Ig	Immunoglobulin
TG2	Tissue transglutaminase 2
Vh/CrD	Villous height crypt depth ratio

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have a gluten-dependent disorder and benefit from a gluten-free diet despite normal small-bowel villous structure.

Methods

The open study was performed at the Departments of Pediatrics and Gastroenterology and Alimentary Tract Surgery in Tampere University Hospital. The study protocol was approved by the ethical committee of Tampere University Hospital, and all the families gave written informed consent. The EmA-positive patient group comprised 59 consecutive children who were referred from primary health care because of suspicion of celiac disease (**Figure 1**; available at www.jpeds.com). After baseline examinations, all participants underwent upper gastrointestinal endoscopy with general anesthesia and small-bowel mucosal biopsies; and serological samples were taken for further studies. The degree of mucosal damage was further classified according to Marsh criteria.[10] Children who at baseline were positive for EmA but evinced normal small-bowel mucosal structure (Marsh 0-II) comprised the study group. Because there is no consensus on the treatment of such children, a choice either to continue with a normal diet or take an opportunity to place the child on an experimental gluten-free diet was offered to all the families in question. Children who were EmA positive and already had villous atrophy and crypt hyperplasia (Marsh III) comprised the celiac group, and they were placed on a gluten-free diet. After one year in this trial, the baseline investigations were repeated in all the EmA-positive cases. However, because a second gastrointestinal endoscopy after a year on treatment is no longer standard procedure in children,[4] a control biopsy was considered unethical and performed only in children who continued the gluten consumption. To ensure strict adherence to the gluten-free diet, all the families whose child started the dietary treatment were counseled by a trained dietitian.

A total of 17 children and adolescents who were EmA negative and investigated because of gastrointestinal symptoms or signs of malabsorption and found to have a normal small-bowel mucosal villous structure were used in histological comparisons (control group).

Small-bowel Mucosal Morphology and Inflammation

The small-bowel mucosal morphology was determined from at least 3 separate biopsies sections taken from the distal duodenum and graded according to Marsh criteria, as described previously.[3] Cases with patchy small-bowel mucosal villous atrophy were classified as having celiac disease.[19] Furthermore, the mucosal morphology was evaluated in detail by measuring the villous height-crypt depth ratio (Vh/CrD) as a mean of 5 villous-crypt pairs; a ratio <2.0 was regarded as compatible with celiac disease.[20] Immunohistochemical stainings were performed with 5-μm–thick frozen sections from small-bowel mucosal biopsy specimens. CD3+ intraepithelial lymphocytes (IELs) were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, California) and $\gamma\delta$+ IELs with T-cell receptor-γ antibody (Endogen, Woburn, Massachusetts). Positive IELs were counted with a 100× flat field light microscope objective throughout the surface epithelium and expressed as cells/mm of epithelium.[21] In our laboratory, the correlation coefficient for intraobserver variation for CD3+ and $\gamma\delta$+ IELs has been 0.95 and 0.98, and for interobserver variation it has been 0.92 and 0.98, respectively.[21]

Intestinal Transglutaminase 2-Targeted Autoantibody Deposits

The serum EmA are known to be targeted against tissue transglutaminase 2 (TG2), and the antibodies are produced locally in the small-bowel mucosa.[22] In patients with celiac disease who are untreated, these antibodies target the extracellular TG2 below the basement membrane along the villous and crypt epithelium and around mucosal vessels, whereas in subjects without celiac disease, only endogenous immunoglobulin A (IgA) is detected inside the plasma and epithelial cells.[23] These mucosal autoantibody deposits (IgA deposits), which have been shown to possess an excellent specificity for untreated celiac disease,[23-27] were measured in this study as a marker of small-bowel mucosal antibody production. The deposition was determined from 5-μm–thick unfixed frozen mucosal sections by using direct immunofluorescence with fluorescein isothiocyanate-labeled rabbit antibody against human IgA (DAKO AS, Glostrup, Denmark) at a dilution of 1:40 in phosphate-buffered saline, pH 7.4. The co-localization of IgA deposits with TG2 was confirmed with double-staining for IgA and for TG2 with monoclonal mouse antibodies against TG2 (CUB7402; NeoMarkers, Fremont, California), followed by rhodamine-conjugated anti-mouse immunoglobulin antibodies (DAKO).[25] The deposits were further graded from negative to strong positive (+++) on the basis of their intensity in the villous-crypt area, as previously described.[25]

Serology and HLA Genotype

Serum IgA class EmA titers were determined by using an indirect immunofluorescence method with human umbilical cord as substrate; a dilution ≥1:5 was considered to be positive.[28] Positive sera were further diluted 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000, and 1:4000. Serum IgA class TG2-ab levels were investigated with an enzyme-linked immunosorbent assay (Celikey; Phadia, Freiburg, Germany) according to the manufacturer's instructions; values ≥5.0 U were considered to be positive. In the case of selective IgA deficiency, the EmA and TG2-ab values were determined by measuring corresponding antibodies in IgG class.[29] The HLA genotype was determined with single-nucleotide polymorphisms tagging the celiac disease-associated HLA haplotypes, as previously described.[30]

Statistics

Quantitative data were expressed as means or medians and ranges. Depending on the variable measured, the 2-tailed Student t test, Mann-Whitney U test, or χ^2 test in cross tabulations