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ORIGINAL ARTICLE

Simultaneous Examination of Eosinophil Infiltration in Esophageal Mucosa and Muscle in Patients with Achalasia: Direct Biopsy of the Esophageal Muscle at Per-oral Endoscopic Myotomy

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Abstract

Background The relationship between eosinophilic esophagitis (EoE) and achalasia is not completely understood. There have been reports of eosinophilic infiltration of all esophageal layers in patients with achalasia. However, a routine endoscopic biopsy of the muscular layer is usually not feasible. We evaluate the safety and efficacy of muscle layer biopsy during per-oral endoscopic myotomy (POEM) as well as the prevalence of eosinophilic infiltration of the esophageal mucosa and muscular layer in patients with achalasia.

Patients and Methods All enrolled patients had diagnosed achalasia and had simultaneous biopsies of the muscular layer at the middle esophagus and distal esophageal sphincter as well as the mucosal layer of the proximal and distal esophagus during POEM. All POEM procedures took place from August 2018 to December 2018 or September 2019 to November 2019. Various demographic, disease-related, and procedure-related data were collected from chart review. Eosinophilic infiltration in the biopsy specimen was examined.

Key Results Twenty consecutive patients (65% female, age range: 21–84) with a pre-procedure Eckardt score of >6 were enrolled during the study period, with the duration of their achalasia ranging from 1 to 32 years. Eighteen patients had clinical symptomatic improvement after POEM, as defined by an Eckardt score <3. Endoscopic examination did not reveal any signs of eosinophilic esophagitis. Pathologic examination of biopsies revealed eosinophilic infiltration in three of 20 patients (15%) in the distal esophageal mucosa (all <15 eosinophils/HPF) and none in the proximal esophageal mucosa. There was no eosinophilic infiltration in the distal esophageal sphincter and the middle esophageal muscle. No complication was noted due to muscle biopsy.

Conclusions and Inferences Submucosal tunneling during POEM provides a safe access for direct esophageal muscle biopsy. This is the first report of the simultaneous biopsy of the esophageal mucosa and muscle in patients with achalasia. Contrary to all previously published studies, the association of esophageal eosinophilic infiltration and achalasia was not observed in this small sample study. Based on our findings, immune or autoimmune reaction rather than direct eosinophilic infiltration in the muscle is more likely the cause of achalasia.

Keywords POEM · Eosinophilic esophagitis (EoE) · Achalasia · Pathophysiology

Introduction

The majority of achalasia presentations is idiopathic, characterized by impaired relaxation of the lower esophageal sphincter (LES) and absent contractility in the esophageal body while swallowing [1, 2]. Commonly, patients' symptoms manifest as dysphagia and heartburn, but they can also include other symptoms such as epigastric pain, chest pain, regurgitation, cough, odynophagia, and weight loss [3]. Three different subtypes of achalasia were defined by

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high-resolution manometry: type I: 100% failed esophageal peristalsis, type II: 100% failed peristalsis with panesophageal pressurization, and type III: ≥20% premature contractions with spasm [4].

At present, there are a few studies that report abnormal collections of eosinophils or cellular degranulation products in all layers of the esophagus, predominantly in the esophageal muscle, in patients with achalasia [5–7]. A subsequent hypothesis has been raised that achalasia might develop from a muscle-predominant subtype of eosinophilic esophagitis (EoE) [8]. With this, there are discussions that reducing eosinophilic infiltration might prevent further neuronal damage in achalasia. However, biopsy of the muscular layer is usually not feasible in routine endoscopy. Hence, the causal relationship between eosinophilic infiltration and achalasia remains unclear.

Per-oral endoscopic myotomy (POEM) is a minimally invasive therapeutic endoscopic procedure that has shown promising clinical success in the treatment of achalasia [1]. The procedure includes mucosal incision starting from the mid-esophagus creating a submucosal tunnel to the gastric cardia and then a selective myotomy of the circular muscle inside the submucosal tunnel. This access to the muscular layer of the esophagus allows for real-time direct biopsies of the muscular layer. In this study, we prospectively evaluated the efficacy/safety of muscle layer biopsy during POEM procedure as well as the prevalence of eosinophilic infiltration in the esophageal mucosa and muscular layer in patients with achalasia. If there is an association of esophageal eosinophilic infiltration and achalasia in some patients, we can start steroid therapy specifically for those patients.

Materials and Methods

Patients

Patients with achalasia referred to our center were assessed by following routine clinical protocol, including timed barium swallow, chest computed tomography, standard high-resolution manometry [9, 10], and esophagogastroduodenoscopy as needed. Consecutive patients who had simultaneous biopsies (with regular cold biopsy forceps) of the muscular layer of the middle esophagus and distal esophageal sphincter as well as distal and proximal mucosal during POEM procedure from August 2018 to December 2018 and then September 2019 to November 2019 were enrolled in this study. During the two periods, all consecutive patients were enrolled and did not skip any patients. General patients' characteristics, Eckardt scores before and after POEM [11], type of achalasia, details of the procedure, and intraoperative/postoperative complications were all collected. Adverse events were graded according to the American Society for Gastrointestinal Endoscopy (ASGE) lexicon's severity grading system [12].

All patients provided written consent to participate in this study before the POEM. The present study was approved by the Emory University Institutional Review Board.

Per-oral Endoscopic Myotomy

All the procedures were performed by QC and assisted by an advanced endoscopic fellow. All procedures were performed under anesthesia and CO2 insufflation. A gastroscope (GIF-H190; Olympus, Tokyo, Japan) with a transparent distal cap attachment (MH-588; Olympus, Japan) was used at our endoscopy suite for the procedure. Procedure methods and evaluation were described in our previous study [13]. All the patients were placed on a clear liquid diet for 72 h before the procedure and kept NPO the night before planned POEM. First, a routine upper endoscopy was performed. For each POEM, a mucosal bleb was created in the posterior esophageal wall 10–15 cm above the gastroesophageal junction (GEJ), and a 2-cm longitudinal mucosal incision was made to reveal submucosal layer. Mucosotomy was performed by a Hybrid knife (ERBE, Germany) or a triangle tip knife (Olympus, Japan), extending a submucosal tunnel to approximately 2 cm into the gastric cardia. Once the cardia was reached, a circular myotomy was performed in a retrograde fashion starting in cardia extending proximally into the middle esophagus. The mucosotomy was then closed by placement of endoclips (Micro-Tech, Nanjing, China).

Direct Biopsy of the Esophageal Muscle and Mucosa

During POEM, simultaneous esophageal mucosal and muscle tissue biopsies were obtained from all the patients included in the study (Fig. 1). To distinguish gastroesophageal reflux disease from eosinophilic esophagitis, the proximal and distal mucosa was biopsied and examined separately for the number of eosinophilic cells. Accordingly, a direct biopsy of the middle (about 10 cm proximal to the cardia) and distal esophageal muscle (about at the distal sphincter) inside the submucosal tunnel was performed, and the samples were examined for the number of eosinophilic cells separately as well. All the biopsies were placed into separate jars.

Postoperatively, if patients had no complaints and had benign clinical examination, they were given trial of clear liquid diet and were discharged home in the next day. If chest or abdominal pain persisted in the next day, an esophagogram with gastrografin was performed. If the results were normal, the patient would trial a liquid diet prior to discharge. Patients were followed up at 1 month and 6 months after POEM.

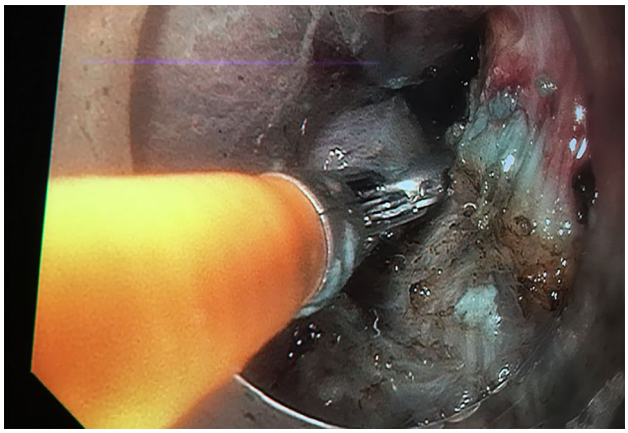

Fig. 1 Direct biopsy (via cold forceps) of the distal esophageal sphincter

Outcome Measures

Technical success was defined as the successful completion of the POEM procedure along with biopsy from the muscular and mucosal layer with cold biopsy forceps. Clinical success was defined as the Eckardt score <3 after the POEM procedure. All biopsy specimens (both from mucosa and muscular layer) were analyzed to evaluate and quantify eosinophils within the biopsy specimen. Adverse events were also recorded in the periprocedure period.

Histopathology and Quantification of Eosinophils

Biopsy specimens of the muscular and mucosal layers were placed in processing cassettes, immersed in formalin, dehydrated through a serial alcohol gradient, and embedded in paraffin wax blocks before slicing into pieces on glass slides. The specimens were routinely stained with hematoxylin and eosin (H&E).

The number of eosinophils per high-power field was examined and counted by light microscopy (Olympus BX 40 America, Melville, NY, USA).

The quantification of eosinophils value was averaged by two fields with the highest concentration of eosinophil infiltration. Esophageal eosinophils infiltration was defined as >15 eosinophils/HPF. Slides of the esophageal tissues from the patients were reviewed independently by two pathologists who were blinded to the clinical information. Any inconsistency of opinions would be decided jointly after discussion by both of them.

Statistical Analysis

Continuous variables were expressed by mean values ± standard deviation (range), and categorical variables were represented by the number of cases (ratio %). Results for continuous variables were evaluated with a two-sample independent t test or Wilcoxon rank-sum test for comparisons, and results for categorical variables were evaluated with Fisher's exact test for comparisons.

Statistical analysis was performed by a statistician blinded to the clinical outcomes with the SPSS 24.0 software (SPSS Inc., Chicago, IL) or Stata v15.1 software. Probability values <0.05 were considered significant.

Results

Demographic and Clinical Characteristics of Patients with Achalasia

Twenty consecutive patients in the study period (65% female, mean age 60 years, age range: 21–74 years) were enrolled. The duration of achalasia ranged from 8 months to 32 years. Of these 20 patients, one patient had type I achalasia, nine patients had type II achalasia, three patients had type III achalasia, one patient had esophagogastric junction outflow obstruction (EGJOO), five patients could not complete manometry but had assumed achalasia based on clinical history and findings on barium swallow, and one additional patient had nutcracker esophagus but was also included based on its similar documented association with eosinophilic invasion. Prior to POEM, all patients had Eckardt scores greater than 6, with a mean of 8.55. None had any documented infectious disease or any prior diagnosis of eosinophilic esophagitis (Table 1).

Seven patients (35%) had prior botulinum injection, 13 patients (65%) had prior through the wire balloon dilation, three patients (15%) had prior Heller's myotomy, one patient (5%) had prior POEM, and two patients (10%) had G-tube. None of the interventions were performed within 6 months before the POEM procedure (Table 1).

The POEM was performed successfully in all the patients with a technical success rate of 100%. Overall, 18 of the 20 patients had a significant symptom improvement after POEM with an Eckardt score of less than 3. At 6-month follow-up, the clinical success rate was 90%.

POEM and Biopsy Safety

The average length of myotomy was 7.8 (range 2 to 14) cm, and the average operation time was 48 min (range 33 to 73) min. The average length of hospital stay was 1.65 (range 1 to 7) (Table 2). Endoscopic examination did not reveal any signs of eosinophilic esophagitis. Endoscopic muscular layer and mucosal biopsies could be obtained in all 20 patients with cold forceps biopsy without any difficulties. There were no bleeding, mucosal injuries, or postoperative fistulas in

Table 1 Baseline characteristics

Number of patients	20	
Gender (F/M)	13:7	65%:35%
Age, years (mean ± SD, range)	60.26	(21–74)
Symptom duration, years (mean ± SD, range)	7.75	(0.67–32)
Race (N, %)		
African-American	4	20%
Native American	1	5%
Asian	1	5%
Caucasian	13	65%
Unknown	1	5%
Manometry (N, %)		
Achalasia type I	1	5%
Achalasia type II	9	45%
Achalasia type III	3	15%
Incomplete manometry (cannot bear) and diagnosed with barium swallow or EndoFLIP	5	25%
EGJOO manometry	1	5%
Nut cracker	1	5%
Prior therapy		
Botox injection	7	35%
Balloon dilation	13	65%
Heller	3	15%
POEM	1	5%
G-Tube	2	10%
BMI (kg/m2) before (mean ± SD, range)	28.5 ± 7.63	(17.0–41.9)
BMI (kg/m2) after (mean ± SD, range)	28.9 ± 7.53	(17.0–43.2)

F Female, *M* male, *SD* standard deviation, *N* number, *G-tube* gastric feeding tube

Table 2 Intraoperative and postoperative clinical outcomes

Procedure time (minutes)	48 ± 9.73	(33–73)
Length of hospital stay (days)	1.65 ± 1.42	(1–7)
Myotomy length (mean ± SD)	7.8 ± 2.79	(2–14)
Post-procedure Eckardt scores (mean ± SD, range) *	2.68 ± 2.30	(0–6)
Pre-procedure Eckardt scores (mean ± SD, range)	8.55 ± 1.53	(6–11)

SD: standard deviation

*Compared with pre-procedure Eckardt scores, $P = 0.00$

the submucosal tunnel. No intraoperative and postoperative complications were noted due to muscle biopsy (Fig. 1).

Histologic Evaluation of Esophageal Biopsies

Pathologic examination of biopsies revealed eosinophils infiltration in 3/20 (15%) of patients in the distal esophageal mucosa (all less than 15 eosinophils/HPF) with no evidence of eosinophils infiltration in the proximal esophageal mucosa. Those three patients who had a few eosinophils in the distal esophageal mucosa reported reflux symptoms and had no endoscopic stigmata of EoE. There was no evidence of eosinophilic infiltration in the distal esophageal sphincter and the middle esophageal muscle biopsies (Fig. 2). Pathologists were not informed about the study.

Discussion

Previous histologic studies of the muscular layer of the esophagus have been limited due to the inability to access the deeper layers underneath the mucosa and submucosa during routine endoscopy. However, through the creation of a submucosal tunnel, POEM exposes the esophageal muscle, making biopsy of the muscle feasible. To our knowledge, this is the first study of simultaneous biopsy of the esophageal mucosa and direct muscle biopsy in patients with idiopathic achalasia through submucosal tunneling during POEM procedure. Our study demonstrates that direct biopsy during POEM is a feasible and safe way to evaluate the histopathological changes of mucosal and muscular layer intuitively. The direct biopsy of both the distal and proximal esophageal muscle was 100% successful without adverse events.

The interaction between achalasia and eosinophilic esophagitis is an unknown topic. The mechanism that eosinophilia infection could cause achalasia is unknown. Very few retrospective studies reported evidence of esophageal eosinophils and/or degranulation products infiltrating in the esophageal muscle in patients with achalasia [5–7]. In a study with 96 patients with achalasia, eosinophilia was evaluated in distal and mid-esophageal biopsy specimens obtained before and after laparoscopic Heller myotomy, and esophageal eosinophilic infiltration was presented in a significant number of operated subjects [14]. Another study indicated that in primary esophageal dysmotility, esophageal eosinophilic infiltration was shown in the muscle layer, but not in the epithelial layer [15].

Since those limited studies illustrated the eosinophil infiltration in patients with achalasia presented predominantly in the muscle layer, researchers proposed the hypothesis that achalasia might develop from a muscle-predominant subtype of eosinophilic esophagitis (EoE) [8]. A more recent study found dense eosinophilic numbers existed in the muscular layer, but not in the mucosa, in patients with jackhammer and nutcracker esophagus [15, 16]. In another study conducted by Jin H. et al., esophageal mucosa and muscle samples were obtained during POEM in 28 patients with achalasia. Eosinophils infiltrated into the muscularis externa in 24 cases (85.7%) and into the muscularis propria in eight

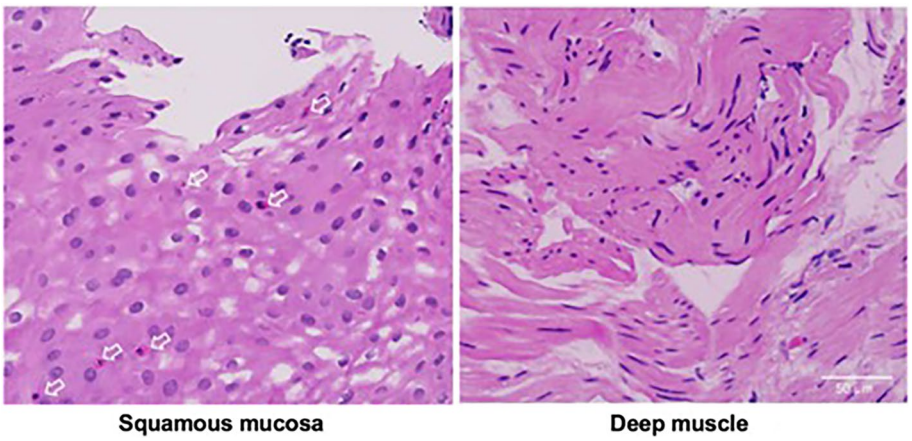

Fig. 2 No eosinophils infiltration in the distal esophageal sphincter nor the middle esophageal muscle biopsies: eosinophils present in squamous epithelium (<15 Eosinophils/HPF), but not in deep muscle (eosinophils are marked by the arrow)

cases (28.6%), and degranulation products of eosinophils such as eosinophil major basic protein and eosinophil-derived neurotoxin were detected in 24 cases (86%) [17]. However, these studies did not examine mucosal eosinophilic infiltration.

There are several pieces of evidence suggesting EoE could induce achalasia-like disorder by releasing multiple cytokines and cationic proteins affecting the contractile function of esophageal smooth muscle [18–24]. Moreover, a few studies have reported that steroids treatments aiming at normalizing eosinophil numbers have corrected esophageal dysmotility symptoms in EoE patients. However, idiopathic achalasia responds poorly to steroids treatments [15, 23, 24]. The mechanism for idiopathic esophageal achalasia is still unclear. In our study, we simultaneously obtained esophageal mucosal (including proximal and distal esophagus) and muscle tissue (including middle and distal esophagus) from all the patients during POEM. Histologic examination did not reveal any eosinophils infiltration in the esophageal muscle layer, so an association of esophageal eosinophilic infiltration and achalasia was not observed in this study. There are several potential mechanisms that might explain the discrepancy of our results from previous studies. First, different biopsy methods may yield different results. However, biopsy of the muscular layer is usually not feasible in routine endoscopic procedures. We biopsied the muscle directly at POEM and presented reliable sample for pathologic examination. Second, the difference in the study population may contribute to the difference in the result. It was reported that PPIs inhibit esophageal epithelial cells from releasing eosinophil chemoattractant (eotaxin-3) in some in vitro studies [18–21]. However, we did observe some eosinophilic cells in the distal mucosa.

There were a few limitations in the study: The number of patients was small, one patient had nutcracker esophagus, and five patients did not have esophageal manometry to confirm the diagnosis of achalasia. And also, since the prior study showed a huge difference in the percent of eosinophilia infiltration in achalasia (18–24), we had difficult time to use a reference to calculate the sample size. Although the limitation of our study was the relatively small number of subjects, our results showed that biopsy of the esophageal muscle at POEM was 100% success and safe. Our study did not show any eosinophilic cells in the esophageal muscle in any patients; therefore, we concluded that there is very weak, if any, association between eosinophils infiltration in the esophageal muscle and achalasia. Rather, immune, autoimmune causes may be more likely the mechanism for idiopathic achalasia.

In conclusion, submucosal tunneling during POEM provides safe access for direct esophageal muscle biopsy. This is the first report of the simultaneous biopsy of the esophageal mucosa and muscle in patients with idiopathic achalasia. In contrast to all previously published studies, the association of esophageal eosinophils infiltration and achalasia was not observed in this study. Based on our findings in this small study, immune or autoimmune reaction rather than direct eosinophilic infiltration in the muscle is more likely the cause of achalasia. Further multicenter studies with large sample size are needed to confirm the finding.

Key Points

1. To our knowledge, this is the first study to perform simultaneous biopsy of the esophageal mucosa and direct esophageal muscle in patients with idiopathic achalasia through submucosal tunneling during a POEM procedure. This study demonstrates that muscle biopsy during POEM is a safe way to directly evaluate the histopathological changes of esophageal muscle and mucosa.
2. The relationship between achalasia and eosinophilic infiltration is unknown. Though its association was suggested in previous publications, it was not demonstrated in our study. Therefore, it is more likely that immune

or autoimmune processes are responsible for achalasia rather than direct eosinophilic infiltration.

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Compliance with Ethical Standards

Conflicts of interest Qiang Cai, MD, PhD, FACG, is a guarantor of the article. Specific author contributions: Huimin Chen, MD, PhD, searched the literature and wrote the original draft; Lucie F. Calderon, MD, Rushikesh Shah, MD, Wei Zheng, MD, Yue Xue, MD, and Steve Keilin, MD, searched the endoscopic photographs, revised the manuscript, and approved the final draft. Liang Xia, MD, PhD, Wei Wang, MD, Lianyong Li, MD, PhD, Baiwen Li, MD, PhD, and Steve Keilin, MD, revised the original draft and approved the final draft. Qiang Cai, MD, PhD, FACG, designed the concept and format of the article, provided endoscopic photographs, and revised and approved the final draft.

Informed consent Informed consent was obtained from the patients for the publication of their information and imaging.

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