A new method in the diagnosis of reflux esophagitis: confocal laser endomicroscopy

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Background: The diagnosis of GERD is made by using a combination of clinical symptoms, pH study, endoscopy, and histology. Histologic changes include basal cell hyperplasia and papillary elongation. Confocal laser endomicroscopy (CLE) enables surface and subsurface imaging of living cells in vivo at ×1000 magnification and up to 250 μm below the tissue surface. In the esophagus, the distance between the surface to papillary (S-P) tip can be measured by using CLE.

Objective: To measure the S-P distance in the esophagus in patients with reflux esophagitis and controls by using CLE and comparing with histologic measurements.

Design: Retrospective analysis of a prospective database.

Setting: Endoscopy unit of a tertiary-care children's hospital.

Patients: This study involved 7 patients (5 female) with a median age of 7.6 years (range 1.8-15.5 years) and median weight of 23 kg (range 13.2-71 kg) and 16 controls with a median age of 12.0 years (range 2.2-15.3 years) and median weight of 38.2 kg (range 10.7-83 kg).

Intervention: S-P distance was measured both by CLE and histology and was corrected for height for both patients and controls and the results compared.

Main Outcome Measurements: To determine if there were significant differences in the S-P distance in patients with esophagitis and controls.

Results: The median confocal and histologic measurements for S-P distance, corrected for patient height, were 0.19 μm/cm (range 0.10-0.49 μm/cm) and 0.58 μm/cm (range 0.29-0.76 μm/cm) and for controls were 0.44 μm/cm (range 0.20-0.93 μm/cm) and 1.07 μm/cm (range 0.76-0.1.57 μm/cm), respectively.

Limitations: Small numbers involved in the study, reliance on only papillary elongation in arriving at a diagnosis.

Conclusion: Measurement of the S-P distance by CLE will enable real-time diagnosis of GERD-related esophagitis during ongoing endoscopy.

GERD in children is defined as a condition that develops when reflux of stomach contents causes troublesome symptoms and or complications.[1] The diagnosis of GERD is established by a combination of clinical symptoms, endoscopy, histology, and pH studies. The prevalence of symptoms suggestive of GERD in adults in the developed world is high, with 20% reporting heartburn and/or acid regurgitation on a weekly basis[2-4] and 40% having them at

Abbreviations: CLE, confocal laser endomicroscopy; NERD, non-erosive reflux disease; S-P, surface to papillary.

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least once a month. In infants and children, however, the prevalence of regurgitation is only 5% to 8%.[5,6] However, one particular questionnaire-based study in adolescents reported that 40% had at least one esophageal symptom suggestive of GERD.[7] Endoscopic features of esophagitis include the presence of erosions, ulcerations, exudates, and strictures, and in extreme cases, development of Barrett's esophagus and adenocarcinoma. Only 40% of patients with reflux symptoms have endoscopic features of esophagitis; the rest are diagnosed as having non-erosive reflux disease (NERD).[8,9]

Traditionally, the presence of inflammatory cells in the lamina propria was considered a hallmark of reflux esophagitis. In 1970, Ismail-Beigi et al[10] described the presence of basal cell hyperplasia and papillary elongation in a majority of patients with reflux esophagitis. Basal cell hyperplasia is present when the basal cell zone occupies >15% of the thickness of the mucosa. Papillary elongation is defined as when the subepithelial papilla extends to >67% of the thickness. In 1996, Tobey et al[11] proposed dilation of intercellular spaces as a feature of esophagitis. Thus, a combination of increased inflammatory cells in the lamina propria, basal cell hyperplasia, papillary elongation, and the presence of dilated intercellular spaces are now considered to be the histologic signs of esophagitis.

Confocal laser endomicroscopy (CLE) enables surface and subsurface imaging of living cells in vivo at ×1000 magnification and up to 250 μm below the tissue surface in 4-μm steps. In the esophagus, the surface squamous epithelial cells with prominent nuclei are well-visualized (Fig. 1A). At a deeper plane, capillary loops from a papilla are made out (Fig. 1B). The distance from the surface to the papillary (S-P) tip can be measured by using CLE in vivo during ongoing endoscopy (Fig. 2). A similar measurement is possible on histology (Fig. 3A and B).

It is reasonable to assume that papillary elongation in reflux esophagitis should lead to a decrease in the distance between the surface and the papillary tip. We postulated that by measuring the S-P distance by CLE, we could predict the presence of esophagitis and differentiate the normal esophagus from the inflamed esophagus. The aims of this study were 2-fold—primarily to ascertain whether there were significant differences in the S-P distance measured by CLE and histology between patients with esophagitis and controls and second to clarify whether the measurements between the two methods are comparable.

METHODS

We recruited 23 patients (15 female) needing upper GI endoscopy for various indications, including recurrent abdominal pain, failure to thrive, suspected inflammatory bowel disease, and celiac disease. 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 >18 years; previous documented adverse reaction/allergy to sodium fluorescein or acriflavine hydrochloride. All patients were admitted on the day of the procedure. All procedures occurred with patients under general anesthetic as is normal practice in our institution for pediatric GI endoscopy.

Take-home Message

- Confocal laser endomicroscopy would contribute in making a real-time endoscopic diagnosis of GERD. The measurement of surface-to-papillary distance is a new method in the diagnosis of reflux-associated esophagitis.

The endoscopic procedure using the confocal endomicroscope (EC3870CILK; Pentax, Tokyo, Japan) has been well-described[12] and summarized as follows. After duodenal intubation, 0.05 to 0.1 mL/kg of 10% fluorescein sodium solution was administered intravenously and flushed adequately with normal saline solution. On withdrawing the endoscope to the lower end of the esophagus, we sprayed acriflavine 0.05% on the surface of the esophageal mucosa by using a spray catheter. CLE image acquisition was performed by placing the tip of the endoscope in direct contact with the surface of the esophageal mucosa. Using gentle suction to stabilize the mucosa, we then actuated image acquisition and focal plane z-axis scanning depth by using two discrete handpiece control buttons. The imaging depth below the tissue surface can be dynamically controlled by the operator. With each deeper plane, the focal plane of the confocal microscope moves by 4 μm, and consequently the image obtained is approximately 4 μm deeper than the previous one. Consecutive confocal images were then obtained from the esophagus sequentially at different planes from the surface to the maximum permissible depth. The S-P distance in micrometers was calculated by counting the number of images obtained from the surface until the first capillary loop was first detected and multiplying by a factor of 4.

Same-site mucosal specimens were obtained by using standard biopsy forceps. Biopsy specimens were fixed in 10% formalin solution, processed for paraffin embedding, and cut at 5 μm. Sections were stained with hematoxylin and eosin. The histologic images were reviewed by an experienced pediatric GI pathologist (M.C.). S-P measurements were obtained from the histologic images of all patients.

Statistical methods

All statistical analyses were performed by using SPSS 15.0 for Windows software package (SPSS, Inc, Chicago, Ill). Median, range, and standard deviation were calcu-

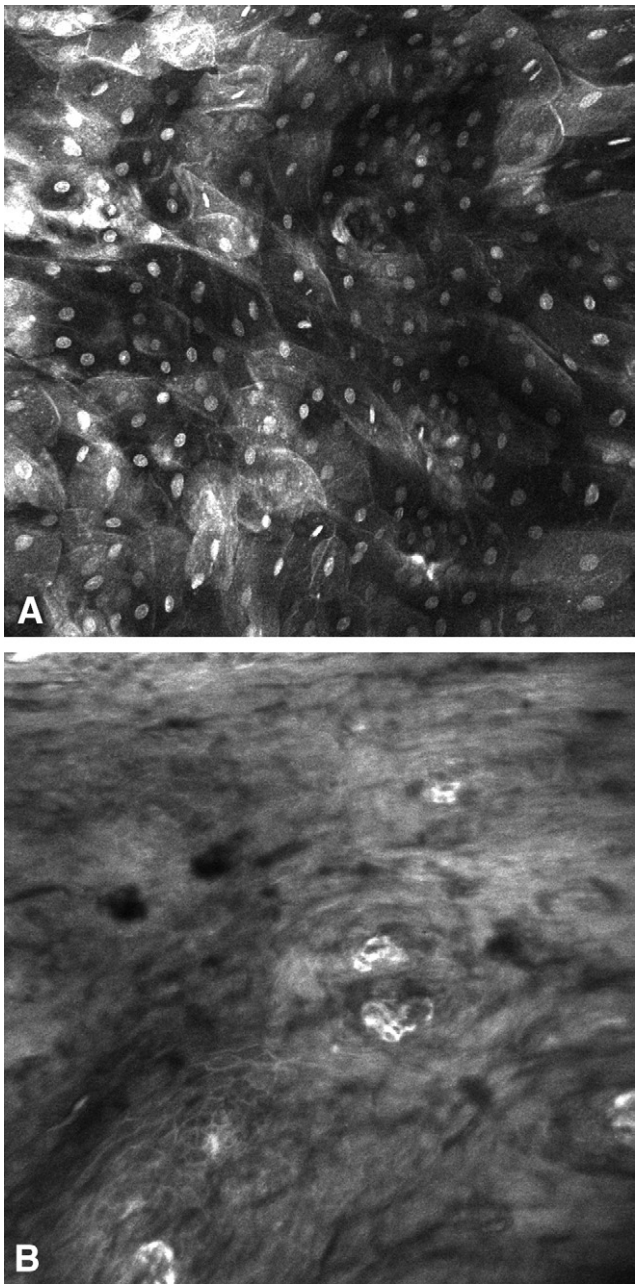

Figure 1. A, Confocal image of the surface of the esophageal mucosa. **B,** Confocal image showing a papillary loop on a deeper plane.

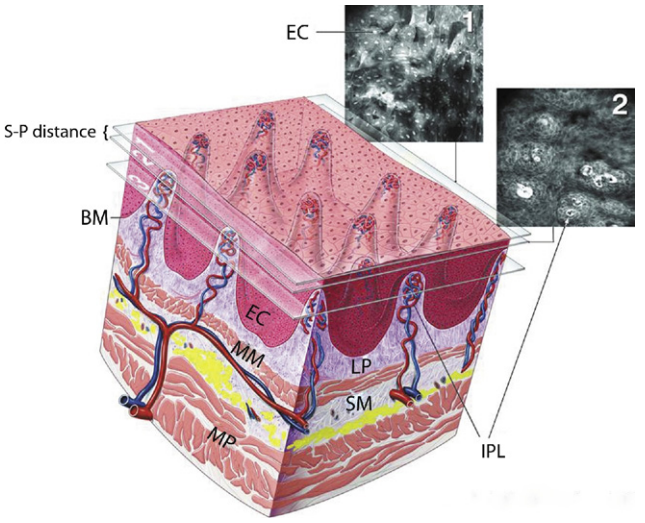

Figure 2. Confocal measurement of surface to papillary tip.

lated. We used the Mann-Whitney U test, a nonparametric 2-sample test, to compare the results between the patient and control groups. The exact significance was determined, and $P < .05$ was considered significant.

RESULTS

Seven of the 23 patients had histologic features consistent with esophagitis, including basal cell hyperplasia, papillary elongation, and increase in inflammatory cells in the lamina propria and thus were deemed the patient group. However,

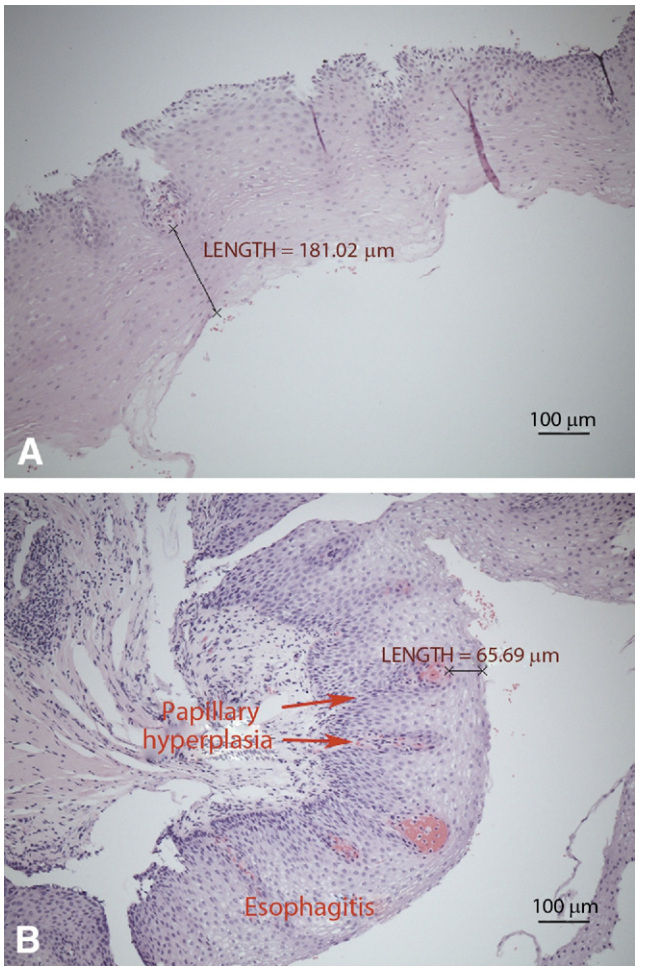

Figure 3. A, Histologic image showing measurement of the surface-papillary distance in normal esophagus. **B,** Histologic image showing measurement of the surface-papillary distance in esophagitis.

TABLE 1. Confocal laser endomicroscopy and histologic measurements in patient group

		CLE measurement		Histologic measurement	
Patient	Height, cm	Actual, μm	Corrected for height, (μm/cm)	Actual, μm	Corrected for height, (μm/cm)
1	114	12	0.10	33.29	0.29
2	86	32	0.37	65.69	0.76
3	145	20	0.13	100.62	0.69
4	150	24	0.16	89.67	0.59
5	123	60	0.49	92.96	0.75
6	101	20	0.19	47.07	0.46
7	166	80	0.48	88.58	0.53

CLE, Confocal laser endomicroscopy.

TABLE 2. CLE and histologic measurements in control group

		CLE measurement		Histologic measurement	
Control	Height, cm	Actual, μm	Corrected for height, (μm/cm)	Actual, μm	Corrected for height, (μm/cm)
1	87	44	0.50	67.51	0.77
2	118	32	0.27	105.97	0.89
3	171	76	0.44	150.3	0.87
4	151	128	0.84	157.22	1.03
5	156	46	0.29	161.64	1.03
6	150	140	0.93	138.67	0.92
7	109	72	0.65	125.48	1.14
8	164	32	0.20	125.92	0.76
9	116	52	0.44	158.53	1.36
10	146	36	0.24	177.49	1.21
11	161	64	0.39	122.54	0.76
12	138	80	0.57	181.02	1.30
13	155	112	0.72	236.17	1.52
14	142	52	0.36	158.24	1.11
15	163	148	0.90	230.09	1.41
16	87	24	0.27	136.98	1.57

CLE, Confocal laser endomicroscopy.

none of the 7 patients at endoscopy had endoscopic changes suggestive of reflux esophagitis. The remaining 16 patients with no esophagitis on histology were included in the control group. The S-P measurements for both histology and CLE were corrected for height to account for the variation in thickness of the esophageal mucosa for different ages. Seven patients (5 female), with a median age of 7.6 years (range 1.8-15.5 years) and median weight of 23 kg (range 13.2-71 kg) and 16 controls with a median age of 12.0 years (range 2.2-15.3 years) and median weight of 38.2 kg (range 10.7-83 kg) were thus included in the study. The actual CLE and histologic measurements for patients and controls as well as measurements corrected for height are given in Tables 1 and 2, respectively.

The median CLE measurement for S-P distance corrected for height for patients was 0.19 μm/cm (range

0.10-0.49 μm/cm; standard deviation [SD] 0.16) and for controls was 0.44 μm/cm (range 0.20-0.93 μm/cm; SD 0.24). The Mann-Whitney U test statistic was 25.0, and $P = .019$.

The median histologic measurement for S-P distance corrected for height for patients was 0.58 μm/cm (range 0.29-0.76 μm/cm; SD 0.17) and for controls was 1.07 μm/cm (range 0.76-0.1.57 μm/cm; SD 0.26). The Mann-Whitney U test statistic was 0.0, and $P < .001$.

DISCUSSION

There is no definite test for the diagnosis of GERD. A combination of clinical symptoms, pH study, endoscopy, and histology are used to establish a diagnosis.[13] Mucosal breaks seen on endoscopy were long considered synonymous with GERD. Several classifications were developed, and presently the modified Los Angeles classification is used by endoscopists to grade erosive esophagitis.[14] However, it has been shown that only 40% of patients presenting with symptoms of GERD have typical features of esophagitis on endoscopy.[8,13,15,16] The remaining patients with symptoms of reflux disease but without any endoscopic signs are diagnosed as having NERD, and the diagnosis has relied on pH study and histology. A third of patients with NERD can have normal 24 hour pH monitoring.[17]

Some studies using histology alone in diagnosing GERD have reported a sensitivity of 17% to 62%.[18-20] One recent study, however, showed a sensitivity of 84% and specificity of 85% when a combination of basal cell zone hyperplasia, papillary elongation, dilation of intercellular spaces, and increase in inflammatory cells in the lamina propria was used to make a diagnosis.[21]

In our study, the diagnosis of esophagitis was initially established on histology. Then S-P distance was measured for both the patient and control groups after adequate orientation of the histology slides. In view of the wide variation in the ages in both groups and to account for the possible differences in the thickness of normal esophageal mucosa, the S-P distance was corrected for height. To our knowledge, this is the first study looking at S-P distance measurements on histology or by CLE. Our study shows that there is a significant difference in the S-P distance between the patients with histology-proven esophagitis and those without when either of the CLE or histologic methods were used, even though the actual measurements did not exactly match. With the CLE method, $P = .019$, and with histology, $P < .001$.

Limitations of this study include reliance on one particular feature (papillary elongation) in arriving at a diagnosis, small numbers involved, and the variation in the ages of the study groups. The lack of agreement in the actual score between the histologic and CLE measurements could be explained by the different methods used to measure the S-P distance. Further, histologic processing would affect the thickness of the mucosa. Also, the depth of each plane of the confocal image (calculated as 4 μm) is based on the fact that the confocal microscope in the endoscope moves by 4-μm steps. This conversion factor may not be accurate.

The major advantage of the CLE method is the capacity to make a real-time, in vivo diagnosis of esophagitis. This study suggests that reflux-related esophagitis may be diagnosed by using CLE. Although this method may not be the most accurate and definitive method to diagnose esophagitis, it certainly would add to the diagnostic armamentarium of reflux-related esophagitis. Further large studies with clinical, pH study, endoscopic, and histologic correlations may help to clarify whether this technique is indeed helpful in making an instant diagnosis at endoscopy of reflux-associated esophagitis.

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