

ORIGINAL ARTICLE

Distinguishing gastroesophageal reflux disease and eosinophilic esophagitis in adults: The role of esophageal mucosal immunoglobulin G4

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Key words

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Abstract

Background and Aim: Eosinophilic esophagitis (EoE) and gastroesophageal reflux disease (GERD) can be difficult to distinguish as many of their clinical and histological features overlap. Preliminary data suggest a potential association between EoE and immunoglobulin G4 (IgG4) but not GERD. This study aimed to examine the role of esophageal mucosal IgG4 staining when differentiating EoE from GERD.

Methods: Esophageal biopsy specimens from patients with proven EoE and GERD were evaluated, and immunohistochemical staining for IgG4 was performed by an experienced gastrointestinal pathologist blinded to the clinical and endoscopic data. The results on IgG4 staining were then correlated with clinical, endoscopic, and histological features.

Results: Sixty patients were included in the study, with 30 EoE (38.8 ± 12.8 years, 23 M:7 F) and 30 GERD (50.7 ± 14.3 years, 14 M:16 F) patients. The prevalence of a positive intercellular IgG4 stain was significantly higher in the EoE patients than those with GERD (23/29 vs 2/30; $P < 0.0001$). Positive IgG4 stain had the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 77%, 93%, 92%, and 80% for predicting the diagnosis of EoE, respectively. In both EoE and GERD patients, correlation was found between positive IgG4 staining and food bolus obstruction, dysphagia to solids, reflux, fixed rings, Barrett's esophagus, hiatus hernia, and esophagitis. In EoE patients, positive IgG4 staining was not correlated with the type of symptoms, endoscopic findings, histological findings, proton pump inhibitor therapy, or history of allergy/atopy.

Conclusion: Given the high specificity and PPV of positive IgG4 staining in esophageal biopsies for EoE, this can be a useful marker to distinguish the disease from GERD.

Introduction

Eosinophilic esophagitis (EoE) is a clinicopathological condition characterized by an antigen-driven immunologic process that manifests clinically with symptoms of esophageal dysfunction and histologically with eosinophilic inflammation.^{1,2} According to the EoE diagnostic criteria, other diseases associated with esophageal eosinophilia must be excluded before a diagnosis of EoE can be made, with the main differential being gastroesophageal reflux disease (GERD).^{1,3,4} It is important to distinguish between EoE and GERD as their pathogenesis, natural history, monitoring, and treatment differ.⁵ This can be challenging as many of their clinical and histological features overlap.^{5,6} Given that the prevalence of GERD in the general population is approximately 20%, it is inevitable that there will be a high probability for EoE and GERD to coexist.⁶

The exact pathophysiology of EoE is not fully comprehended.^{7–9} Significant evidence shows that EoE is an allergen

(T helper type 2 [Th2] cell)-mediated response.⁹ This response was previously thought to have been triggered by antigen-specific immunoglobulin E (IgE) as 50–75% of EoE patients are atopic.^{9,10} However, this conclusion has been questioned after a study showed that omalizumab (an anti-IgE antibody) failed to improve symptoms or esophageal eosinophilic counts in patients with EoE.¹¹ This finding was further supported by the discovery that there was a 45-fold increase of immunoglobulin G4 (IgG4) in esophageal tissue, as well as serum levels of IgG4, that appeared to react to specific foods, suggesting that EoE is an IgG4-associated and not an IgE-induced allergy.¹¹ Subsequently, Zuberbeg et al. showed that immunohistochemical staining of esophageal tissue with IgG4 could help distinguish EoE from GERD, given that 76% of EoE cases were positive for intrasquamous IgG4, and none of the GERD cases were positive.¹² The aim of this study was to examine the role of esophageal mucosal IgG4 staining in differentiating EoE from GERD.

Methods

This study is a retrospective review of prospectively collected databases of patients who were referred to the Department of Gastroenterology and Hepatology at the Royal Adelaide Hospital for assessment and treatment of EoE and GERD over a 3-year period. Our department is the largest tertiary referral hospital for these two disorders in South Australia. Consecutive patients with either EoE or GERD who fulfilled the inclusion and exclusion criteria during this period were included in the study until the target number was reached. Inclusion criteria for patients with GERD were: 18–80 years of age, typical symptoms of GERD responsive to proton pump inhibitor (PPI) therapy, evidence of esophagitis on endoscopy with supportive esophageal biopsy specimens, and eosinophil count <10/hpf. Inclusion criteria for patients with EoE were: 18–80 years of age, symptoms of esophageal dysfunction, and ≥ 15 eosinophils/hpf. Exclusion criteria were history of severe respiratory; cardiovascular, hepatic, hematological, and/or renal disease; chronic alcohol abuse; medications that may influence gastrointestinal function; previous gastrointestinal surgery; and other cause of eosinophilia. This study was approved by the Royal Adelaide Hospital Research Ethics Committee (reference number: HREC/17/RAH/376).

Protocol. Our unit has prospectively collected electronic databases on all patients who were referred for assessment and treatment of EoE and GERD as part of ongoing clinical trials and audits in these areas. These databases have records of patient demographics, clinical presentation, medications, past medical history, investigations, and treatment that were originally extracted from both paper and electronic medical records. Similarly, endoscopic and histological data were linked to the databases via an electronic system. From these databases, 30 consecutive EoE and GERD patients who fulfilled the inclusion/exclusion criteria were included in the study. Tissue specimens from esophageal mucosal biopsies of all patients were then retrieved and prospectively stained for IgG4. The slides were reviewed by an independent experienced gastrointestinal pathologist blinded to the clinical and endoscopic data.

Assessment of esophageal mucosal IgG4. The presence of an esophageal mucosal IgG4 stain was assessed using an automated immunohistochemistry technique through the Ventana BenchMark Ultra platform and the commercially available mouse IgG4 monoclonal antibody (Cell Marque, MRQ-44). Sections of paraffin wax-embedded tissue (4 μ m thin) were mounted on coated slides, dewaxed, and rehydrated using standard techniques. Antigen retrieval was performed according to the Ventana protocol. Appropriate negative controls were performed for each batch of slides.

IgG4 immunohistochemistry was scored positive when a strong signal was present in the intercellular spaces of the esophageal squamous-lined mucosa. Weak and focal staining or a complete absence of signals between squamous cells was recorded as a negative test result. Weak staining was defined as a very low strength of signal generated by the detection system, which was difficult or impossible to distinguish from artefactual background staining. Focal staining was defined as staining present in intercellular spaces in less than 2% of squamous cells present in the biopsy sample.

Definitions. Dysphagia was defined as difficulty in swallowing solid food. Food bolus obstruction was defined as a food bolus requiring endoscopic removal. Typical reflux symptoms were defined as heartburn, regurgitation, and/or epigastric pain. Dysphagia to solids was an accepted symptom for GERD patients provided it was also associated with one or more of the typical reflux symptoms as previously detailed. History of allergy/atopy included asthma, hay fever, and food allergy.

Statistical analysis. Based on the data published by Zukerberg *et al.*,¹² a sample size of 30 cases (15 EoE and 15 GERD) was required to achieve a power of 95% and α of 0.001. Data were expressed as mean \pm SEM, assessed for normality. Binary outcomes were compared using appropriate statistical techniques (Fisher's exact test). A *P* value of <0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism 8[®].

Results

Sixty patients were included in the study, with 30 EoE and 30 GERD cases. The patients with GERD were older with almost equal gender representation, compared to the younger, male-predominant EoE patients. Other demographics and clinical characteristics of the two groups are summarized in Table 1.

The prevalence of a positive intercellular IgG4 stain was significantly higher in EoE patients than those with GERD (23/30 vs 2/30; *P* < 0.0001, Fig. 1). A positive IgG4 stain had

Table 1 Demographics and clinical characteristics of all EoE and GERD patients

	EoE (<i>n</i> = 30)	GERD (<i>n</i> = 30)
Mean age (years)	38.8 \pm 12.8	50.7 \pm 14.3
Gender	23 M:7 F	14 M:16 F
Symptoms		
Food bolus obstruction	25	2
Dysphagia to solids	24	10
Reflux symptoms	5	26
Histological findings		
Elongated papillae	12	16
Eosinophilic microabscesses	4	0
Mucosal edema	10	11
Basal cell hyperplasia	20	24
Eosinophil count/hpf (range)	16–50	0–13
Endoscopic findings		
Fixed rings	20	2
White plaques	8	1
Longitudinal furrows	18	2
Stricture	5	2
Barrett's esophagus	0	6
Hiatus hernia	5	17
Esophagitis	3	30
Medications		
Proton pump inhibitor (PPI)	12	10
History of allergy/atopy	10	4

EoE, eosinophilic esophagitis.

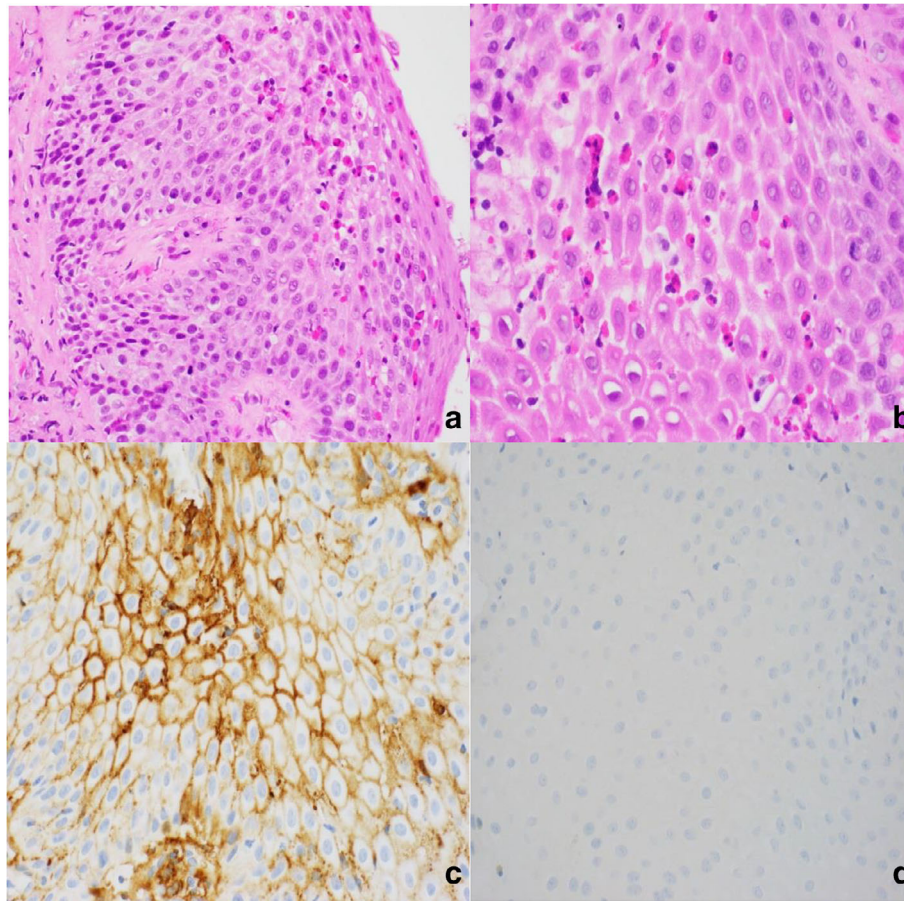


Figure 1 (a) EoE. (b) EoE with intercellular edema. (c) EoE with positive IgG4. (d) EoE with negative IgG4. EoE, eosinophilic esophagitis; IgG4, immunoglobulin G4.

sensitivity, specificity, PPV, and NPV of 77%, 93%, 92%, and 80% for predicting the diagnosis of EoE, respectively.

A statistically significant correlation was found between positive esophageal IgG4 staining with food bolus obstruction, dysphagia

to solids, and fixed rings. No correlation was found between positive esophageal IgG4 staining with elongated papillae, eosinophilic microabscesses, basal cell hyperplasia, white plaques, longitudinal furrows, or the presence of a stricture. (Table 2).

Table 2 Correlation of esophageal IgG4 staining with clinical and endoscopic characteristics in EoE and GERD patients ($n = 60$)

	Present in IgG4 positive	Present in IgG4 negative	<i>P</i> value
Symptoms			
Food bolus obstruction	18/25 (72%)	10/35 (27%)	0.0015
Dysphagia to solids	20/25 (80%)	12/35 (34%)	0.0006
Histological findings			
Elongated papillae	11/25 (44%)	16/35 (46%)	>0.999
Eosinophilic microabscesses	4/25 (16%)	0/35 (0%)	0.1217
Basal cell hyperplasia	16/25 (64%)	27/35 (77%)	0.8004
Endoscopic findings			
Fixed rings	16/25 (64%)	5/35 (14%)	0.0003
White plaques	5/25 (20%)	3/35 (9%)	0.4697
Longitudinal furrows	12/25 (48%)	6/35 (17%)	0.0546
Stricture	3/25 (12%)	2/35 (6%)	0.5650

EoE, eosinophilic esophagitis; GERD, gastroesophageal reflux disease.

Discussion

To our knowledge, the current study is the largest to date examining the prevalence of IgG4-positive stains in patients with EoE and GERD. Although we confirm that IgG4 stain is significantly more prevalent in EoE than GERD, the specificity is not 100% and is consistent with most previous studies.^{11–16} In the current study, less than 10% of GERD patients had a positive IgG4 stain, and up to a quarter of EoE patients had a negative IgG4 stain. Overall, our study suggests that the use of IgG4 stain has a positive predictive value of 92% for distinguishing EoE from GERD, which can be valuable in the clinical assessment of undifferentiated presentation.

The exact role that IgG4 plays in the pathogenesis of EoE is yet uncertain, and caution has been suggested in shifting the focus too early away from IgE.¹⁷ Similarities have been noted between EoE- and IgG4-related disorders (IgG4-RD), such as the development of submucosal fibrosis.¹³ However, obliterative phlebitis, which is often seen in IgG4-RD, is not seen in EoE.¹³ Other similarities are responsiveness to steroids; a predilection to males; and an association with atopy, eosinophilic infiltration, IgG4 plasma cells, and granular IgG4 deposits.¹⁴ IgG4 levels in EoE, however, are lower and more localized than in IgG4-RD, potentially due to a smaller affected tissue compartment.¹⁴ Thus, EoE is hypothesized to be associated with IgG4 and not related to IgG4.¹⁴

We observed that IgG4 staining was able to distinguish between EoE and GERD with a moderate sensitivity of 77% and a high specificity of 93%. This is similar to a study that showed a sensitivity and specificity of 88% and 100%, respectively.¹² Only one study to date has shown that IgG4 staining had a poor sensitivity of 48% for diagnosing EoE; however, the specificity remained high at 100%.¹⁵ Serum IgG4 levels and local IgG4 plasma cells expression were found to be elevated in EoE compared to GERD and reduced with topical steroid therapy, suggesting that IgG4 may be a marker of disease activity.¹⁴ It is important to distinguish between EoE and GERD as their pathogenesis, natural history, monitoring, and treatment differ.⁵ This can be challenging as many of their clinical and histological features overlap.^{5,6} Our results suggest that IgG4 staining can be used as an adjunct to help differentiate between EoE and GERD as previously proposed.¹⁴

This is the first study to our knowledge that has shown positive IgG4 staining in the GERD cohort (7% [2/30]). These two patients have been confirmed, on repeat examination of their medical records, to not meet criteria for a diagnosis of EoE. Both were females in their 50s who presented with dysphagia to solids and reflux. Only one was on PPI therapy at the time of biopsy but had had a previous esophageal biopsy off treatment, which did not show any eosinophils. All esophageal biopsy specimens from these patients showed occasional (<6/hpf) eosinophils only. Both had a history of asthma, which could explain this result as IgG4 reactivity can be falsely positive in atopic individuals.¹⁷

Nearly a quarter of our EoE patients (7/30) were negative for IgG4, and only two of these patients were on PPI therapy at the time of esophageal biopsy. In both cases, there was still active inflammation, with eosinophil counts of greater than 20/hpf. Interestingly, 26% (6/23) of IgG4-positive EoE patients did not have positive stains in all esophageal biopsy specimens. This may reflect the patchy disposition of the EoE disease process and was observed in a previous pediatric study.¹⁵ This highlights the importance of obtaining sufficient esophageal biopsies along the whole length of

the esophagus to maximize the diagnostic yield. The most recent EoE consensus suggests two to four mucosal biopsies of the proximal and distal esophagus.¹ Gonsalves *et al.* reported a diagnostic sensitivity of 55% with one esophageal biopsy, which increased to 100% with five esophageal biopsies.¹⁸

Our results were supportive of a correlation between positive IgG4 staining with food bolus obstruction, dysphagia to solids, and fixed rings. However, no correlation was found between positive IgG4 staining with elongated papillae, eosinophilic microabscesses, basal cell hyperplasia, white plaques, longitudinal furrows, or the presence of a stricture. Little data currently exist for comparison. A study using a cohort of both adults and children with EoE showed a strong association between distal IgG4 staining and basal zone hyperplasia (P 0.003).¹⁵ Pediatric EoE patients with active esophagitis have been shown to be associated with increased levels of IgG4-positive plasma cells, particularly in those with a food allergy.¹³ Esophageal IgG4 levels in children have also been found to correlate with peak eosinophil count; mean histologic grade; and esophageal IL4, IL13, and IL10 and had strong associations with a subset of the EoE transcriptome.¹⁶ As our study cohort consists purely of adults, comparisons with the aforementioned studies may not be appropriate as the EoE disease process has been shown to be different in adults and children, with progression from an inflammatory to a fibrostenotic phenotype.^{19,20}

Although a limitation of our study is its retrospective nature, cases were included from a pre-existing database of EoE and GERD patients selected based on strict criteria listed above. The paper and electronic medical records of these cases were also examined to ensure that the inclusion criteria were fulfilled.

Conclusion

In conclusion, the prevalence of positive IgG4 staining in esophageal biopsy specimens of EoE patients is significantly higher than GERD and can be used as an adjunct to help differentiate between the two entities. More studies are required to determine the exact role of IgG4 in the pathogenesis and treatment of EoE.

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