Prospective study of the role of duodenal bulb biopsies in the diagnosis of celiac disease

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Background: Studies have demonstrated that villous atrophy in celiac disease is patchy and have suggested that duodenal bulb biopsies aid in diagnosis.

Objective: To determine the role of the addition of duodenal bulb biopsies to distal duodenum (D2) biopsies in the diagnosis of celiac disease.

Design: Prospective, case-control study.

Setting: Tertiary referral hospital.

Patients: Patients undergoing upper endoscopy with biopsy for diagnosis or follow-up of celiac disease and control patients.

Interventions: Blinded review of duodenal biopsy samples.

Main Outcome Measurements: Increasing the yield as well as accuracy of the histologic diagnosis of celiac disease with the addition of bulb biopsies.

Results: Of 128 patients enrolled in the study, 67 had celiac disease. Of 1079 biopsy specimens, only 319 (30%) were adequate for complete histologic analysis, resulting in 40 celiac patients and 40 control patients for analysis. Of the 40 celiac patients, 35 (87.5%) had atrophy in either the bulb or D2, 30 (75%) exhibited atrophy at both sites with an identical grade of atrophy seen in 18 patients (45%). Fourteen patients (35%) had identical types of Marsh lesions in both biopsy sites. Twelve patients (30%) had atrophy detected in the bulb, D2, or both, but lacked intraepithelial lymphocytes and thus could not be assigned a Marsh grade. Five patients (13%) had a diagnosis of celiac disease based on findings in the bulb biopsy only.

Limitations: Small sample size and study performed in an academic medical center.

Conclusions: Our study confirms the patchy nature of villous atrophy as well as intraepithelial lymphocytosis in biopsy specimens from individuals with celiac disease. Adding duodenal bulb biopsies to our sampling regimen increased the diagnostic yield of celiac disease. (Gastrointest Endosc 2010;72:758-65.)

The diagnosis of celiac disease requires the presence of characteristic histologic alterations in biopsy specimens taken from the descending duodenum, which are classified according to Marsh (or modified Marsh) criteria.[1-4]

Traditionally, gastroenterologists have avoided biopsies of the duodenal bulb because of potential confounding histopathologic alterations caused by acid-induced damage, gastric metaplasia, Brunner gland hyperplasia, or the

Abbreviations: D2, second part of the duodenum/distal duodenum; IEL, intraepithelial lymphocyte.

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presence of lymphoid follicles.[5] However, several recent studies demonstrated the patchy nature of villous atrophy, with changes restricted to the duodenal bulb in some patients.[6-11] A limitation of these studies has been the lack of adequate control groups and a lack of direct comparison of the histologic findings in both the descending duodenum and duodenal bulb.

In our study, we assessed whether the addition of duodenal bulb biopsies to distal duodenum biopsies would increase the diagnostic yield of celiac disease. In addition, we also assessed the prevalence of celiac disease–associated histologic alterations of the duodenal bulb in a group of control patients with other GI symptoms.

Take-home Message

- The presence of villous atrophy and intraepithelial lymphocytes is very patchy in celiac disease. Performing duodenal bulb biopsies in patients undergoing evaluation for celiac disease will increase the diagnostic yield.

MATERIALS AND METHODS

Patients and sampling

From July 2008 until March 2009, we prospectively evaluated patients undergoing EGD for the diagnosis or follow-up of celiac disease and control subjects. Three endoscopists performed the procedures. All patients had biopsies of the second part of the duodenum (D2) (at least 4 biopsy specimens) and the bulb (at least 2 biopsy specimens) with standard needle biopsy forceps. There were no attempts to orient the biopsy samples that were placed in formalin by the endoscopy assistant. The study was approved by the Institutional Review Board of Columbia University.

Histologic assessment

Two GI pathologists who were blinded to the indication for endoscopy and patient identifiers evaluated the biopsy specimens. The orientation of the biopsy specimens was ascertained to evaluate the presence and degree of villous atrophy and crypt hyperplasia and determine the villous-to-crypt ratio. Specimens were considered adequate for assessment if they had at least 1 well-oriented biopsy sample with 3 consecutive, well-aligned villous-crypt units. Villous-to-crypt ratios were assessed to determine the grade of mucosal atrophy, none (≥4:1), partial (2:1 or 3:1), subtotal (1:1), and total (<1:1) as well as the Marsh grade. An increase in the number of intraepithelial lymphocytes (IELs) was graded in a semiquantitative manner: mild (1 IEL per 3-5 epithelial cells), moderate (1 per 2 epithelial cells), or marked (≥1 per epithelial cell). The distribution of IELs was further classified as patchy (involving <50% of the villi in a biopsy specimen with interspersed areas demonstrating normal numbers of IELs) or diffuse (>50% of all villi showing a homogeneous increase in IELs). Assignment of a Marsh grade required the presence of an increase in IELs with or without villous atrophy and/or crypt hyperplasia (Table 1). The extent of lamina propria inflammation was also graded and neutrophils, if present, were noted. Gastric surface metaplasia was classified as focal, multifocal, or extensive, and the percentage of total biopsy epithelium exhibiting gastric metaplasia was quantified as less than 5%, 5% to 25%, 25% to 50%, or more than 50%. Subepithelial collagen was evaluated to determine any increase, and the presence and location of Brunner's glands (mucosal and/or submucosal) were determined (a note was made if the biopsy was superficial and the submucosa could not be evaluated). The histologic criteria for diagnosis of celiac disease used for this study required the presence of increased IELs with or without villous atrophy and/or crypt hyperplasia in at least 1 site (bulb or duodenum), without any other obvious cause.

Statistical analysis

Categorical variables were expressed as percentages. Continuous variables were expressed as means. The χ^2 test was used to compare categorical variables, and differences were considered significant if $P < .05$. Sensitivities were reported with 95% confidence intervals.

RESULTS

Among the 128 patients enrolled in the study (Table 2), 70% were female; 67 had celiac disease (22 newly diagnosed and 45 were undergoing follow-up endoscopy), and 61 were control patients (indications for EGD, Table 2).

There were 128 matched-pair biopsy samples (D2 and bulb) with a total of 1079 biopsy samples. The median number of biopsy samples obtained was greater for D2 than for the bulb (Table 3). Only 319 (30%) of the biopsy samples were considered adequate for analysis of the villous-to-crypt ratio. After exclusion of patients with inadequate biopsy specimens, 40 celiac and 40 control patients with adequate paired biopsy samples remained (Table 3, Fig. 1).

Of the 40 celiac patients, 35 had atrophy in either the bulb or D2 (Fig. 2), and 30 had atrophy at both sites. An identical grade of atrophy in the bulb and D2 was seen in 18 patients (45%) (3 had total, 3 had subtotal, and 12 had partial atrophy at both sites). Five patients (12.5%) had no atrophy (all follow-up biopsy samples from patients on a gluten-free diet), and 17 (42%) had differing grades of atrophy between the bulb and D2.

When we compared the Marsh grades of biopsy samples from the 2 locations, 14 patients (35%) had identical grades (3a, 3b, 3c, or 2) in all biopsy sites (Fig. 3). Three

TABLE 1. Modified Marsh classification scheme

	Type					
	0	1	2	3a	3b	3c
IELs*	<40	>40	>40	>40	>40	>40
Crypts	Normal	Normal	Hypertrophic	Hypertrophic	Hypertrophic	Hypertrophic
Villi	Normal	Normal	Normal	Mild atrophy	Marked atrophy	Absent

IELs, Intraepithelial lymphocytes.
*Values are intraepithelial lymphocytes/100 epithelial cells.

TABLE 2. Study population and indications for endoscopy*

	Celiac	Control
Male, no. (%)	18 (14)	20 (16)
Female, no. (%)	49 (38)	41 (32)
Age, y, mean (range)	45 (19-82)	44 (16-87)
Follow-up of celiac disease	45	0
Diarrhea	19	21
Positive celiac serology	18	0
Abdominal pain	18	28
Exclusion of celiac disease	15	25
Anemia	12	9
Family history of celiac disease	7	0
Symptoms of refractory celiac disease	6	0
Heartburn/GERD	10	21
Dysphagia	2	0
Abnormal CT of GI tract	2	2
Heme-positive stool	2	0
Dyspepsia	0	2
Follow-up of Barrett's esophagus	0	4

*All patients had more than 1 indication for EGD. In the celiac group, 2 patients had Enteropathy-associated T-cell lymphoma (EATL), 2 had dermatitis herpetiformis, and 2 had a B-cell lymphoma. In the control group, 2 patients had human immunodeficiency virus.

patients had no ascribable Marsh grade (normal appearance) and were among the patients who had no atrophy present. Therefore, 17 patients (42.5%) had both equal atrophy and Marsh histology in both the bulb and D2. In addition, there were 12 patients (30%) who had atrophy present in either the bulb, D2, or both, but could not be assigned a Marsh grade because of a lack of increase in IELs (Table 4). Of these 12 patients, 3 had a recent diagnosis of celiac disease, and 9 had previously received the diagnosis and were having follow-up endoscopy. Of the 3 patients with a new diagnosis, the diagnosis in 2 patients was based on positive pathology findings only on bulb biopsy samples. There were 11 patients with discordant findings between the bulb and D2 (Table 5). Eight of the 17 patients did not have differences in Marsh grades (ie, 3a to 3b) that would have changed the diagnosis. However, 3 patients received a diagnosis of celiac disease based on the abnormal architecture with villous atrophy and IELs in the bulb only. Therefore, a total of 5 patients (13%) received a diagnosis of celiac disease based on findings only from the bulb biopsy samples.

On analyzing the presence of increased IELs, we noted that in the group of patients with celiac disease, 50% had an equal increase in IELs in the bulb and D2. Nine patients (22.5%) had no increase in IELs in the bulb and D2. Of the remaining 11, 4 had increased IELs only in D2 and 7 had increased IELs only in the bulb. Gastric metaplasia was identified more frequently in the bulb, whereas the distribution of Brunner's glands was similar in the bulb and D2 (Table 6). Only 1 patient had slightly increased subepithelial collagen in both the bulb and D2 (insufficient for a diagnosis of collagenous sprue).

In the control group, 80% of patients had concordant histopathologic findings in the bulb and D2, 29 had no villous atrophy (Marsh 0), and 3 had partial atrophy. Biopsy samples from 8 (20%) patients showed differences in the grade of atrophy between the bulb and duodenum, with all 8 having partial atrophy in the bulb and none in D2. Of these 8 patients with differences in atrophy grade, 7 could not be ascribed a Marsh grade because of a lack of increase in IELs, and 1 patient had Marsh 3a grade in the bulb and Marsh 1 grade in D2.

Eight (20%) of the control patients had increased IELs, with 7 of these patients demonstrating a mild increase in both the bulb and D2, and 1 patient with a slight increase in the bulb, but not in D2. Of the 8 patients with increased IELs, 2 also had villous atrophy. One patient had *Helicobacter pylori* gastritis and had also recently received a diagnosis of acquired immunodeficiency syndrome and profuse diarrhea (Marsh grade 3a lesions in the bulb and D2). Diagnostic workup for an etiologic factor could not

TABLE 3. Analysis of biopsy number and adequacy for assessment of villous atrophy

		D2		Bulb	
	Total	Celiac	Control	Celiac	Control
No. of biopsy samples	1079	388	347	183	161
Median no. of biopsy samples	4	6	5	3	2
Adequate samples	319 (30%)	120 (31%)	104 (30%)	47 (26%)	48 (30%)
Patients with no adequate samples	48	4	5	24	18

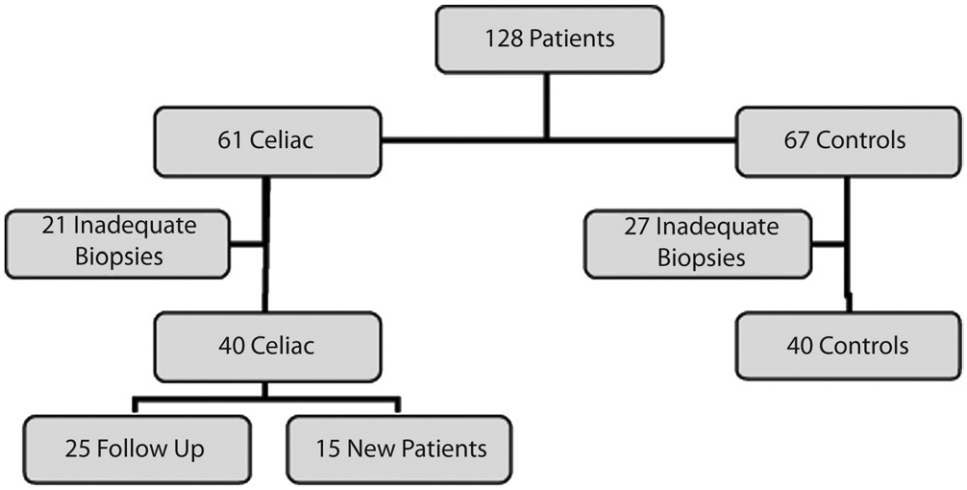

Figure 1. Flow diagram of study.

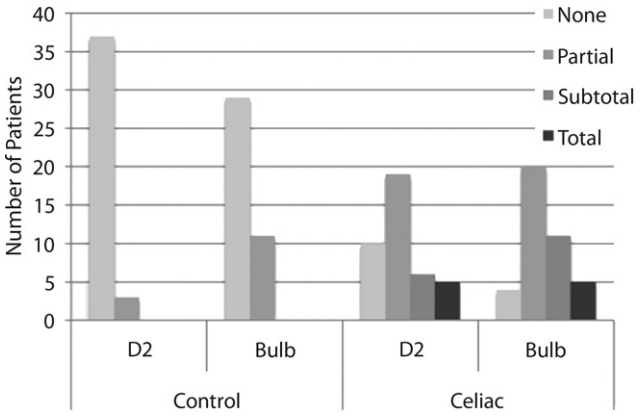

Figure 2. Frequency distribution of grade atrophy in bulb and D2 in celiac patients and controls.

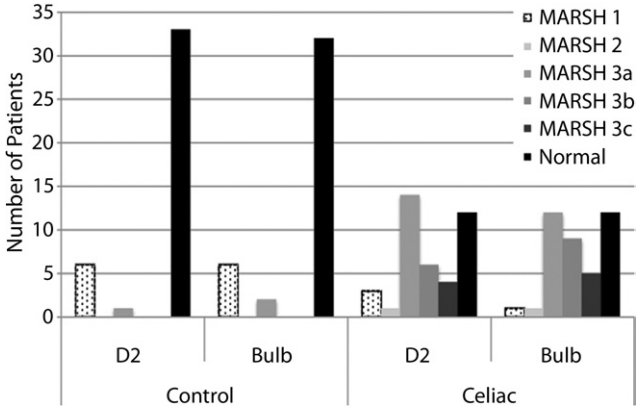

Figure 3. Frequency distribution of MARSH lesions in bulb and D2 in celiac patients and controls.

identify an infectious cause of diarrhea, which eventually resolved after the initiation of antiretroviral therapy and restoration of the CD4 count to more than 200. The other patient had GERD (Marsh grade 3a in the bulb and Marsh grade 1 in D2).

Differences in the presence of gastric metaplasia and Brunner's glands are shown in Table 6. None of the control patients had any increase in subepithelial collagen.

Among the 48 patients from whom biopsy samples were obtained, either of the bulb or D2 and were inadequate for analysis of villous atrophy, 11 had increased IELs to varying degrees (14 mild, 6 moderate or marked) in either the bulb (50% mild, 25% moderate or marked) or D2 (70% mild, 25% moderate or marked), whereas 9 patients had concordant degrees of IEL increases in the bulb and D2. All 20 of these patients had celiac disease. Furthermore, 13 patients had at least 1 adequately oriented biopsy sample in either the bulb

TABLE 4. Celiac patients with no ascribable Marsh score because of a lack of IELs

			Adequate pieces		V:C ratio		IELs		Atrophy		Marsh	
Patient	New or FU	Serology	Bulb	D2	Bulb	D2	Bulb	D2	Bulb	D2	Bulb	D2
1	FU	Neg	1	2	2:1	3:1	Not increased	Mild patchy	Partial	Partial	N/A	3a
2	FU	Pos	1	2	2:1	2:1	Not increased	Mild, patchy	Partial	Partial	N/A	3a
3	New	Pos	1	3	1:1	1:1	Not increased	Mild patchy	Subtotal	Subtotal	N/A	3b
4	FU	Neg	1	3	3:1	2:1	Not increased	Not increased	Partial	Partial	N/A	N/A
5	FU	Neg	1	3	3:1	3:1	Not increased	Not increased	Partial	Partial	N/A	N/A
6	FU	Neg	1	2	3:1	4:1	Not increased	Not increased	Partial	None	N/A	N/A
7	FU	Neg	1	2	1:1	2:1	Not increased	Not increased	Subtotal	Partial	N/A	N/A
8	FU	Pos	1	1	4:1	4:1	Mild, diffuse	Not increased	None	None	1	N/A
9	FU	Pos	1	3	2:1	<1:1	Not increased	Not increased	Partial	Total	N/A	N/A
10	FU	Neg	1	1	3:1	4:1	Not increased	Not increased	Partial	None	N/A	N/A
11*	New	Neg	1	3	1:1	3:1	Mild, diffuse	Not increased	Subtotal	Partial	3b	N/A
12*	New	Pos	1	4	3:1	4:1	Moderate diffuse	Not increased	Subtotal	None	3b	N/A

D2, Second part of the duodenum/distal duodenum; *FU*, follow-up; *IELs*, intraepithelial lymphocytes; *N/A*, not available; *Neg*, negative; *Pos*, positive; *V:C*, villous: crypt ratio.
*Patients with a diagnosis of celiac disease only by findings in duodenal bulb biopsies.

TABLE 5. Celiac patients with adequate biopsy samples with discordant findings

			Adequate samples		V:C ratio		IELs		Atrophy		Marsh	
Patient	New or FU	Serology	Bulb	D2	Bulb	D2	Bulb	D2	Bulb	D2	Bulb	D2
1	New	Pos	1	1	<1:1	2:1	Mod, patchy	Mod, diffuse	Total	Partial	3c	3a
2	FU	Neg	1	1	1:1	3:1	Mild, diffuse	Mild, diffuse	Subtotal	Partial	3b	3a
3	New	Pos	1	2	2:1	1:1	Mild, diffuse	Mod, diffuse	Partial	Subtotal	3a	3b
4	New	Pos	1	1	3:1	1:1	Mild, patchy	Mild, diffuse	Partial	Subtotal	3a	3b
5	FU	Pos	1	2	1:1	3:1	Mild, diffuse	Mild, diffuse	Subtotal	Partial	3b	3a
6	New	Pos	1	1	1:1	<1:1	Mild, diffuse	Mild, diffuse	Subtotal	Total	3b	3c
7	FU	Pos	1	1	1:1	3:1	Marked, diffuse	Mild, diffuse	Subtotal	Partial	3b	3a
8	New	Pos	1	2	<1:1	1:1	Mod, diffuse	Mod, diffuse	Total	Subtotal	3c	3b
9*	FU	Neg	1	2	3:1	4:1	Mild, patchy	Mild, patchy	Partial	None	3a	1
10*	New	Neg	1	2	1:1	4:1	Mod, diffuse	Mild, patchy	Subtotal	None	3b	1
11*	New	Pos	1	3	2:1	4:1	Mod, diffuse	Mild, diffuse	Partial	None	3a	1

D2, Second part of the duodenum/distal duodenum; *FU*, follow-up; *IELs*, intraepithelial lymphocytes; *Mod*, moderate; *Neg*, negative; *Pos*, positive; *V:C*, villous: crypt ratio.
*Patients diagnosed with celiac disease only by findings in duodenal bulb biopsies.

or D2 demonstrating villous atrophy (Marsh grade 3a or 3b), all of whom had celiac disease.

To assess whether the presence of gastric metaplasia affected the assessment of other histologic alterations, we found that among the celiac and control patients, there was no significant association between the presence of gastric metaplasia and Marsh score for the bulb ($P = .311$) or D2 biopsies ($P = .13$).

TABLE 6. Histologic features of celiac and control biopsies

	IELS			
	Not increased	Mild	Moderate	Marked
Celiac				
Bulb	12	20	5	3
D2	12	23	4	1
Control				
Bulb	32	8	0	0
D2	33	7	0	0

	Gastric metaplasia						
	None	Focal <5%	Focal 5%-25%	Multifocal <5%	Multifocal 5%-25%	Multifocal 25%-50%	Extensive >50%
Celiac							
Bulb	19	8	0	6	6	1	
D2	31	5	0	4			
Control							
Bulb	24	5	1	1	5	2	2
D2	37	2	0	0	1	0	0

	Brunner's glands			
	None	Mucosal/submucosal	Mucosal only	Submucosal only
Celiac				
Bulb	5	30	3	2
D2	4	30	4	2
Control				
Bulb	0	37	3	0
D2	9	23	4	4

D2, Second part of the duodenum/distal duodenum; *IELs*, intraepithelial lymphocytes.

Overall, biopsy samples from D2 alone had a sensitivity of 60% (95% CI, 43.3%-74.7%) and a specificity of 97.5% (95% CI, 85.2%-99.8%), while biopsy samples from the bulb alone had a sensitivity of 65% (95% CI, 48.3%-78.9%) and a specificity of 95% (95% CI, 81.8%-99.1%) for diagnosing celiac disease. Combining D2 and bulb samples, the sensitivity and specificity for diagnosing celiac disease was 72.5% (95% CI, 55.8%-84.8%) and 95% (95% CI, 81.7%-99.1%), respectively.

DISCUSSION

We observed an increased rate of diagnosing celiac disease (13%) by including duodenal bulb biopsies in our prospective study. In addition, we found significant histologic variability among biopsy samples of the descending duodenum and duodenal bulb. Among patients with celiac disease, only 48% of the individuals with adequate biopsy samples had identical degrees of atrophy between the bulb and D2. Moreover, 20% of the control patients had villous atrophy in either D2 or the bulb biopsy samples, and 20% had increased IELS. To our knowledge, this is the first study to compare duodenal bulb biopsy samples in celiac and control populations.

The current criterion standard for diagnosing celiac disease is the presence of mucosal alterations, ie, increased IELs and crypt hyperplasia with or without mucosal atrophy and lamina propria inflammation in distal duodenal biopsy samples along with supportive patient symptoms and serologic studies.[3] The diagnosis of celiac disease also requires demonstration of an improvement after gluten withdrawal. The pathologic criterion standard for diagnosing celiac disease remains imperfect because it is clear that patients can have celiac disease in the absence

of crypt hyperplasia and villous atrophy, characteristic serologic findings can be absent at diagnosis, and other diseases can manifest the histologic features of celiac disease. Controversy also exists over the location of biopsy samples and the number of biopsies required to diagnose celiac disease. Earlier studies demonstrated no difference in the quality of biopsy samples obtained from the jejunum versus duodenum, and no differences based on forceps size.[12,13] Vogelsang et al[7] described 2 adult patients in whom the diagnosis of celiac disease could only be established by taking biopsy samples from the duodenal bulb. These patients had normal findings on the biopsy samples of the distal duodenum. Studies in both children and adults with increased endomysial antibody or tissue transglutaminase antibody titers have also confirmed the patchy nature of villous atrophy and the value of duodenal bulb biopsies to increase the diagnostic yield of celiac disease.[8-14] Multiple duodenal biopsy samples, including those taken from the duodenal bulb, were recently recommended by a group of investigators who found that celiac disease in children is not only patchy throughout the duodenum, but there can also be significant variability in the severity of the disease in a single biopsy sample.[15] In our study, fewer biopsy samples were taken from the bulb than from the descending duodenum, and this led to a high rate of inadequate diagnostic interpretations of the bulb biopsy samples. Future studies should investigate the incremental yield of taking 4 to 5 biopsy samples from the bulb to maximize the chance of adequate orientation and avoid areas of peptic injury. A recent retrospective study of nonoriented biopsy specimens found variability among 25% of the duodenal biopsy specimens and concluded that 4 biopsy specimens were needed to confirm the diagnosis of celiac disease with 100% confidence.[16] None of these studies had control populations with which compare the frequency of abnormal duodenal bulb biopsy findings.

Our findings also confirm the significant histologic variability among the different biopsy sites. Of the celiac population, only 48% of the patients with adequate biopsy samples had identical degrees of atrophy between the bulb and D2. In addition, 5 patients (13%) had celiac disease associated histologic alterations only in biopsy specimen from the duodenal bulb. Of these 5 patients, 4 were patients with a new diagnosis of celiac disease and 1 was a patient undergoing follow-up examination for known celiac disease.

In our control group, only 2 patients (5% of those with adequate biopsy samples) were incorrectly diagnosed based on histologic findings alone as having celiac disease. One patient had acquired immunodeficiency syndrome and *H pylori* gastritis (a known cause of increased IELs in the duodenum).[17] The second patient was undergoing endoscopy for reflux disease and had negative celiac serologies. Such patients have also been described in recent studies analyzing disorders associated with increased IELs and normal villous architecture.[18]

Gastric metaplasia of varying degrees was noted in 25% of the bulb or D2 region biopsies, suggestive of peptic injury. Gastric metaplasia resulted in a lower estimation of intraepithelial lymphocytosis. However, in the vast majority of patients, the presence of gastric metaplasia (or Brunner's glands) did not interfere with the assessment of villous atrophy grade.

Perhaps the most striking finding of our study was the large number of inadequate biopsy specimens. Using strict criteria, we found that only 30% of biopsy specimens were considered adequate for diagnosis. This is despite the fact that we had 4 or more biopsy samples (median) taken from D2 in both celiac patients and controls and 2 or 3 biopsy samples from the bulb of controls and celiac patients, respectively (Table 3). Our specimens were not oriented before fixation, which is a time-consuming additional step not commonly practiced in most endoscopy units in North America and is reflective of everyday practice.[16]

Limitations of our study included the relatively small sample size of adequately oriented biopsy pieces, the academic medical setting that may have biased our patient selection and inclusion of patients already on a gluten-free diet. Furthermore, human leukocyte antigen and serology results were not available for all patients.

In summary, our study confirms the patchy nature not only of villous atrophy, but also IELs in biopsy samples from individuals with celiac disease. The current criterion standard for diagnosing celiac disease requires histologic evidence of characteristic mucosal abnormalities in distal duodenal biopsy samples along with supportive patient symptoms and serologic studies. However, by adding duodenal bulb biopsies, we were able to increase the diagnostic yield of celiac disease. We thus recommend that all patients being evaluated for celiac disease have biopsy samples obtained from the duodenal bulb in addition to D2. Biopsy of normal-appearing D2 has been advocated for routine endoscopy.[19] Adding bulb biopsies to this biopsy routine would decrease the number of misclassified or nondiagnosed cases. The low yield of adequately oriented biopsy samples for assessment of villous atrophy argues for increasing the number of biopsy samples taken and also readdresses the issue of orientation of small-bowel biopsies.

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