Role of Confocal Endomicroscopy in the Diagnosis of Celiac Disease

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ABSTRACT

Background and Aims: Confocal laser endomicroscopy (CLE) is a recent development that enables surface and subsurface imaging of living cells in vivo at 1000× magnification. The aims of the present study were to define confocal features of celiac disease (CD) and to evaluate the usefulness of the CLE in the diagnosis of CD in children in comparison to histology.

Patients and Methods: Nine patients (8 girls) with a median age of 8.35 years (range 2–12.66 years) and a median weight of 28.3 kg (range 11–71 kg) were suspected with CD and 10 matched controls underwent oesophagogastroduodenoscopy using the confocal laser endomicroscope (EC3870CILK; Pentax, Tokyo, Japan). Histologic sections were compared with the confocal images of the same site by 2 experienced paediatric histopathologists and endoscopists, all of whom were blinded to the diagnosis.

Results: The median procedure time was 17 minutes (range 8–25 minutes). Confocal features of CD were defined and a score was developed. A total of 1384 confocal images were collected from 9 patients and 10 controls. Five images from each patient and control were selected and compared with the biopsy specimen of the same site. The sensitivity, specificity, and positive predictive value for the confocal images in comparison to the histology were 100%, 80%, and 81%. The kappa inter-observer agreement between the 2 endoscopists was 0.769 ($P = 0.018$) and between the 2 histopathologists was 0.571 ($P = 0.05$).

Conclusions: Confocal endomicroscopy offers the prospect of diagnosis of CD during ongoing endoscopy. It also enables targeting biopsies to abnormal mucosa and thereby increasing the diagnostic yield, especially when villous atrophy is patchy in the duodenum.

Key Words: celiac disease, endomicroscopy, gastrointestinal, histology, paediatric

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Celiac disease (CD) is an immune-mediated disorder that occurs following ingestion of gluten present in wheat, barley, and rye, in genetically susceptible individuals and characterised by small intestinal mucosal damage. The prevalence of CD is estimated to be 1 in 100 (1–3). Serological markers for the diagnosis of CD include raised levels of immunoglobulin A (IgA) anti-endomysial antibodies and tissue transglutaminase antibodies. When combined, they give positive and negative predictive values above 95% (4). However, the revised European Society of Paediatric Gastroenterology, Hepatology, and Nutrition criteria for the diagnosis of CD stipulates the presence of villous atrophy, crypt hyperplasia with increased intraepithelial lymphocytes on a small intestinal mucosal biopsy, and an unequivocal clinical response to a gluten-free diet (5). Thus, duodenal biopsy and histological assessment at present is mandatory to make a diagnosis of CD.

Confocal laser endomicroscopy permits in vivo assessment of gastrointestinal (GI) mucosal structure at cellular levels; in addition, this technique avoids crush artefact from the grasp biopsy forceps and artefactual changes from histopathological processing.

We hypothesised that confocal endomicroscopy may be a valid tool for in vivo diagnosis of CD. The aims were, first, to define the confocal features of CD and, second, to compare endomicroscopic and histologic findings of normal and CD in a blinded manner.

PATIENTS AND METHODS

Patients

Nine patients (8 girls) with a suspected diagnosis of CD based on raised anti-endomysial and tissue transglutaminase antibodies (except for 1 patient with X-linked agammaglobulinaemia and a low IgA level) and suggestive symptoms, with a median age of 8.35 years (range 2–12.66 years), and a median weight of 28.3 kg (range 11–71 kg), were prospectively enrolled for the study. Three patients presented with abdominal pain, 1 with diarrhoea, 1 with failure to thrive, and 1 with weight loss. One patient had type 1 diabetes mellitus and 3 patients had a family history of CD. Ten patients (6 girls) with a median age of 7.59 years (range 1.8–13.8 years) and with a median weight of 29.2 kg (range 12.6–63 kg) needing upper GI endoscopy for various GI conditions, such as presumed gastroesophageal reflux, nonspecific upper abdominal pain, suspected peptic ulcer disease, and inflammatory bowel disease, were enrolled as controls.

Written informed consent was obtained from parents, and where age and competency appropriate, from each patient and control, before the examination. The study protocols were reviewed and approved by the South Sheffield Regional Ethics Committee. Patient exclusion criteria were as follows: inability to give signed informed consent; age older than 18 years; and previous documented adverse reaction or allergy to fluorescein sodium or acriflavine hydrochloride.

All of the patients were admitted on the day of the procedure. All procedures occurred under general anaesthetic condition, as is the normal practice in our institution for paediatric GI endoscopy.

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Confocal Endomicroscope

The Pentax EC3870CILK endoscope (Fig. 1) has a 5-mm diameter miniaturised confocal microscope integrated into the distal tip of the endoscope. The diameter of the distal tip and insertion tube of the endoscope is 12.8 mm. In addition to the integrated confocal microscope, the distal tip also contains a colour charge-coupled device camera that enables simultaneous confocal microscopy with standard video endoscopy, air- and water-jet nozzles, 2 light guides, a 2.8-mm working channel, and an auxiliary water-jet channel. During CLE, the laser delivers an excitation wavelength of 488 nm at a maximum laser output of 1 mW to the tissue (typically, 300–700 μW). Confocal images can then be collected either at 1024 × 1024 pixels (0.8 frames/second) or at 1024 × 512 pixels (1.6 frames/second). The optical sections have a 475 μm × 475 μm field of view, with a lateral resolution of 0.7 μm, an axial resolution of 7.0 μm, and imaging depth (z axis) range of 0 to 250 μm below the tissue surface in 4-μm steps. The imaging depth below the tissue surface can be dynamically controlled by the operator. CLE magnifies images 1000-fold.

Endoscopic Procedure

Confocal laser endomicroscopy was performed by a single experienced endoscopist (M.T.), who had completed the Mainz CLE training programme before patient recruitment, using the confocal laser endomicroscope (EC3870CILK). Following duodenal

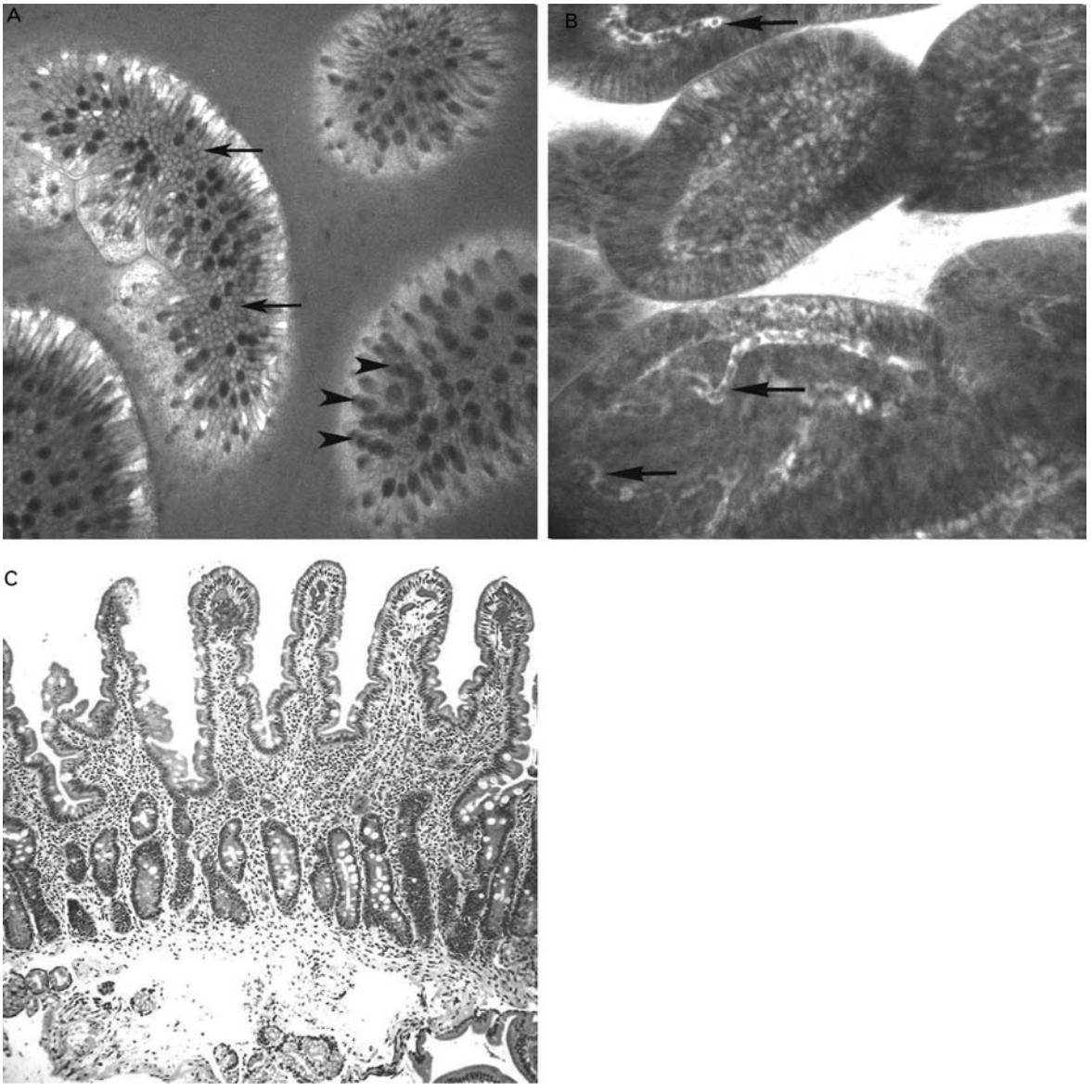

FIGURE 1. Normal duodenum. A, Confocal image of a normal duodenum showing long, slender villi with honeycomb appearance of the duodenal enterocytes (arrows) and numerous goblet cells (arrowheads). B, Confocal image of a normal duodenum at a deeper plane showing microvasculature in the lamina propria (arrows). C, Histology of a normal duodenum.

intubation, 0.05 to 0.1 mL/kg of 10% fluorescein sodium was administered intravenously and flushed adequately with normal saline. Acriflavine hydrochloride (0.05%) was applied directly to the duodenal mucosa using a spray catheter. Confocal laser endomicroscopic image acquisition was performed by placing the tip of the colonoscope in direct contact with the target tissue site. Using gentle suction to stabilize the mucosa, image acquisition and focal plane z-axis scanning depth was then actuated using 2 discrete handpiece control buttons. Confocal images were then obtained from the third part of the duodenum sequentially at different planes from the surface to the maximum permissible depth.

Same-site mucosal specimens were obtained using standard biopsy forceps. Biopsies were fixed in 10% formalin, processed for paraffin embedding, and cut at 5 μm. Sections were stained with haematoxylin and eosin (H & E).

Confocal and corresponding histologic images of both normal and CD were jointly reviewed by 2 endoscopists, trained in confocal image assessment, and 2 experienced paediatric GI histopathologists (M.C., C.E.). Confocal features of CD were described and a confocal score was devised (Table 1).

The 5 best confocal images were subsequently selected for each biopsy specimen taken from both patients and controls. Both the histopathologists and the endoscopists, who were blinded to the histologic specimen and endoscopic images, respectively, assessed and reported the results. Modified Marsh criteria (6) were used to grade the histologic specimen and confocal images were graded for CD according to the criteria shown in Table 1.

Statistical Analysis

All statistical analyses were performed by using SPSS 15.0 for Windows software package (SPSS Inc, Chicago, IL) with input from the statistical unit of Sheffield University. The sensitivity, specificity, positive predictive value, and *P* value were calculated. In calculating these values, in which there was a difference in scoring between the histopathologists and endoscopists, a higher score was taken into account. Kappa coefficient was used to compare and to calculate the interobserver agreement between the endoscopists and the histopathologists.

TABLE 1. Confocal scoring for celiac disease (type 3a/b)

Shape of villi	Long slender and distinct (normal)—0
	Broad with some distortion—1
	Broad with total destruction of villous architecture—2
Pattern of surface epithelial enterocytes	Evenly shaped and distribution of the villous epithelial cells (normal)—0
	Some distortion of the cellular architecture of the villous epithelium—1
	Gross distortion of the cellular architecture of the villous epithelium with loss of the honeycomb pattern—2
Goblet cells	Normal—0
	Decreased—1
	Absent—2
Infolding of villi	Absent—0
	Present—2
Intervillous bridging	Absent—0
	Present—2

RESULTS

Nine patients and 10 controls underwent oesophagogastroduodenoscopy. The median procedure time was 17 minutes (range 8–25 minutes). The youngest to undergo the procedure in this study was 1.8 years old with a weight of 11 kg. Forty-four biopsies were taken in total (25 from patient arm and 19 from control arm). Also, 771 and 502 images were collected from patients and controls, respectively.

Duodenum in Normal Controls and Celiac Disease

Confocal features of the small intestine have been described (7). Duodenal villi appear slender, long, discrete, and finger-like with the surface having hexagonal-shaped enterocytes giving a honeycomb appearance, interspersed with goblet cells (Fig. 1A). At a deeper plane, the single layer of brush border columnar epithelial cells lining the lamina propria is well visualised. The lamina propria demonstrates capillary vasculature in the stroma (Fig. 1B). Crypts are not normally visible. These features correspond well with histology (Fig. 1C).

On confocal imaging, in CD with subtotal villous atrophy (Marsh type 3a/b) (Fig. 2A), the duodenal villi are broad with loss of the hexagonal pattern of the surface epithelium and a decrease in goblet cells. A characteristic feature observed was linking between adjacent villi giving an appearance of the villi being ''sticky.'' Furthermore, the villi appear to be folded onto themselves. In contrast, in CD with total villous atrophy (Marsh type 3c on histology), on confocal imaging, villi are absent and crypts are visible with dense cellular infiltration in the surrounding stroma (Fig. 3A) similar to histology (Fig. 3B). These features were taken into consideration while devising the confocal score. A score of zero was indicative of a normal duodenum, whereas a higher score, especially those closer to 10, was suggestive of CD.

In the present study, of the 9 patients with CD, both histologists agreed on the following: 3 patients had total villous atrophy (type 3c) and 4 had marked villous atrophy (type 3b). One histopathologist graded the remaining 2 patients as 3b and the second histopathologist graded them as 3a and 3c (Table 2). Thus, both histopathologists concurred on the diagnosis of CD, although they differed in the categorisation of villous atrophy. All 9 patients were rightly identified as having CD by both the endoscopists. The 2 endoscopists agreed with each other as to the Marsh grade in 8 of the 9 cases (89%). Five of the cases were rated as 3c and 3 were rated as 3b by both endoscopists. The 1 discordant case was rated as 3b by 1 endoscopist but 3c by another. In the 3 patients rated as 3b by both endoscopists, a score of 4 to 9, out of a possible 10, was given by the endoscopists. An interrater reliability analysis using the kappa statistic was performed to determine consistency among raters. The interrater reliability for the endoscopists was found to be kappa = 0.769 ($P = 0.018$) and for the histopathologists was kappa = 0.571 ($P = 0.05$).

Among the 10 controls, all were reported as normal by both histopathologists. The 2 endoscopists were in agreement in 8 of the 10 controls (80%). In the 2 discordant cases, the mucosa was considered normal by 1 endoscopist but not by the other, with a score of less than 3.

A conservative approach to calculating the sensitivity and specificity of the confocal approach is to treat the 2 ''abnormal'' ratings of the control as a final diagnosis of ''abnormal'' (ie, false positives) despite the fact that there was disagreement between the

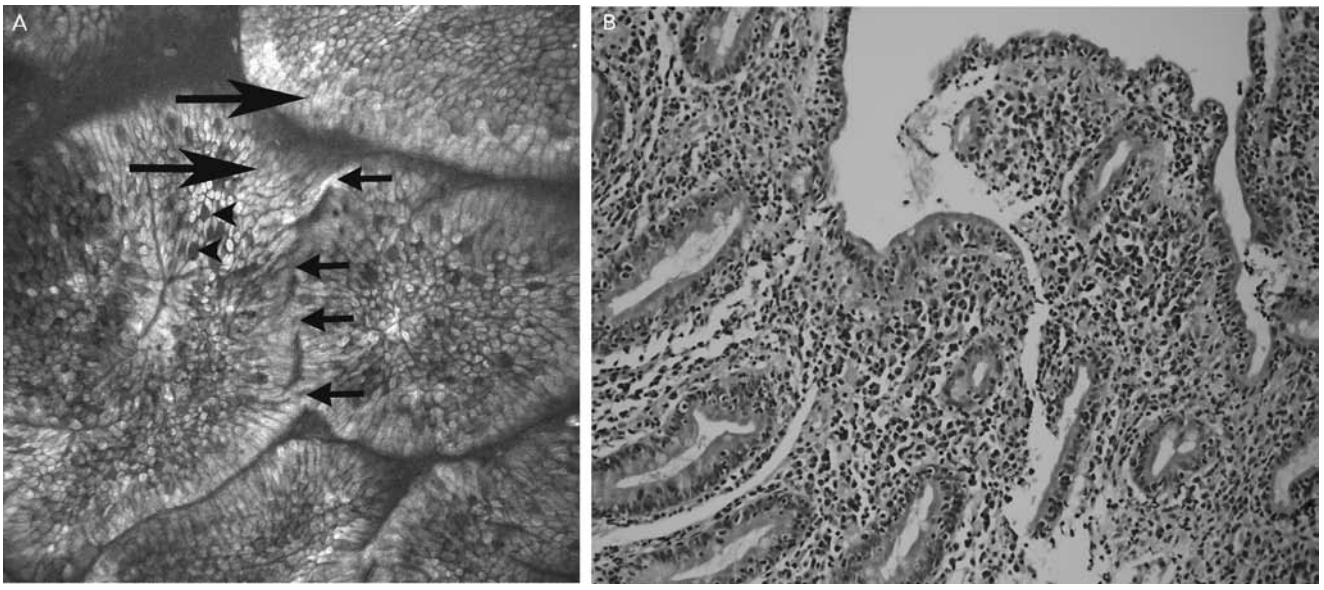

FIGURE 2. Celiac disease type 3b. A, Confocal image showing presence of broad duodenal villi with loss of the cellular architecture (large arrows) of the surface epithelium, decrease in goblet cells (arrowheads) and intervillous bridging (small arrows). B, Comparative histology.

endoscopists. Thus, the sensitivity, specificity, and positive predictive value for confocal endomicroscopy in comparison to the histology were 100%, 80%, and 81%, respectively.

DISCUSSION

Celiac disease is now considered to affect at least 1% of the Western population (1–3). Serological markers have been used to screen patients with suspected CD and with a combined specificity and sensitivity in excess of 95% (4). IgA deficiency can, however, lead to false-negative serological results (8). A correlation exists between the degree of duodenal atrophy and the occurrence and magnitude of positive serological results (9).

Endoscopic findings in CD include mosaic pattern of the duodenal mucosa, and scalloping and loss of the duodenal folds (10–12). Other endoscopic methods described, such as immersion technique (13) and video capsule endoscopy (14), claim to have a high degree of specificity and sensitivity. The pitfall of these methods in the diagnosis of CD has been the reliance on macroscopic appearances of the small intestinal mucosa. The gold standard, however, for the diagnosis of CD, is the presence of villous atrophy at histology on a duodenal or jejunal biopsy specimen and subsequent improvement in symptoms while on a gluten-free diet.

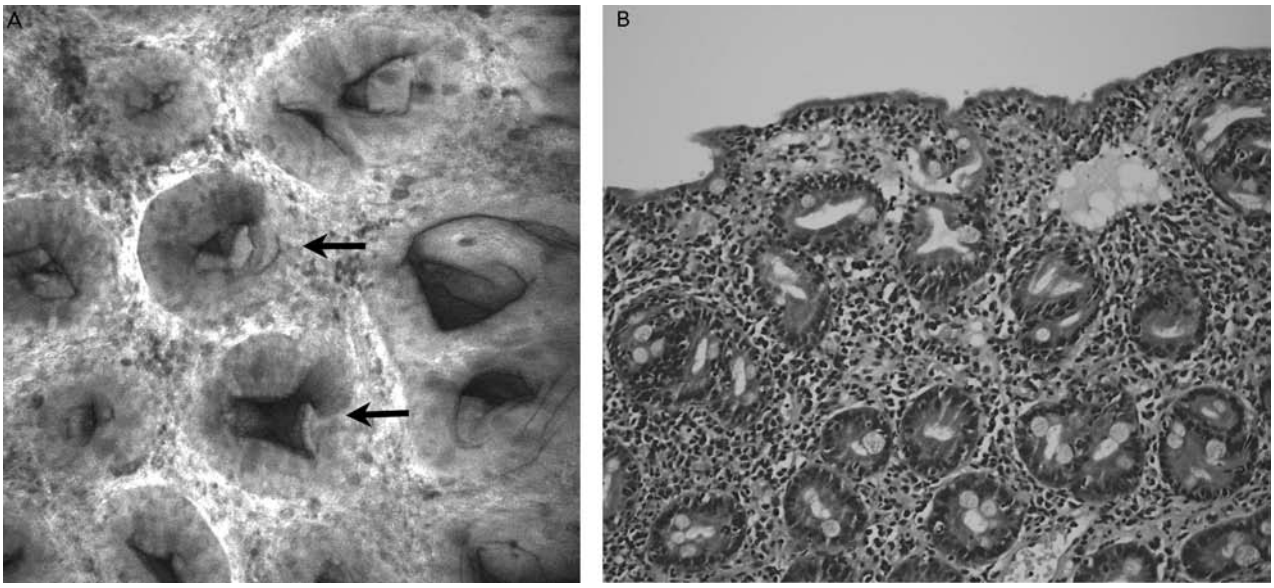

FIGURE 3. Celiac disease type 3c. A, Confocal image showing total absence of villi and enlarged crypts (arrows). B, Comparative histology.

TABLE 2. Comparison between the histologists and endoscopists in the assessment of patients with celiac disease; histological grading by modified Marsh criteria and confocal scoring by confocal celiac score

Patients	Endo 1	Endo 2	Histo 1*	Histo 2*
1	TVA	TVA	3b	3b
2	TVA	TVA	3c	3c
3	TVA	TVA	3b	3b
4	PVA (9)	PVA (8)	3b	3b
5	PVA (10)	TVA	3c	3c
6	PVA (4)	PVA (5)	3b	3b
7	TVA	TVA	3b	3c
8	TVA	TVA	3c	3c
9	PVA (7)	PVA (5)	3b	3a

The confocal score is given in brackets. Endo1 = endoscopist 1; Endo2 = endoscopist 2; Histo1 = histopathologist 1; Histo2 = histopatholo-histopathologist 2; PVA = partial villous atrophy on confocal laser endomicroscopy with confocal score in brackets; TVA = total villous atrophy on confocal laser endomicroscopy.

* Modified Marsh criteria.

Confocal laser endomicroscopy with 1000-fold magnification has been used in the in vivo diagnosis of GI malignancies (15) and other pathologies (16). So far, there have been only 3 reports of its use in CD in adults (17–19). The confocal celiac score for villous atrophy described by Leong et al (19) takes into account 2 features: blunting of villi and presence of less than 5 villi. In contrast, we have described detailed features of villous atrophy including unique ones such as intervillous bridging, distortion of surface architecture, and infolding of villi. These features are indicative of villous damage and consequent loss of continuity of the mucosal surface. Furthermore, the confocal celiac score describes the ratio of abnormal images against total images. Deeper images tend to blur. Consequently, detailed features of villous atrophy as described in this article could be difficult to recognise in images obtained at a deeper level. Furthermore, in our experience, the number of villi in the image remains constant irrespective of the depth when the images are collected from 1 particular site. Therefore, we did not take a ratio of images with abnormal changes against the total number to calculate the confocal score.

The findings of enlarged villi attributed to the expansion of extracellular matrix (20), loss of cellular architecture, decreased goblet cells, and mucosal damage that are easily identified on CLE. These findings correspond to Marsh type 3a/b on histology. Marsh type 1 is defined as an increase in intraepithelial lymphocytes. However, it is, at least at the present time, not possible to definitively foretell an increase in intraepithelial lymphocytes by the confocal method. Because the maximum depth obtained is only 250 μm, crypt hyperplasia (Marsh type 2) is difficult to appreciate unless there is total or near-total villous atrophy. Moreover, a definite diagnosis of CD is made only with a Marsh 3a or more on histology. Hence, comparative confocal features corresponding to Marsh grades type 1 and type 2 were not described and these patients were not included in the study group.

We have, in this study, validated the usefulness of this technique in diagnosing CD and differentiating it from normal in a small series of patients. Of the 9 patients with CD, 5 patients had total villous atrophy with absence of villi and crypt hypertrophy (type 3c). Thus, the absence of villi together with visible enlarged crypts on confocal imaging would indicate an unambiguous diagnosis of type 3c CD. Four had subtotal villous atrophy with characteristic findings (type 3b). Our study suggests that a score of ≥ 4 is suggestive of CD whereas a score ≤ 3 is indicative of a normal duodenum. Specificity and positive predictive value appear less in this study due to the stringent criteria used to define normal and abnormal. If we take a confocal score of ≥ 4 as abnormal, the specificity obtained would be 100%.

There have been reports of a patchy duodenal involvement in CD (21). This had led to suggestions to obtain several biopsies from different sites of the duodenum including the duodenal bulb in adults (22). Similar findings have been replicated in studies in children (23).

Confocal laser endomicroscopy could find application in targeting biopsies to the abnormal areas and, thus, potentially increase the diagnostic yields of CD. A corollary may be a decrease in the number of biopsies needed. Similarly in latent CD, in which the duodenal mucosa is normal on random biopsies, with positive serology, a role for CLE exists to target biopsies. This would, in the long run, save costs involved in processing and assessing of unnecessary biopsy specimen.

CONCLUSIONS

In conclusion, we have shown that CLE used by experienced operators can distinguish normal duodenal mucosa from CD in almost all cases. This paves the way for larger studies aiming to clarify the place of this technique in making a reliable diagnosis, in limiting the number of biopsies, and for same-day treatment initiation in those cases that appear conclusive of CD while awaiting biopsy results.

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