Bulb biopsies for the diagnosis of celiac disease in pediatric patients

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Background: Celiac disease (CD) is a gluten-dependent enteropathy. The current standard for diagnosing CD involves obtaining 4 biopsy samples from the descending duodenum. It has been suggested that duodenal bulb biopsies may also be useful.

Objective: To assess the utility of bulbar biopsies for the diagnosis of CD in pediatric patients.

Design: Prospective study.

Setting: Single center.

Patients: Forty-seven consecutively enrolled pediatric patients with celiac serologies and a clinical suspicion of CD.

Interventions: All patients underwent EGD, and 4 biopsy samples were obtained from the duodenal bulb and 4 from the descending duodenum of each child.

Main Outcome Measurements: The pathologist blindly reported the Marsh histological grade for the diagnosis of CD of the bulb and descending duodenum.

Results: The diagnosis of CD was histologically confirmed in 89.4% (42/47) of the cases of biopsy samples obtained from the descending duodenum and in all 47 obtained from the bulb. In 35 patients (74.5%), histology was the same in the bulb and duodenum; in 11 (23.4%) cases, the grade of atrophy was higher in the bulb than in the descending duodenum, and 5 (10.6%) had bulb histology positive for CD but negative duodenal findings. One child (2.1%) had a higher histological grade in the duodenum than in the bulb. The diagnostic gain with bulbar biopsies was 10.6%.

Limitations: Small sample and absence of a comparison group (asymptomatic children with normal CD antibodies).

Conclusions: We suggest examining 4 biopsy samples from the duodenal bulb and 4 from the descending duodenum to improve diagnostic accuracy of CD. (Gastrointest Endosc 2010;72:564-8.)

Celiac disease (CD) is a gluten-dependent enteropathy characterized by chronic small intestinal inflammation and villous atrophy. CD has many atypical manifestations, and endoscopic findings can include a mosaic pattern of the duodenal mucosa, reduction or loss of duodenal folds, and scalloping of the valvulae conniventes.[1] However, endoscopic signs alone are not considered sensitive or specific for the diagnosis of CD. Accordingly, guidelines published by the North American Society for Pediatric Gastroenterology Hepatology and Nutrition state that "confirmation of the diagnosis of CD requires an intestinal biopsy in all cases."[2] In particular, the current internationally accepted

Abbreviations: CD, celiac disease; HC, hypertrophic crypt; IEL, intraepithelial lymphocyte; M, Marsh histological grade.

DISCLOSURE: *All authors disclosed no financial relationships relevant to this publication.*

0016-5107/$36.00
doi:10.1016/j.gie.2010.05.021

Received September 15, 2009. Accepted May 14, 2010.

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standard for the diagnosis of CD is 4 biopsy samples from the 4 quadrants of the descending duodenum.[3]

Pais et al[4] recently published the results of a study in which they examined 247 patients to determine how many duodenal biopsy specimens were needed to diagnose CD. They concluded that only 2 specimens led to confirmation of CD in 90% of cases and that 4 descending duodenal biopsy specimens led to 100% confidence in the diagnosis.

Comparison of biopsy specimens from the second, third, and fourth parts of the duodenum, the ligament of Treitz, and the proximal jejunum has shown that each site is suitable for diagnosing CD.[5] Because mucosal specimens taken from the distal duodenal or jejunal mucosa are strongly correlated, biopsy samples from the second or third part of the duodenum are considered adequate to obtain material for histological interpretation.[6]

The question of added utility of obtaining bulbar biopsy specimens has been less studied. Two articles (an extensive article and a case report) discussed the utility of bulb biopsy specimens for diagnosing CD in adults in addition to the standard 4 from the descending duodenum. Other studies since then have indicated the utility of duodenal bulb biopsies for the diagnosis of CD.[7,8]

The diagnostic accuracy of endoscopy in children with clinically suspected CD is particularly important. Performing endoscopy in children involves an elaborate process of ensuring adequate and safe sedation and generally uses limited and expensive health care resources, including access to pediatric endoscopists and anesthesia support. Pediatric endoscopists have an obligation to make or refute the diagnosis of CD with certainty in their young patients.

The aim of this prospective study was to assess the utility of bulbar biopsies for confirming the diagnosis of CD in a series of pediatric patients with clinical and serological indicators of the disease.

Take-home Message

- In this series of patients, 23.4% had a higher histological atrophy grade in the bulb than the duodenum. A total of 10.6% of patients with celiac disease (CD) had negative descending duodenum histology with bulb biopsy specimens positive for CD. If no bulb biopsy specimens had been taken, children would not have had a correct diagnosis of CD.

MATERIALS AND METHODS

From May 1, 2008, to March 31, 2009, a total of 47 children (14 boys and 33 girls; age 8.1 ± 4.59 years) with suspected CD because of positive anti-endomysium IgA and/or antitissue transglutaminase antibodies IgA and IgG were prospectively and consecutively enrolled. Their main clinical symptoms were iron-deficiency anemia, diarrhea, abdominal distention, and short stature.

All patients underwent EGD (EG 1840-EG 2940; Pentax, Hamburg, Germany) during which 4 mucosal biopsy samples were obtained from the duodenal bulb and 4 from the descending duodenum. We chose to take the same number of biopsy samples from both the bulb and the descending duodenum to reduce the chances that absolute numbers of biopsy specimens from either location could explain a difference in diagnostic utility. All endoscopies were performed with the patient under deep sedation with Propofol (Propofol B. Braun 1%; Melsungen, Germany), and patients were not allowed any solid or liquid foods in the 8 hours before the procedure. All patients with positive celiac serologies were screened for diabetes mellitus.

Biopsy specimens were fixed in 10% formalin and stained with hematoxylin and eosin. A blinded pathologist reported the Marsh histological grade (M)[9] for the diagnosis of CD in the bulb and descending duodenum.

The study protocol was approved by the hospital's ethics committee, and the patients' legal representatives gave informed consent for the procedures and data collection for scientific purposes.

Descriptive statistics are used to present the results of this exploratory pilot study investigating the utility of bulbar biopsies for the diagnosis of CD. Given the nature of the study, a formal calculation of study power was not made.

RESULTS

Table 1 summarizes the patients' main characteristics. EGD and specimen collection were successful in all 47 children. No procedure- or sedation-related complications were encountered during the endoscopy or in the 24 hours after the procedure, and all patients restarted oral intake the same day as the examination.

The diagnosis of CD was histologically confirmed in all 47 patients positive for celiac serologies. Confirmation was obtained in 89.4% (42/47) of the cases with biopsy specimens from the descending duodenum and in 100% of the cases when the diagnosis was made from specimens taken from the duodenal bulb.

Histological patterns in the descending duodenum in patients with positive serology for CD

In 5 of the 47 cases, biopsy specimens from the descending duodenum were negative for CD (M 0). Among the 42 children in whom the diagnosis of CD was confirmed from descending duodenal biopsy samples, 2 showed only intraepithelial lymphocytes (IELs) (corresponding to M 1); in 2 others, the diagnosis was based on IELs plus hypertrophic crypts (HCs) (corresponding to M 2), whereas in the remaining 38 cases, the diagnosis was

TABLE 1. Serological features and symptoms of 47 children with suspected celiac disease

Features and symptoms	Patients, no. (%)
EmA IgA positive	47 (100)
tTG-Ab IgA positive	47 (100)
tTG-Ab IgG positive	47 (100)
Iron-deficiency anemia	36 (76.6)
Diarrhea	25 (53.2)
Abdominal distention	15 (31.9)
Short stature	9 (19.1)

EmA IgA, Anti-endomysium IgA; *tTG-Ab*, anti-tissue transglutaminase antibody.

TABLE 2. Histological patterns of the bulb and the descending duodenum

Histology (Marsh grade)	Bulbar histology (47 patients)	Descending duodenum histology (47 patients)
No diagnosis (M 0)	0 (0%)	5 (10.6%)
Intraepithelial lymphocytes (M 1)	3 (6.4%)	2 (4.3%)
Intraepithelial lymphocytes + hypertrophic crypts (M 2)	2 (4.3%)	2 (4.3%)
Intraepithelial lymphocytes + hypertrophic crypts + villous atrophy (M 3a,b,c)	42 (89.4%)	38 (80.8%)

M, Marsh histological grade.

based on IELs plus HCs and villous atrophy (M 3a,b,c) (Table 2).

Histological patterns in the duodenal bulb in patients with positive serology for CD

All the bulbar biopsy samples provided histological evidence of CD. In 3 cases, the diagnosis was based on the presence of IELs (M 1), in 2, it was based on IELs plus HCs (M 2), and in 42, it was based on IELs plus HCs and villous atrophy (M 3a,b,c) (Table 2).

Comparison of descending duodenum and duodenal bulb biopsy specimens in patients with positive serology for CD

Thirty-five patients (74.4%) had the same histology in the bulb and descending duodenum: 1 patient had M 1 (2.9%), 6 had M 3a (17.1%), 10 had M 3b (28.6%), and 18 had M 3c (51.4%). In 12 cases, however, the histological findings differed in the bulb and the distal duodenum: in 1 patient (2.2% of the total cohort), the grade of atrophy in the descending duodenum (M 3b) was higher than that in the bulb (M 3a), whereas in all the other 11 patients (23.4%), the opposite was true. In 5 of these 11 patients, bulb histology was positive for CD (2 with pattern M 1, 2 with pattern M 2, and 1 with pattern M 3b), whereas the duodenal biopsies were negative for CD (M 0). In the other 6 patients, biopsy samples from both duodenal sites showed atrophy, but the histological grade was higher for those taken from the duodenal bulb than for those from the descending duodenum (Table 3).

TABLE 3. Bulb and duodenal histology (different or same) in the 47 patients

Histology (Marsh grade)	Patients, no. (%)
Bulb positive/duodenal negative	5 (10.6)
Bulb > duodenal	6 (12.8)
Bulb = duodenal	35 (74.5)
Duodenal > bulb	1 (2.1)

The diagnostic gain with bulbar biopsies compared with descending duodenum biopsies alone was 10.6%.

DISCUSSION

In our series of 47 patients with clinical and serological indicators of CD, 23.4% had a higher grade of histological atrophy in the bulb than in the descending duodenum, and 10.6% did not show histological signs of CD on biopsy samples from the descending duodenum, although bulb biopsy samples were positive for CD. If no bulb biopsy samples had been taken from this latter group, it would not have been possible to make the diagnosis of CD correctly. Considering that mucosal specimens from the distal duodenal or jejunal mucosa are strongly correlated, that these biopsy specimens provide adequate material for histological interpretation,[4] and that studies on the usefulness of bulbar biopsies in the diagnosis of CD have been inconclusive, we decided to compare bulbar and duodenal histology in patients with suspected CD.

The higher grade of bulb atrophy in 23.4% of patients in our series might be explained by the fact that the duodenal bulb is particularly rich in lymphatic structures[10] and is the first portion to be reached by gluten.[8]

The limitations of this study are its small sample size, the absence of a comparison group (asymptomatic children with normal CD antibodies), and the lack of inclusion in the study of children with a family history of CD or other autoimmune disorders.

In 2001, Vogelsang et al,[8] after finding 2 cases of CD in which biopsy samples from the duodenal bulb were diag-

TABLE 4. Symptoms, hemoglobin concentration, and red blood cell count in patients with a Marsh 1 grade celiac disease diagnosis

Patient	March histology grade		Symptoms	Hb (g/dL)	RBC count ($\times 10^9$/L)
	Bulb	Descending duodenum			
1	1	1	Occasional diarrhea	12.3	4.36
2	1	0	Bloating	11.8	4.38
3*	1	0	No	15.4	5.25

Hb, Hemoglobin; *RBC,* red blood cell.
*Patient 3 was tested for serological CD antibodies because of a family history of CD.

nostic, retrospectively analyzed biopsy samples from the descending duodenum and duodenal bulb of 51 patients with suspected or diagnosed CD. The number of IELs was, on average, higher in the descending part of the duodenum, although the difference was not statistically significant, and the conclusion was that most patients with CD show similar mucosal changes in biopsy samples from the descending duodenum and from the bulb.

Traditionally, biopsy samples from the duodenal bulb have not been recommended on the assumption that histological findings in specimens from this area may be difficult to interpret. Compared with the distal duodenum, the bulb has more Brunner's glands and lymphoid tissue and may show gastric metaplasia.[11] The villi in the bulb may also be shorter and broader,[12,13] and some authors maintain that villi in the bulb may be blunted or even absent over Brunner's glands.[14,15] Furthermore, duodenitis from other causes can interfere with the interpretation of villous atrophy in this region.

In fact, the duodenal bulb is still not considered a useful site for target biopsies for the diagnosis of CD, even though this site has rarely been reported to be the only one showing reliable histological changes in adults and children with CD.[16] It is also already known that normal subjects have normal histology of the bulb and descending duodenum.[17]

Brocchi et al[7] presented a case in which the diagnosis of CD was based only on biopsy samples from the duodenal bulb, and Bonamico et al[16] described 5 children with descending duodenum biopsy samples negative for CD in whom the first diagnosis of the disease was possible only after subsequent bulbar biopsies.

Bonamico et al[18] also conducted a large population study on 665 children, randomized into 2 groups on the basis of the suspicion of CD because of positive antibodies. Of these 665 children, 16 (2.4%) had positive CD antibodies and histological lesions in the bulb compatible with CD, but a histologically normal mucosal pattern in the descending duodenum, with normal villi, normal CD3 lymphocyte count, and no HCs. We found a much higher frequency of patients with bulb-positive but descending duodenum-negative biopsy samples (10.6%). Considering the patchy histological distribution of CD, this difference could perhaps be explained by the higher number of biopsy samples taken from our patients. However, while this paper was in preparation, Weir et al[19] published the results of their study, reporting on 101 children from whom biopsy samples were taken from both the duodenal bulb and the second portion of the duodenum; in 10 cases (9.9%), only the duodenal bulb biopsy samples were diagnostic of CD. This is remarkably similar to our findings.

It is possible that the majority of cases with negative duodenal biopsy samples and positive bulbar ones have a low Marsh grade. This could explain why the symptoms in this subgroup of patients were mild (Table 4) compared with those of patients with marked villous atrophy. However, in a recent study by Prasad et al[20] of 52 children from whom bulbar and descending duodenum biopsy samples were taken, no significant differences were found between the histology in the 2 sites, leading to the conclusion that the diagnosis of CD can be made even if biopsy samples are taken from the duodenal bulb rather than the distal duodenum or jejunum.

Despite reports in which the diagnosis of CD was obtained with the aid of bulb biopsies,[6,7,21] Ravelli et al,[22] in 110 untreated CD patients, found no cases in which biopsy samples from the descending duodenum were negative for CD, but bulb biopsy samples were positive.

The importance of making or refuting a diagnosis of CD cannot be overstated. Although an early correct diagnosis of CD in pediatric patients is translated into a gain of weight; the disappearance of CD-related symptoms; reestablishment of a normal hemoglobin concentration, mean cell volume, and red blood cell count; and prevention of potentially fatal complications such as lymphoma and jejunoileal ulcerative disease, the diagnosis currently involves committing a child to a lifetime of a gluten-free diet, which has been associated with a negative impact on the quality of life, and the diagnosis must not, therefore, be applied without the histological certainty that the child has the disease. Conversely, if the diagnosis is missed during endoscopy, the child risks continuing to have symptoms, possibly requiring a repeat endoscopy in the future.

In conclusion, although further studies are needed to confirm our results in patients with positive CD antibodies, we suggest taking biopsy samples from both the duodenal

bulb and the descending duodenum to maximize the diagnostic yield and make the diagnosis of CD more certain. In our study, 4 biopsy samples from each site enabled the diagnosis of CD to be confirmed in 100% of the cases.

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