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Duodenal Bulb Biopsies in Celiac Disease: A Multicenter Study

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ABSTRACT

Objectives: Celiac disease (CD)–related lesions have been reported in duodenal bulb biopsies, sometimes the bulb mucosa being the only one affected. The aim was to verify in a significant series whether histological lesions are always present in the bulb of celiac patients, what is the prevalence of lesions when isolated to the bulb, and if similar lesions are present in nonceliac subjects.

Methods: We studied 665 children with CD (241 males, range 9 months–15 years, 8 months), at diagnosis on a gluten-containing diet, and 348 age- and sex-matched gastroenterological controls submitted to upper endoscopy for gastroenterological complaints. During endoscopy, multiple biopsies (1 bulb and 4 distal duodenum samples) were taken. Anti-endomysium antibodies were evaluated by immunofluorescence method, anti–human tissue–transglutaminase antibodies by an enzyme-linked immunosorbent assay or radioimmunoassay. Human leukocyte antigen-DRB1, -DQA1, and -DQB1 genes were typed by polymerase chain reaction sequence–specific primers repeat method.

Results: In all of the patients with CD, histological lesions were present in the bulb sample; in 16 of them, the lesions were present only in the bulb. Patchy villous atrophy was found in 20 children. All of the patients with CD were anti-endomysium and/or anti-transglutaminase positive. The controls showed neither autoantibody positivity nor mucosal changes compatible with CD.

Conclusions: This study demonstrated that CD-related histological lesions are always present in the bulb; sometimes this specific site is the only one affected. Therefore, we suggest taking 2 biopsies from the bulb and 2 from the distal duodenum for CD diagnosis. ***JPGN 47:618–622, 2008.*** **Key Words:** Celiac disease—Duodenal bulb biopsy.

Celiac disease (CD) is an autoimmune disorder characterized by a gluten-dependent enteropathy. The characteristic lesions (increase of intraepithelial lymphocytes, crypt hyperplasia, and villous atrophy) are generally searched for in the distal duodenum or proximal jejunum, according to the 1970 recommendation of the European Society for Paediatric Gastroenterology (1). Twenty years later, the Working Group of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (2) recommended carrying out the small intestinal mucosal biopsy "always with a biopsy capsule rather than through an endoscope," to obtain adequate specimens for histological examination, and the use of endoscopic grasp forceps was discouraged. The more recent guideline of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) suggests that "multiple endoscopic biopsies should be obtained from the more distal segments of the duodenum" for the presence of Brunner gland, which could interfere in the evaluation of the villous crypt ratio in the proximal duodenum (3,4).

We have demonstrated for the first time that at the diagnosis of CD, the histologically characteristic lesions

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are also present in the bulbar area; in 4 cases of 95, the bulbous mucosa was the only area affected by this enteropathy (5).

To throw a definitive light on this topic, we decided to perform a multicentre study with the following aims:

1. To verify, in a larger number of children with CD, whether histological lesions in CD are always present in the bulb mucosa
2. To evaluate the prevalence in children with CD who show lesions only in the duodenal bulb
3. To investigate whether CD-related mucosal lesions are present in the duodenal bulb mucosa of subjects submitted to upper endoscopy who are not affected by CD.

MATERIALS AND METHODS

We studied 665 children and adolescents with CD (241 males and 424 females, ages 9 months–15 years, 8 months, median age 5 years, 3 months) living in various Italian regions (group 1). The first 95 patients with CD had been collected in the Paediatric Department of the University ''La Sapienza'' of Rome (5); the other patients were enrolled consecutively from September 2003 to September 2005 under the auspices of the Italian Society of Paediatric Gastroenterology, Hepatology, and Nutrition from various centers in the northern, central, southern, and insular Italian regions, thus making our sample fairly representative of the entire Italian population. CD was diagnosed according to the revised ESPGHAN (2) and NASPGHAN (3) criteria.

A total of 348 children and adolescents also were enrolled (179 males and 169 females, ages 9 months–16 years, 4 months, median age 6 years, 2 months) as a control group. They underwent upper endoscopy for failure to thrive, abdominal pain, or vomiting, but their results did not indicate CD (group 2).

The screening tests included total immunoglobulin (Ig) A; IgA and IgG anti-gliadin antibodies, tested by an enzyme-linked immunosorbent assay (Alfagliatest, Eurospital, Trieste, Italy); IgA and IgG anti-endomysium antibodies (EMA), tested by an indirect immunofluorescence method (Eurospital) using as substrate sections from the distal portion of monkey's esophagus; IgA and IgG anti-tissue transglutaminase autoantibodies (anti-tTGAbs) evaluated with an enzyme-linked immunosorbent assay (Eurospital and Menarini, Firenze, Italy) or with a quantitative radioimmunoprecipitation assay using human recombinant ^{35}S-methionine–labeled tTG (6).

Human leukocyte antigen-DRB1, -DQA1, and -DQB1 genes were typed by polymerase chain reaction sequence–specific primers using commercial kits (Dynal, Bromborough, UK).

After fasting overnight, all of the patients underwent upper endoscopy with multiple biopsies following sedation or narcosis. An Olympus (Melville, NY) XP 10 gastroscope was used in children younger than 5 years, and an Olympus GIF E was used in older children. During each endoscopy, 1 sample from the duodenal bulb and 3 or 4 samples from the distal duodenum were taken.

To obtain a well-oriented sample, we laid each biopsy on filter paper and oriented it so that the luminal surface was uppermost. Each bioptic sample was fixed in 10% formalin and separately embedded in paraffin blocks. The sections were serially cut, stained with hematoxylin and eosin, and assessed under light microscopy. The histological adequacy of samples was assessed as described by Branski et al (7) and by Gottrand et al (8).

The histological lesions of the intestinal mucosa were evaluated according to the Marsh classification, as modified by Oberhuber et al (9): type 0 = normal mucosa; type 1 = infiltrative (with $\geq$40 intraepithelial lymphocytes/100 epithelial cells); type 2 = crypt hyperplasia; type 3a = mild villous atrophy; type 3b = marked villous flattening; and type 3c = total villous atrophy.

Immunohistochemistry with anti-CD3 monoclonal antibody (Dako, Copenhagen, Denmark) was performed in 306 patients representative of all of the participating groups. According to the review of Dickson et al (10), we considered 30 intraepithelial lesions (IELs)/100 epithelial cells to be the normal value.

A gluten-free diet was suggested for patients with CD and a clinical and serological follow-up was initiated; in patients who did not show diffuse lesions of the intestinal mucosa, DQ2/DQ8 heterodimers were typed and a second biopsy was suggested after 1 year of a gluten-free diet. The study was performed according to the Declaration of Helsinki. Informed consent was obtained from the parents for their children.

RESULTS

All 665 children and adolescents with CD (group 1) showed mucosal changes compatible with CD in the duodenal bulb. Of them, 16 (2.4%), 5 boys and 11 girls, ages 1 year, 8 months to 12 years, 11 months (median age 6 years, 7 months) had histological lesions in the duodenal bulb, but a typical-appearing mucosa in the distal duodenum (group 1A); CD3 count of biopsies of the distal duodenum was always <30 IELs. Patchy villous atrophy was detected in 20 (3%) patients, 8 boys and 12 girls, ages 1 year, 8 months to 16 years (median age 6 years, 2 months) (group 1B).

As reported in Table 1, all of the group 1A and group 1B children were EMA and/or anti-tTGAbs positive; 18 of 19 (95%) patients carried the DQ2 and/or DQ8 heterodimers. Only 2 of 36 group 1A and group 1B patients (CD relatives) were clinically silent.

Diffuse histological lesions were present in all of the remaining patients with CD (group 1C). The types of lesions observed on the bulb, according to the Marsh modified classification (9), in the 3 groups of patients with CD, are shown in Table 2. In particular, type 1 lesions (Fig. 1) were detected in 1 of group 1A and group 1B and in 23 group 1C patients; type 2 lesions were present in 1 group 1A and in 19 group 1C subjects. Overall, the most frequent lesions present in the 3 groups were the most severe degrees of villous atrophy (Oberhuber types 3b and 3c) as shown in Figures 2 and 3, respectively. None of the 348 nonceliac gastroenterological controls (group 2) had similar mucosal changes in the duodenal bulb.

TABLE 1. *Clinical and laboratory data of patients with CD with histological lesions only in the duodenal bulb area (group 1A) and of children with patchily distributed lesions (group 1B)*

Patients (no.)	Group 1A (16)			Group 1B (20)			Total (36)		
EMA IgA positive									
No.	14			18			32		
%	87.5			90			88.9		
IgA anti-tTGAbs positive (ELISA/RIA)									
No.	16			20			36		
%	100			100			100		
HLA-DQ2 +	6			7			13		
DQ8+	3			1			4		
DQ2/DQ8+				1			1		
DQ2/DQ8−				1			1		
Clinical manifestation of CD	T	A	S	T	A	S	T	A	S
	12	3	1	18	1	1	30	4	2

CD = celiac disease; EMA = anti-endomysium antibodies; anti-tTGAbs = anti-transglutaminase; ELISA = enzyme-linked immunosorbent assay; RIA = radioimmunoassay; HLA = human leukocyte antigen; T = typical CD manifestation (gastrointestinal signs and symptoms); A = atypical CD manifestation (nongastrointestinal signs and symptoms); S = silent presentation of CD.

The follow-up of group 1A and 1B patients showed the disappearance of CD-related symptoms and the normalization of serum autoantibodies. In 7 of 16 group 1A children (including the 2 patients with type 1 and 2 lesions) and in 5 of 20 group 1B children, who underwent a control biopsy after 1 year or more following the start of a gluten-free diet, the mucosa showed a normal histological aspect. In particular, the DQ2-/DQ8- patient belonging to group 1B was EMA positive, and antibodies became negative after 6 months of a gluten-free diet. Permission for a second biopsy was denied by the parents.

DISCUSSION

We already have demonstrated (5) that bulbar mucosa in children with CD always shows CD-related histological alterations; sometimes this mucosa is the only one affected at the diagnosis of the disease, and not only during gluten challenge, as Vogelsang et al (11) reported in 2 adult females.

To confirm these data, we decided to enlarge the previous series of 95 children and adolescents to achieve a statistically significant number of subjects. We collected 570 more children and adolescents, with a total number of 665 from 8 Italian centers, and all of them showed some degree of histological lesions in the bulb mucosa.

The results of the multicenter study confirm our previous observation (5) that in CD, histological lesions always are present in the bulb mucosa. In a remarkable percentage (2.5%) of patients, this area was the only one affected. In these patients, all of the samples taken in the distal duodenum showed normal villi and the count of IELs, made by CD3, was always <30 lymphocytes/100 epithelial cells. Recently, many studies on IEL counts have been published; however, to date, there is no agreement about the choice of cutoff value for normal subjects. Some papers proposed a normal value lower than 25 lymphocytes/100 epithelial cells (12,13). Other authors described an increase of duodenal IELs in association with mucosal changes that mimic CD (14–16). In the present study, in accordance with the recent review

TABLE 2. *Histological lesions of the bulb according to the Oberhuber classifications (10) in the 3 groups of patients with CD*

Patients (no.)	Group 1A (16)	Group 1B (20)	Group 1C (629)
Type 1 (%)	1 (6.25)	1 (5)	23 (3.67)
Type 2 (%)	1 (6.25)	0	19 (3.02)
Type 3a (%)	1 (6.25)	1 (5)	76 (12.08)
Type 3b (%)	4 (25)	5 (25)	240 (38.15)
Type 3c (%)	9 (56.25)	13 (65)	271 (43.08)

CD = celiac disease; group 1A: only bulbar lesions; group 1B: lesions patchily distributed; group 1C: diffuse lesions.

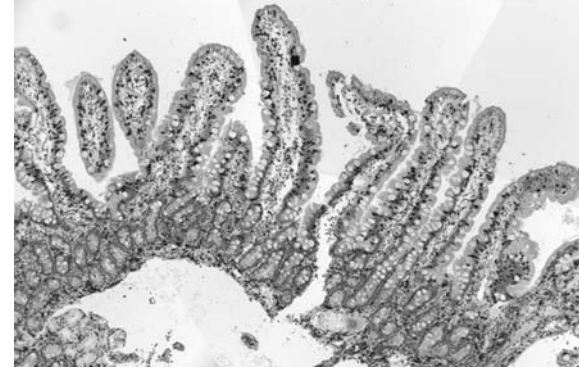

FIG. 1. Normal villous architecture with an increase of intraepithelial lesions (type 1) stained with CD3 of the duodenal bulb in a patient with celiac disease. Hematoxylin and eosin, original magnification ×100.

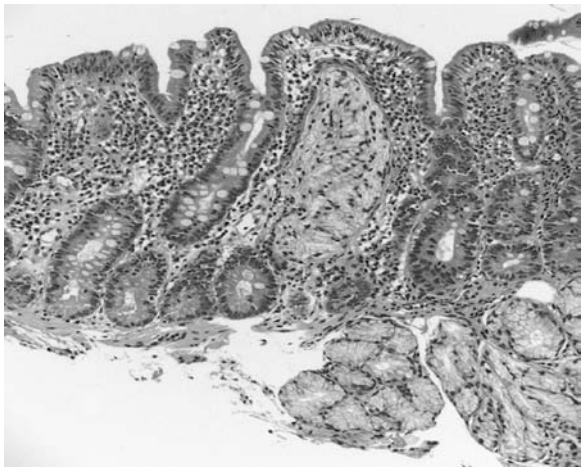

FIG. 2. Marked villous atrophy (type 3b) of the duodenal bulb in a patient with celiac disease. Hematoxylin and eosin, original magnification ×100.

of Dickson et al (10), we have chosen the cutoff of 30 IELs/100 epithelial cells.

Isolated bulb lesions have also been reported by Brocchi et al (17), in a symptomatic adult female patient with negative serology. Also, Ravelli et al (18) in a recent study reported on 110 children with CD, all of them presenting with histological lesions in the duodenal bulb. Interestingly, in these patients total villous atrophy was found more frequently in the distal duodenum and the proximal jejunum than in more proximal areas. A possible explanation has not been provided.

The histological lesions in the duodenal bulb could be explained by the fact that this intestinal portion is particularly rich in lymphatic structures (4) and the first one to be reached by gluten (11). We can speculate that patients considered to potentially have CD could have

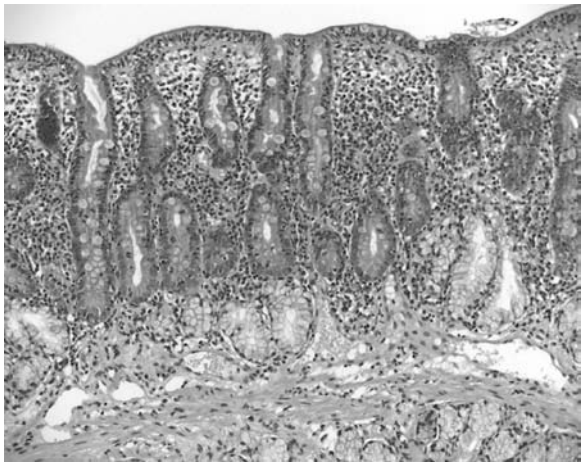

FIG. 3. Total villous atrophy (type 3c) of the duodenal bulb in a patient with celiac disease. Hematoxylin and eosin, original magnification ×100.

isolated bulbar lesion, but they did not undergo the bulbar biopsies.

The percentage of isolated bulb lesions in our multicenter study was lower (2.4%) than in our previous article (4.2%). In this study, there is evidence of the phenomenon in which enlarging the number of observations following a pilot study tends to decrease the prevalence of a particular variable. The results of early pilot studies for other aspects of CD, such as the association with Down syndrome and Turner syndrome, including the former (19,20), were partially modified by enlarging the series (21,22).

The detection of villous atrophy patchily distributed in children is generally referred to as cow's milk protein intolerance (23), whereas its presence already is described in adults affected by CD (24). In the present multicenter study, we observed a patchy villous atrophy in a small percentage of children with CD, demonstrating that, although infrequent, the possibility of patchy distribution of mucosal lesion in this pathology must be taken into consideration.

The diagnosis of CD in our patients without diffuse lesions is supported by the clinical signs, prevalence of symptoms, and presence of CD-related autoantibodies. In fact, we found 88.9% of them were EMA positive and all of them were anti-tTGAbs positive, underlining the higher sensitivity of these last autoantibodies (25,26). In addition, the normalization of antibody serum levels on a gluten-free diet and of the bulb mucosa in patients undergoing intestinal biopsy, including those with Marsh type 1 and type 2 lesions, confirm the diagnosis of CD.

The absence of lesions suggesting CD in the duodenal bulb in all of the gastroenterological control subjects emphasizes the importance of performing intestinal biopsy in this area. We can suggest a useful diagnostic flowchart for CD diagnosis and for CD screening in subjects who undergo endoscopy for other reasons. If we suspect the disease (from the clinical observation, the serology, and the patient belonging to at-risk groups), then we advise taking multiple biopsies, at least 2 from the bulb and 2 from the distal duodenum in each endoscopic procedure. If the bioptic samples show CD-compatible lesions, then the gluten-free diet should be initiated. In addition, to avoid missing diagnosis of a silent form of CD (27), when upper endoscopies are performed for indications different from CD, we suggest taking at least 1 bioptic sample of bulb. In these patients, if we found CD-related bulbar lesions, then EMA and/or anti-tTGAbs would be evaluated, and in positive cases, gluten-free diet initiated. In fact, it is well known that undetected CD may cause many harmful complications: those related to malabsorption, such as iron-deficient anemia (28) and osteopathy (29), and those associated with the autoimmune process underlying the disease, such as type 1 diabetes mellitus (30) and thyreopathy (31). In addition, infertility (32), low-birth-weight infants

(33), dilated cardiomyopathy (34), and other forms of heart failure (35) have been observed in patients with CD. Therefore, making an elusive diagnosis of celiac disease could compensate for the effort of performing an additional bulbar biopsy.

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