

Pharyngeal mucosa brushing does not correlate with disease activity in patients with eosinophilic esophagitis



Hedieh Saffari, PhD^a, Krista Baer, BSc^b,
Kathleen K. Boynton, MD^b, Gerald J. Gleich, MD^c, and
Kathryn A. Peterson, MD^b

Clinical Implications

- Analysis of pharyngeal mucosa samples by eosinophil peroxidase assay could not demonstrate the evidence of eosinophil's inflammation in patients with eosinophilic esophagitis.

TO THE EDITOR:

Minimally invasive methods are under study to detect the inflammation in eosinophilic esophagitis (EoE), a chronic, atopic disease characterized by activation of eosinophils and deposition of eosinophil granule proteins, including eosinophil peroxidase (EPO), into the esophageal mucosa, accompanied by symptoms of esophageal dysfunction.¹ Currently, diagnosis and monitoring disease require multiple esophageal biopsies obtained at endoscopy that are associated with costs due to the procedure concomitant, sedation, and days lost from work. The current criterion standard of peak eosinophil counts of more than or equal to 15 eosinophils/hpf on esophageal epithelium may not reflect disease activity because of the patchy nature of eosinophil infiltration, where up to 5 biopsies are needed to accurately identify eosinophils.^{2,3} Despite its relatively recent description, EoE has an estimated prevalence of 56.7/100,000 persons, and an estimated cost of \$1.4 billion a year.⁴ In EoE, eosinophils undergo cytolysis and release granule proteins, including EPO, major basic protein 1 and 2, eosinophil-derived neurotoxin, and eosinophil cationic protein, into both the epithelial and lamina propria layers. Therefore, measurements of eosinophil granule proteins in esophageal mucosa may reflect disease activity better than histologic identification of eosinophils.^{5,6}

In EoE, the esophageal-stratified squamous epithelium becomes highly activated.⁷ Other sites of squamous epithelium such as the bronchus and skin in such patients are commonly affected as well with up to 70% of patients reporting asthma and up to 60% reporting eczema.⁸ The oral mucosa, however, remains an enigma in EoE. Oropharyngeal tissue is an additional site of the mucosal-associated lymphoid tissue, lined by squamous mucosa, and exposed to the same antigens that trigger EoE in patients. Tissue found along the oral mucosa could provide clinicians a mechanism by which to diagnose and evaluate EoE.

Squamous mucosa similar to esophageal mucosa lines the oropharyngeal cavity. Measurement of EPO using a colorimetric EPO assay is a simple and quantitative method to detect the eosinophil-associated inflammation from esophageal mucosal brushing from patients with EoE.⁹ In this study, we determined whether analyses of brushings from the pharyngeal mucosa differentiates patients with EoE from normal individuals.

TABLE I. Demographic characteristics of patients

Patient no.	Age (y)/sex	PPI treatment	Disease state
1	65/F	No	Normal
2	64/F	No	Normal
3	41/M	Yes	Normal
4	61/M	No	Normal
5	19/M	No	Normal
6	39/F	Yes	Normal
7	31/M	No	Normal
8	52/F	No	Resolved EoE
9	58/F	Yes	Resolved EoE
10	33/F	Yes	Resolved EoE
11	67/F	Yes	Resolved EoE
12	40/F	Yes	Resolved EoE
13	32/F	No	Resolved EoE
14	55/F	Yes	Barrett esophagus
15	61/M	Yes	GERD
16	60/M	No	GERD
17	55/F	Yes	GERD
18	75/M	No	GERD
19	35/M	Yes	GERD
20	53/F	No	GERD
21	40/F	No	GERD
22	50/M	Yes	EoE
23	33/F	Yes	EoE
24	43/F	Yes	EoE
25	34/F	Yes	EoE
26	20/M	Yes	EoE
27	20/F	Yes	EoE
28	23/F	No	EoE
29	52/M	Yes	EoE

F, Female; GERD, gastroesophageal reflux disease; M, male; PPI, proton pump inhibitor.

We have previously validated a simple, rapid method to assess eosinophil-associated mucosal inflammation by quantifying EPO from esophageal mucosal brush specimens.⁹ Here, we tested the hypothesis that measurements of EPO in pharyngeal brushing specimens from patients with EoE permit disease detection.

With approval from the University of Utah Institutional Review Board (institutional review board no. 52527), pharyngeal brushing samples were collected before endoscopy from 29 patients with active EoE (n = 8) and non-EoE (n = 21) using sterile polyester swabs (reference no. 25-8061PD; Medical Product Company LLC). Before endoscopy for biopsy collection, patients were asked to permit swabbing of the bilateral sides of their posterior pharynxes. Polyester swabs were immediately placed into a container for analysis. The 8 patients identified as active EoE had peak eosinophil counts greater than 15 per hpf in biopsy specimen from at least 1 segment of the esophagus (proximal, mid, or distal). Patients without EoE included 6 patients with resolved EoE (patients who had been previously diagnosed with EoE, but the biopsy specimens obtained at the time of brushing did not demonstrate eosinophil counts greater than 15 eos/hpf), 7 normal controls

Patient No.	Disease State of Tissue	Peak Eosinophil Counts	EPO Assay Buccal Brushing: Absorbance @ 492 nm	EPO Assay Buccal Brushing: Color
1	Normal	0	0.145	
2	Normal	0	0.074	
3	Normal	0	0.038	
4	Normal	0	0.088	
5	Normal	0	0.003	
6	Normal	0	0.404	
7	Normal	0	0.375	
8	Resolved EoE	0	0.106	
9	Resolved EoE	0	0.227	
10	Resolved EoE	0	0.401	
11	Resolved EoE	3	0.255	
12	Resolved EoE	10	0.432	
13	Resolved EoE	10	0.167	
14	Barrett's Esophagus	3	0.24	
15	GERD	0	0.611	
16	GERD	0	0.649	
17	GERD	0	1.138	
18	GERD	0	1.154	
19	GERD	0	1.195	
20	GERD	0	1.293	
21	GERD	0	1.341	
22	EoE	30	0.162	
23	EoE	35	0.232	
24	EoE	59	0.28	
25	EoE	60	0.08	
26	EoE	65	0.656	
27	EoE	68	0.767	
28	EoE	70	1.114	
29	EoE	100	0.323	

FIGURE 1. Patient clinical presentations including esophagogastroduodenoscopy (EGD) findings, peak eosinophil counts by histopathology, and pharyngeal brushing analyses including EPO assay absorbance measurements and the visualized colors.

(patients who presented with esophageal symptoms but had normal esophagi on endoscopic assessment), 7 patients with symptoms of gastroesophageal reflux disease, and 1 patient with Barrett esophagus (see Table I, the demographic data).

Pharyngeal brushing samples were assayed immediately by EPO assay as described previously.⁹ Briefly, the brushing samples were incubated for 30 minutes in freshly prepared EPO detection reagent (800 μ L 5 mM α -phenylenediamine in Tris-HCl buffer [pH 8], 100 μ L sodium cyanide [15 mM], and 1.25 μ L 30% hydrogen peroxide) followed by addition of 100 μ L stop solution (4 mol sulfuric acid). The resulting color was noted, and absorbance was measured at 492 nm (SmartSpec Plus; Bio-Rad, Hercules, Calif). The absorbance units were compared with the peak eosinophil counts obtained from histopathological examination of esophageal biopsy specimens. Comparison between groups for findings in histopathology was made by nonparametric Mann Whitney *U* test.

Figure 1 summarizes the peak eosinophil counts from histological observations and absorbance measurements at 492 nm for the 29 patients. By using different cutoff values (ranges from 0.1 to 1.3 in value), we could not find a cutoff value with both high sensitivity and specificity. At low cutoff values, the sensitivity was high (>80%), but the specificity was low (<20%) and

at high cutoff values opposite results were obtained. No cutoff value satisfactorily differentiated EoE from non-EoE. Therefore, the EPO assay failed to quantitatively detect the disease state from the pharyngeal brushing specimens. Notably, the high absorbance readings (>1 absorbance unit, orange color on EPO assay) were from patients without EoE diagnosed with gastroesophageal reflux disease (see Figure 1, patient nos. 17-21), suggesting that the refluxate may interfere with the EPO assay.

In conclusion, our previous study showed that the EPO assay of esophageal mucosal brushings closely reflected EoE disease activity with high sensitivity and specificity (100% and 83%, respectively, area under curve of 0.991). However, the results from the pharyngeal brushing specimens did not correlate well with disease activity and disease diagnosis, indicating that pharyngeal sampling cannot replace esophageal biopsy identification of eosinophil-associated inflammation, despite similar squamous epithelium.

^aDepartment of Dermatology, University of Utah, Salt Lake City, Utah

^bDivision of Gastroenterology, Department of Internal Medicine, University of Utah, Salt Lake City, Utah

^cDepartment of Dermatology and Internal Medicine, University of Utah, Salt Lake City, Utah

The research was conducted through University of Utah, School of Medicine.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication November 21, 2016; revised February 23, 2017; accepted for publication March 17, 2017.

Available online May 3, 2017.

Corresponding author: Hedieh Saffari, PhD, Department of Dermatology, University of Utah, 4A330 School of Medicine, 30 N, 1900 E, Salt Lake City, UT 84132. E-mail: Hedieh.Saffari@utah.edu.

2213-2198

© 2017 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaip.2017.03.012>

REFERENCES

- Furuta GT, Katzka DA. Eosinophilic esophagitis. *N Engl J Med* 2015;373:1640-8.
- Saffari H, Peterson KA, Fang JC, Teman C, Gleich GJ, Pease LF 3rd. Patchy eosinophil distributions in an esophagectomy specimen from a patient with eosinophilic esophagitis: implications for endoscopic biopsy. *J Allergy Clin Immunol* 2012;130:798-800.
- Gonsalves N, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirano I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. *Gastrointest Endosc* 2006;64:313-9.
- Dellon ES, Jensen ET, Martin CF, Shaheen NJ, Kappelman MD. Prevalence of eosinophilic esophagitis in the United States. *Clin Gastroenterol Hepatol* 2014;12:589-596.e1.
- Saffari H, Hoffman LH, Peterson KA, Fang JC, Leiferman KM, Pease LF 3rd, et al. Electron microscopy elucidates eosinophil degranulation patterns in eosinophilic esophagitis. *J Allergy Clin Immunol* 2014;133:1728-34.
- Kephart GM, Alexander JA, Arora AS, Romero Y, Smyrk TC, Talley NJ, et al. Marked deposition of eosinophil-derived neurotoxin in adult patients with eosinophilic esophagitis. *Am J Gastroenterol* 2010;105:298-307.
- Mulder DJ, Gander S, Hurlbut DJ, Soboleski DA, Smith RG, Justinich CJ. Multiple squamous hyperplastic-fibrous inflammatory polyps of the oesophagus: a new feature of eosinophilic oesophagitis? *J Clin Pathol* 2009;62:845-6.
- Dellon ES, Liacouras CA. Advances in clinical management of eosinophilic esophagitis. *Gastroenterology* 2014;147:1238-54.
- Saffari H, Leiferman KM, Clayton F, Baer K, Pease LF, Gleich GJ, et al. Measurement of inflammation in eosinophilic esophagitis using an eosinophil peroxidase assay. *Am J Gastroenterol* 2016;111:933-9.

Reproduced with permission of copyright owner. Further reproduction prohibited without permission.