A Comprehensive Assessment of Eosinophilic Oesophagitis

By

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A dissertation submitted to The University of Adelaide

In candidature for the degree of

Doctor of Philosophy in the Faculty of Health and Medical Sciences

May 2023

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PUBLICATION CITATIONS

Abstracts and poster presentations

1. Wong S, Ellison S, Haj Ali S, Hawkes J, Collinson J, O'Neill T, Ruszkiewicz A, Moore D, Holloway RH, Nguyen NQ. Natural history of eosinophilic esophagitis in children and adults. (2018), Luminal Clinical. Journal of Gastroenterology and Hepatology, 33: 117-138.
2. Wong S, Nguyen NQ, Ruszkiewicz A. Can IgG4 be Used as an Adjunctive Marker in the Diagnosis of Eosinophilic Oesophagitis? Gastroenterology May 2019, Volume 156, Issue 6, S-720.
3. Wong S, Ellison S, Hawkes J, Ruszkiewicz A, Holloway RH, Moore D, Nguyen NQ. Eosinophilic Oesophagitis in Children and Adults: Is the Bimodal Presentation a Reflection of Different Stages of the Same Disease? Gastroenterology May 2019, Volume 156, Issue 6, S-720.
4. Wong S, Tippett M, Safaeian R, Ruszkieicz A, Nguyen NQ. A Comprehensive Assessment of Eosinophilic Oesophagitis Using High-Resolution Manometry, Mucosal Biopsy and Endosocpic Ultrasound in Adults. Gastroenterology May 2019, Volume 156, Issue 6, S-719.

Journal publications

1. Wong S, Ruszkiewicz A, Holloway RH, Nguyen NQ. Gastro-oesophageal reflux disease and eosinophilic oesophagitis: What is the relationship? World J Gastrointest Pathophysiol. 2018 Oct 25;9(3):63-72. doi: 10.4291/wjgp.v9.i3.63. PMID: 30386667; PMCID: PMC6209579.
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ABBREVIATIONS USED

As this thesis is based on published works, a range of editorial requirements including British English or American English spelling had to be met.

EoE	eosinophilic oesophagitis/eosinophilic esophagitis
GORD/GERD	gastro-oesophageal reflux disease/gastro-esophageal reflux disease
IgG4	immunoglobulin G4
PPV	positive predictive value
NPV	negative predictive value
EUS	endoscopic ultrasound
PPI(s)	proton-pump inhibitor(s)
PPI-REE	proton-pump inhibitor responsive oesophageal eosinophilia
LOS	lower oesophageal sphincter
TLOSRs	transient lower oesophageal sphincter relaxations
Th1	T-helper type 1
Th2	T-helper type 2
IL	interleukin
TSLP	thymic stromal lymphopoetin
/hpf	per high powered field
H_2RA	histamine-receptor antagonist
IgE	immunoglobulin E
IgG4-RD	IgG4-related disorders
BMI	body mass index
HRM	high resolution manometry
GOJ/GEJ	gastro-oesophageal junction/gastro-esophageal junction

EREFS	EoE Endoscopic Reference Score
FLIP	Endoluminal Functional Imaging System

ABSTRACT

Introduction

Eosinophilic oesophagitis (EoE) is an uncommon disease that has recently been experiencing an increase in incidence and prevalence. Due to the similarities, it is challenging to distinguish EoE from its primary differential diagnosis, gastro-oesophageal reflux disease (GORD). The lack of a diagnostic adjunct further compounds this issue, although immunoglobulin G4 (IgG4) staining has recently shown some potential. The onset of disease has been established in both children and adults, but there is limited understanding regarding the natural history of this condition. Furthermore, an incomplete comprehension of EoE pathogenesis has led to uncertainty regarding the correlation between its clinical and endoscopic variables.

Aims

This thesis aimed to:

1. Evaluate the role of oesophageal mucosal IgG4 staining as a diagnostic adjunct for EoE.
2. Investigate the natural history of childhood-onset and adult-onset EoE.
3. Characterise and differentiate the oesophageal endoscopic appearance, wall thickness, histology, and motility in adults with EoE and GORD.
4. Identify markers of disease progression in EoE.

Methods

A literature review was performed, highlighting the differences and potential relationship between EoE and GORD. Subsequently, a retrospective analysis was completed examining the role of oesophageal mucosal IgG4 staining in differentiating EoE from GORD. Next, a cross-sectional, questionnaire-based study compared the characteristics and disease progression between childhood-onset and adult-onset EoE. Following this, a prospective, interventional study characterising and comparing the anatomy, histology, and motor function in EoE and GORD subjects was accomplished. Finally, a longitudinal study was carried out to assess the potential changes in the oesophageal wall of EoE subjects.

Results

The prevalence of positive IgG4 stain was higher in the EoE compared to GORD with high sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Most of those with childhood-onset and adult-onset EoE were found to have a long duration of symptoms before and after the diagnosis despite treatment. Childhood-onset EoE was progressive from childhood to adulthood but was associated with more inflammatory-type symptoms post transition. Although total oesophageal wall thickness was comparable between EoE and GORD, the thickness of the submucosa in the distal oesophagus was higher in EoE. Positive correlations were found between dysphagia score and distal total oesophageal wall thickness, as well as disease duration and distal submucosal thickness only in EoE. Lastly, distal total oesophageal wall thickness increased over time in EoE independent of dysphagia score and eosinophil count.

Conclusions

IgG4 staining in oesophageal biopsies was a valuable marker for distinguishing EoE from GORD. EoE appears to be a progressive, chronic disease, the onset of which may occur in childhood or adulthood. Inflammatory-type symptoms persisted in those with childhood-onset EoE. Distal oesophageal wall thickness correlates positively with dysphagia score in EoE but not GORD due to the composition of the submucosa, which is identifiable via endoscopic ultrasound (EUS). Distal total oesophageal wall thickness increased with time in EoE, but this was not associated with a change in dysphagia or eosinophil count.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree of diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Stephanie Siau Ping Wong

May 2023

ACKNOWLEDGMENTS

Firstly, I would like to sincerely thank my supervisors Associate Professor Andrew Ruszkiewicz and Professor Nam Quoc Nguyen for their expertise and support in helping me complete this degree.

I would like to acknowledge all the doctors, nurses, clerical staff and research assistants of The Department of Gastroenterology and Hepatology, Royal Adelaide Hospital as well as all the co-authors of the manuscripts for their assistance and contributions in completing the studies in this thesis. Special mention to Professor Richard Holloway, Dr Vinh-An Phan, Dr Tsai-Wing Ow, Dr Amanda Lim, Mr Marcus Tippett, Ms Romina Safaeian and Mr Joshua Zobel.

I will be forever grateful to the patients who selflessly gave up their time to participate in the studies.

Last, but certainly not least, I would like to thank my husband Dr Kian Lim, family, and friends for their unwavering encouragement and support.

CHAPTER 1: THESIS OUTLINE

This thesis is mainly based on published research works that were conducted and completed during the candidature.

Chapter 2 includes a literature review article highlighting the differences and potential relationship between EoE and GORD [Manuscript: Wong *et al*. "Gastro-oesophageal reflux disease and eosinophilic oesophagitis: What is the relationship?", published in *World Journal of Gastrointestinal Pathophysiology*, 2018].

Chapter 3 describes a retrospective analysis examining the role of oesophageal mucosal immunoglobulin G4 (IgG4) staining in differentiating EoE from GORD [Manuscript: Wong *at al*. "Distinguishing gastroesophageal reflux disease and eosinophilic esophagitis in adults: The role of esophageal mucosal immunoglobulin G4", published in *Journal of Gastroenterology and Hepatology Open*, 2020]. The techniques used in this study include extraction of clinical data from medical records, histological evaluation of biopsy specimens, and immunohistochemistry staining for IgG4.

Chapter 4 is a study assessing the natural history of EoE. It is a cross-sectional, questionnaire-based study comparing the characteristics and disease progression between childhood-onset and adult-onset EoE [Manuscript: Wong *et al*. "Characteristics and progression of childhood-onset and adult-onset eosinophilic esophagitis", published in *Journal of Gastroenterology and Hepatology*, 2021]. The technique used in this study is the development of a questionnaire.

Chapter 5 contains a prospective, interventional study characterising and comparing the anatomy, histology, and motor function in EoE and GORD subjects [Manuscript: Wong *et al*. "Distal esophageal wall thickness correlates with dysphagia in adult patients with eosinophilic esophagitis", published in *Esophagus*, 2022]. The techniques used in this study incorporate upper gastrointestinal endoscopy, endoscopic ultrasound, histological evaluation of biopsy specimens, and high-resolution manometry.

Chapter 6 is a longitudinal study exploring the alterations in the oesophageal wall of EoE subjects [Manuscript: Wong *et al*. "Increase in distal esophageal wall thickness with time in adult patients with eosinophilic esophagitis", published in *Journal of Gastroenterology and Hepatology Open*, 2023]. The techniques used in this study include upper gastrointestinal endoscopy, endoscopic ultrasound, and histological evaluation of biopsy specimens.

Chapter 7 examines the conclusions of the thesis, its impact on current clinical management and considers future research directions.

1.1 Aims and objectives

The overarching aims of this thesis were to evaluate the natural history of EoE and to comprehensively characterise and differentiate EoE and GORD.

Research objectives:

1. To evaluate the role of oesophageal mucosal IgG4 staining in differentiating EoE from GORD.
2. To explore the natural history of childhood-onset and adult-onset EoE and elucidate any differences.
3. To compare the dysphagia score, oesophageal wall thickness, oesophageal mucosal histology, and oesophageal motility between EoE and GORD.
4. To highlight any correlation between dysphagia score, oesophageal wall thickness, oesophageal mucosal histology, and oesophageal motility in EoE and GORD.
5. To assess for any markers of disease progression in adults with EoE.

CHAPTER 2: LITERATURE REVIEW

2.1 Overview

This chapter contains a published literature review article that describes the current definition and initial recognition of EoE. It outlines the differentiating factors of EoE and GORD regarding pathogenesis, epidemiology, clinical presentation, diagnosis, and treatment. The final section discusses proposed hypotheses to explain the relationship between EoE and GORD. This literature review provided an in-depth understanding of the current difficulties clinicians face in distinguishing the two disease processes and allowed us to form a large proportion of our research objectives.

2.2 Specific Author Contributions

Statement of Authorship

Title of Paper	Gastro-oesophageal reflux disease and eosinophilic oesophagitis: What is the relationship?
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	**Wong S**, Ruszkiewicz A, Holloway RH, Nguyen NQ. Gastro-oesophageal reflux disease and eosinophilic oesophagitis: What is the relationship? World J Gastrointest Pathophysiol. 2018;9(3):63-72.

Principal Author

Name of Principal Author (Candidate)	Stephanie Wong		
Contribution to the Paper	Drafting of entire manuscript, submission of manuscript for publication		
Overall percentage (%)	85		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	11/6/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Andrew Ruszkiewicz		
Contribution to the Paper	Critical revisions of manuscript		
Signature		Date	11/6/2021

Name of Co-Author	Richard Holloway		
Contribution to the Paper	Critical revisions of manuscript		
Signature		Date	25/6/2021

Name of Co-Author	Nam Q Nguyen		
Contribution to the Paper	Critical revisions of manuscript		
Signature		Date	16/6/2021

2.3 Published work 1: Gastro-oesophageal Reflux Disease and Eosinophilic Oesophagitis: What is the Relationship?

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Running title: Relationship between reflux and eosinophilic oesophagitis.

Author contributions: Wong S contributed to drafting of manuscript; Ruszkiewicz A, Holloway R and Nguyen N contributed to critical revisions of manuscript.

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Conflict-of-interest statement: There is no conflict of interest to disclose from all authors.

Abstract

Eosinophilic oesophagitis (EoE) and gastro-oesophageal reflux disease (GORD) are the most common causes of chronic oesophagitis and dysphagia associated with oesophageal mucosal eosinophilia. Distinguishing between the two is imperative but challenging due to overlapping clinical and histological features. A diagnosis of EoE requires clinical, histological and endoscopic correlation whereas a diagnosis of GORD is mainly clinical without the need for other investigations. Both entities may exhibit oesophageal eosinophilia at a similar level making a histological distinction between them difficult. Although the term proton-pump inhibitor responsive oesophageal eosinophilia has recently been retracted from the guidelines, a relationship between EoE and GORD still exists. This relationship is complex as they may coexist, either interacting bidirectionally or are unrelated. This review aims to outline the differences and potential relationship between the two conditions, with specific focus on histology, immunology, pathogenesis and treatment.

Keywords

Relationship; Pathogenesis; Eosinophilic oesophagitis; Histological features; Gastro-oesophageal reflux disease

Core tip: The relationship between gastro-oesophageal reflux disease and eosinophilic oesophagitis is complex as they may coexist, either interacting bidirectionally or are unrelated. This review aims to outline the differences and potential relationship between the two conditions, with specific focus on histology, immunology, pathogenesis and treatment.

Introduction

Eosinophilic oesophagitis (EoE) is a clinicopathological condition characterised by an antigen-driven immunologic process that manifests clinically with symptoms of oesophageal dysfunction and histologically by eosinophilic inflammation[(1)]. The first case report of oesophageal eosinophilia can be traced back as far as 1962 by Schreiber[(2)], followed by the first published case series of EoE as a distinct clinicopathological condition in 1993 by Attwood *et al*[(3)]. In 2007, the first consensus recommendation by an international expert panel

for the diagnosis and treatment of EoE was published[4]. This consensus was recently updated in 2017[5].

The recognition of EoE has increased so swiftly that it is now thought to be the most frequent eosinophilic gastrointestinal disorder as well as the second most common cause of chronic oesophagitis and dysphagia after gastro-oesophageal reflux disease (GORD)[6]. Although it is still an uncommon disease, the prevalence has been increasing over the past few years with an estimated prevalence in the general population of 13-49 cases/100,000 persons[5, 7]. This is also in keeping with an increasing incidence of EoE estimated at 1-20 cases/100,000 persons[5, 7]. Various hypotheses have been considered for this phenomenon particularly that of an increase in the recognition of the disease and an increase in volume of endoscopies performed[8-10]. However, two population-based studies have shown that the incidence and cumulative prevalence of EoE has indeed increased more than the rate of annual endoscopies during the observation period[11, 12]. This, therefore, argues in favour of a true rise in the incidence and prevalence of the disease.

Attwood *et al*[3] first characterized EoE as a distinct entity from GORD in 1993 where patients with more than 20 eosinophils per high power field and dysphagia in the absence of endoscopic oesophagitis and a normal 24-hour pH testing were proposed to have EoE. According to the diagnostic criteria for EoE, other diseases associated with oesophageal eosinophilia must be excluded before a diagnosis of EoE is made (**Table 2.1**), with the main differential being GORD[1, 13, 14]. It is important to distinguish between EoE and GORD as their pathogenesis, natural history, monitoring, and treatment differ[15]. This is challenging as many of their clinical and histological features overlap[15, 16]. Given the prevalence of GORD in the general population is approximately 20%, it is inevitable that there will be a high probability for EoE to co-exist with GORD[16].

Prior to the 2017 consensus, a lack of response to a 2-month course of a proton-pump inhibitor (PPI) was required to exclude PPI-responsive oesophageal eosinophilia (PPI-REE) and confirm the diagnosis of EoE[1]. Patients with PPI-REE presented symptomatically like a typical EoE patient, had GORD diagnostically excluded and exhibited a clinicopathologic response to PPI therapy[1]. Recent evidence, however, indicate that differentiating PPI-REE from EoE is counterintuitive as their phenotypic, molecular, mechanistic, and therapeutic features cannot be reliably distinguished[15, 17-20]. Also, there was no definition regarding the extent of clinical and histological response required to diagnose PPI-REE[13, 15]. Thus, the most

recent consensus has retracted the term PPI-REE and considers PPI therapy as a therapeutic agent, rather than a diagnostic criterion[5]. The term 'PPI-responsive EoE' has been proposed to replace the now defunct PPI-REE[20].

Despites the fact that PPI responders are now considered to be within the EoE continuum, a relationship between EoE and GORD still exists[5]. Studies have suggested that up to 30-40% of EoE patients may be PPI responsive, either due to a reduction in acid secretion in patients with co-existent GORD or by means of other still unknown anti-inflammatory mechanisms[21, 22]. PPI therapy may also be helpful in patients with EoE as the altered oesophagus may be predisposed and more sensitive to acid exposure[23]. This review aims to outline the factors that differentiate between EoE and GORD as well as to evaluate the complex relationship between the two entities in term of pathophysiology and immunology.

Pathogenesis

The main pathogenic mechanism of GORD is increased transient lower oesophageal sphincter (LOS) relaxations (TLOSRs), leading to excessive reflux of gastric acid to the lower oesophageal mucosa[24]. Other potential mechanistic factors that can increase acid reflux to the oesophagus are impaired LOS resting pressure, impaired oesophageal acid clearance, delayed gastric emptying and anatomical factors, such as a hiatus hernia[24]. More recently, impaired mucosal resistance and increased visceral hypersensitivity to acid have also been reported to predispose to GORD[24]. Histologically, it was thought that erosive changes in the distal oesophagus developed due to direct chemical-induced injury of the oesophageal mucosa and death of surface cells[25]. Such injury has been shown to provoke a T-helper Type 1 (Th1) inflammatory response, activating mostly granulocytes and lymphocytes[25]. Thus, it is intriguing that oesophageal eosinophilia can occasionally be seen in GORD, and the underlying mechanism remains unclear[26]. A study showing that GORD may also be a cytokine-mediated disease lead to the discovery that oesophageal squamous cells from EoE and GORD patients exhibit similar levels of eotaxin-3 (a chemokine that attracts eosinophils) when stimulated by T-helper Type 2 (Th2) cytokines; production of which is typical of an allergic disorder[10, 15, 22, 26, 27]. This suggests that GORD may be driven to a Th2 inflammatory response when the appropriate stimulus is present leading to oesophageal eosinophilia[26]. Low intraluminal baseline impedance has been shown to be associated with dilatation of intercellular spaces and

increased acid exposure in patients with GORD[28]. However, whether this damage can lead to exposure of food allergens and subsequently a Th2 response is unknown[26, 29, 30].

Although the exact pathophysiology of EoE is not fully understood, substantial evidence exists to show that EoE is an allergen (Th2 cell)-mediated response in genetically predisposed individuals (**Figure 2.1**)[10, 31, 32]. Defects in the oesophageal barrier are thought to facilitate the entry of food allergens or swallowed aeroallergens into the oesophageal epithelium which trigger a Th2 response and lead to mast cell activation and release of mediators such as interleukin (IL)-5, which is a known eosinophil activator[10, 22]. Activated eosinophils then release cytotoxic granules which contribute to cell death and tissue damage in these patients[10, 33, 34]. The gene coding for eotaxin-3, *CCL26* is overexpressed in the oesophagus of patients with EoE compared to healthy controls, which correlates with the increased levels of IL-5 and IL-13 in the oesophagus and blood of EoE patients[35, 36]. The development of EoE may also be associated with a genetic predisposition[10]. Hereditary collagen disorders such as Marfan and Ehlers-Danlos syndromes are the most frequent associations of EoE with an incidence of about one percent[21]. In patients with atopic dermatitis, a loss of function mutation in the gene filaggrin (2282del4) is overexpressed in EoE patients compared with healthy controls[37]. Filaggrin is a key structural, keratin-binding protein that plays an important role in the maturation of skin as an epithelial barrier by preventing keratin proteolysis[37]. EoE has been shown in paediatric patients to be associated with variants at chromosome 5q22 encompassing the gene *TSLP* (thymic stromal lymphopoetin), which encodes a cytokine that controls dendritic cell-mediated Th2-cell responses[21, 38]. More recently, EoE susceptibility locus was found at 2p23 which encodes *CAPN14*, which is upregulated on exposure to IL-13[39]. However, the exact impact of these genetic abnormalities on the pathogenesis of EoE is uncertain.

Epidemiology and Clinical Presentation

A few epidemiological differences exist between GORD and EoE. GORD is typically diagnosed in the second to fifth decade of life[20]. In contrast, EoE has a bimodal age presentation, with one peak in childhood and the second in the third and fourth decade with the mean age of diagnosis of 38 years[1, 33, 40]. Furthermore, whilst there is no gender preponderance in GORD, EoE affects males three times more than females[1, 41, 42]. Both conditions have been more frequently reported in Caucasians compared with other ethnicities[1, 8, 41, 43]. It should be

noted that the prevalence of GORD is much higher than that of EoE, ranging between 10-20% in the Western population as compared to less than 1% for EoE[8, 9, 40, 41]. Obesity has been shown to associated with GORD whereas EoE is strongly associated with atopic diseases such as asthma, food allergy, eczema, environmental allergies and chronic rhinitis[1, 8, 10, 31, 44].

GORD has been defined by the Montreal Classification as a condition that occurs due to retrograde flow of gastric contents into the oesophagus that lead to troublesome symptoms, which are typically heartburn and regurgitation[45, 46]. Other less common symptoms include chest pain, dysphagia, dyspepsia, epigastric pain, nausea, bloating, belching, chronic cough, asthma, laryngitis, and other respiratory symptoms[45-48]. Whilst dysphagia is infrequent in GORD, it is the most common presenting symptom for EoE along with food bolus impaction[1, 10, 49]. Approximately 50% of patients who present with food bolus impaction and up to 15% of patients who undergo endoscopy for non-obstructive dysphagia will have EoE[6, 50]. Although some EoE patients report GORD symptoms, they may respond poorly to PPIs[51]. Fifty to eighty percent of EoE patients have a prior history of atopic symptoms[21]. Other non-specific symptoms include chest pain, heartburn, regurgitation, dyspepsia, nausea and vomiting, odynophagia, abdominal pain and non-specific throat symptoms[1, 10, 31, 33, 49, 52].

Diagnosis

A diagnosis of GORD is usually based on clinical symptoms, typically heartburn and regurgitation, in a patient who is responsive to PPI therapy[46]. Thus, upper endoscopy, routine biopsies from the distal oesophagus and ambulatory pH testing are not usually required in a patient with typical GORD symptoms in the absence of alarm symptoms such as dysphagia, odynophagia and weight loss[16, 44, 46]. The diagnosis of EoE on the other hand, relies on a correlation between clinical symptoms, endoscopic and histological features as there is no one pathognomonic feature of EoE[10, 13]. According to the most recent consensus, it requires the presence of ≥ 15 intraepithelial eosinophils per high power field in one or more oesophageal mucosal biopsies in combination with symptoms of oesophageal dysfunction[5]. However, this definition may be too simplified as the diagnosis of EoE may be established with a lower intraepithelial eosinophil count if there is strong clinical suspicion and other histological features associated with eosinophilic inflammation are present[1, 10]. Given that excessive accumulation of eosinophils in tissues is a common finding in numerous gastrointestinal disorders, other causes of oesophageal eosinophilia (**Table 2.1**) should also be excluded,

particularly GORD[1, 14]. The following diagnostic features that may be found in GORD and EoE and may help distinguish between the two entities are summarised in **Table 2.2**.

Endoscopic Oesophageal Features: Relevant endoscopic findings of GORD are erosive oesophagitis, peptic strictures, a hiatus hernia, and Barrett's oesophagus[15, 16, 46]. Endoscopy has a high specificity for diagnosing GORD particularly when erosive oesophagitis is seen and the Los Angeles classification is used[53]. However, most patients with GORD will have normal endoscopies[15, 16]. In contrast, endoscopic oesophageal features of EoE patients are trachealization, felinization, whitish exudates, longitudinal furrows, oedema, diffuse oesophageal narrowing, narrow-calibre oesophagus and oesophageal lacerations secondary to passage of the endoscope[1, 10, 13, 16, 54] (**Figure 2.2**). Loss of mucosal vascular pattern has also been reported[55]. These features, however, are not pathognomonic for EoE and thus histological correlation is required[1, 10]. Normal endoscopic findings have been reported in up to 30% of patients with EoE[10, 13].

Histological features: Patients with GORD may exhibit oesophageal eosinophilia, typically less than 10 per high power field (/hpf) as compared to ≥15/hpf for EoE[1, 10, 15, 56] (**Figure 2.3**). The presence of additional histological features of eosinophilic microabscesses, eosinophil degranulation, basal cell hyperplasia, papillary lengthening, superficial layering of eosinophils, extracellular eosinophil granules, intracytoplasmic keratinocyte vacuolation, dilated intracellular spaces or lamina propria fibrosis are more supportive of a diagnosis of EoE[1, 10, 13, 16, 57]. Although some of these additional histological features have been reported in biopsy specimens of patients with GORD, they are less commonly found as compared to EoE[10, 13, 16, 57]. Recently, Zuckerberg et al[17] showed that immunohistochemical staining of oesophageal tissue with immunoglobulin G4 (IgG4) could help distinguish EoE from GORD, given that 76% of EoE cases were positive for intrasquamous IgG4 and none of the GORD cases were positive. The distribution of oesophageal eosinophilia may also be helpful in distinguishing the two conditions, with diffuse oesophageal eosinophilia more suggestive of EoE and distal oesophageal eosinophilia of GORD[16]. Thus, it is important to biopsy at least 2 regions of the oesophagus and accurately label the site of oesophageal biopsies.

Oesophageal Motor Function: Oesophageal manometry is of limited use in the diagnosis of GORD and EoE given that findings have so far been non-specific[1, 13, 58]. Oesophageal motility disorders found in patients with GORD have a similar type and prevalence to patients with EoE ranging between 4-87%[14, 21, 33]. However, in cases where dysphagia is the main symptom, it

is important to perform manometric assessment to exclude major and minor disorders of peristalsis which can sometimes mimic symptoms of GORD and EoE[18, 33]. The duration of EoE has been shown to be longer in those with abnormal oesophageal motility[59].

Treatment

The initial management of GORD usually involves a combination of lifestyle interventions and medical therapy with the aim of eliminating symptoms, repairing any existing oesophageal mucosal injury and preventing further inflammatory injury[46, 60]. Lifestyle interventions of weight loss (particularly if BMI > 25 or recent weight gain) and head of bed elevation have been proven to reduce symptoms and improve oesophageal pH values[61, 62]. Other lifestyle interventions such as avoidance of late evening meals and cessation of alcohol, tobacco, chocolate, caffeine, spicy foods, citrus and carbonated drinks lack evidence and are not routinely recommended[46]. Medical therapy such as antacids, histamine-receptor antagonists (H_2RA) or PPI therapy should then be considered in patients failing lifestyle interventions alone[46, 60]. PPI therapy is effective in 70-80% of patients and has been shown to be superior to H_2RAs in regard to healing rates and decreased relapse rates[63]. Surgical therapy is as effective as medical therapy and may be contemplated in GORD patients who wish to discontinue medications, are non-compliant, have side-effects associated with medications, have a large hiatus hernia or have refractory oesophagitis and symptoms despite optimal medical therapy[46].

The choice of initial treatment for EoE patients on the other hand is made on an individualized basis as PPI therapy, topical steroids and dietary therapy can all be considered as first-line therapeutic options[5]. All EoE patients should receive treatment to improve quality of life, prevent oesophageal remodelling secondary to active eosinophilic inflammation and prevent oesophageal injury due to the disease or endoscopic intervention[64]. 30-40% of EoE patients may be responsive to PPIs, either due to a reduction in acid secretion in patients with co-existent GORD or by means of other still unknown anti-inflammatory mechanisms[21, 22]. EoE patients can also be treated with topical steroids as it has been shown to improve symptoms and reduces oesophageal eosinophilia[21, 65]. Viscous steroids have been shown to be more effective than nebulized steroids possibly due to greater mucosal contact time compared with the latter[66]. A recent meta-analysis of seven randomized controlled trials concluded that although there was an increased risk of asymptomatic oesophageal candidiasis with topical

steroid therapy, it is considered safe with no evidence of adrenal suppression[67]. Dietary therapy is based on the fact that the majority of EoE patients have food allergies that may contribute to the pathogenesis of the disease[22, 68]. There are 3 strategies of dietary therapy: an amino acid-based formula/elemental diet, a targeted elimination diet guided by allergy testing, and an empiric elimination diet[22, 65, 68]. All diets should be followed for a minimum of 6 weeks and its efficacy evaluated via symptoms as well and oesophageal biopsies[65, 69].

Oesophageal dilation, either via through-the-scope balloons or by Savary bougies can lead to long-lasting symptom improvement in EoE patients with stricturing disease or impaired oesophageal distensibility due to subepithelial fibrosis[21, 22]. Clinical improvement post dilation occurred in 75% of patients[70]. A meta-analysis evaluating the clinical efficacy and safety of oesophageal dilation in these patients showed that it is a safe procedure with a < 1% rate of serious complications[70]. However, it does not result in a decreased in eosinophil infiltration or histologic improvement and thus should not be used as a sole therapeutic option in these patients[5, 71]. Several other treatment options for EoE have been assessed namely Montelukast (leukotriene receptor antagonist), Infliximab (anti-tumour necrosis factor), Mepolizumab (anti-IL-5), Azathioprine or 6-mercaptopurine, Reslizumab (IL-5 neutralizing antibody), Omalizumab (anti-immunoglobulin E (anti-IgE)), QAX576 (anti-IL-13) and OC000459 (prostaglandin D2 receptor antagonist)[34, 64, 72-80]. Although studies of these agents have shown changes in the biological behaviour of EoE disease markers, they have not yet displayed sufficient clinical benefit for widespread use[81].

Relationship between Eosinophilic Oesophagitis and Gastroesophageal Reflux Disease

The interaction between EoE and GORD is complex and may be bidirectional[5]. An approximate prevalence of GORD in the general population of 20% is sufficiently high enough to make the coexistence of EoE and GORD plausible[16]. In patients with refractory GORD symptoms, EoE was found in approximately 4%[10, 56]. 4 hypotheses to account for interactions between oesophageal eosinophilia and GORD have been proposed: eosinophilia as a marker of GORD; GORD and EoE coexist but are unrelated, EoE contributes or causes GORD; and GORD contributes to or causes EoE[16, 20, 82, 83].

1. **Eosinophilia as a marker of GORD:** GORD is thought to cause a mild eosinophilia in the absence of EoE[16, 82]. Acid exposure was thought to cause oesophageal injury which results in chronic inflammation, including the presence of oesophageal eosinophils that are

recruited via an increase in expression of adhesion molecules, release of chemokines that attract eosinophils and increase in blood flow[16]. However, the role of these adhesion molecules and chemokines in the pathogenesis of GORD is yet unclear[16]. A study also showed that dense oesophageal eosinophilia in GORD was uncommon[3].

2. **GORD and EoE coexist but are unrelated:** As mentioned above, due to a high prevalence of GORD in the general population, the coexistence of EoE and GORD due to chance alone is plausible[16, 83]. Oesophageal pH studies have shown that 25-50% of EoE patients have increased oesophageal acid exposure, thus supporting the notion that the two entities can coexist[1, 16].
3. **EoE contributes or causes GORD:** This hypothesis is based on the fact that eosinophils secrete a number of agents that affect the integrity of the mucosal barrier and the function of oesophageal smooth muscle as well as producing a direct cytotoxic effect on the mucosa[16, 84]. Remodelling effect in EoE may contribute to increased acid exposure due to effects on the lower oesophageal sphincter or impaired oesophageal clearance of refluxed contents[16, 20].
4. **GORD contributes to or causes EoE:** An unproven hypothesis has suggested that GORD may contribute to the pathogenesis of EoE by causing changes in the integrity of the oesophageal mucosa, promoting trans-epithelial allergen permeation followed by allergic immune activation[5, 85].

Conclusion

The relationship between EoE and GORD is complex as they are different entities that may coexist. Distinguishing between the two remains challenging given that they have multiple overlapping features. At present, the combination of clinical, endoscopic, and histological features, as well as response to PPI therapy, may help to differentiate the two conditions. Further studies into the immuno-pathophysiology are needed to elucidate more objective diagnostic testing that can reliably differentiate between the two disease processes.

TABLES

Table 2.1 Diseases associated with oesophageal eosinophilia.

GORD
Eosinophilic gastrointestinal diseases
Atopy
Coeliac disease
Crohn's disease
Oesophageal infections
Hypereosinophilic syndrome
Achalasia
Drug hypersensitivity
Vasculitis
Pemphigoid vegetans
Connective tissue disease
Graft-versus-host-disease
Oesophageal atresia

GORD: Gastro-oesophageal reflux disease.

Table 2.2 Diagnostic features of gastro-oesophageal reflux disease and eosinophilic oesophagitis.

	GORD	EoE
Endoscopic	Erosive oesophagitis Peptic strictures Hiatus hernia Barrett's oesophagus	Trachealization Felinization Whitish exudates Longitudinal furrows Oedema Diffuse oesophageal narrowing Narrow-calibre oesophagus Oesophageal lacerations Loss of mucosal vascular pattern
Histological	Eosinophilia <10/hpf	Eosinophilia ≥15/hpf Eosinophilic microabscesses Eosinophil degranulation Basal cell hyperplasia Papillary lengthening Superficial layering of eosinophils Extracellular eosinophil granules Intracytoplasmic keratinocyte vacuolation Dilated intracellular spaces Lamina propria fibrosis Positive intrasqamous IgG4
Motor function	Non-specific	Non-specific

FIGURES

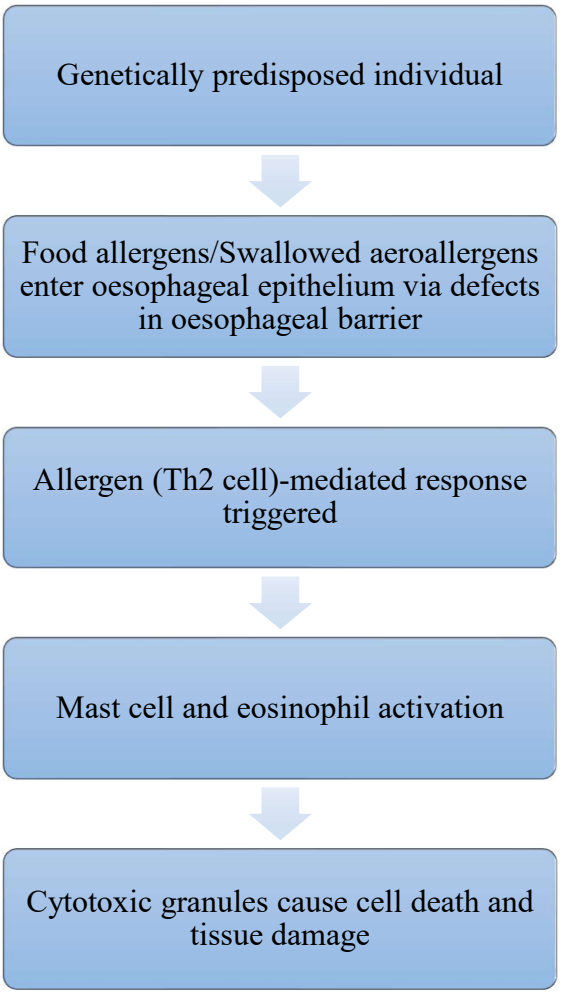

Figure 2.1 Proposed pathogenesis of eosinophilic oesophagitis.

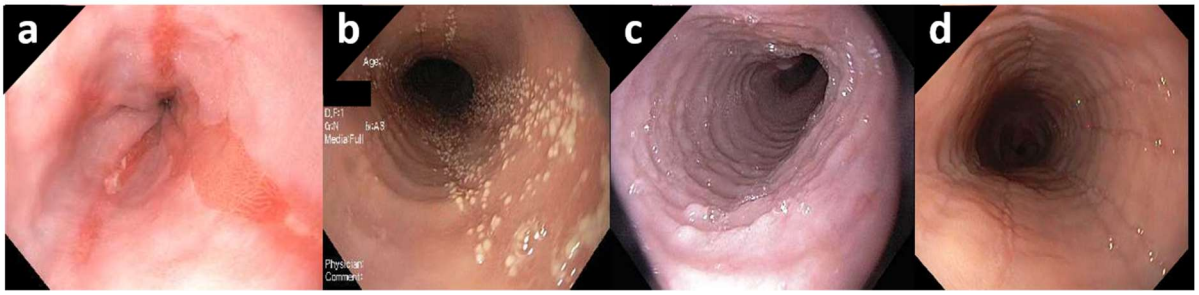

Figure 2.2 Endoscopic changes in patients with gastro-oesophageal reflux disease and eosinophilic oesophagitis. A: Erosive oesophagitis of GORD; B: White exudates in eosinophilic oesophagitis; C: mucosal rings or trachealization in eosinophilic oesophagitis; D: longitudinal furrows in eosinophilic oesophagitis.

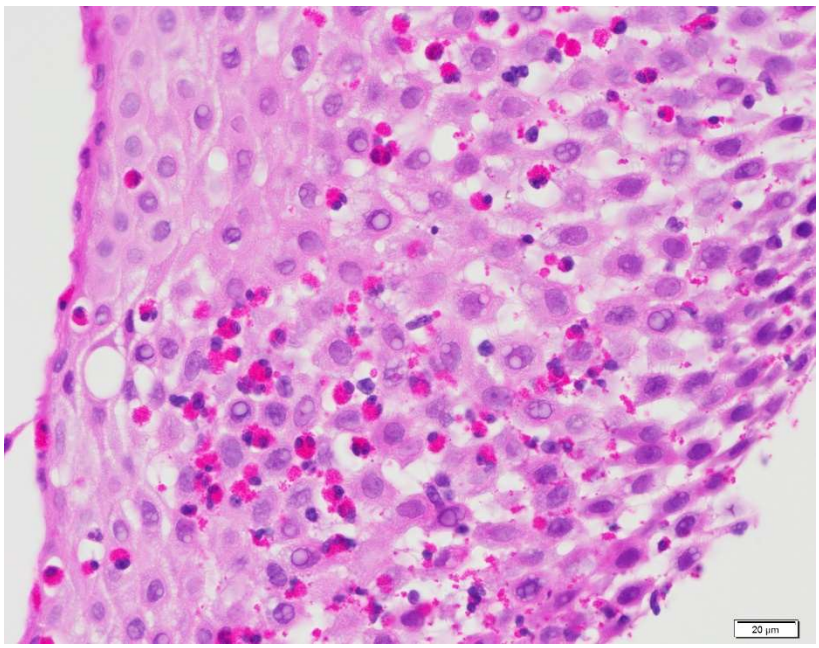

Figure 2.3 Histological specimen from the oesophagus (luminal aspect on left) of an eosinophilic oesophagitis patient showing marked oedema and numerous intraepithelial eosinophils in the oesophageal squamous mucosa, which are also seen in the superficial component of the mucosa.

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CHAPTER 3: OESOPHAGEAL MUCOSAL IMMUNOGLOBULIN G4

3.1 Overview

This chapter contains a published article assessing the potential role of oesophageal mucosal IgG4 staining to help differentiate EoE from GORD. As emphasised in the previous chapters, it may be difficult for clinicians to distinguish between the two disease processes. An accurate diagnosis is important in medicine to improve patient outcomes and ensure safety. This study was performed to ascertain the potential of IgG4 staining as a diagnostic adjunct to distinguish between the two disease processes.

3.2 Specific Author Contributions

Statement of Authorship

Title of Paper	Distinguishing gastroesophageal reflux disease and eosinophilic esophagitis in adults: The role of esophageal mucosal immunoglobulin G4
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	**Wong S**, Smith G, Ruszkiewicz A, Nguyen N.Q. (2020), Distinguishing gastroesophageal reflux disease and eosinophilic esophagitis in adults: The role of esophageal mucosal immunoglobulin G4. JGH Open. doi:10.1002/jgh3.12327

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Contribution to the Paper	Ethics submission, data acquisition, analysis of data, drafting of entire manuscript, submission of manuscript for publication		
Overall percentage (%)	75		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

5. the candidate's stated contribution to the publication is accurate (as detailed above);
6. permission is granted for the candidate in include the publication in the thesis; and
7. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Data analysis and critical revisions of manuscript		
Signature		Date	14/6/2021

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Contribution to the Paper	Study and concept design, data analysis and critical revisions of manuscript		
Signature		Date	15/6/2021

3.3 Published work 2: Distinguishing gastroesophageal reflux disease and eosinophilic esophagitis in adults: The role of esophageal mucosal immunoglobulin G4

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Disclosure statement:

No conflict of interest to disclose from all authors.

Keywords:
Adults, Eosinophilic oesophagitis, gastroesophageal reflux disease, immunoglobulin G4.

ABSTRACT

Background: Eosinophilic esophagitis (EoE) and gastroesophageal reflux disease (GERD) can be difficult to distinguish as many of their clinical and histological features overlap. Preliminary data suggests a potential association between EoE and immunoglobulin G4 (IgG4), but not GERD. This study aimed to examine the role of esophageal mucosal IgG4 staining in 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 from 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, 23M:7F) and 30 GERD (50.7±14.3 years, 14M:16F) patients. The prevalence of 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 GORD patients, correlation was found between positive IgG4 staining with food bolus obstruction, dysphagia to solids, reflux, fixed rings, Barrett's oesophagus, hiatus hernia and oesophagitis. 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 characterised by an antigen-driven immunologic process that manifests clinically with symptoms of esophageal dysfunction and histologically by 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 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 co-exist.[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) since 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 oesophageal eosinophilic counts in patients with EoE.[11] This finding was also coupled with the discovery that there was a 45-fold increase of IgG4 in esophageal tissue as well as serum levels of IgG4 that appeared to react to specific foods, suggesting that EoE is IgG4-associated and not an IgE-induced allergy.[11] Subsequently, Zukerberg et al showed that immunohistochemical staining of oesophageal 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: age 18-80 years of age, typical symptoms of GERD responsive to proton

pump inhibitor (PPI) therapy, evidence of oesophagitis on endoscopy with supportive oesophageal biopsy specimens and eosinophil count <10 per high powered field (/hpf). Inclusion criteria for patients with EoE were: age 18-80 years of age, symptoms of oesophageal dysfunction and ≥15 eosinophils/hpf. Exclusion criteria were history of severe respiratory, cardiovascular, hepatic, haematological 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 record of patient demographics, clinical presentation, medications, past medical history, investigations and treatment which 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 into 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 from the clinical and endoscopic data.

Assessment of esophageal mucosal IgG4

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

IgG4 immunohistochemistry was scored as 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 signal 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 around less than 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 detailed prior. 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 was expressed as mean±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, as compared to the younger, male predominant EoE patients. Other demographics and clinical characteristics of the 2 groups are summarised in **Table 3.1**.

The prevalence of positive intercellular IgG4 stain was significantly higher in EoE patients than those with GERD (23/30 vs. 2/30; $P<0.0001$, **Figure 3.1**). A 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.

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 micro-abscesses, basal cell hyperplasia, white plaques, longitudinal furrows or the presence of a stricture. (**Table 3.2**)

DISCUSSION

To our knowledge, the current study is the largest to date in examining the prevalence of IgG4 positive stain 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 as 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 hypothesised to be IgG4-associated and not IgG4-related.[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 which 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 was 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 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 2 of these patients were on PPI therapy at the time of esophageal biopsy. In both cases, there were 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 had been observed in a previous paediatric 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 2 to 4 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 5 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 exists at present 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] Paediatric 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, oesophageal IL4, IL13 and IL10, and had strong associations with a subset of the EoE transcriptome.[16] As our study cohort consists purely of adults, comparison 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 as 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.

TABLES

Table 3.1: Demographics and clinical characteristics of all eosinophilic esophagitis and gastroesophageal reflux disease patients.

	EoE (n=30)	**GERD (n=30)**
Mean age (years)	38.8±12.8	50.7±14.3
Gender	23M:7F	14M:16F
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 oedema	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 oesophagus	0	6
Hiatus hernia	5	17
Oesophagitis	3	30
Medications		
Proton-pump inhibitor (PPI)	12	10
History of allergy/atopy	10	4

Table 3.2: Correlation of esophageal IgG4 staining with clinical and endoscopic characteristics in eosinophilic esophagitis and gastroesophageal reflux disease patients (n=60).

	Present in IgG4 positive	Present in IgG4 negative	P 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

FIGURE

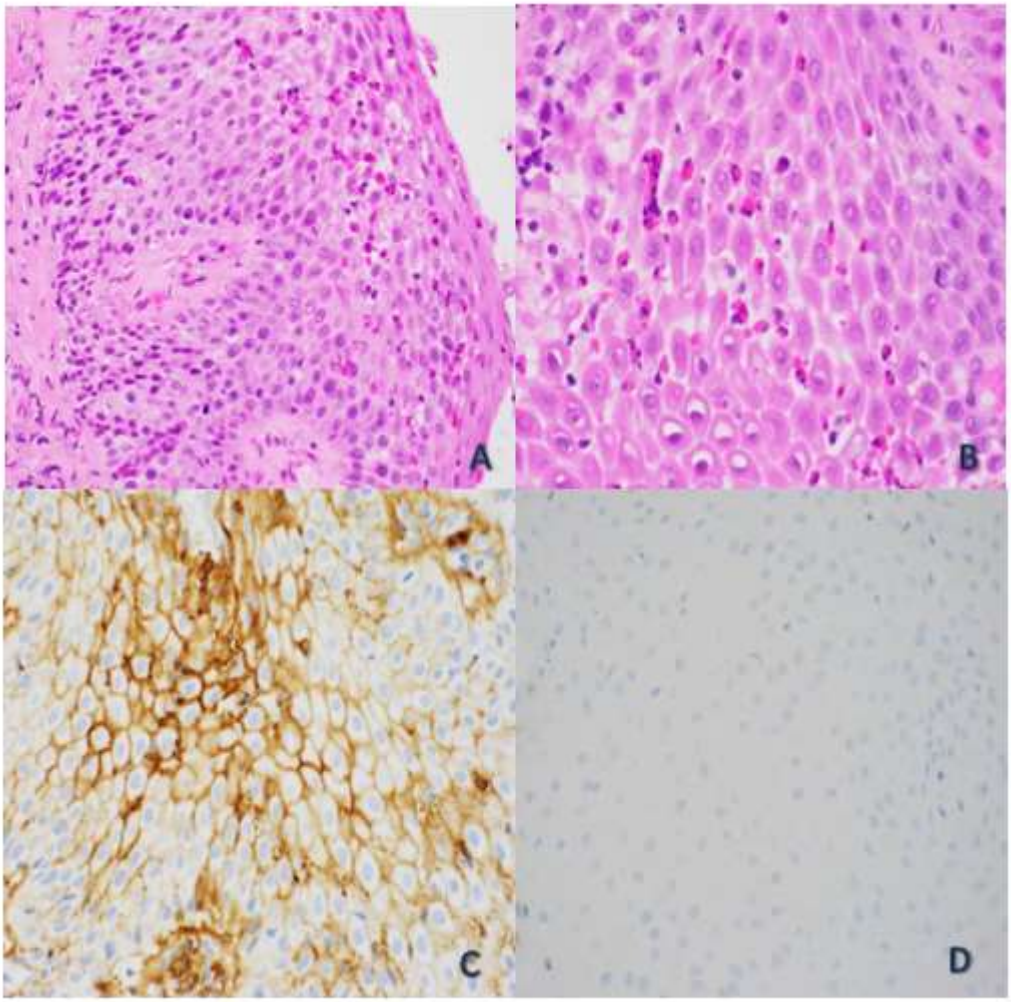

Figure 3.1. Histological assessment of esophageal mucosa: Eosinophilic esophagitis (EoE), B: EoE with intercellular oedema. C: EoE with positive IgG4. D: EoE with negative IgG4.

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CHAPTER 4: CHILHOOD-ONSET AND ADULT-ONSET EOSINOPHILIC OESOPHAGITIS

4.1 Overview

This chapter contains a published article evaluating the characteristics of children and adults with EoE. A further comparison focused on those with childhood-onset and adult-onset EoE was performed. The natural history of EoE, particularly during the transition from childhood to adulthood, has long confounded clinicians. A paucity of research data in this area has prevented strong evidence-based recommendations in treatment guidelines. This study attempts to clarify whether childhood-onset and adult-onset EoE are separate disease processes or are chronically progressive.

4.2 Specific Author Contributions

Statement of Authorship

Title of Paper	Characteristics and Progression of Childhood-Onset and Adult-Onset Eosinophilic Esophagitis
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Wong S, Ellison S, Haj Ali S, Hawkes J, Collinson J, O'Neill T, Ruszkiewicz A, Moore D, Holloway RH, Nguyen NQ. Characteristics and progression of childhood-onset and adult-onset eosinophilic esophagitis. J Gastroenterol Hepatol. 2022 Jan;37(1):69-74. doi: 10.1111/jgh.15660. Epub 2021 Aug 31. PMID: 34374118.

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Contribution to the Paper	Data acquisition, data analysis, drafting of entire manuscript, submission of manuscript for publication		
Overall percentage (%)	60		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

8. the candidate's stated contribution to the publication is accurate (as detailed above);
9. permission is granted for the candidate in include the publication in the thesis; and
10. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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4.3 Published work 3: Characteristics and progression of childhood-onset and adult-onset eosinophilic esophagitis

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Conflict-of-interest-statement: No conflict of interest to disclose from all authors.

ABSTRACT

Background: The prevalence and incidence of eosinophilic esophagitis (EoE) has been increasing over recent years. However, the natural history remains incompletely understood particularly the differences in disease characteristics and progression of childhood-onset and adult-onset EoE. **Aims:** To evaluate the disease characteristics and progression of childhood-onset and adult-onset EoE. **Methods:** A cross-sectional, questionnaire-based study, on 87 adults and 67 children from 2 major tertiary hospitals in South Australia was conducted. Data of those who were diagnosed with EoE between 1999 and 2018 were collected and correlated with medical records. **Results:** Of the 87 adults with EoE, 34 (39%) were diagnosed at the age of <18 years (childhood-onset EoE). Reflux symptoms were more common in childhood-onset EoE whereas asthma was more common in adult-onset EoE. The median duration of symptoms prior to diagnosis of EoE was >1-4 years in childhood-onset disease (44%) and ≥10 years in adult-onset disease (34%). Food impaction was significantly more common on initial presentation in those with adult-onset EoE whereas weight loss was more common in childhood-onset EoE. At the time of questionnaire, regurgitation, abdominal pain, and bloating were more common in childhood-onset EoE. Those with childhood-onset EoE were more likely to have multiple symptoms at questionnaire when compared to their adult-onset counterparts. In both groups, 15% (5/34 childhood-onset EoE and 8/53 adult-onset EoE) were asymptomatic at the time of questionnaire. **Conclusion:** Childhood-onset EoE appears to be a progressive disease from childhood to adulthood, however with more inflammatory-type symptoms post transition compared to those with adult-onset EoE.

INTRODUCTION

Eosinophilic esophagitis (EoE) is a clinicopathological condition characterised by an antigen-driven immunologic process that manifests clinically with symptoms of oesophageal dysfunction and histologically by eosinophilic inflammation.[1, 2] It has a bimodal age presentation, with one peak in childhood and the second in the third and fourth decade with a mean age of diagnosis of 38 years.[1, 3, 4] Current estimated prevalence and incidence in the general population is 13-49 cases/100,000 persons and 1-20 cases/100,000 persons, respectively.[2, 5] EoE is now thought to be the most frequent eosinophilic gastrointestinal disorder and the second most common cause of chronic esophagitis and dysphagia after gastroesophageal reflux disease (GERD).[6] An increasing prevalence of food bolus impaction has been shown to be associated with an increased prevalence of EoE and a reduction in peptic strictures.[7] Various hypotheses have been considered for this phenomenon particularly that of an increase in the recognition of the disease and an increase in the volume of endoscopies performed.[7-9] However, two population-based studies have shown that the incidence and cumulative prevalence of EoE has increased more than the rate of annual endoscopies during the observation period in keeping with a true rise in the incidence and prevalence of the disease.[10, 11]

The natural history of EoE is incompletely understood, particularly whether EoE worsens, stays the same or remits during transition from childhood to adulthood.[12] Studies in adults suggest that EoE is a chronic disease with persistence of dysphagia and long-term complications including oesophageal fibrosis.[8, 10, 12] A study that investigated the clinical outcome of EoE patients diagnosed as children concluded that most of the children had resolution or improvement of symptoms as young adults.[13] It is unclear as to why there are phenotypic differences in EoE and whether they indicate different responses to therapy or prognoses.[14] We hypothesise that EoE is a chronic single disease entity that may present in childhood or adulthood. The aims of this study were to compare the characteristics and disease progression between childhood-onset and adult-onset EoE.

MATERIALS AND METHODS

Study population and design

This cross-sectional, questionnaire-based study was performed at the Royal Adelaide Hospital and The Women's and Children's Hospital in Adelaide, South Australia. The study protocol was approved by both the Royal Adelaide Hospital Research and Ethics Committee and the

Women's and Children's Health Network Human Research Ethic Committee (reference number: HREC/14/WCHN/87). All patients with a diagnosis of EoE between 1999 and 2018 at both hospitals were included. Identified patients were sent an invitation letter, information sheet, consent form (signed by legal guardian if aged less than 18 years) and the questionnaire along with a reply-paid envelope. This package was resent to patients who did not respond to the initial invitation. No further correspondence was initiated if a response was not received following this second attempt. Exclusion criteria were patients who did not given written informed consent, and incomplete questionnaires/data.

The questionnaire incorporated questions regarding demographics, past medical history, allergy, family history, and a detailed history of eosinophilic esophagitis (Appendix A Children and Appendix B Adults). Data collected from questionnaires were correlated with medical records, in particular the date of diagnosis and treatment history.

Definitions

A diagnosis of EoE was defined as ≥15 eosinophils per high powered field (/hpf) with symptoms of oesophageal dysfunction (such as food bolus impaction, dysphagia and vomiting) and exclusion of other causes of oesophageal eosinophilia. Adulthood was defined as age ≥18 years and childhood as age <18 years.

Statistical Analysis

Descriptive statistics were used to describe the results. Categorical data were compared by Fisher's exact test and continuous data were compared by Student's t-test. Statistical significance was determined by a *P* value of less than 0.05. Analyses were performed using GraphPad Prism statistical software, version 8 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Of the 446 sent questionnaires, 87/280 adults and 67/166 children returned completed questionnaires. (**Figure 4.1**)

Comparison of adults with childhood-onset and adult-onset EoE

Of the 87 adults with EoE, 34 (39%) were diagnosed at less than 18 years of age (childhood-onset EoE). The differences between adults with childhood-onset EoE and adult-onset EoE are summarised in **Table 4.1**. At the time of completing the questionnaire, adults with childhood-

onset EoE were significantly younger than those with adult-onset EoE both at time of questionnaire and at the age of diagnosis of EoE (P <0.0001). Reflux symptoms were more common in childhood-onset EoE whereas asthma was more common in adult-onset EoE. There were no differences between the two groups with respect to personal history of allergy, family history of allergy and family history of EoE. The most frequently reported duration of symptoms prior to the diagnosis of EoE was >1-4 years in childhood-onset disease (44%) and ≥10 years in adult-onset disease (34%). Food impaction was significantly more common as an initial presentation in those with adult-onset EoE whereas weight loss was more common in childhood-onset EoE **(Figure 4.2)**. At the time of questionnaire, regurgitation, abdominal pain, and bloating were more common in childhood-onset EoE. Those with childhood-onset EoE were more likely to have multiple symptoms when compared to their adult-onset counterparts. **(Figure 4.3)** Equal proportions of those with childhood-onset (5/34, 15%) and adult-onset EoE (8/53, 15%) were asymptomatic at the time of questionnaire.

Comparison between children and adults with EoE

The differences between children and adults with EoE are summarised in **Table 4.2**. The median age of diagnosis was 5±4.5 years for children and 25±18.5 years for adults. Of note, dysphagia to solids and food impaction were significantly more common on presentation in adults than children, whereas this was the opposite for vomiting and abdominal pain. Adults were also more likely to experience multiple symptoms initially compared to children. However, at the time of questionnaire, the most common symptom in both groups was dysphagia to solids, although food impaction in adults remained significantly more common than in children. Retching/Vomiting and abdominal pain remained more common in children at the time of questionnaire.

DISCUSSION

Our study describes the differences in disease characteristics and progression in patients with childhood-onset versus adult-onset EoE. Our data shows that those with childhood-onset EoE, confirmed to be significantly younger at time of diagnosis and questionnaire, experienced more inflammatory-type symptoms, but there was no difference in the presence of continued symptoms into adulthood. Most patients in both groups recalled experiencing symptoms for years prior to the diagnosis of EoE. This is consistent with what is known about the natural

history of EoE where patients often describe a history of symptoms that began years prior to their diagnosis.[15, 16] EoE has been considered to be food allergy predominant in paediatric EoE and airway allergy predominant in adult EoE.[17] Our data reflects this, where GORD was found to be more common in childhood-onset disease, whereas asthma was more common in adult-onset disease.

Patients with adult-onset EoE had a significantly higher rate of presenting with food impaction compared to those with childhood-onset EoE. This significance receded in the transition from childhood to adulthood. A further comparison of adults and children with EoE (**Table 4.2**) echoed this finding, where dysphagia to solids ultimately became the most common symptom in both groups. This change supports the theory that EoE progresses from an inflammatory to a fibrostenotic phenotype due to development of subepithelial fibrosis in the oesophagus.[10, 16, 18] Post transition into adulthood, however, those with childhood-onset EoE continued to have a significantly higher incidence of multiple and inflammatory-type symptoms, namely regurgitation, abdominal pain, and bloating. This new and interesting finding suggests that although fibrosis eventually develops in childhood-onset EoE, the inflammatory component remains significant enough to contribute to ongoing symptoms.

At the time of questionnaire, only 15% of both childhood-onset and adult-onset cohorts were asymptomatic. Thus, 85% of our patients with childhood-onset EoE continued to have symptoms into adulthood. Studies that have looked in particular at transition of EoE from childhood to adulthood have shown that childhood-onset EoE was associated with a reduced quality of life and persistent symptoms into adulthood.[12, 19] This contrasts with more recent findings which concluded that those with childhood-onset EoE had improvement or resolution of symptoms as adults.[13] We believe that our data adds impact to the theory that childhood-EoE is a progressive condition and not a different disease entity in children and adults.

A strength of our study is that this is a multicentre analysis of childhood-onset and adult-onset EoE with a higher-than-average response rate (35%, 154/446). Although our study was limited by recall bias, all data obtained from the questionnaires were correlated with medical records. Also, given the structure of our questionnaire where the duration of disease is expressed as a range rather than a single time value, logistic regression to assess associations between disease duration and other variables was not possible.

CONCLUSIONS

Childhood-onset EoE appears to be a progressive disease from childhood to adulthood, however with more inflammatory-type symptoms post transition compared to those with adult-onset EoE.

TABLES

Table 4.1: Comparison of demographics, concomitant medical conditions, history of allergy and family history in adults with childhood-onset and adult-onset eosinophilic oesophagitis

	Childhood-onset (n=34)	Adult-onset (n=53)	P-value
Age at questionnaire (years)	20± 4.4	47 ± 13.7	**<0.0001**
Age at diagnosis of EoE (years)	14 ± 4.6	38± 13.2	**<0.0001**
Gender (Male: Female)	24M:10F	41M:12F	
Body mass index (BMI; kg/m^2)	18 ± 39	25 ± 48	
Country of birth	97% Australian 3% Other	87% Australian 13% Other	
Concomitant medical conditions			
GORD	53%	19%	**0.0019**
Asthma	12%	38%	**0.0129**
Personal history of allergy	77%	77%	1.0000
Family history of allergy	59%	38%	0.0776
Family history of EoE	9%	9%	1.0000
Duration of symptoms prior to diagnosis			
0-7 days	12%	25%	
>7-30 days	-	-	
>30 days-1 year	15%	2%	
>1-4 years	44%	25%	
>5-9 years	21%	15%	
≥10 years	9%	34%	
Initial symptoms			
Dysphagia-solids	76%	91%	0.1208
Dysphagia-liquids	24%	25%	1.0000
Heartburn	41%	28%	0.2486
Food impaction	30%	85%	**<0.0001**
Regurgitation	44%	32%	0.2662
Chest pain	29%	19%	0.3011
Retching/Vomiting	26%	32%	0.6373
Weight loss	29%	8%	**0.0142**
Abdominal pain	35%	17%	0.0723
Bloating	32%	15%	0.0677
Multiple symptoms	97%	94%	1.0000

Current symptoms			
Dysphagia-solids	41%	43%	1.0000
Dysphagia-liquids	32%	13%	0.0555
Heartburn	38%	21%	0.0894
Food impaction	24%	23%	1.0000
Regurgitation	35%	11%	**0.0131**
Chest pain	32%	15%	0.0677
Retching/Vomiting	18%	6%	0.1452
Weight loss	6%	6%	1.0000
Abdominal pain	21%	11%	**0.0068**
Bloating	32%	9%	**0.0104**
Multiple symptoms	68%	34%	**0.0494**

Table 4.2: Patient demographics, concomitant medical conditions, history of allergy and family history of adults and children with eosinophilic oesophagitis.

	Children (n=67)	Adults (n=87)	P-value
Age at questionnaire (years)	13 ± 4.1	32 ± 16.3	
Age at diagnosis of EoE (years)	5 ± 4.5	26 ± 18.4	
Gender (Male: Female)	57M:10F	65M:22F	
Body mass index (BMI; kg/m^2)	18 ± 39	25 ± 48	
Country of birth	99% Australian 1% Other	91% Australian 9% Other	
Concomitant medical conditions			
GORD	39%	32%	0.4008
Asthma	39%	28%	0.1662
Personal history of allergy	88%	77%	0.0936
Family history of allergy	78%	46%	**<0.0001**
Family history of EoE	4%	9%	0.3505
Duration of symptoms prior to diagnosis			
0-7 days	3%	20%	
>7-30 days	1%	-	
>30 days-1 year	25%	7%	
>1-4 years	57%	32%	
>5-9 years	10%	17%	
≥10 years	3%	24%	
Initial symptoms			
Dysphagia-solids	63%	85%	**0.0023**
Dysphagia-liquids	28%	24%	0.5821
Heartburn	28%	33%	0.5993
Food impaction	25%	63%	**0.0001**
Regurgitation	-	37%	-
Chest pain	25%	23%	0.8494
Retching/Vomiting	64%	30%	**<0.0001**
Weight loss	19%	16%	0.6708
Abdominal pain	43%	24%	**0.0151**
Bloating	-	22%	-
Multiple symptoms	78%	95%	**<0.0001**
Current symptoms			
Dysphagia-solids	45%	43%	0.8700
Dysphagia-liquids	9%	21%	0.0715

Heartburn	28%	28%	1.0000
Food impaction	7%	23%	**0.0141**
Regurgitation	-	21%	-
Chest pain	19%	22%	0.8417
Retching/Vomiting	27%	10%	**0.0100**
Weight loss	6%	6%	1.0000
Abdominal pain	34%	15%	**0.0068**
Bloating	-	18%	-
Multiple symptoms	54%	46%	0.4166

FIGURES

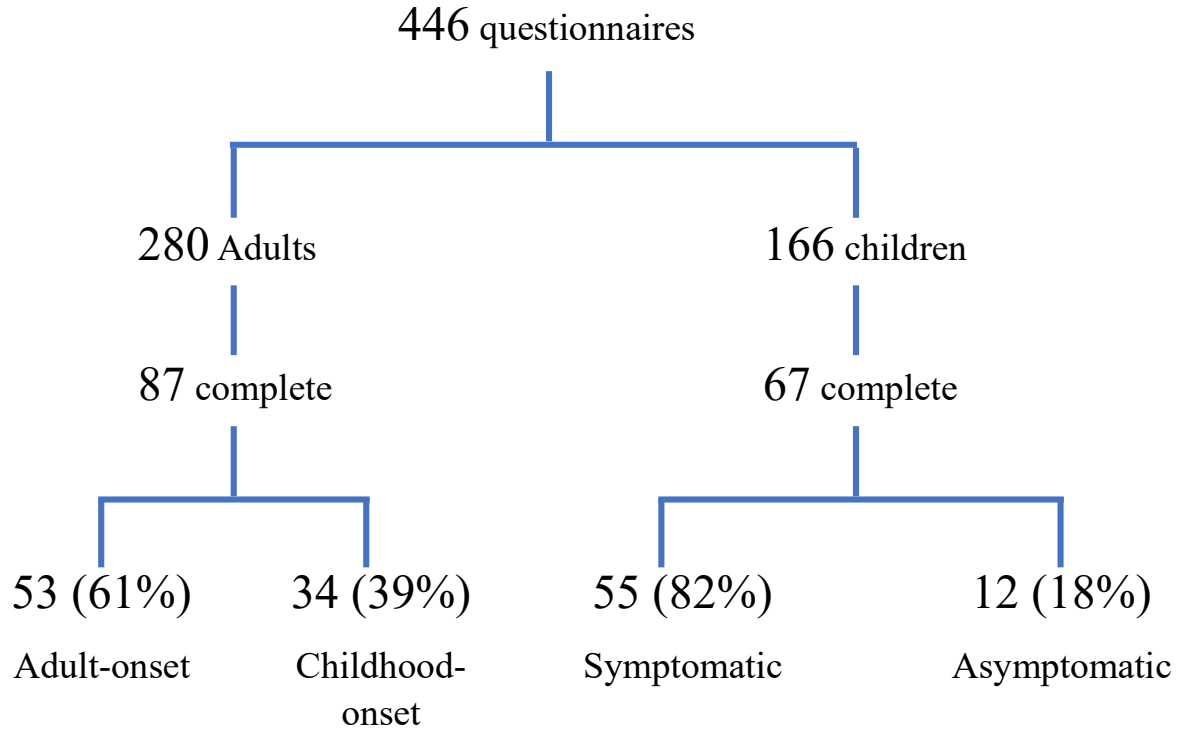

Figure 4.1: Study population.

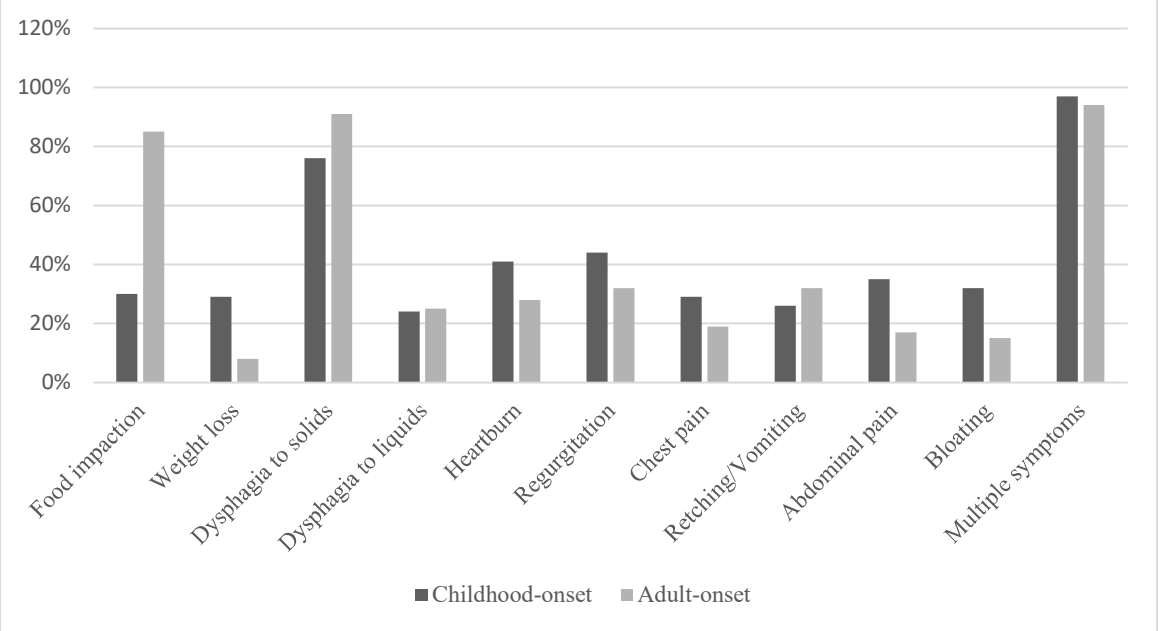

Figure 4.2: Initial presenting eosinophilic oesophagitis symptoms at the time of diagnosis in adults with childhood-onset and adult-onset eosinophilic oesophagitis.

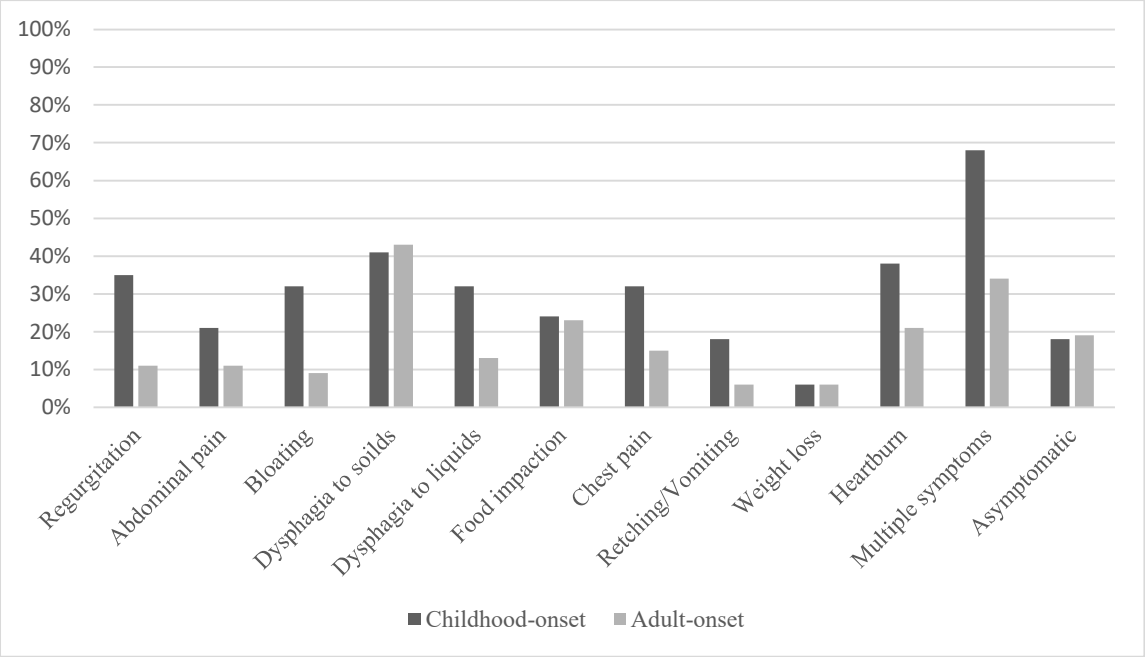

Figure 4.3: Current eosinophilic oesophagitis symptoms at the time of questionnaire in adults with childhood-onset and adult-onset eosinophilic oesophagitis.

Supporting information

Eosinophilic oesophagitis child questionnaire

Questionnaire: Natural history of eosinophilic esophagitis

Age of child: _____

Gender: M / F

Weight: _____

Height:_____

Country of Birth: _____

Please tick one box:

☐ I agree to the accessing of my child's medical records for the purpose of this study.

☐ I do not agree to the accessing of my child's medical records for the purpose of this study.

Signed _____ Dated _____

1. Does your child have any of the following medical condition?

Reflux disease YES / NO

Diabetes mellitus YES / NO

Bronchial asthma YES / NO

Others, please specify _____

Is your child on any medication
at present? YES / NO

If yes, please specify _____

_____

_____

2. Does your child have a history of allergy?

Allergy history YES / NO

If yes, is the allergy involving:

Food YES / NO

Medication YES / NO

Skin/eczema YES / NO

Asthma YES / NO

Hay fever YES / NO

3. Is there a family history of allergy?

Is there any family member with
allergy or history of allergy? YES / NO

If yes, please specify: _____

_____

Is there any family member
diagnosed with eosinophilic esophagitis? YES / NO

If yes, please specify: _____

_____

4. Social history?

Is there any family member
living in the same house who smokes? YES /NO

5. Details about your child's medical condition of eosinophilic esophagitis

At what age was your child diagnosed with eosinophilic esophagitis? _____

At what age did your child experience the first symptom/s that may be attributed to eosinophilic esophagitis? _____

How long did your child experience the symptoms before the diagnosis was made?

_____

What were your child's initial presenting symptoms?

Difficulty in swallowing:	
Food (solids)	YES / NO
Fluids	YES / NO
Heartburn	YES / NO
Food impaction*[1]	YES / NO
Chest pain	YES / NO
Vomiting	YES / NO
Weight loss	YES / NO
Abdominal pain/discomfort	YES / NO
Failure to thrive	YES / NO

Currently, does your child have any of the following symptoms?

Difficulty in swallowing:	
Food (solids)	YES / NO
Fluids	YES / NO
Heartburn	YES / NO
Food impaction	YES / NO
Vomiting	YES / NO
Chest pain	YES / NO
Weight loss	YES / NO
Abdominal pain/discomfort	YES / NO
Failure to thrive	YES / NO

How long have the symptom/s been present? _____

---

*[1] Food getting stuck requiring endoscopy to push it down or remove it

6. Details about your child's treatment and symptom progression

What treatment has been given to your child after the diagnosis was made?

Diet modification YES / NO
If yes, please tick the box where appropriate:
□Food exclusion □Elemental diet □Others (please specify_____)

Proton pump inhibitor YES / NO
(LOSEC, SOMAC, ZOTON, NEXIUM)

Steroids (oral or inhaler) YES / NO

Antacids YES / NO

Endoscopic dilatation YES / NO

Other treatment YES / NO

If yes, please specify _____

Did your child's symptoms improve after the treatment? YES / NO

If yes, which were the symptoms that improved?

Difficulty in swallowing:
Food (solids) YES / NO
Fluids YES / NO

Heartburn YES / NO

Food impaction YES / NO

Chest pain YES / NO

Vomiting YES / NO

Weight loss YES / NO

Abdominal pain/discomfort YES / NO

Failure to thrive YES / NO

If no, what were the changes to your child's treatment?

Please specify: _____

Did the changes to your child's treatment help the symptoms? YES / NO

If the symptom/s have reduced,
how long after treatment did you notice it? _____

Currently, is your child receiving any treatment? YES / NO

If yes, what is/are the treatment?

Proton pump inhibitor YES / NO

Steroids (oral or inhaler) YES / NO

Antacids YES / NO

Endoscopic dilatation YES / NO

Diet modification YES / NO
If yes, please tick the box where appropriate:
□Food exclusion □Elemental diet □Others (please specify _____)

Other treatment YES / NO

If yes, please specify _____

If no, what are the reasons?

My child doesn't have any more symptoms YES / NO

My child's symptoms persist but are bearable YES / NO

My child's symptoms persist and are problematic,
but he/she can't be bothered to take medication YES / NO

If your child has associated allergy (skin or airway or other organ), has it increased, reduced or remained the same after the treatment? _____

Did your child have any skin testing to identify the source of his/her allergy? YES / NO

If yes, did elimination of the source of allergy improve his/her symptoms related to:

Asthma YES / NO

Eosinophilic esophagitis YES / NO

Skin eczema YES / NO

Hay fever YES / NO

Food allergy YES / NO

Eosinophilic oesophagitis adult questionnaire

Questionnaire: Natural history of eosinophilic esophagitis

Age: _____

Gender: M / F

Weight: _____

Height: _____

Country of Birth: _____

Please tick one box:

☐ I agree to the accessing of my medical records for the purpose of this study.

☐ I do not agree to the accessing of my medical records for the purpose of this study.

Signed _____ Dated _____

1. Do you suffer from any of the following medical condition?

Reflux disease	YES / NO
Diabetes mellitus	YES / NO
High blood pressure	YES / NO
Ischemic heart disease	YES / NO
Bronchial asthma	YES / NO
Chronic obstructive pulmonary disease	YES / NO

Others, please specify _____

Are you on any medication at present? YES /NO

If yes, please specify _____

_____

2. Do you have a history of allergy?

Allergy history YES / NO

If yes, is the allergy involving:

Food YES / NO

Medication YES / NO

Skin/eczema YES / NO

Asthma YES / NO

Hay fever YES / NO

3. Do you have a family history of allergy?

Do you have any family member with
allergy or history of allergy YES / NO

If yes, please specify: _____

_____

Do you have any family member
diagnosed with eosinophilic esophagitis YES / NO

If yes, please specify: _____

_____

4. Social history?

Occupation: _____

Do you smoke? YES / NO

Do you regularly drink alcohol? YES / NO

5. Details about your medical condition of eosinophilic esophagitis

At what age were you diagnosed with eosinophilic esophagitis?
□ ≤ 20 years □ 21-40 years □ 41-60 years □ ≥ 61 years

At what age did you experience the first symptom/s that may be attributed to eosinophilic esophagitis?
□ ≤ 20 years □ 21-40 years □ 41-60 years □ ≥ 61 years
How long did you experience the symptoms before the diagnosis was made? _____

What were your initial presenting symptoms?

Difficulty in swallowing:	
Food (solids)	YES / NO
Fluids	YES / NO
Heartburn	YES / NO
Food impaction*[1]	YES / NO
Regurgitation	YES / NO
Chest pain	YES / NO
Retching	YES / NO
Weight loss	YES / NO
Abdominal pain/discomfort	YES / NO
Bloating	YES /NO

Currently, do you have any of the following symptoms?

Difficulty in swallowing:	
Food (solids)	YES / NO
Fluids	YES / NO
Heartburn	YES / NO
Food impaction	YES / NO
Regurgitation	YES / NO
Chest pain	YES / NO
Retching	YES / NO
Weight loss	YES / NO
Abdominal pain/discomfort	YES / NO
Bloating	YES / NO

How long have the symptom/s been present? _____

***1** Food getting stuck with a need for endoscopy to push it down or remove it

6. Details about your treatment and symptom progression

What treatment has been given to you after the diagnosis was made?

Diet modification YES / NO

If yes, please tick the box where appropriate: □ Food exclusion □ Elemental diet _____ □ Others (please specify)

Proton pump inhibitor (LOSEC, SOMAC, ZOTON, NEXIUM) YES / NO

Steroids (oral or inhaler) YES / NO

Antacids YES / NO

Endoscopic dilatation YES / NO

Other treatment YES / NO

If yes, please specify _____

Did your symptoms improve after the treatment? YES / NO

If yes, which were the symptoms that improved?

Difficulty in swallowing:

Food (solids) YES / NO

Fluids YES / NO

Heartburn YES / NO

Food impaction YES / NO

Regurgitation YES / NO

Chest pain YES / NO

Retching YES / NO

Weight loss YES / NO

Abdominal pain/discomfort YES / NO

Bloating YES / NO

If no, what were the changes to your treatment?

Please specify: _____

Did the changes to your treatment YES / NO

help the symptoms?

If the symptom/s have reduced,
How long after treatment did you notice it? _____

Currently, are you receiving any treatment? YES / NO

If yes, what is/are the treatment?

Proton pump inhibitor YES / NO

Steroids (oral or inhaler) YES / NO

Antacids YES / NO

Endoscopic dilatation YES / NO

Diet modification YES / NO
If yes, please tick the box where appropriate: □ Food exclusion
□ Elemental diet
□ Others (please specify_____)
Other treatment YES / NO

If yes, please specify _____

If no, what are the reasons?
I don't have any more symptoms YES / NO

My symptoms persist but are bearable YES / NO

My symptoms persist and are problematic,

but I can't be bothered to take medication YES / NO

If you have associated allergy (skin or airway or other organ), has it increased, reduced or remained the same after the treatment? _____

Did you have any skin testing to identify the source of your allergy? YES / NO

If yes, did elimination of the source of allergy improve your symptoms related to?

Asthma YES / NO

Eosinophilic esophagitis YES / NO

Skin eczema YES / NO

Hay fever YES / NO

Food allergy YES / NO

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CHAPTER 5: OESOPHAGEAL WALL APPEARANCE, THICKNESS, HISTOLOGY, AND MOTILITY

5.1 Overview

This chapter contains a published article assessing the relationship between different characteristics of oesophageal anatomy and function in EoE and GORD. Thus far, conflicting conclusions have been reached regarding the correlation between symptoms and histological and endoscopic findings in EoE. Additionally, only a few studies evaluating oesophageal wall thickness in EoE have been conducted, but none have compared their findings with that of GORD patients. Therefore, given that GORD is the primary differential diagnosis for EoE, this study endeavoured to ascertain if any differences or correlations exist between these variables and compare them with GORD.

5.2 Specific Author Contributions

Statement of Authorship

Title of Paper	Distal Oesophageal Wall Thickness Correlates with Dysphagia in Adult Patients with Eosinophilic Esophagitis
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Wong S, Tippett M, Zobel J, Safaeian R, Holloway RH, Ruszkiewicz A, Nguyen NQ. Distal esophageal wall thickness correlates with dysphagia in adult patients with eosinophilic esophagitis. Esophagus. 2022 Oct;19(4):554-559. doi: 10.1007/s10388-022-00924-7. Epub 2022 Jun 6. PMID: 35666332.

Principal Author

Name of Principal Author (Candidate)	Stephanie Wong		
Contribution to the Paper	Patient recruitment, data acquisition, data analysis, drafting of entire manuscript, submission of manuscript for publication		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	01/11/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

11. the candidate's stated contribution to the publication is accurate (as detailed above);
12. permission is granted for the candidate in include the publication in the thesis; and
13. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Marcus Tippett		
Contribution to the Paper	Data acquisition		
Signature		Date	02/11/2021

Name of Co-Author	Joshua Zobel		
Contribution to the Paper	Ethics submission, data analysis		
Signature		Date	08/11/2021

Name of Co-Author	Romina Safaeian		
Contribution to the Paper	Ethics submission, patient recruitment		
Signature		Date	08/11/2021

Name of Co-Author	Richard H Holloway		
Contribution to the Paper	Study concept design, critical revisions of manuscript		
Signature		Date	08/11/2021

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5.3 Published work 4: Distal Oesophageal Wall Thickness Correlates with Dysphagia in Adult Patients with Eosinophilic Esophagitis

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ABSTRACT

Background: Thickening of the esophageal wall in patients with eosinophilic esophagitis (EoE) and gastro-esophageal reflux disease (GERD) has been shown in studies using endoscopic ultrasound (EUS). We hypothesise that transmural inflammation in EoE results in prominent esophageal wall thickening compared with the mucosal inflammation in GERD. The aim of this study was to compare the relationship among dysphagia, endoscopic appearance, wall thickness, histology, and motility in EoE and GORD. **Methods:** EoE and GERD patients were prospectively studied between February 2012 and April 2021. Patients were studied on 2 separate occasions with endoscopy, EUS and mucosal biopsies, followed by high-resolution manometry. Epidemiology and dysphagia data were obtained. **Results:** A total of 45 patients (31 EoE, 14 GERD) were included. There were no significant differences in age, sex, duration of disease and presence of esophageal motility disorders. EoE patients had a higher dysphagia score ($P<0.001$), EREFS score ($P<0.001$) and peak eosinophil count ($P<0.001$) compared with GERD patients. Thickness of the submucosa in the distal esophagus in EoE was significantly higher than GERD ($P=0.003$) and positively correlated with duration of disease ($P=0.01$, $R=0.67$). Positive correlation was also found between dysphagia score and distal total esophageal wall thickness ($P=0.03$, $R=0.39$) in EoE patients. No correlation was found between these variables in GERD patients. **Conclusion:** Distal esophageal wall thickness positively correlates with dysphagia score in EoE but not GERD. This appears to be related to the composition of the submucosa which can be identified using EUS.

INTRODUCTION

Eosinophilic esophagitis (EoE) is a clinicopathological disorder characterised by an immunologic, antigen-driven process that manifests clinically with symptoms of esophageal dysfunction and histologically by eosinophilic inflammation.[1] The prevalence and incidence, which have been increasing over the past few years, are estimated to be 13-49 cases/100,000 persons and 1-20 cases/100,000 persons, respectively in the general population.[2, 3] EoE is thought to be the most frequent eosinophilic gastrointestinal disorder and the second most common cause of chronic esophagitis and dysphagia after gastro-esophageal reflux disease (GERD), which is one of its main differential diagnoses.[1, 4-6] Distinguishing between EoE and GERD may be challenging as many of their clinical and histological features overlap.[7, 8]

As a result of chronic inflammation caused by inflammatory cell infiltration of the esophageal mucosa, fibrosis may be induced in the wall which leads to remodelling of the deeper layers of the esophagus in both EoE and GERD.[9, 10] This is supported by the few endoscopic ultrasound (EUS) studies that have been performed showing significant thickening of the esophageal wall in EoE.[10-14] We hypothesise that transmural inflammation in EoE results in more prominent esophageal wall thickening compared with the mainly mucosal inflammation in GERD. The aim of this study was to comprehensively characterise the relationships among dysphagia, endoscopic appearance, wall thickness, histology and motility in the oesophagi of patients with EoE and compare it with GERD.

MATERIALS AND METHODS

Study population and design

This prospective, comprehensive clinicopathological study was performed at the Royal Adelaide Hospital, the largest adult tertiary referral hospital in South Australia and was approved by the Royal Adelaide Hospital Research Ethics Committee (protocol number: 111233). Patients between 18 and 70 years of age with a diagnosis of EoE or GERD were identified from pre-existing databases and recruited at their outpatient clinic/endoscopy appointments or via an invitation package. This invitation package contained an invitation letter, information sheet and the investigators' contact details should they wish to participate. No further correspondence was initiated if a response was not received following this attempt. Written informed consent was obtained from all patients. Exclusion criteria were history of

severe respiratory, cardiovascular, hepatic and/or renal disease, chronic alcohol abuse or epilepsy, medications that may influence gastrointestinal function, anti-coagulation therapy, gastrointestinal surgery, history of recent or recurrent epistaxis, known history of major psychiatric disorders, pregnant/breast-feeding women and inability to given written informed consent.

Definitions

A diagnosis of EoE was defined as ≥15 eosinophils per high powered field (/hpf) with symptoms of esophageal dysfunction and exclusion of other causes of esophageal eosinophilia. On the other hand, GERD was defined as a clinical diagnosis in a patient with typical symptoms (heartburn and regurgitation) responsive to proton-pump inhibitor (PPI) therapy and either a positive pH study or an endoscopy with biopsies confirming reflux disease.

Protocol

Recruited patients were studied initially with completion of a dysphagia score and an endoscopy with endoscopic ultrasound and mucosal biopsies. This was followed by assessment with high-resolution manometry (HRM) at a separate session ≥7 days after the initial endoscopy.

Symptom evaluation

Dysphagia was assessed using a modified version of a non-validated dysphagia score used by Straumann et al. in a randomised placebo-controlled trial of oral viscous budesonide in adult EoE patients.[15] This score assessed frequency of dysphagia ranging from none (0) to several times per day (5) and intensity of dysphagia ranging from unhindered swallowing (0) to obstruction requiring endoscopic intervention (5). Total scores ranged from 0 to 10.

Endoscopy and endoscopic ultrasound

All endoscopies were performed by either one of the two gastroenterology investigators (SW and NN) with EUS experience using conscious sedation with midazolam, fentanyl and/or propofol. A full endoscopic inspection of the upper gastrointestinal tract to the second part of the duodenum was first performed with a standard gastroscope (Olympus® 180, Japan). Endoscopic features of EoE were graded according to the EoE Endoscopic Reference Score (EREFS).[16] After completion of the endoscopic examination, the wall thickness of the oesophagus was evaluated with an Olympus® UM-S20-20R miniature probe that was passed

through the accessory channel of the gastroscope. The ultrasound probe was connected to Olympus® EU-ME1 ultrasound system. Thickness of the esophageal wall was measured at the proximal (≥20cm above gastro-esophageal junction (GEJ)), mid (10-20cm above GEJ) and distal (5cm above GEJ) segments. Esophageal wall thickness was measured in a contracted/non-distended state in all study patients to avoid distortion caused by the presence of longitudinal furrows and ensure that distensibility of the esophagus was constant/controlled. In addition to the total wall thickness, measurements of the mucosa and submucosa were also taken.

Histological evaluation

A total of 10 biopsies were then collected from the oesophagus (n=2 from each segment; proximal, mid and distal esophagus), stomach (n=2) and duodenum (n=2) after the endoscopic ultrasound measurements. Duodenal and gastric biopsies were taken to rule out other causes of esophageal eosinophilia. Biopsies obtained were evaluated after fixation in formaldehyde and hematoxylin-eosin staining. Peak eosinophil count was analysed per high power field (x400). All biopsies were examined by a single gastrointestinal pathology investigator (AR) who was blinded from the clinical and endoscopic data.

High-resolution manometric (HRM) assessment

Esophageal motor function was assessed using ManoScan 360™ high-resolution manometry system (Given Imaging) along with a ManoScan™ ESO catheter by a single technician (MT) investigator. After the catheter had been calibrated, topical anaesthetic spray (Co-Phenylcaine) and gel (Lignocaine 2%) was applied to one of the patient's nostrils after a 3-hour fast. In the upright posture, the catheter was intubated with the subject taking small sips of water to pass the assembly into the stomach. The subjects were then positioned in the left lateral position. A 3-minute resting period was observed including a 30 second period to assess basal sphincter pressure. Swallowing exercises were then performed which consisted of 10 x 5mls water swallows, 3 x 10mls multiple rapid swallows and 2 x 200mls cup of water. HRM data was analysed using ManoView™ software. Interpretation of the results were done according to The Chicago Classification version 4.0 by one of the gastroenterology investigators (SW) with motility experience.[17]

Statistical Analysis

Based on data published by Muroi et al.[12], a sample size of 38 cases was required to achieve a difference of 20% with power of 90% and α of 0.05. Descriptive statistics were used to describe the results with normality assessed using the Kolmogorov-Smirnov test. Mann-Whitney U test was used to evaluate the parameters between the two study groups. Spearman's rank correlation coefficient (Spearman r) was used to detect any significant correlation between variables in each study group. Statistical significance was determined by a *P* value of less than 0.05. Analyses and graph construction were performed using IBM®SPSS® software, version 28 and GraphPad® Prism software, version 9.

RESULTS

Demographics and clinical characteristics

A total of 45 patients (31 EoE and 14 GERD) were included in the study. The demographics and clinical characteristics of the 2 groups are summarised in **Table 5.1**. There were no significant differences in age, sex, duration of disease and presence of esophageal motility disorders. EoE patients had a higher dysphagia score, EREFS score and peak eosinophil count in all esophageal segments compared with GERD patients (**Figure 5.1**). Conversely, a higher proportion of GERD patients were on medical therapy as our EoE cohort had mostly refractory disease.

Differences in esophageal wall thickness between EoE and GERD

The differences in the esophageal wall thickness measurements, assessed by EUS, between the EoE and GERD are summarised in **Table 5.2**. Only the thickness of the submucosa in the distal esophagus of EoE patients was found to be significantly higher than that of patients with GORD (P=0.003).

Inter-relationship between esophageal wall thickness, symptoms, histology, and motility

In patients with EoE, there was a positive correlation between dysphagia score and distal total esophageal wall thickness (P=0.03, R=0.39) (**Figure 5.2**). EoE disease duration was not found to correlate with dysphagia score or distal total esophageal wall thickness. A positive correlation, however, was found between duration of disease and distal submucosa (P=0.01,

R=0.67), distal mucosa (P=0.03, R=0.5), mid submucosa (P=0.045, R=0.55) and proximal mucosa (P=0.01, R=0.64) thickness in EoE patients.

The above correlations in EoE were not observed in GORD, in particular, dysphagia score in GERD did not correlate with distal total oesophageal wall thickness (P=0.86, R=0.08).

DISCUSSION

Our study describes the differences in the endoscopic appearance, wall thickness, histology, and motility between patients with EoE and GERD. Our data shows that although there was no difference in total oesophageal wall thickness between the 2 entities, distal submucosa thickness was higher in EoE than GERD. Additionally, positive correlation was found between dysphagia score and distal total oesophageal wall thickness and disease duration and distal submucosal thickness in EoE patients. No correlation was found between these variables in GERD patients.

Contradictory to our hypothesis, the similarity found in total esophageal wall thickness indicates that the inflammation and subsequent remodelling process of the esophagus is comparable in both EoE and GERD. Previous EUS studies in adult and paediatric patients with EoE showing an increase in total esophageal wall thickness involving the mucosa, submucosa and muscularis propria, were performed using either healthy or asymptomatic EoE patients as the control group.[11-13] Our data indicates that the inflammatory infiltration mainly involves the distal submucosa of the esophagus in EoE, whereas this is evenly spread throughout the affected oesophageal layers in GERD. Thus, the correlation found between dysphagia score and distal total oesophageal wall thickness in EoE appears to be due to the composition of the distal submucosa.

There were no significant differences in age, sex and duration of disease indicating that our study cohort was well matched. A higher proportion of GERD patients were on active medical therapy as the majority of EoE patients were refractory to treatment based on peak eosinophil count obtained during endoscopy. The differences between the 2 groups regarding dysphagia score, EREFS score, and peak eosinophil count were expected findings given GERD patients less commonly present with dysphagia, have either normal or characteristic endoscopic findings such as erosive esophagitis, peptic strictures, a hiatus hernia and Barrett's esophagus, and exhibit esophageal eosinophilia, typically less than 10 per high power field.[7, 8, 18, 19]

Our study did not show any dissimilarity in the presence of esophageal motility disorders between EoE and GERD patients. Manometric irregularities occur in an estimated 20-76% of patients with EoE, namely patterns of weak or failed peristalsis, pan-esophageal pressurization, high intrabolus pressure and achalasia.[20-26] The prevalence of manometric abnormalities in EoE appears to increase with longer disease duration, but thus far no correlation has been found with either severity of dysphagia or endoscopic appearance of the oesophagus.[21, 24] Esophageal motility disorders in GERD patients have a similar type and prevalence to those with EoE ranging between 4-87%.[5, 27, 28] These data suggest that esophageal dysmotility is not a major contributor to symptoms and that they result primarily from the mechanical changes to the esophageal wall as a consequence of inflammation and fibrosis. Given this, we believe that the role of HRM to assist in the diagnosis and differentiation of EoE and GERD is limited.

The strength of our study is that it is the first, prospective, comprehensive clinicopathological study comparing EoE and GERD patients. We also had adequate sample size based on power calculation. Our study has several limitations, the first of which is that it was performed in a single institution. Although there was recall bias, our cohort was age and sex matched. Most of our EoE patients were untreated or had refractory disease and thus our findings may not be applicable to those who are treated. We were also unable to describe the histology of the submucosa given that endoscopically obtained biopsies are not able to penetrate in this layer. Finally, quantitative data on the range of normal oesophageal wall is lacking, hence we were unable to compare our data with a standardised normal.

CONCLUSIONS

Distal esophageal wall thickness positively correlates with dysphagia score in EoE but not GERD. This appears to be related to the composition of the submucosa which can be identified using EUS.

TABLES

Table 5.1: Demographics and clinical characteristics of eosinophilic esophagitis and gastroesophageal reflux disease patients.

	EoE (n=31)	GORD (n=14)	P-value
Age, median (IQR), years	41 (26)	54 (26)	0.056
Sex (Male: Female)	24M:7F	6M: 8F	0.067
Duration of disease, median (IQR), years	2 (5)	3 (4.25)	0.459
Medications, n (%)	15 (48%)	14 (100%)	**<0.001**
PPI, n	10	14	
Steroids, n	5	0	
Refractory to therapy, n (%)	11 (73%, 7 PPI, 4 Steroids)	-	
Dysphagia total score, median (IQR)	3 (3)	0	**<0.001**
EREFS total score, median (IQR)	2 (3)	0	**<0.001**
Fixed rings, n (%)	24 (77%)	0	
White plaques, n (%)	11 (35%)	0	
Longitudinal furrows, n (%)	26 (84%)	1 (7%)	
Strictures, n (%)	3 (10%)	0	
Other endoscopic findings			
Oesophagitis, (%)	3 (10%)	4 (29%)	
Hiatus hernia, n (%)	1 (3%)	8 (57%)	
Barrett's, n (%)	0	1 (7%)	
HRM			0.787
Normal, n	16	8	
Ineffective Oesophageal Motility, n	6	4	
Did not attend, n	9	2	
Peak eosinophil count/hpf, median (IQR)			
Proximal	26 (42)	0 (1)	**<0.001**
Mid	30 (36)	0 (1)	**<0.001**
Distal	28 (32)	0 (1)	**<0.001**

Table 5.2: Comparison of oesophageal wall thickness in eosinophilic esophagitis and gastroesophageal reflux disease patients.

Oesophageal thickness	EoE (n=31)	GORD (n=14)	P-value
Proximal, mean (±SD), mm			
Total	2.7 (±0.88)	3.0 (±1.07)	0.472
Submucosa	1.5 (±0.96)	1.6 (±0.55)	0.342
Mucosa	1.1 (±0.33)	1.2 (±0.28)	0.412
Mid, mean (±SD), mm			
Total	3.1 (±1.21)	3.2 (±1.06)	0.631
Submucosa	1.7 (±0.71)	1.5 (±0.81)	0.308
Mucosa	1.2 (±0.48)	1.1 (±0.29)	0.555
Distal, mean (±SD), mm			
Total	4.1 (±1.79)	4.0 (±0.86)	0.881
Submucosa	2.5 (±1.11)	1.4 (±0.59)	**0.003**
Mucosa	1.4 (±0.5)	1.3 (±0.52)	0.332

FIGURES

Figure 5.1. Comparison of endoscopic appearances, distal oesophageal wall thickness and histology between eosinophilic oesophagitis (a-d) and reflux oesophagitis (e-h). D1= Total oesophageal wall thickness, D2= Combined submucosa and mucosa thickness, D3= Mucosa thickness. Thickness of the submucosa was obtained by subtracting D3 from D2.

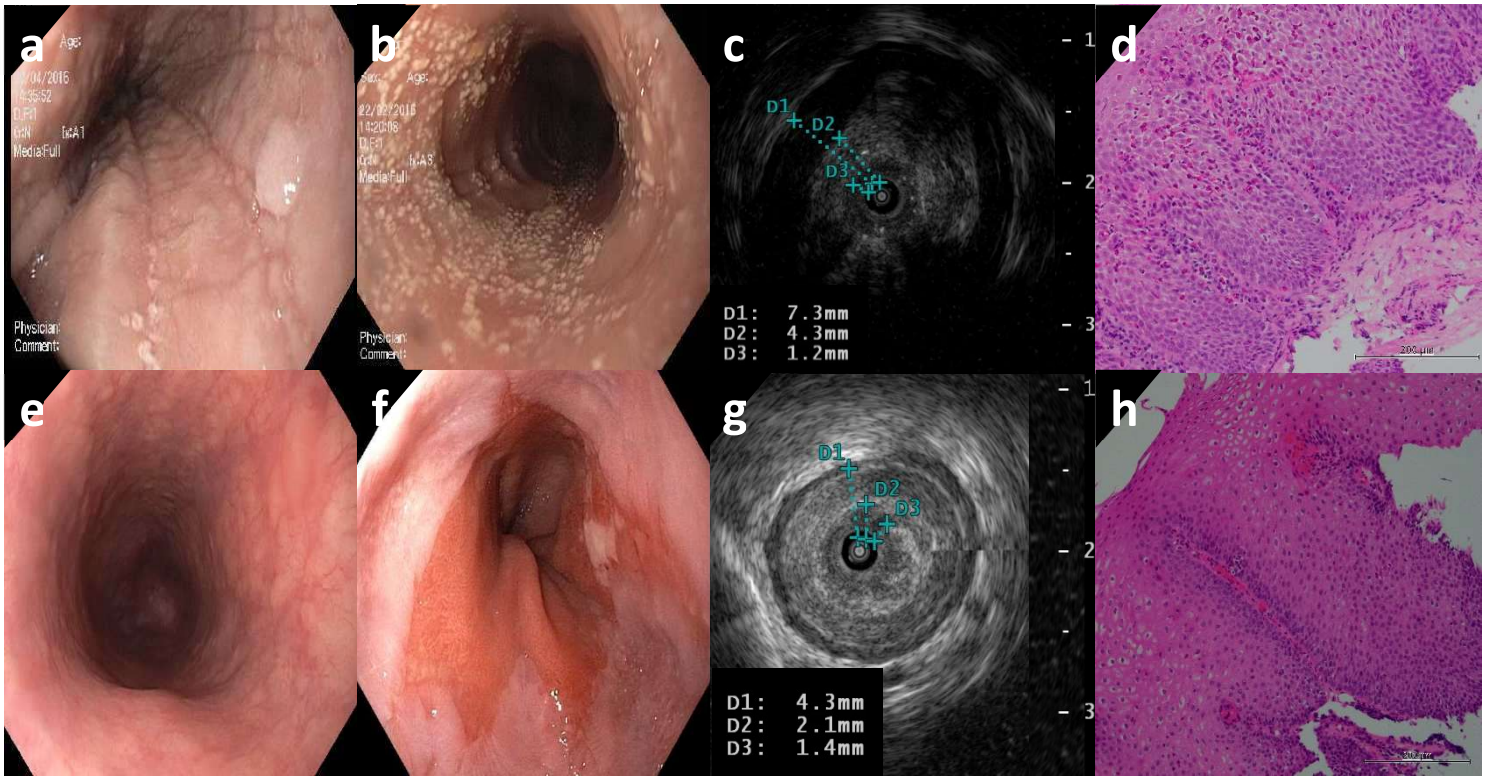
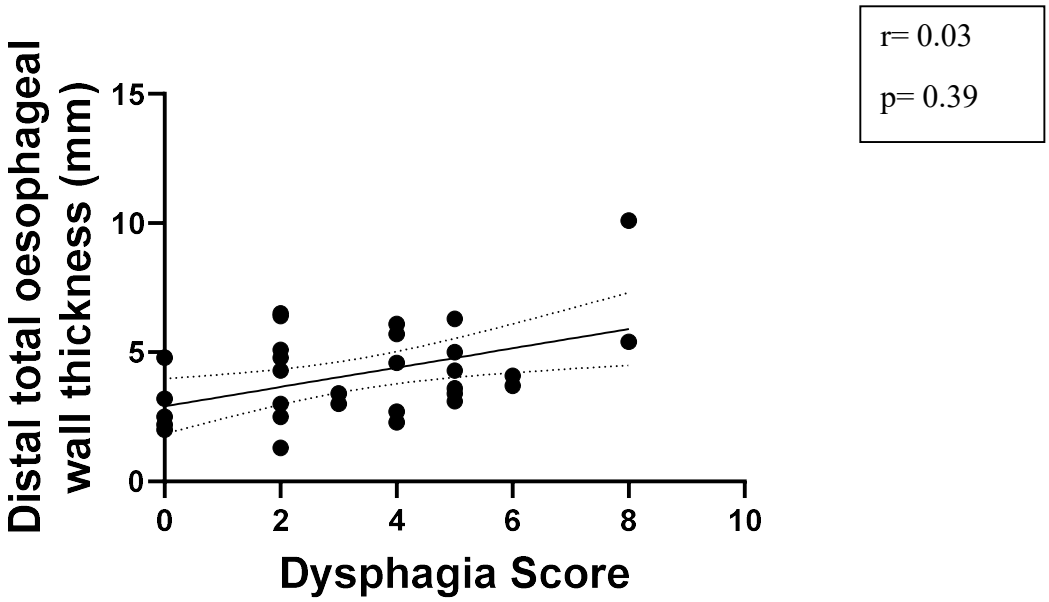


Figure 5.2. Correlation between dysphagia score and total distal oesophageal wall thickness of EoE patients.



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CHAPTER 6: LONGITUDINAL ASSESSMENT OF OESOPHAGEAL WALL THICKNESS

6.1 Overview

This chapter contains a submitted manuscript detailing a prospective evaluation of adult patients with EoE. Two distinct phenotypes have been proposed in EoE, namely inflammatory and fibro-stenotic. However, little is known about the factors that influence the inception or progression of each phenotype. Thus, we sought to assess the endoscopic appearance, wall thickness, histology, and dysphagia score of EoE longitudinally.

6.2 Specific Author Contributions

Statement of Authorship

Title of Paper	Distal esophageal Wall Thickness Increases with Time in Adult Patients with Eosinophilic Esophagitis
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Wong S, Safaeian R, Zobel J, Holloway RH, Ruszkiewicz A, Nguyen NQ. Increase in distal esophageal wall thickness with time in adult patients with eosinophilic esophagitis. JGH Open. 2023 Feb 1;7(3):178-181. doi: 10.1002/jgh3.12866. PMID: 36968573; PMCID: PMC10037037.

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Contribution to the Paper	Patient recruitment, data acquisition, data analysis, drafting of entire manuscript, submission of manuscript for publication		
Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

14. the candidate's stated contribution to the publication is accurate (as detailed above);
15. permission is granted for the candidate in include the publication in the thesis; and
16. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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6.3 Published work 5: Increase in Distal Esophageal Wall Thickness with Time in Adult Patients with Eosinophilic Esophagitis

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Keywords:

Endoscopic ultrasound; eosinophilic esophagitis, esophageal wall thickness; dysphagia; eosinophil count

ABSTRACT

Background: Eosinophilic esophagitis (EoE) is a chronic disease which may progress to a fibro-stenotic phenotype due to esophageal sub-epithelial fibrosis. Esophageal wall thickening in patients with EoE has been demonstrated in a few studies using endoscopic ultrasound (EUS). The aim of this study was to longitudinally assess the endoscopic appearance, wall thickness, histology, and dysphagia score of EoE patients. **Methods:** Patients with EoE were recruited and studied between February 2012 and April 2021. Patients were evaluated on 2 separate occasions at least 12 months apart with endoscopy, endoscopic ultrasound, and esophageal mucosal biopsies. A dysphagia score and epidemiology data were also assessed. **Results:** A total of 16 EoE patients were included with a mean follow-up duration of 2.2±1.2 years. In 14/16 (88%) patients, the total wall thickness of the distal esophagus significantly increased (P 0.0012) due to thickening of the muscularis propria (P 0.0218). However, only 1/14 (7%) patient had an increase in dysphagia score with 8/14 (57%) and 5/14 (36%) having stable and reduced dysphagia score respectively. No differences were found in the total thickness of other esophageal regions, dysphagia score, endoscopic appearance, and eosinophil count. **Conclusion:** Distal esophageal wall thickness increases with time in EoE patients, independent of dysphagia score and eosinophil count.

INTRODUCTION

Eosinophilic esophagitis (EoE), which has been increasing in prevalence and incidence, is most frequent eosinophilic gastrointestinal disorder and the second most common cause of chronic esophagitis and dysphagia after gastro-esophageal reflux disease (GERD).[1-5] The natural history of EoE is incompletely understood and it is yet unclear as to whether phenotypic variations exist or if these differences indicate a different disease pattern of responsiveness to therapy or prognosis.[6, 7] Current data indicates that EoE is a chronic, progressive disease with persistence of dysphagia and long-term complications such as stricture formation, food impaction, narrow-calibre esophagus and esophageal perforation.[6, 8-11]

The prevalence of fibrotic strictures has been shown to increase with increasing duration of disease as well as diagnostic delay suggesting that the natural history of EoE is a progression from an inflammatory to a fibro-stenotic phenotype due to development of subepithelial fibrosis in the esophageal wall.[9, 12, 13] Studies using endoscopic ultrasound (EUS) studies in EoE patients have been able demonstrate significant thickening of the esophageal wall due to this remodelling process.[14-18] We thus hypothesise that the esophageal wall thickness in EoE increases with time and can be detected using EUS. The aim of this study was to longitudinally assess the endoscopic appearance, wall thickness, histology, and dysphagia score of EoE patients.

MATERIALS AND METHODS

This longitudinal study was performed at the Royal Adelaide Hospital and was approved by the Royal Adelaide Hospital Research Ethics Committee (protocol number: 111233).

In the initial assessment study, patients between 18 and 70 years of age with a diagnosis of EoE (defined as ≥15 eosinophils/high powered field with symptoms of oesophageal dysfunction and exclusion of other causes of oesophageal eosinophilia) were retrieved from a pre-existing database and enrolled at their outpatient clinic/endoscopy appointments or via an invitation letter.[19] Enrolled patients underwent an endoscopy with endoscopic ultrasound and mucosal biopsies and completed a dysphagia score.[19] Esophageal wall thickness was measured in a contracted state to avoid distortion caused by longitudinal furrows and ensure constant distensibility of the esophagus.[19] A non-validated, modified version of the dysphagia score used by Straumann et al. in a randomised placebo-controlled trial of oral viscous budesonide in adult EoE patients was used in the study.[20] This score assessed frequency [none (0) to several times per day (5)] and intensity of dysphagia [unhindered swallowing (0) to obstruction

requiring endoscopic intervention (5)] with total scores ranging from 0 to 10.[19] These patients were then invited to return for a follow-up endoscopy with endoscopic ultrasound and mucosal biopsies along with completion of a dysphagia score ≥1 year after their initial assessment.
All endoscopies and endoscopic ultrasounds were performed by either one of the two gastroenterology investigators (SW and NN).

Statistical Analysis

Descriptive statistics were used to describe the results with normality assessed using the Kolmogorov-Smirnov test. Paired T test was used to evaluate the parameters between the two assessments. Spearman's rank correlation coefficient (Spearman r) was used to detect any significant correlation between variables in each study group. Statistical significance was determined by a *P* value of less than 0.05. Analyses and graph construction were performed using IBM®SPSS® software, version 28 and GraphPad® Prism software, version 9.

RESULTS

Demographics and clinical characteristics

A total of 16 EoE patients were included in the study with a mean follow-up duration of 2.2±1.2 years. The demographics and clinical characteristics of our patient cohort are summarised in **Table 6.1**.

Progression of oesophageal wall thickness

Esophageal wall thickness measurements obtained via EUS are summarised in **Table 6.2**. In 14/16 (88%) patients, the total thickness of the distal esophageal wall significantly increased over the 2.2±1.2 years (P=0.0012) due to thickening of the muscularis propria layer (P=0.0218). Of these, the majority had persistent dysphagia [9/14 (64%)], with only one of these patients' having an increase in dysphagia score. Only 5 patients (36%) who had an increase in wall thickness experienced a reduction in dysphagia score [from 4(3) to 0(3)] (**Figure 6.1**). Of the 2 patients that did not exhibit thickening of the esophageal wall, their dysphagia scores remained stable at 5 and 8 respectively.
The muscularis propria layer of the mid esophagus was thicker (P=0.0259) on follow-up assessment, but total thickness only showed a trend towards statistical significance (P=0.0542). No correlation was found between dysphagia score and proximal (P=0.78, R=-0.08), mid (P=0.58, R=0.15) and distal (P=0.14, R=0.39) esophageal wall thickness at follow-up

assessment. There was also no significant correlation between the change in dysphagia score and distal esophageal wall thickness at follow-up assessment (P=0.41, R=-0.22).

DISCUSSION

This is the first longitudinal study to assess esophageal wall thickness, endoscopic appearance, histology, and dysphagia score in adult EoE patients over a mean duration of 2.2 years. Our data shows that distal total esophageal wall thickness significantly increased over time due to thickening of the muscularis propria layer in 88% of patients and was independent of dysphagia score. No significant difference was found in EREFS score, eosinophil count, and total wall thickness in the mid and proximal esophagus between the initial and follow-up assessment.

Previous studies assessing the esophageal wall in EoE patients have shown significant thickening involving the mucosa, submucosa and muscularis propria on EUS supporting the occurrence of a chronic remodelling process.[14-16, 18, 21] Additionally, an assessment of full thickness esophageal samples in 12 EoE patients showed that histological changes and mediators of EoE pathogenesis were present in both the submucosa and muscularis propria.[22] Our study shows that remodelling and development of esophageal wall thickening appears to be progressive with disease duration but is limited to the muscularis propria layer. This layer may thus be the source of esophageal non-compliance and stiffness seen in studies using Endoluminal Functional Imaging System (FLIP).[23, 24]

The precise mechanism of dysphagia in EoE patients without strictures is unclear.[7, 25] It is hypothesized that the dysphagia is due to the remodelling process as described in the previous paragraph which leads to irreversible structural changes and subsequent loss of function.[9, 25, 26] The correlation between severity of symptoms and histological and endoscopic findings are unclear given conflicting study results.[1, 7, 9, 25, 27-29] Relying on symptoms alone is therefore inadequate to allow for either a diagnosis or assessment of efficacy of therapy.[1] Standard esophageal biopsies may also be an insufficient way of assessing overall disease severity given the changes of EoE involve the sub epithelium of the oesophagus.[30] Our finding that esophageal wall thickening progresses independent of dysphagia score and eosinophil count is thus not unexpected given this poor correlation and the lack of a readily available, validated symptom score for EoE.

The strength of our study is that it is a comprehensive, longitudinal study assessing esophageal wall thickness in adult EoE patients. The main limitation of our study is the small sample size where Type II errors may occur. We did not have a standardised treatment protocol,

however most of our patients (approximately 70%) were not on treatment due to non-compliance. The duration of our study may not have been adequate to allow changes in the esophageal wall thickness to have an impact on the symptom of dysphagia. Additionally, biopsies of the submucosa and muscularis propria were unobtainable and thus we were unable to depict the histological findings of this layer.

CONCLUSIONS

Distal esophageal wall thickness increases with time in EoE patients, independent of dysphagia score and eosinophil count. Larger studies are required to confirm this finding and assess its impact on clinical management of these patients.

TABLES

Table 6.1: Demographics and clinical characteristics.

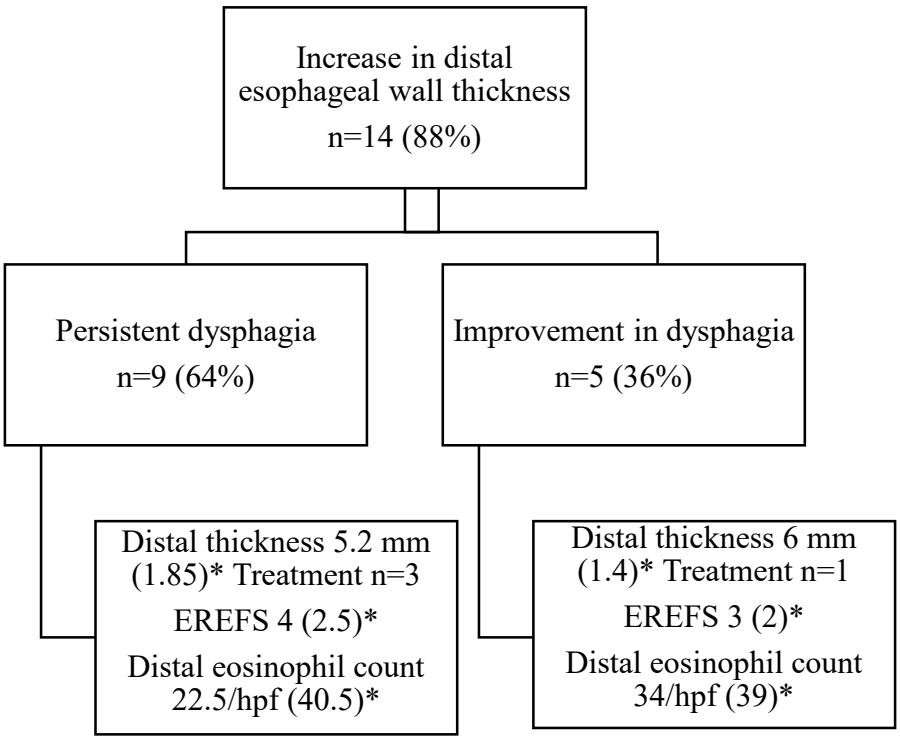
	Initial Assessment	Follow-up Assessment	P-value
Age, median (IQR) years	43.5 (28)	45.4 (60.5)	
Gender (Male: Female)	14M:2F		
Duration of disease, (median) IQR years	2.5 (7)		
Medications, n (%)	6 (38%)	5 (31%)	0.7505
PPI, n	6	5	
Steroids, n	1	2	
Refractory to therapy, n (%)	5 (83%)	2 (40%)	
Dysphagia score, median (IQR)	4 (3.75)	3 (5)	0.8945
EREFS total, median (IQR)	2.5 (2.75)	3 (2.75)	0.5805
Fixed rings, n (%)	12 (75%)	14 (88%)	
White plaques, n (%)	7 (44%)	8 (50%)	
Longitudinal furrows, n (%)	15 (94%)	13 81%)	
Peak eosinophil count/hpf, median (IQR)	18 (25.5)	22.5 (25)	0.9248
Proximal	25 (19.5)	25.5 (24)	0.3636
Mid	23.5 (16)	30 (37.5)	0.8168
Distal			
Other histological findings, n			
Eosinophil abscess	2	3	
Basal zone hyperplasia	13	13	
Dilated intracellular spaces	4	2	
Lamina propria fibrosis	1	1	
Eosinophil surface layering	6	6	

*EREFS= Endoscopic Reference Score

Table 6.2: Esophageal wall thickness.

Esophageal wall thickness	Initial Assessment	Follow-up Assessment	P-value
Proximal, median (IQR), mm			
Total	2.6 (1.55)	3.15 (1.7)	0.0555
Muscularis propria	2 (0.3)	2.1 (1.6)	0.8955
Submucosa	1.55 (0.55)	1.3 (0.825)	0.5567
Mucosa	0.8 (0.9)	1.2 (0.6)	0.6452
Mid, median (IQR), mm			
Total	2.85 (1.65)	3.65 (1.1)	0.0542
Muscularis propria	1.3 (1.2)	1.7 (1.1)	**0.0259**
Submucosa	1.85 (1.45)	1.45 (0.7)	0.4888
Mucosa	1.1 (0.6)	1 (0.4)	0.9510
Distal, median (IQR), mm			
Total	3.9 (2.3)	5.6 (2)	**0.0012**
Muscularis propria	1.3 (1.3)	2.4 (0.8)	**0.0218**
Submucosa	2.4 (1.33)	2.7 (1.85)	0.5711
Mucosa	1.3 (1.1)	1.3 (0.35)	0.6470

FIGURE



* Median (IQR)

Figure 6.1. Characteristics of patients showing an increase in distal esophageal wall thickness at follow-up assessment based on dysphagia score.

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CHAPTER 7: CONCLUSIONS

7.1 Key outcomes, significance, and limitations

1. Oesophageal mucosal IgG4 staining can be used as a diagnostic adjunct to differentiate EoE from GORD.

   The difficulty of differentiating EoE from GORD has been discussed previously in this thesis. A need for an alternative diagnostic test to assist with this was apparent. We thus performed a study showing that positive IgG4 staining had a high specificity and PPV for EoE and thus can be used as an adjunct to distinguish EoE from GORD in clinical practice. A limitation of this study was that it was a retrospective analysis.

2. Childhood- and adult-onset EoE are not distinct entities but a progressive disease.

   An important finding from this thesis is that most of those with childhood-onset EoE continued to have symptoms into adulthood. Only 3 previous studies assessing this transition period have been published, 2 of which support our conclusion. [1-3] Given this and the multicentre nature of our data, we conclude that childhood-EoE is a progressive disease and not a different disease entity in children and adults. Limitations of this study include recall bias and the inability to perform logistic regression, given the structure of our questionnaire.

3. Adults with childhood-onset EoE continue to have inflammatory symptoms.

   Due to ongoing inflammation and subsequent development of subepithelial fibrosis in the oesophagus, EoE is thought to progress from an inflammatory to a fibrostenotic phenotype. [4-6] However, our study found that those with childhood-onset EoE continued to have a higher incidence of inflammatory-type symptoms in conjunction with fibrotic-type symptoms after the transition to adulthood. This is the first study illustrating this interesting finding suggesting that a strong inflammatory component persists in adults with childhood-onset EoE. The limitations of this study are described in the paragraph above.

4. The thickness of the distal oesophageal wall appears to correlate with dysphagia in adults with untreated or refractory EoE.

To our knowledge, this is the first study to show a positive correlation between distal oesophageal wall thickness and dysphagia in EoE. This finding suggests that oesophageal wall inflammation with subsequent development of fibrosis and reduced compliance may play a role in the mechanism of dysphagia in these patients. Limitations of this study include a single-centre investigation, recall bias and the inability to obtain submucosal oesophageal biopsies.

5. The distal oesophageal wall thickness gradually increases in untreated EoE, irrespective of dysphagia score and eosinophil count.

   To our knowledge, this is the first longitudinal study to show that the thickness of the oesophageal wall increases independent of the severity of dysphagia and underlying inflammation in untreated EoE patients. Thus, early intervention to reduce inflammation may be imperative to prevent chronic symptoms and fibrotic complications. The limitations of this study include small sample size, non-standardised treatment protocol, short duration of follow-up and the inability to obtain oesophageal biopsies beyond the mucosal layer.

7.2 Implications on clinical practice

1. Incorporating the use of oesophageal mucosal IgG4 staining in indistinguishable cases of EoE and GORD.

   The Gastroenterology and Hepatology Department at the Royal Adelaide Hospital now uses IgG4 staining of oesophageal mucosal biopsies in difficult-to-distinguish cases of EoE and GORD since the publication of this manuscript. We are currently composing a Central Adelaide Local Health Network Organisational Wide Instruction on the diagnosis, management, and referral pathway for EoE. We will be incorporating this recommendation in the diagnostic algorithm.

2. Children with EoE require ongoing monitoring and treatment into adulthood.

   As we have shown in this thesis, childhood-onset EoE progresses into adulthood. This highlights the need for these patients to be transitioned from paediatric to adult specialist care for ongoing monitoring and treatment. Currently, no formal handover process occurs

between the paediatric and adult hospitals in South Australia. The creation of a panel of interested specialists to facilitate this should be considered.

7.3 Future research directions

1. Clarify the role of IgG4 in the pathogenesis of EoE.

   Current evidence indicates increased production of IgG4 in EoE and that oesophageal and serum IgG4 levels can be normalised with dietary and medical therapy. [11-15] However, the exact role of IgG4 in the pathogenesis of EoE is still limited, with the leading hypothesis being rapid immune complex deposition in the setting of high levels of local food antigen exposure. [16] Future research to determine the exact role of IgG4 in EoE could significantly impact treatment guidelines.

2. Identify risk factors and the proportion of those progressing from childhood-onset to adult-onset EoE.

   Early recognition of those at risk of progression would help streamline the transition of care from childhood to adulthood. Additionally, the ability to identify those with risk factors may assist in determining whether any preventative measures can be deployed.

3. Determine the normal range of oesophageal wall thickness.

   Quantitative data on the range of normal oesophageal wall thickness is deficient. Additional research to establish this is needed so that future endoscopic ultrasound studies can be compared to age- and sex-specific standards.

4. Standardise endoscopic oesophageal wall ultrasound measurements and techniques.

   Endoscopic ultrasound measurements and techniques varied widely during our literature review. Therefore, a standardised approach should be defined to allow for accurate regulation and comparison of future research endeavours.

5. Verify oesophageal wall thickness findings and their impact on clinical management.

   The usefulness of endoscopic ultrasound in monitoring disease activity in EoE patients is alluded to but not established in our study results. Larger multicentre studies are required to determine whether routine use of endoscopic ultrasound has a role in the management of these patients.

6. Establish treatment targets in EoE.

   In the absence of robust randomised controlled data on therapy and outcome of care, the development of the international consensus guidelines has been an important milestone in EoE treatment. [7, 8] Overall treatment goals are alleviating symptoms, preventing disease progression, improving quality of life, and reversing existing complications. [9] However, defining therapeutic endpoints based on symptom and histological improvement is complicated. [10] This is because symptoms of EoE can sometimes be non-specific and alleviated by dietary modifications which can be difficult to quantify. [9, 10] Also, there is little data looking specifically at the degree to which eosinophil density needs to be reduced to prevent or reverse oesophageal injury. [10] Another barrier to drug development in EoE is the previous absence of a disease severity index and the lack of predictive ability of the future likelihood of complicated EoE disease. [7]

7.4 Conclusion

Oesophageal mucosal IgG4 staining is a useful adjunct in the diagnosis of EoE and has been incorporated into clinical practice at our hospital. Supplementary information is required to clarify the precise role of IgG4 in the pathogenesis of this disease, as this may impact how we manage EoE in the future. Childhood-onset EoE appears primarily to be a progressive disease into adulthood with a high incidence of inflammatory-type symptoms. Given this, there is a need for these patients to have ongoing specialist input with consideration of a transition panel to expedite this. Further research to identify possible risk factors for progression may help to streamline this process. Dysphagia in adults with untreated or refractory EoE correlates with the thickness of the distal oesophageal wall, but there is a need for additional studies to ascertain normal ranges and standardise techniques before more research is performed to validate our results. The thickness of the distal oesophageal wall increased in untreated EoE

regardless of dysphagia score and eosinophil count. However, more extensive studies are required for corroboration before integrating EUS into clinical practice. Lastly, more robust data is needed overall in EoE to assist with establishing treatment targets to prevent morbidity in these patients and reduce the burden on the healthcare system.

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Appendix 1

World Journal of Gastrointestinal Pathophysiology

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World J Gastrointest Pathophysiol 2018 October 25; 9(3): 63-72

DOI: 10.4291/wjgp.v9.i3.63

ISSN 2150-5330 (online)

MINIREVIEWS

Gastro-oesophageal reflux disease and eosinophilic oesophagitis: What is the relationship?

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Author contributions: Wong S contributed to drafting of manuscript; Ruszkiewicz A, Holloway RH and Nguyen NQ contributed to critical revisions of manuscript.

Conflict-of-interest statement: There is no conflict of interest to disclose from all authors.

Manuscript source: Invited manuscript

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Received: August 2, 2018
Peer-review started: August 3, 2018
First decision: August 24, 2018
Revised: September 17, 2018
Accepted: October 11, 2018
Article in press: October 11, 2018
Published online: October 25, 2018

Abstract

Eosinophilic oesophagitis (EoE) and gastro-oesophageal reflux disease (GORD) are the most common causes of chronic oesophagitis and dysphagia associated with oesophageal mucosal eosinophilia. Distinguishing between the two is imperative but challenging due to overlapping clinical and histological features. A diagnosis of EoE requires clinical, histological and endoscopic correlation whereas a diagnosis of GORD is mainly clinical without the need for other investigations. Both entities may exhibit oesophageal eosinophilia at a similar level making a histological distinction between them difficult. Although the term proton-pump inhibitor responsive oesophageal eosinophilia has recently been retracted from the guidelines, a relationship between EoE and GORD still exists. This relationship is complex as they may coexist, either interacting bidirectionally or are unrelated. This review aims to outline the differences and potential relationship between the two conditions, with specific focus on histology, immunology, pathogenesis and treatment.

Key words: Relationship; Pathogenesis; Eosinophilic oesophagitis; Histological features; Gastro-oesophageal reflux disease

Core tip: The relationship between gastro-oesophageal reflux disease and eosinophilic oesophagitis is complex as they may coexist, either interacting bidirectionally or are

unrelated. This review aims to outline the differences and potential relationship between the two conditions, with specific focus on histology, immunology, pathogenesis and treatment.

Wong S, Ruszkiewicz A, Holloway RH, Nguyen NQ. Gastro-oesophageal reflux disease and eosinophilic oesophagitis: What is the relationship? *World J Gastrointest Pathophysiol* 2018; 9(3): 63-72 Available from: URL: http://www.wjgnet.com/2150-5330/full/v9/i3/63.htm DOI: http://dx.doi.org/10.4291/wjgp.v9.i3.63

INTRODUCTION

Eosinophilic oesophagitis (EoE) is a clinicopathological condition characterised by an antigen-driven immunologic process that manifests clinically with symptoms of oesophageal dysfunction and histologically by eosinophilic inflammation[1]. The first case report of oesophageal eosinophilia can be traced back as far as 1962 by Schreiber[2], followed by the first published case series of EoE as a distinct clinicopathological condition in 1993 by Attwood *et al*[3] In 2007, the first consensus recommendation by an international expert panel for the diagnosis and treatment of EoE was published[4]. This consensus was recently updated in 2017[5].

The recognition of EoE has increased so swiftly that it is now thought to be the most frequent eosinophilic gastrointestinal disorder as well as the second most common cause of chronic oesophagitis and dysphagia after gastro-oesophageal reflux disease (GORD)[6]. Although it is still an uncommon disease, the prevalence has been increasing over the past few years with an estimated prevalence in the general population of 13-49 cases/100000 persons[5,7]. This is also in keeping with an increasing incidence of EoE estimated at 1-20 cases/100000 persons[5,7]. Various hypotheses have been considered for this phenomenon particularly that of an increase in the recognition of the disease and an increase in volume of endoscopies performed[8-10]. However, two population-based studies have shown that the incidence and cumulative prevalence of EoE has indeed increased more than the rate of annual endoscopies during the observation period[11,12]. This, therefore, argues in favour of a true rise in the incidence and prevalence of the disease.

Attwood *et al*[3] first characterized EoE as a distinct entity from GORD in 1993 where patients with more than 20 eosinophils per high power field and dysphagia in the absence of endoscopic oesophagitis and a normal 24-h pH testing were proposed to have EoE. According to the diagnostic criteria for EoE, other diseases associated with oesophageal eosinophilia must be excluded before a diagnosis of EoE is made (Table 1), with the main differential being GORD[1,13,14]. It is important to distinguish between EoE and GORD as their pathogenesis, natural history, monitoring and treatment differ[15]. This is challenging as many of their clinical and histological features overlap[15,16]. Given the prevalence of GORD in the general population is approximately 20%, it is inevitable that there will be a high probability for EoE to co-exist with GORD[16].

Table 1 Diseases associated with oesophageal eosinophilia

GORD
Eosinophilic gastrointestinal diseases
Atopy
Coeliac disease
Crohn's disease
Oesophageal infections
Hypereosinophilic syndrome
Achalasia
Drug hypersensitivity
Vasculitis
Pemphigoid vegetans
Connective tissue disease
Graft-versus-host-disease
Oesophageal atresia

GORD: Gastro-oesophageal reflux disease.

Prior to the 2017 consensus, a lack of response to a 2-mo course of a proton-pump inhibitor (PPI) was required exclude PPI-responsive oesophageal eosinophilia (PPI-REE) and confirm the diagnosis of EoE[1]. Patients with PPI-REE presented symptomatically like a typical EoE patient, had GORD diagnostically excluded and exhibited a clinicopathologic response to PPI therapy[1]. Recent evidence, however, indicate that differentiating PPI-REE from EoE is counterintuitive as their phenotypic, molecular, mechanistic and therapeutic features cannot be reliably distinguished[15,17-20]. Also, there was no definition regarding the extent of clinical and histological response required to diagnose PPI-REE[13,15]. Thus, the most recent consensus has retracted the term PPI-REE and considers PPI therapy as a therapeutic agent, rather than a diagnostic criterion[5]. The term "PPI-responsive EoE" has been proposed to replace the now defunct PPI-REE[20].

Despites the fact that PPI responders are now considered to be within the EoE continuum, a relationship between EoE and GORD still exists[5]. Studies have suggested that up to 30%-40% of EoE patients may be PPI responsive, either due to a reduction in acid secretion in patients with co-existent GORD or by means of other still unknown anti-inflammatory mechanisms[21,22]. PPI therapy may also be helpful in patients with EoE as the altered oesophagus may be predisposed and more sensitive to acid exposure[23]. This review aims to outline the factors that differentiate between EoE and GORD as well as to evaluate the complex relationship between the two entities in term of pathophysiology and immunology.

PATHOGENESIS

The main pathogenic mechanism of GORD is increased

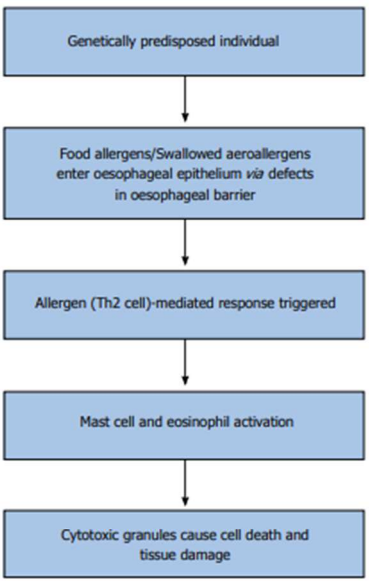

Figure 1 Proposed pathogenesis of eosinophilic oesophagitis.

transient lower oesophageal sphincter (LOS) relaxations (TLOSRs), leading to excessive reflux of gastric acid to the lower oesophageal mucosa[24]. Other potential mechanistic factors that can increase acid reflux to the oesophagus are impaired LOS resting pressure, impaired oesophageal acid clearance, delayed gastric emptying and anatomical factors, such as a hiatus hernia[24]. More recently, impaired mucosal resistance and increased visceral hypersensitivity to acid have also been reported to predispose to GORD[24]. Histologically, it was thought that erosive changes in the distal oesophagus developed due to direct chemical-induced injury of the oesophageal mucosa and death of surface cells[25]. Such injury has been shown to provoke a T-helper Type 1 (Th1) inflammatory response, activating mostly granulocytes and lymphocytes[25]. Thus, it is intriguing that oesophageal eosinophilia can occasionally be seen in GORD, and the underlying mechanism remains unclear[26]. A study showing that GORD may also be a cytokine-mediated disease lead to the discovery that oesophageal squamous cells from EoE and GORD patients exhibit similar levels of eotaxin-3 (a chemokine that attracts eosinophils) when stimulated by T-helper Type 2 (Th2) cytokines; production of which is typical of an allergic disorder[10,15,22,26,27]. This suggests that GORD may be driven to a Th2 inflammatory response when the appropriate stimulus is present leading to oesophageal eosinophilia[26]. Low intraluminal baseline impedance has been shown to be associated with dilatation of intercellular spaces and increased acid exposure in patients with GORD[28]. However, whether this damage can lead to exposure of food allergens and subsequently a Th2 response is unknown[26,29,30].

Although the exact pathophysiology of EoE is not fully understood, substantial evidence exists to show that EoE is an allergen (Th2 cell)-mediated response in genetically predisposed individuals (Figure 1)[10,31,32]. Defects in the oesophageal barrier are thought to facilitate the entry of food allergens or swallowed aeroallergens into the oesophageal epithelium which trigger a Th2 response and lead to mast cell activation and release of mediators such as interleukin (IL)-5, which is a known eosinophil activator[10,22]. Activated eosinophils then release cytotoxic granules which contribute to cell death and tissue damage in these patients[10,33,34]. The gene coding for eotaxin-3, *CCL26* is overexpressed in the oesophagus of patients with EoE compared to healthy controls, which correlates with the increased levels of IL-5 and IL-13 in the oesophagus and blood of EoE patients[35,36]. The development of EoE may also be associated with a genetic predisposition[10]. Hereditary collagen disorders such as Marfan and Ehlers-Danlos syndromes are the most frequent associations of EoE with an incidence of about one percent[21]. In patients with atopic dermatitis, a loss of function mutation in the gene filaggrin (2282del4) is overexpressed in EoE patients compared with healthy controls[37]. Filaggrin is a key structural, keratin-binding protein that plays an important role in the maturation of skin as an epithelial barrier by preventing keratin proteolysis[37]. EoE has been shown in paediatric patients to be associated with variants at chromosome 5q22 encompassing the gene *TSLP* (thymic stromal lymphopoetin), which encodes a cytokine that controls dendritic cell-mediated Th2-cell responses[21,38]. More recently, EoE susceptibility locus was found at 2p23 which encodes *CAPN14*, which is upregulated on exposure to IL-13[39]. However, the exact impact of these genetic abnormalities on the pathogenesis of EoE is uncertain.

EPIDEMIOLOGY AND CLINICAL PRESENTATION

A few epidemiological differences exist between GORD and EoE. GORD is typically diagnosed in the second to fifth decade of life[20]. In contrast, EoE has a bimodal age presentation, with one peak in childhood and the second in the third and fourth decade with the mean age of diagnosis of 38 years[1,33,40]. Furthermore, whilst there is no gender preponderance in GORD, EoE affects males three times more than females[1,41,42]. Both conditions have been more frequently reported in Caucasians compared with other ethnicities[1,8,41,43]. It should be noted that the prevalence of GORD is much higher than that of EoE, ranging between 10%-20% in the Western population as compared to less than 1% for EoE[8,9,40,41]. Obesity has been shown to associated with GORD whereas EoE is strongly associated with atopic diseases

Table 2 Diagnostic features of gastro-oesophageal reflux disease and eosinophilic oesophagitis

	GORD	EoE
Endoscopic	Erosive oesophagitis	Trachealization
	Peptic strictures	Felinization
	Hiatus hernia	Whitish exudates
	Barrett's oesophagus	Longitudinal furrows
		Oedema
		Diffuse oesophageal narrowing
		Narrow-calibre oesophagus
		Oesophageal lacerations
		Loss of mucosal vascular pattern
Histological	Eosinophilia < 10/hpf	Eosinophilia ≥ 15/hpf
		Eosinophilic microabscesses
		Eosinophil degranulation
		Basal cell hyperplasia
		Papillary lengthening
		Superficial layering of eosinophils
		Extracellular eosinophil granules
		Intracytoplasmic keratinocyte vacuolation
		Dilated intracellular spaces
		Lamina propria fibrosis
		Positive intrasqamous IgG4
Motor function	Non-specific	Non-specific

GORD: Gastro-oesophageal reflux disease; EoE: Eosinophilic oesophagitis.

such as asthma, food allergy, eczema, environmental allergies and chronic rhinitis[1,8,10,31,44].

GORD has been defined by the Montreal Classification as a condition that occurs due to retrograde flow of gastric contents into the oesophagus that lead to troublesome symptoms, which are typically heartburn and regurgitation[45,46]. Other less common symptoms include chest pain, dysphagia, dyspepsia, epigastric pain, nausea, bloating, belching, chronic cough, asthma, laryngitis and other respiratory symptoms[45-48]. Whilst dysphagia is infrequent in GORD, it is the most common presenting symptom for EoE along with food bolus impaction[1,10,49]. Approximately 50% of patients who present with food bolus impaction and up to 15% of patients who undergo endoscopy for non-obstructive dysphagia will have EoE[6,50]. Although some EoE patients report GORD symptoms, they may respond poorly to PPIs[51]. Fifty to eighty percent of EoE patients have a prior history of atopic symptoms[21]. Other non-specific symptoms include chest pain, heartburn, regurgitation, dyspepsia, nausea and vomiting, odynophagia, abdominal pain and non-specific throat symptoms[1,10,31,33,49,52].

DIAGNOSIS

A diagnosis of GORD is usually based on clinical symptoms, typically heartburn and regurgitation, in a patient who is responsive to PPI therapy[46]. Thus, upper endoscopy, routine biopsies from the distal oesophagus and ambulatory pH testing are not usually required in a patient with typical GORD symptoms in the absence of alarm symptoms such as dysphagia, odynophagia and weight loss[16,44,46]. The diagnosis of EoE on the other hand, relies on a correlation between clinical symptoms, endoscopic and histological features as there is no one pathognomonic feature of EoE[10,13]. According to the most recent consensus, it requires the presence of ≥ 15 intraepithelial eosinophils per high power field in one or more oesophageal mucosal biopsies in combination with symptoms of oesophageal dysfunction[5]. However, this definition may be too simplified as the diagnosis of EoE may be established with a lower intraepithelial eosinophil count if there is strong clinical suspicion and other histological features associated with eosinophilic inflammation are present[1,10]. Given that excessive accumulation of eosinophils in tissues is a common finding in numerous gastrointestinal disorders, other causes of oesophageal eosinophilia (Table 1) should also be excluded, particularly GORD[1,14]. The following diagnostic features that may be found in GORD and EoE and may help distinguish between the two entities are summarised in Table 2.

Endoscopic oesophageal features

Relevant endoscopic findings of GORD are erosive oesophagitis, peptic strictures, a hiatus hernia and Barrett's oesophagus[15,16,46]. Endoscopy has a high specificity for diagnosing GORD particularly when erosive oesophagitis is seen and the Los Angeles classification is used[53]. However, most patients with GORD will have normal endoscopies[15,16]. In contrast, endoscopic oesophageal features of EoE patients are trachealization, felinization, whitish exudates, longitudinal furrows, oedema, diffuse oesophageal narrowing, narrow-calibre oesophagus and oesophageal lacerations secondary to passage of the endoscope[1,10,13,16,54] (Figure 2). Loss of

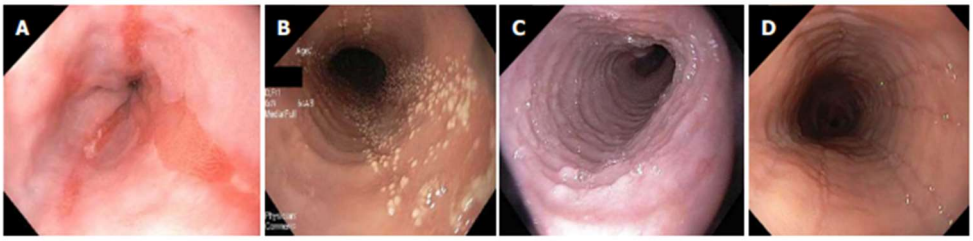

Figure 2 Endoscopic changes in patients with gastro-oesophageal reflux disease and eosinophilic oesophagitis. A: Erosive oesophagitis of gastro-oesophageal reflux disease; B: White exudates in eosinophilic oesophagitis (EoE); C: Mucosal rings or trachealization in EoE; D: Longitudinal furrows in EoE.

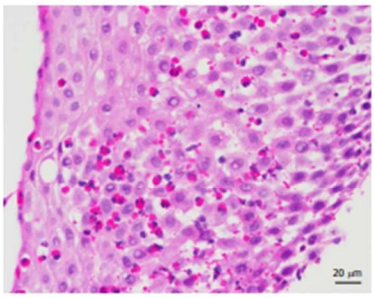

Figure 3 Histological specimen from the oesophagus (luminal aspect on left) of an eosinophilic oesophagitis patient showing marked oedema and numerous intraepithelial eosinophils in the oesophageal squamous mucosa, which are also seen in the superficial component of the mucosa.

mucosal vascular pattern has also been reported[55]. These features however, are not pathognomonic for EoE and thus histological correlation is required[1,10]. Normal endoscopic findings have been reported in up to 30% of patients with EoE[10,13].

Histological features

Patients with GORD may exhibit oesophageal eosinophilia, typically less than 10 per high power field as compared to ≥ 15 per high power field for EoE[1,10,15,56] (Figure 3). The presence of additional histological features of eosinophilic microabscesses, eosinophil degranulation, basal cell hyperplasia, papillary lengthening, superficial layering of eosinophils, extracellular eosinophil granules, intracytoplasmic keratinocyte vacuolation, dilated intracellular spaces or lamina propria fibrosis are more supportive of a diagnosis of EoE[1,10,13,16,57]. Although some of these additional histological features have been reported in biopsy specimens of patients with GORD, they are less commonly found as compared to EoE[10,13,16,57]. Recently, Zukerberg *et al*[17] showed that immunohistochemical staining of oesophageal tissue with IgG4 could help distinguish EoE from GORD, given that 76% of EoE cases were positive for intrasqamous IgG4 and none of the GORD cases were positive. The distribution of oesophageal eosinophilia may also be helpful in distinguishing the two conditions, with diffuse oesophageal eosinophilia more suggestive of EoE and distal oesophageal eosinophilia of GORD[16]. Thus, it is important to biopsy at least 2 regions of the oesophagus and accurately label the site of oesophageal biopsies.

Oesophageal motor function

Oesophageal manometry is of limited use in the diagnosis of GORD and EoE given that findings have so far been non-specific[1,13,58]. Oesophageal motility disorders found in patients with GORD have a similar type and prevalence to patients with EoE ranging between 4%-87%[14,21,33]. However, in cases where dysphagia is the main symptom, it is important to perform manometric assessment to exclude major and minor disorders of peristalsis which can sometimes mimic symptoms of GORD and EoE[18,33]. The duration of EoE has been shown to be longer in those with abnormal oesophageal motility[59].

TREATMENT

The initial management of GORD usually involves a combination of lifestyle interventions and medical therapy with the aim of eliminating symptoms, repairing any existing oesophageal mucosal injury and preventing further inflammatory injury[46,60]. Lifestyle interventions of weight loss (particularly if BMI > 25 or recent weight gain) and head of bed elevation have been proven to reduce symptoms and improve oesophageal pH values[61,62]. Other lifestyle interventions such as avoidance of late evening meals and cessation of alcohol, tobacco, chocolate, caffeine, spicy foods, citrus and carbonated drinks lack evidence and are not routinely recommended[46]. Medical therapy such as antacids, histamine-receptor antagonists (H_2RA) or PPI therapy should then be considered in patients failing lifestyle interventions alone[46,60]. PPI therapy is effective in 70%-80% of patients and has been shown to be superior to H_2RAs in regard to healing rates and decreased relapse rates[63]. Surgical therapy is as effective as medical therapy and may be contemplated in GORD patients who wish to discontinue medications, are non-compliant, have side-effects associated with medications, have a

large hiatus hernia or have refractory oesophagitis and symptoms despite optimal medical therapy[46].

The choice of initial treatment for EoE patients on the other hand is made on an individualized basis as PPI therapy, topical steroids and dietary therapy can all be considered as first-line therapeutic options[5]. All EoE patients should receive treatment to improve quality of life, prevent oesophageal remodelling secondary to active eosinophilic inflammation and prevent oesophageal injury due to the disease or endoscopic intervention[64]. 30%-40% of EoE patients may be responsive to PPIs, either due to a reduction in acid secretion in patients with co-existent GORD or by means of other still unknown anti-inflammatory mechanisms[21,22]. EoE patients can also be treated with topical steroids as it has been shown to improve symptoms and reduces oesophageal eosinophilia[21,65]. Viscous steroids have been shown to be more effective than nebulized steroids possibly due to greater mucosal contact time compared with the latter[66]. A recent meta-analysis of seven randomized controlled trials concluded that although there was an increased risk of asymptomatic oesophageal candidiasis with topical steroid therapy, it is considered safe with no evidence of adrenal suppression[67]. Dietary therapy is based on the fact that the majority of EoE patients have food allergies that may contribute to the pathogenesis of the disease[22,68]. There are 3 strategies of dietary therapy: An amino acid-based formula/elemental diet, a targeted elimination diet guided by allergy testing, and an empiric elimination diet[22,65,68]. All diets should be followed for a minimum of 6 wk and its efficacy evaluated *via* symptoms as well and oesophageal biopsies[65,69].

Oesophageal dilation, either *via* through-the-scope balloons or by Savary bougies can lead to long-lasting symptom improvement in EoE patients with structuring disease or impaired oesophageal distensibility due to subepithelial fibrosis[21,22]. Clinical improvement post dilation occurred in 75% of patients[70]. A meta-analysis evaluating the clinical efficacy and safety of oesophageal dilation in these patients showed that it is a safe procedure with a < 1% rate of serious complications[70]. However, it does not result in a decreased in eosinophil infiltration or histologic improvement and thus should not be used as a sole therapeutic option in these patients[5,71]. Several other treatment options for EoE have been assessed namely Montelukast (leukotriene receptor antagonist), Infliximab (anti-tumour necrosis factor), Mepolizumab (anti-IL-5), Azathioprine or 6-mercaptopurine, Reslizumab (IL-5 neutralizing antibody), Omalizumab (anti-IgE), QAX576 (anti-IL-13) and OC000459 (prostaglandin D2 receptor antagonist)[34,64,72-80] Although studies of these agents have shown changes in the biological behaviour of EoE disease markers, they have not yet displayed sufficient clinical benefit for widespread use[81].

RELATIONSHIP BETWEEN EoE AND GASTROESOPHAGEAL REFLUX DISEASE

The interaction between EoE and GORD is complex and may be bidirectional[5]. An approximate prevalence of GORD in the general population of 20% is sufficiently high enough to make the coexistence of EoE and GORD plausible[16]. In patients with refractory GORD symptoms, EoE was found in approximately 4%[10,56]. Four hypotheses to account for interactions between oesophageal eosinophilia and GORD have been proposed: Eosinophilia as a marker of GORD; GORD and EoE coexist but are unrelated, EoE contributes or causes GORD; and GORD contributes to or causes EoE[16,20,82,83].

Eosinophilia as a marker of GORD

GORD is thought to cause a mild eosinophilia in the absence of EoE[16,82]. Acid exposure was thought to cause oesophageal injury which results in chronic inflammation, including the presence of oesophageal eosinophils that are recruited *via* an increase in expression of adhesion molecules, release of chemokines that attract eosinophils and increase in blood flow[16]. However, the role of these adhesion molecules and chemokines in the pathogenesis of GORD is yet unclear[16]. A study also showed that dense oesophageal eosinophilia in GORD was uncommon[3].

GORD and EoE coexist but are unrelated

As mentioned above, due to a high prevalence of GORD in the general population, the coexistence of EoE and GORD due to chance alone is plausible[16,83]. Oesophageal pH studies have shown that 25%-50% of EoE patients have increased oesophageal acid exposure, thus supporting the notion that the two entities can coexist[1,16].

EoE contributes or causes GORD

This hypothesis is based on the fact that eosinophils secrete a number of agents that affect the integrity of the mucosal barrier and the function of oesophageal smooth muscle as well as producing a direct cytotoxic effect on the mucosa[16,20]. Remodelling effect in EoE may contribute to increased acid exposure due to effects on the LOS or impaired oesophageal clearance of refluxed contents[16,20].

GORD contributes to or causes EoE

An unproven hypothesis has suggested that GORD may contribute to the pathogenesis of EoE by causing changes in the integrity of the oesophageal mucosa, promoting trans-epithelial allergen permeation followed by allergic immune activation[5,84].

CONCLUSION

The relationship between EoE and GORD is complex as

they are different entities that may coexist. Distinguishing between the two remains challenging given that it has multiple overlapping features. At present, the combination of clinical, endoscopic and histological features, as well as response to PPI therapy, may help to differentiate the two conditions. Further studies into the immunopathophysiology are needed to elucidate more objective diagnostic testing that can reliably differentiate between the two disease processes.

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P- Reviewer: Abdel-Hamid SMM, Akiho H, Mann O, Venu RP
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Huang Y

Appendix 2

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doi:10.1002/jgh3.12327

ORIGINAL ARTICLE

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

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Key words
adults, eosinophilic esophagitis, gastroesophageal reflux disease, immunoglobulin G4.

Accepted for publication 5 March 2020.

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Declaration of conflict of interest: No conflict of interest to disclose from all authors.

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, Zukerbeg 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 μm 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 ± 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 (n = 30)	GERD (n = 30)
Mean age (years)	38.8 ± 12.8	50.7 ± 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.

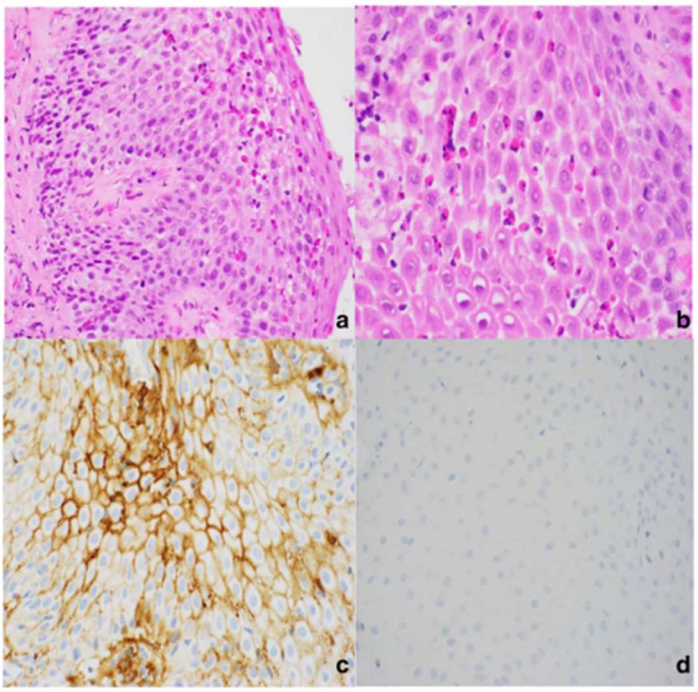

Figure 1 (a) EoE. (b) EoE with intercellular edema. (c) EoE with positive IgG4. (d) EoE with negative IgG4. EoE, eosinophilic esophagitis; IgG4, immunoglobulin G4.

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	*P* 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 nature of EoE and the need to biopsy multiple areas 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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Appendix 3

Journal of Gastroenterology and Hepatology JGHF
doi:10.1111/jgh.15660

ORIGINAL ARTICLE - GASTROENTEROLOGY (CLINICAL)

Characteristics and progression of childhood-onset and adult-onset eosinophilic esophagitis

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Key words
adult-onset eosinophilic esophagitis, childhood-onset eosinophilic esophagitis, eosinophilic esophagitis, esophageal disorders.

Accepted for publication 3 August 2021.

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Declaration of conflict of interest: No conflict of interest to disclose from all authors.

Abstract

Background and Aim: The prevalence and incidence of eosinophilic esophagitis (EoE) has been increasing over recent years. However, the natural history remains incompletely understood particularly the differences in disease characteristics and progression of childhood-onset and adult-onset EoE. The aim of this study was to evaluate the disease characteristics and progression of childhood-onset and adult-onset EoE.

Methods: A cross-sectional, questionnaire-based study, on 87 adults and 67 children from 2 major tertiary hospitals in South Australia was conducted. Data of those who were diagnosed with EoE between 1999 and 2018 were collected and correlated with medical records.

Results: Of the 87 adults with EoE, 34 (39%) were diagnosed at the age of < 18 years (childhood-onset EoE). Reflux symptoms were more common in childhood-onset EoE, whereas asthma was more common in adult-onset EoE. The median duration of symptoms prior to diagnosis of EoE was > 1–4 years in childhood-onset disease (44%) and ≥ 10 years in adult-onset disease (34%). Food impaction was significantly more common on initial presentation in those with adult-onset EoE, whereas weight loss was more common in childhood-onset EoE. At the time of questionnaire, regurgitation, abdominal pain, and bloating were more common in childhood-onset EoE. Those with childhood-onset EoE were more likely to have multiple symptoms at questionnaire when compared with their adult-onset counterparts. In both groups, 15% (5/34 childhood-onset EoE and 8/53 adult-onset EoE) were asymptomatic at the time of questionnaire.

Conclusion: Childhood-onset EoE appears to be a progressive disease from childhood to adulthood, however with more inflammatory-type symptoms post transition compared to those with adult-onset EoE.

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 by eosinophilic inflammation.[1,2] It has a bimodal age presentation, with one peak in childhood and the second in the third and fourth decade with a mean age of diagnosis of 38 years.[1,3,4] Current estimated prevalence and incidence in the general population is 13–49 cases/100 000 persons and 1–20 cases/100 000 persons, respectively.[2,5] EoE is now thought to be the most frequent eosinophilic gastrointestinal disorder and the second most common cause of chronic esophagitis and dysphagia after gastroesophageal reflux disease (GORD).[6] An increasing prevalence of food bolus impaction has been shown to be associated with an increased prevalence of EoE and a reduction in peptic strictures.[7] Various hypotheses have been considered for this phenomenon particularly that of an increase in the recognition of the disease and an increase in the volume of endoscopies performed.[7–9] However, two population-based studies have shown that the incidence and cumulative prevalence of EoE has increased more than the rate of annual endoscopies during the observation period in keeping with a true rise in the incidence and prevalence of the disease.[10,11]

The natural history of EoE is incompletely understood, particularly whether EoE worsens, stays the same or remits during transition from childhood to adulthood.[12] Studies in adults suggest that EoE is a chronic disease with persistence of dysphagia and long-term complications including esophageal fibrosis.[8,10,12] A study that investigated the clinical outcome of EoE patients diagnosed as children concluded that most of the children had resolution or improvement of symptoms as young adults.[13] It is unclear as to why there are phenotypic differences in EoE and whether they indicate different responses to therapy or prognoses.[14] We hypothesize that EoE is a chronic single disease entity that may present in childhood or adulthood. The aims of this

study were to compare the characteristics and disease progression between childhood-onset and adult-onset EoE.

Materials and methods

Study population and design. This cross-sectional, questionnaire-based study was performed at the Royal Adelaide Hospital and The Women's and Children's Hospital in Adelaide, South Australia. The study protocol was approved by both the Royal Adelaide Hospital Research and Ethics Committee and the Women's and Children's Health Network Human Research Ethic Committee (reference number: HREC/14/WCHN/87). All patients with a diagnosis of EoE between 1999 and 2018 at both hospitals were included. Identified patients were sent an invitation letter, information sheet, consent form (signed by legal guardian if aged less than 18 years), and the questionnaire along with a reply-paid envelope. This package was resent to patients who did not respond to the initial invitation. No further correspondence was initiated if a response was not received following this second attempt. Exclusion criteria were patients who did not given written informed consent, and incomplete questionnaires/data.

The questionnaire incorporated questions regarding demographics, past medical history, allergy, family history, and a detailed history of EoE (Appendix A Children and Appendix B Adults). Data collected from questionnaires were correlated with medical records, in particular the date of diagnosis and treatment history.

Definitions. A diagnosis of EoE was defined as $\geq$ 15 eosinophils/high powered field with symptoms of esophageal dysfunction (such as food bolus impaction, dysphagia, and vomiting) and exclusion of other causes of esophageal eosinophilia. Adulthood was defined as age $\geq$ 18 years and childhood as age $<$ 18 years.

Statistical analysis. Descriptive statistics were used to describe the results. Categorical data were compared by Fisher's exact test, and continuous data were compared by Student's *t*-test. Statistical significance was determined by a P value of less than 0.05. Analyses were performed using GRAPHPAD PRISM statistical software, Version 8 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Of the 446 sent questionnaires, 87/280 adults and 67/166 children returned completed questionnaires (Fig. 1).

Comparison of adults with childhood-onset and adult-onset eosinophilic esophagitis. Of the 87 adults with EoE, 34 (39%) were diagnosed at less than 18 years of age (childhood-onset EoE). The differences between adults with childhood-onset EoE and adult-onset EoE are summarized in Table 1. At the time of completing the questionnaire, adults with childhood-onset EoE were significantly younger than those with adult-onset EoE both at time of questionnaire and at the age of diagnosis of EoE ($P < 0.0001$). Reflux symptoms were more common in childhood-onset EoE, whereas asthma was more common in adult-onset EoE. There were no differences between the two groups with respect to personal history of allergy, family history of allergy, and family history of EoE. The most frequently reported duration of symptoms prior to the diagnosis of EoE was $>$ 1–4 years in childhood-onset disease (44%) and $\geq$ 10 years in adult-onset disease (34%). Food impaction was significantly more common as an initial presentation in those with adult-onset EoE, whereas weight loss was more common in childhood-onset EoE (Fig. 2). At the time of questionnaire, regurgitation, abdominal pain, and bloating were more common in childhood-onset EoE. Those with childhood-onset EoE were more likely to have multiple symptoms when compared with their adult-onset counterparts (Fig. 3). Equal proportions of those with childhood-onset (5/34, 15%) and adult-onset EoE (8/53, 15%) were asymptomatic at the time of questionnaire.

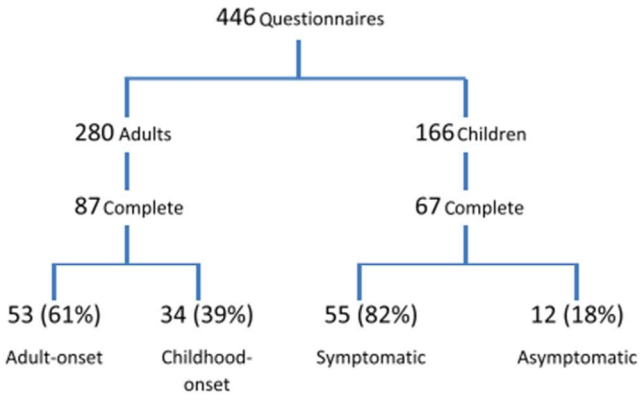

Figure 1 Study population.

Table 1 Comparison of demographics, concomitant medical conditions, history of allergy, and family history in adults with childhood-onset and adult-onset EoE

Variable	Childhood-onset (n = 34)	Adult-onset (n = 53)	P value
Age at questionnaire—median years (IQR)	20 (18–23.5)	47 (35–57)	< **0.0001**
Age at diagnosis of EoE—median years (IQR)	13.5 (8.25–15)	40 (29–53)	< **0.0001**
Gender (Male:Female)	24M:10F	41M:12F	
Body mass index (median BMI; kg/m^2) (IQR)	23.9 (21.9–25.9)	25.5 (23.4–29.4)	
Country of birth	97% Australian	87% Australian	
	3% Other	13% Other	
Concomitant medical conditions			
GORD	53%	19%	**0.0019**
Asthma	12%	38%	**0.0129**
Personal history of allergy	77%	77%	1.0000
Family history of allergy	59%	38%	0.0776
Family history of EoE	9%	9%	1.0000
Duration of symptoms prior to diagnosis			
0–7 days	12%	25%	
> 7–30 days	—	—	
> 30 days to 1 year	15%	2%	
> 1–4 years	44%	25%	
> 5–9 years	21%	15%	
≥ 10 years	9%	34%	
Initial symptoms			
Dysphagia to solids	76%	91%	0.1208
Dysphagia to liquids	24%	25%	1.0000
Heartburn	41%	28%	0.2486
Food impaction	30%	85%	< **0.0001**
Regurgitation	44%	32%	0.2662
Chest pain	29%	19%	0.3011
Retching/Vomiting	26%	32%	0.6373
Weight loss	29%	8%	**0.0142**
Abdominal pain	35%	17%	0.0723
Bloating	32%	15%	0.0677
Multiple symptoms	97%	94%	1.0000
Current symptoms			
Dysphagia to solids	41%	43%	1.0000
Dysphagia to liquids	32%	13%	0.0555
Heartburn	38%	21%	0.0894
Food impaction	24%	23%	1.0000
Regurgitation	35%	11%	**0.0131**
Chest pain	32%	15%	0.0677
Retching/Vomiting	18%	6%	0.1452
Weight loss	6%	6%	1.0000
Abdominal pain	21%	11%	**0.0068**
Bloating	32%	9%	**0.0104**
Multiple symptoms	68%	34%	**0.0494**

BMI, body mass index; EoE, eosinophilic esophagitis; GORD, gastroesophageal reflux disease; IQR, interquartile range.

Comparison between children and adults with eosinophilic esophagitis. The differences between children and adults with EoE are summarized in Table 2. The median age of diagnosis was 5 ± 4.5 years for children and 25 ± 18.5 years for adults. Of note, dysphagia to solids and food impaction were significantly more common on presentation in adults than children, whereas this was the opposite for vomiting and abdominal pain. Adults were also more likely to experience multiple symptoms initially compared with children. However, at the time of questionnaire, the most common symptom in both groups was dysphagia to solids, although food impaction in adults remained significantly more common than in children. Retching/Vomiting and abdominal pain remained more common in children at the time of questionnaire.

Discussion

Our study describes the differences in disease characteristics and progression in patients with childhood-onset versus adult-onset EoE. Our data show that those with childhood-onset EoE

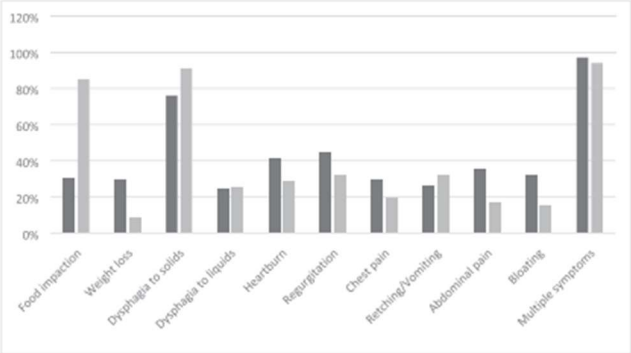

Figure 2 Initial presenting eosinophilic esophagitis symptoms at the time of diagnosis in adults with childhood-onset and adult-onset eosinophilic esophagitis. ■, childhood-onset; ■, adult-onset.

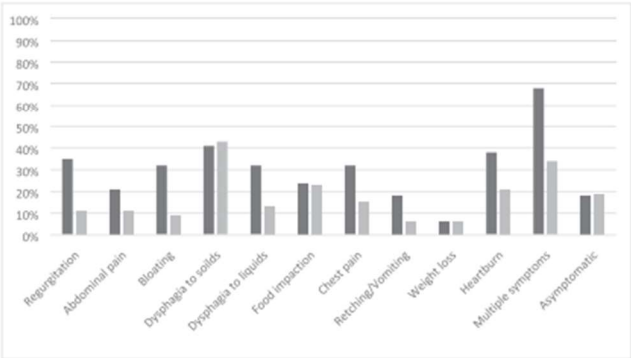

Figure 3 Current eosinophilic esophagitis symptoms at the time of questionnaire in adults with childhood-onset and adult-onset eosinophilic esophagitis. ■, childhood-onset; ■, adult-onset.

confirmed to be significantly younger at time of diagnosis and questionnaire and experienced more inflammatory-type symptoms, but there was no difference in the presence of continued symptoms into adulthood. Most patients in both groups recalled experiencing symptoms for years prior to the diagnosis of EoE. This is consistent with what is known about the natural history of EoE where patients often describe a history of symptoms that began years prior to their diagnosis.[15,16] EoE has been considered to be food allergy predominant in pediatric EoE and airway allergy predominant in adult EoE.[17] Our data reflect this, where gastroesophageal reflux disease was found to be more common in childhood-onset disease, whereas asthma was more common in adult-onset disease.

Patients with adult-onset EoE had a significantly higher rate of presenting with food impaction compared with those with childhood-onset EoE. This significance receded in the transition from childhood to adulthood. A further comparison of adults and children with EoE (Table 2) echoed this finding, where dysphagia to solids ultimately became the most common symptom in both groups. This change supports the theory that EoE progresses from an inflammatory to a fibrostenotic phenotype due to development of subepithelial fibrosis in the esophagus.[10,16,18] Post transition into adulthood, however, those with childhood-onset EoE continued to have a significantly higher incidence of multiple and inflammatory-type symptoms, namely regurgitation, abdominal pain, and bloating. This new and interesting finding suggests that although fibrosis eventually develops in childhood-onset EoE, the inflammatory component remains significant enough to contribute to ongoing symptoms.

At the time of questionnaire, only 15% of both childhood-onset and adult-onset cohorts were asymptomatic. Thus, 85% of our patients with childhood-onset EoE continued to have symptoms into adulthood. Studies that have looked in particular at transition of EoE from childhood to adulthood have shown that childhood-onset EoE was associated with a reduced quality of life and persistent symptoms into adulthood.[12,19] This contrasts with more recent findings which concluded that those with childhood-onset EoE had improvement or resolution of symptoms as adults.[13] We believe that our data adds impact to the theory that childhood-EoE is a progressive condition and not a different disease entity in children and adults.

A strength of our study is that this is a multicenter analysis of childhood-onset and adult-onset EoE with a higher-than-average response rate (35%, 154/446). Although our study was limited by recall bias, all data obtained from the questionnaires were correlated with medical records. Also, given the structure of our

Table 2 Patient demographics, concomitant medical conditions, history of allergy, and family history of adults and children with EoE

Variable	Children (n = 67)	Adults (n = 87)	P value
Age at questionnaire—median years (IQR)	13 (8–15)	32 (22–53)	
Age at diagnosis of EoE—median years (IQR)	5 (2–10)	26 (15–45.5)	
Gender (Male:Female)	57 M:10F	65 M:22F	
Body mass index (median BMI; kg/m^2) (IQR)	19.2 (16.7–22.3)	25 (23–26.9)	
Country of birth	99% Australian	91% Australian	
	1% Other	9% Other	
Concomitant medical conditions			
GORD	39%	32%	0.4008
Asthma	39%	28%	0.1662
Personal history of allergy	88%	77%	0.0936
Family history of allergy	78%	46%	< **0.0001**
Family history of EoE	4%	9%	0.3505
Duration of symptoms prior to diagnosis			
0–7 days	3%	20%	
> 7–30 days	1%	—	
> 30 days to 1 year	25%	7%	
> 1–4 years	57%	32%	
> 5–9 years	10%	17%	
≥ 10 years	3%	24%	
Initial symptoms			
Dysphagia to solids	63%	85%	**0.0023**
Dysphagia to liquids	28%	24%	0.5821
Heartburn	28%	33%	0.5993
Food impaction	25%	63%	**0.0001**
Regurgitation	—	37%	—
Chest pain	25%	23%	0.8494
Retching/Vomiting	64%	30%	< **0.0001**
Weight loss	19%	16%	0.6708
Abdominal pain	43%	24%	**0.0151**
Bloating	—	22%	—
Multiple symptoms	78%	95%	< **0.0001**
Current symptoms			
Dysphagia to solids	45%	43%	0.8700
Dysphagia to liquids	9%	21%	0.0715
Heartburn	28%	28%	1.0000
Food impaction	7%	23%	**0.0141**
Regurgitation	—	21%	—
Chest pain	19%	22%	0.8417
Retching/Vomiting	27%	10%	**0.0100**
Weight loss	6%	6%	1.0000
Abdominal pain	34%	15%	**0.0068**
Bloating	—	18%	—
Multiple symptoms	54%	46%	0.4166

questionnaire where the duration of disease is expressed as a range rather than a single time value, logistic regression to assess associations between disease duration and other variables was not possible.

Conclusions

Childhood-onset EoE appears to be a progressive disease from childhood to adulthood, however with more inflammatory-type symptoms post transition compared to those with adult-onset EoE.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix A. Supporting Information
Appendix B. Supporting Information

Questionnaire: Natural history of eosinophilic esophagitis

Age of child: _____

Gender: M / F

Weight: _____

Height:_____

Country of Birth: _____

Please tick one box:

☐ I agree to the accessing of my child's medical records for the purpose of this study.

☐ I do not agree to the accessing of my child's medical records for the purpose of this study.

Signed _____ Dated _____

1. Does your child have any of the following medical condition?

Reflux disease YES / NO

Diabetes mellitus YES / NO

Bronchial asthma YES / NO

Others, please specify _____

Is your child on any medication at present? YES / NO

If yes, please specify _____

_____

_____

2. Does your child have a history of allergy?

Allergy history YES / NO

If yes, is the allergy involving:

Food YES / NO

Medication YES / NO

Skin/eczema YES / NO

Asthma YES / NO

Hay fever YES / NO

3. Is there a family history of allergy?

Is there any family member with allergy or history of allergy? YES / NO

If yes, please specify: _____

_____

Is there any family member diagnosed with eosinophilic esophagitis? YES / NO

If yes, please specify: _____

_____

4. Social history?

Is there any family member living in the same house who smokes? YES /NO

5. Details about your child's medical condition of eosinophilic esophagitis

At what age was your child diagnosed with eosinophilic esophagitis? _____

At what age did your child experience the first symptom/s that may be attributed to eosinophilic esophagitis? _____

How long did your child experience the symptoms before the diagnosis was made? _____

What were your child's initial presenting symptoms?

Difficulty in swallowing:	
Food (solids)	YES / NO
Fluids	YES / NO
Heartburn	YES / NO
Food impaction*[1]	YES / NO
Chest pain	YES / NO
Vomiting	YES / NO
Weight loss	YES / NO
Abdominal pain/discomfort	YES / NO
Failure to thrive	YES / NO

Currently, does your child have any of the following symptoms?

Difficulty in swallowing:	
Food (solids)	YES / NO
Fluids	YES / NO
Heartburn	YES / NO
Food impaction	YES / NO
Vomiting	YES / NO
Chest pain	YES / NO
Weight loss	YES / NO
Abdominal pain/discomfort	YES / NO
Failure to thrive	YES / NO

How long have the symptom/s been present? _____

*[1] Food getting stuck requiring endoscopy to push it down or remove it

6. Details about your child's treatment and symptom progression

What treatment has been given to your child after the diagnosis was made?

Diet modification YES / NO
If yes, please tick the box where appropriate:
□Food exclusion □Elemental diet □Others (please specify _____)

Proton pump inhibitor YES / NO
(LOSEC, SOMAC, ZOTON, NEXIUM)

Steroids (oral or inhaler) YES / NO

Antacids YES / NO

Endoscopic dilatation YES / NO

Other treatment YES / NO

If yes, please specify _____

Did your child's symptoms improve after the treatment? YES / NO

If yes, which were the symptoms that improved?

Difficulty in swallowing:
Food (solids) YES / NO
Fluids YES / NO

Heartburn YES / NO

Food impaction YES / NO

Chest pain YES / NO

Vomiting YES / NO

Weight loss YES / NO

Abdominal pain/discomfort YES / NO

Failure to thrive YES / NO

If no, what were the changes to your child's treatment?

Please specify: _____

Did the changes to your child's treatment help the symptoms? YES / NO

If the symptom/s have reduced,
how long after treatment did you notice it? _____

Currently, is your child receiving any treatment? YES / NO

If yes, what is/are the treatment?

Proton pump inhibitor YES / NO

Steroids (oral or inhaler) YES / NO

Antacids YES / NO

Endoscopic dilatation YES / NO

Diet modification YES / NO
If yes, please tick the box where appropriate:
□Food exclusion □Elemental diet □Others (please specify_____)

Other treatment YES / NO

If yes, please specify _____

If no, what are the reasons?
My child doesn't have any more symptoms YES / NO
My child's symptoms persist but are bearable YES / NO
My child's symptoms persist and are problematic,
but he/she can't be bothered to take medication YES / NO

If your child has associated allergy (skin or airway or other organ), has it increased, reduced or remained the same after the treatment? _____

Did your child have any skin testing to identify the source of his/her allergy? YES / NO

If yes, did elimination of the source of allergy improve his/her symptoms related to:

Asthma YES / NO
Eosinophilic esophagitis YES / NO
Skin eczema YES / NO
Hay fever YES / NO
Food allergy YES / NO

Questionnaire: Natural history of eosinophilic esophagitis

Age: _____

Gender: M / F

Weight: _____

Height: _____

Country of Birth: _____

Please tick one box:

☐ I agree to the accessing of my medical records for the purpose of this study.

☐ I do not agree to the accessing of my medical records for the purpose of this study.

Signed _____ Dated _____

1. Do you suffer from any of the following medical condition?

Reflux disease	YES / NO
Diabetes mellitus	YES / NO
High blood pressure	YES / NO
Ischemic heart disease	YES / NO
Bronchial asthma	YES / NO
Chronic obstructive pulmonary disease	YES / NO
Others, please specify	_____
Are you on any medication at present?	YES /NO
If yes, please specify	_____

_____

2. Do you have a history of allergy?

Allergy history YES / NO

If yes, is the allergy involving:

Food YES / NO

Medication YES / NO

Skin/eczema YES / NO

Asthma YES / NO

Hay fever YES / NO

3. Do you have a family history of allergy?

Do you have any family member with allergy or history of allergy YES / NO

If yes, please specify: _____

_____

Do you have any family member diagnosed with eosinophilic esophagitis YES / NO

If yes, please specify: _____

_____

4. Social history?

Occupation: _____

Do you smoke? YES / NO

Do you regularly drink alcohol? YES / NO

5. Details about your medical condition of eosinophilic esophagitis

At what age were you diagnosed with eosinophilic esophagitis?
□ ≤ 20 years □ 21-40 years □ 41-60 years □ ≥ 61 years

At what age did you experience the first symptom/s that may be attributed to eosinophilic esophagitis?
□ ≤ 20 years □ 21-40 years □ 41-60 years □ ≥ 61 years
How long did you experience the symptoms before the diagnosis was made? _____

What were your initial presenting symptoms?

Difficulty in swallowing:	
Food (solids)	YES / NO
Fluids	YES / NO
Heartburn	YES / NO
Food impaction*[1]	YES / NO
Regurgitation	YES / NO
Chest pain	YES / NO
Retching	YES / NO
Weight loss	YES / NO
Abdominal pain/discomfort	YES / NO
Bloating	YES /NO

Currently, do you have any of the following symptoms?

Difficulty in swallowing:	
Food (solids)	YES / NO
Fluids	YES / NO
Heartburn	YES / NO
Food impaction	YES / NO
Regurgitation	YES / NO
Chest pain	YES / NO
Retching	YES / NO
Weight loss	YES / NO
Abdominal pain/discomfort	YES / NO
Bloating	YES / NO

How long have the symptom/s been present? _____

*[1] Food getting stuck with a need for endoscopy to push it down or remove it

6. Details about your treatment and symptom progression

What treatment has been given to you after the diagnosis was made?

Diet modification YES / NO
If yes, please tick the box where appropriate: □ Food exclusion □ Elemental diet
□ Others (please specify _____)

Proton pump inhibitor YES / NO
(LOSEC, SOMAC, ZOTON, NEXIUM)

Steroids (oral or inhaler) YES / NO

Antacids YES / NO

Endoscopic dilatation YES / NO

Other treatment YES / NO

If yes, please specify _____

Did your symptoms improve after the treatment? YES / NO

If yes, which were the symptoms that improved?

Difficulty in swallowing:
Food (solids) YES / NO
Fluids YES / NO

Heartburn YES / NO

Food impaction YES / NO

Regurgitation YES / NO

Chest pain YES / NO

Retching YES / NO

Weight loss YES / NO

Abdominal pain/discomfort YES / NO

Bloating YES / NO

If no, what were the changes to your treatment?

Please specify: _____

Did the changes to your treatment help the symptoms? YES / NO

If the symptom/s have reduced,
How long after treatment did you notice it? _____

Currently, are you receiving any treatment? YES / NO

If yes, what is/are the treatment?

Proton pump inhibitor YES / NO

Steroids (oral or inhaler) YES / NO

Antacids YES / NO

Endoscopic dilatation YES / NO

Diet modification YES / NO
If yes, please tick the box where appropriate: □ Food exclusion □ Elemental diet
□ Others (please specify _____)

Other treatment YES / NO

If yes, please specify _____

If no, what are the reasons?

I don't have any more symptoms YES / NO

My symptoms persist but are bearable YES / NO

My symptoms persist and are problematic,
but I can't be bothered to take medication YES / NO

If you have associated allergy (skin or airway or other organ), has it increased, reduced or remained the same after the treatment? _____

Did you have any skin testing to identify the source of your allergy? YES / NO

If yes, did elimination of the source of allergy improve your symptoms related to?

Asthma YES / NO

Eosinophilic esophagitis YES / NO

Skin eczema YES / NO

Hay fever YES / NO

Food allergy YES / NO

Appendix 4

Esophagus
https://doi.org/10.1007/s10388-022-00924-7

ORIGINAL ARTICLE

Distal esophageal wall thickness correlates with dysphagia in adult patients with eosinophilic esophagitis

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Received: 31 January 2022 / Accepted: 6 May 2022

Abstract

Background Thickening of the esophageal wall in patients with eosinophilic esophagitis (EoE) and gastro-esophageal reflux disease (GERD) has been shown in studies using endoscopic ultrasound (EUS). We hypothesise that transmural inflammation in EoE results in prominent esophageal wall thickening compared with the mucosal inflammation in GERD. The aim of this study was to compare the relationship among dysphagia, endoscopic appearance, wall thickness, histology, and motility in EoE and GORD.

Methods EoE and GERD patients were prospectively studied between February 2012 and April 2021. Patients were studied on 2 separate occasions with endoscopy, EUS and mucosal biopsies, followed by high-resolution manometry. Epidemiology and dysphagia data were obtained.

Results A total of 45 patients (31 EoE, 14 GERD) were included. There were no significant differences in age, sex, duration of disease and presence of esophageal motility disorders. EoE patients had a higher dysphagia score ($P<0.001$), EREFS score ($P<0.001$) and peak eosinophil count ($P<0.001$) compared with GERD patients. Thickness of the submucosa in the distal esophagus in EoE was significantly higher than GERD ($P=0.003$) and positively correlated with duration of disease ($P=0.01$, $R=0.67$). Positive correlation was also found between dysphagia score and distal total esophageal wall thickness ($P=0.03$, $R=0.39$) in EoE patients. No correlation was found between these variables in GERD patients.

Conclusion Distal esophageal wall thickness positively correlates with dysphagia score in EoE but not GERD. This appears to be related to the composition of the submucosa which can be identified using EUS.

Keywords Esophageal wall thickness · Dysphagia · Eosinophilic esophagitis · Disease duration · Endoscopic ultrasound

Introduction

Eosinophilic esophagitis (EoE) is a clinicopathological disorder characterised by an immunologic, antigen-driven process that manifests clinically with symptoms of esophageal dysfunction and histologically by eosinophilic inflammation [1]. The prevalence and incidence, which have been increasing over the past few years, are estimated to be 13–49 cases/100,000 persons and 1–20 cases/100,000 persons, respectively, in the general population [2, 3]. EoE is thought to be the most frequent eosinophilic gastrointestinal disorder and the second most common cause of chronic esophagitis and dysphagia after gastro-esophageal reflux disease (GERD), which is one of its main differential diagnoses [1, 4–6]. Distinguishing between EoE and GERD may be challenging as many of their clinical and histological features overlap [7, 8].

As a result of chronic inflammation caused by inflammatory cell infiltration of the esophageal mucosa, fibrosis may be induced in the wall which leads to remodelling of the deeper layers of the esophagus in both EoE and GERD [9, 10]. This is supported by the few endoscopic ultrasound (EUS) studies that have been performed showing significant thickening of the esophageal wall in EoE [10–14]. We

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Published online: 06 June 2022

hypothesise that transmural inflammation in EoE results in more prominent esophageal wall thickening compared with the mainly mucosal inflammation in GERD. The aim of this study was to comprehensively characterise the relationships among dysphagia, endoscopic appearance, wall thickness, histology and motility in the oesophagi of patients with EoE and compare it with GERD.

Materials and methods

Study population and design

This prospective, comprehensive clinicopathological study was performed at the Royal Adelaide Hospital, the largest adult tertiary referral hospital in South Australia and was approved by the Royal Adelaide Hospital Research Ethics Committee (protocol number: 111233). Patients between 18 and 70 years of age with a diagnosis of EoE or GERD were identified from pre-existing databases and recruited at their outpatient clinic/endoscopy appointments or via an invitation package. This invitation package contained an invitation letter, information sheet and the investigators' contact details should they wish to participate. No further correspondence was initiated if a response was not received following this attempt. Written informed consent was obtained from all patients. Exclusion criteria were history of severe respiratory, cardiovascular, hepatic and/or renal disease, chronic alcohol abuse or epilepsy, medications that may influence gastrointestinal function, anti-coagulation therapy, gastrointestinal surgery, history of recent or recurrent epistaxis, known history of major psychiatric disorders, pregnant/breast-feeding women and inability to given written informed consent.

Definitions

A diagnosis of EoE was defined as ≥ 15 eosinophils/high powered field with symptoms of esophageal dysfunction and exclusion of other causes of esophageal eosinophilia. On the other hand, GERD was defined as a clinical diagnosis in a patient with typical symptoms (heartburn and regurgitation) responsive to proton-pump inhibitor (PPI) therapy and either a positive pH study or an endoscopy with biopsies confirming reflux disease.

Protocol

Recruited patients were studied initially with completion of a dysphagia score and an endoscopy with endoscopic ultrasound and mucosal biopsies. This was followed by assessment with high-resolution manometry at a separate session ≥ 7 days after the initial endoscopy.

Symptom evaluation

Dysphagia was assessed using a modified version of a non-validated dysphagia score used by Straumann et al. in a randomised placebo-controlled trial of oral viscous budesonide in adult EoE patients [15]. This score assessed frequency of dysphagia ranging from none (0) to several times per day [5] and intensity of dysphagia ranging from unhindered swallowing (0) to obstruction requiring endoscopic intervention [5]. Total scores ranged from 0 to 10.

Endoscopy and endoscopic ultrasound

All endoscopies were performed by either one of the two gastroenterology investigators (SW and NN) with EUS experience using conscious sedation with midazolam, fentanyl and/or propofol. A full endoscopic inspection of the upper gastrointestinal tract to the second part of the duodenum was first performed with a standard gastroscope (Olympus® 180, Japan). Endoscopic features of EoE were graded according to the EoE Endoscopic Reference Score (EREFS) [16]. After completion of the endoscopic examination, the wall thickness of the oesophagus was evaluated with an Olympus® UM-S20-20R miniature probe that was passed through the accessory channel of the gastroscope. The ultrasound probe was connected to Olympus® EU-ME1 ultrasound system. Thickness of the esophageal wall was measured at the proximal (≥ 20 cm above gastro-esophageal junction (GEJ)), mid (10–20 cm above GEJ) and distal (5 cm above GEJ) segments. Esophageal wall thickness was measured in a contracted/non-distended state in all study patients to avoid distortion caused by the presence of longitudinal furrows and ensure that distensibility of the esophagus was constant/controlled. In addition to the total wall thickness, measurements of the mucosa and submucosa were also taken.

Histological evaluation

A total of 10 biopsies were then collected from the oesophagus ($n = 2$ from each segment; proximal, mid and distal esophagus), stomach ($n = 2$) and duodenum ($n = 2$) after the endoscopic ultrasound measurements. Duodenal and gastric biopsies were taken to rule out other causes of esophageal eosinophilia. Biopsies obtained were evaluated after fixation in formaldehyde and hematoxylin–eosin staining. Peak eosinophil count was analysed per high power field (× 400). All biopsies were examined by a single gastrointestinal pathology investigator (AR) who was blinded from the clinical and endoscopic data.

High-resolution manometric assessment

Esophageal motor function was assessed using ManoScan 360™ high-resolution manometry system (Given Imaging) along with a ManoScan™ ESO catheter by a single technician (MT) investigator. After the catheter had been calibrated, topical anaesthetic spray (Co-Phenylcaine) and gel (Lignocaine 2%) was applied to one of the patient's nostrils after a 3-h fast. In the upright posture, the catheter was intubated with the subject taking small sips of water to pass the assembly into the stomach. The subjects were then positioned in the left lateral position. A 3-min resting period was observed including a 30-s period to assess basal sphincter pressure. Swallowing exercises were then performed which consisted of 10 × 5mls water swallows, 3 × 10mls multiple rapid swallows and 2 × 200mls cup of water. High-resolution manometry data were analysed using ManoView™ software. Interpretation of the results were done according to The Chicago Classification version 4.0 by one of the gastroenterology investigators (SW) with motility experience [17].

Statistical analysis

Based on data published by Muroi et al. [12], a sample size of 38 cases was required to achieve a difference of 20% with power of 90% and α of 0.05. Descriptive statistics were used to describe the results with normality assessed using the Kolmogorov–Smirnov test. Mann–Whitney *U* test was used to evaluate the parameters between the two study groups. Spearman's rank correlation coefficient (Spearman r) was used to detect any significant correlation between variables in each study group. Statistical significance was determined by a *P* value of less than 0.05. Analyses and graph construction were performed using IBM®SPSS® software, version 28 and GraphPad® Prism software, version 9.

Results

Demographics and clinical characteristics

A total of 45 patients (31 EoE and 14 GERD) were included in the study. The demographics and clinical characteristics of the 2 groups are summarised in Table 1. There were no

Table 1 Demographics and clinical characteristics of EoE and GORD patients

	EoE (*n* = 31)	GORD (*n* = 14)	*P* value
Age, median (IQR), years	41 (26)	54 (26)	0.056
Sex (Male: Female)	24 M:7F	6 M: 8F	0.067
Duration of disease, median (IQR), years	2 (5)	3 (4.25)	0.459
Medications, *n* (%)	15 (48%)	14 (100%)	< 0.001
PPI, *ns*	10	14	
Steroids, *n*	5	0	
Refractory to therapy, *n* (%)	11 (73%, 7 PPI, 4 Steroids)	–	
Dysphagia total score, median (IQR)	3 (3)	0	< 0.001
EREFS total score, median (IQR)	2 (3)	0	< 0.001
Fixed rings, *n* (%)	24 (77%)	0	
White plaques, *n* (%)	11 (35%)		
Longitudinal furrows, *n* (%)	26 (84%)	1 (7%)	
Strictures, *n* (%)	3 (10%)	0	
Other endoscopic findings	3 (10%)	4 (29%)	
Oesophagitis, (%)	1 (3%)	8 (57%)	
Hiatus hernia, *n* (%)	0	1 (7%)	
Barrett's, *n* (%)			
HRM			0.787
Normal, *n*	16	8	
Ineffective Oesophageal Motility, *n*	6	4	
Did not attend, *n*	9	2	
Peak eosinophil count/hpf, median (IQR)			
Proximal	26 (42)	0 (1)	< 0.001
Mid	30 (36)	0 (1)	< 0.001
Distal	28 (32)	0 (1)	< 0.001

significant differences in age, sex, duration of disease and presence of esophageal motility disorders. EoE patients had a higher dysphagia score, EREFS score and peak eosinophil count in all esophageal segments compared with GERD patients (Fig. 1). Conversely, a higher proportion of GERD patients were on medical therapy as our EoE cohort had mostly refractory disease.

Differences in esophageal wall thickness between EoE and GERD

The differences in the esophageal wall thickness measurements, assessed by EUS, between the EoE and GERD are summarised in Table 2. Only the thickness of the submucosa in the distal esophagus of EoE patients was found to be significantly higher than that of patients with GORD ($P=0.003$).

Table 2 Comparison of oesophageal wall thickness in EoE and GORD patients

Oesophageal thickness	EoE ($n=31$)	GORD ($n=14$)	P value
Proximal, mean (±SD), mm			
Total	2.7 (±0.88)	3.0 (±1.07)	0.472
Submucosa	1.5 (±0.96)	1.6 (±0.55)	0.342
Mucosa	1.1 (±0.33)	1.2 (±0.28)	0.412
Mid, mean (±SD), mm			
Total	3.1 (±1.21)	3.2 (±1.06)	0.631
Submucosa	1.7 (±0.71)	1.5 (±0.81)	0.308
Mucosa	1.2 (±0.48)	1.1 (±0.29)	0.555
Distal, mean (±SD), mm			
Total	4.1 (±1.79)	4.0 (±0.86)	0.881
Submucosa	2.5 (±1.11)	1.4 (±0.59)	0.003
Mucosa	1.4 (±0.5)	1.3 (±0.52)	0.332

Inter-relationship between esophageal wall thickness, symptoms, histology and motility

In patients with EoE, there was a positive correlation between dysphagia score and distal total esophageal wall thickness ($P=0.03$, $R=0.39$) (Fig. 2). EoE disease duration was not found to correlate with dysphagia score or distal total esophageal wall thickness. A positive correlation, however, was found between duration of disease and distal submucosa ($P=0.01$, $R=0.67$), distal mucosa ($P=0.03$, $R=0.5$), mid-submucosa ($P=0.045$, $R=0.55$) and proximal mucosa ($P=0.01$, $R=0.64$) thickness in EoE patients.

The above correlations in EoE were not observed in GORD, in particular, dysphagia score in GERD did not correlate with distal total oesophageal wall thickness ($P=0.86$, $R=0.08$).

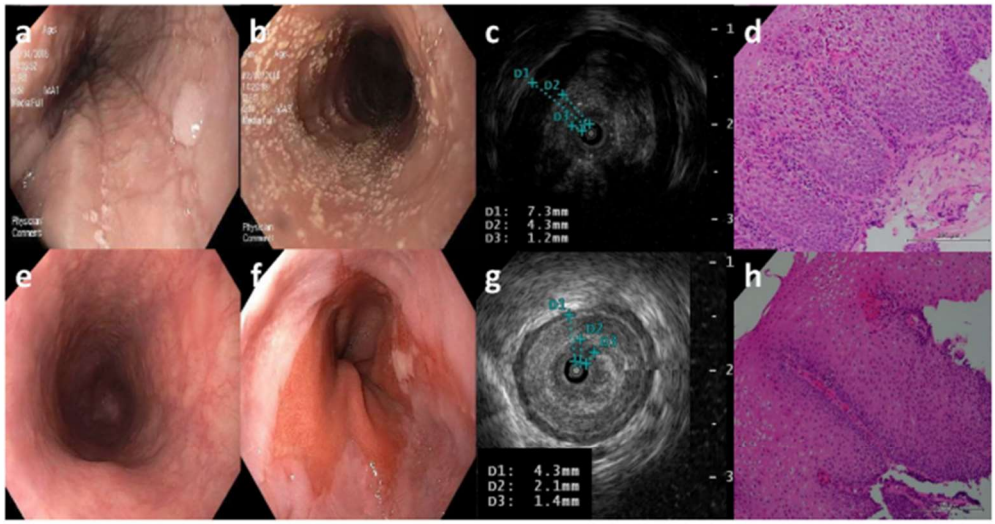

Fig. 1 Comparison of endoscopic appearances, distal oesophageal wall thickness and histology between eosinophilic oesophagitis **a–d** and reflux oesophagitis (**e–h**). D1 = Total oesophageal wall thickness, D2 = Combined submucosa and mucosa thickness, D3 = Mucosa thickness. Thickness of the submucosa was obtained by subtracting D3 from D2

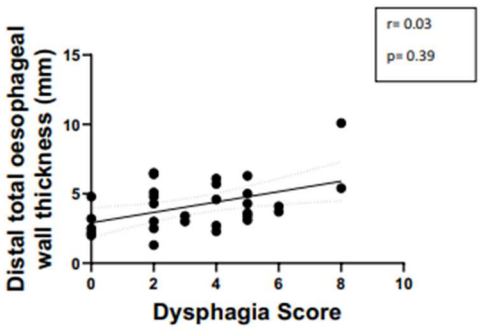

Fig. 2 Correlation between dysphagia score and total distal oesophageal wall thickness of EoE patients

Discussion

Our study describes the differences in the endoscopic appearance, wall thickness, histology and motility between patients with EoE and GERD. Our data show that although there was no difference in total oesophageal wall thickness between the 2 entities, distal submucosa thickness was higher in EoE than GERD. Additionally, positive correlation was found between dysphagia score and distal total oesophageal wall thickness and disease duration and distal submucosal thickness in EoE patients. No correlation was found between these variables in GERD patients.

Contradictory to our hypothesis, the similarity found in total esophageal wall thickness indicates that the inflammation and subsequent remodelling process of the esophagus is comparable in both EoE and GERD. Previous EUS studies in adult and paediatric patients with EoE showing an increase in total esophageal wall thickness involving the mucosa, submucosa and muscularis propria, were performed using either healthy or asymptomatic EoE patients as the control group [11–13]. Our data indicate that the inflammatory infiltration mainly involves the distal submucosa of the esophagus in EoE, whereas this is evenly spread throughout the affected oesophageal layers in GERD. Thus, the correlation found between dysphagia score and distal total oesophageal wall thickness in EoE appears to be due to the composition of the distal submucosa.

There were no significant differences in age, sex and duration of disease indicating that our study cohort was well matched. A higher proportion of GERD patients were on active medical therapy as the majority of EoE patients were refractory to treatment based on peak eosinophil count obtained during endoscopy. The differences between the 2 groups regarding dysphagia score, EREFS score, and peak eosinophil count were expected findings given GERD patients less commonly present with dysphagia, have either normal or characteristic endoscopic findings such as erosive esophagitis, peptic strictures, a hiatus hernia and Barrett's esophagus, and exhibit esophageal eosinophilia, typically less than 10 per high power field [7, 8, 18, 19].

Our study did not show any dissimilarity in the presence of esophageal motility disorders between EoE and GERD patients. Manometric irregularities occur in an estimated 20–76% of patients with EoE, namely patterns of weak or failed peristalsis, pan-esophageal pressurization, high intrabolus pressure and achalasia [20–26]. The prevalence of manometric abnormalities in EoE appears to increase with longer disease duration, but thus far, no correlation has been found with either severity of dysphagia or endoscopic appearance of the oesophagus [21, 24]. Esophageal motility disorders in GERD patients have a similar type and prevalence to those with EoE ranging between 4 and 87% [5, 27, 28]. These data suggest that esophageal dysmotility is not a major contributor to symptoms and that they result primarily from the mechanical changes to the esophageal wall as a consequence of inflammation and fibrosis. Given this, we believe that the role of HRM to assist in the diagnosis and differentiation of EoE and GERD is limited.

The strength of our study is that it is the first, prospective, comprehensive clinicopathological study comparing EoE and GERD patients. We also had adequate sample size based on power calculation. Our study has several limitations, the first of which is that it was performed in a single institution. Although there was recall bias, our cohort was age and sex matched. Most of our EoE patients were untreated or had refractory disease and thus our findings may not be applicable to those who are treated. We were also unable to describe the histology of the submucosa given that endoscopically obtained biopsies are not able to penetrate in this layer. Finally, quantitative data on the range of normal oesophageal wall is lacking, hence we were unable to compare our data with a standardised normal.

Conclusions

Distal esophageal wall thickness positively correlates with dysphagia score in EoE but not GERD. This appears to be related to the composition of the submucosa which can be identified using EUS.

Funding No funding received.

Declarations

Ethical Statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964

Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest All authors have no conflicts of interests to disclose.

Informed consent Informed consent was obtained from all individual participants included in the study.

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Appendix 5

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doi:10.1002/jgh3.12866

ORIGINAL ARTICLE

Increase in distal esophageal wall thickness with time in adult patients with eosinophilic esophagitis

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Key words
dysphagia, endoscopic ultrasound, eosinophil count, eosinophilic esophagitis, esophageal wall thickness.

Accepted for publication 1 January 2023.

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Declaration of conflict of interest: None.

Abstract

Background and Aim: Eosinophilic esophagitis (EoE) is a chronic disease which may progress to a fibro-stenotic phenotype due to esophageal sub-epithelial fibrosis. Esophageal wall thickening in patients with EoE has been demonstrated in a few studies using endoscopic ultrasound (EUS). The aim of this study was to longitudinally assess the endoscopic appearance, wall thickness, histology, and dysphagia score of EoE patients.

Methods: Patients with EoE were recruited and studied between February 2012 and April 2021. Patients were evaluated on two separate occasions at least 12 months apart with endoscopy, EUS, and esophageal mucosal biopsies. The dysphagia score and epidemiology data were also assessed.

Results: A total of 16 EoE patients were included with a mean follow-up duration of 2.2 ± 1.2 years. In 14/16 (88%) patients, the total wall thickness of the distal esophagus significantly increased ($P = 0.0012$) as a result of thickening of the muscularis propria ($P = 0.0218$). However, only 1/14 (7%) patient had an increase in the dysphagia score, while 8/14 (57%) and 5/14 (36%) had a stable and reduced dysphagia score, respectively. No differences were found in the total thickness of other esophageal regions, dysphagia score, endoscopic appearance, and eosinophil count over time.

Conclusion: Distal esophageal wall thickness increases with time in EoE patients, independent of the dysphagia score and eosinophil count.

Introduction

Eosinophilic esophagitis (EoE), which has been increasing in prevalence and incidence, is the most frequent eosinophilic gastrointestinal disorder and the second most common cause of chronic esophagitis and dysphagia after gastro-esophageal reflux disease.[1–5] The natural history of EoE is incompletely understood, and it is yet unclear as to whether phenotypic variations exist or if these differences indicate a different disease pattern of responsiveness to therapy or prognosis.[6,7] Current data indicate that EoE is a chronic, progressive disease with persistence of dysphagia and long-term complications such as stricture formation, food impaction, narrow-caliber esophagus, and esophageal perforation.[6,8–11]

The prevalence of fibrotic strictures has been shown to increase with increasing duration of disease as well as diagnostic delay, suggesting that the natural history of EoE is a progression from an inflammatory to a fibro-stenotic phenotype due to development of subepithelial fibrosis in the esophageal wall.[9,12,13] Studies using endoscopic ultrasound (EUS) studies in EoE patients have been able demonstrate significant thickening of the esophageal wall due to this remodeling process.[14–18] We therefore hypothesized that the esophageal wall thickness in EoE increases with time and can be detected using EUS. The aim of this study was to longitudinally assess the endoscopic appearance, wall thickness, histology, and dysphagia score of EoE patients.

Methods

This longitudinal study was performed at the Royal Adelaide Hospital and was approved by the Royal Adelaide Hospital Research Ethics Committee (protocol number: 111233).

In the initial assessment study, patients between 18 and 70 years of age with a diagnosis of EoE (defined as ≥15 eosinophils/high powered field with symptoms of esophageal dysfunction and exclusion of other causes of esophageal eosinophilia) were retrieved from a pre-existing database and enrolled at their outpatient clinic/endoscopy appointments or via an invitation letter.[19] Enrolled patients underwent an endoscopy with EUS and mucosal biopsies and completed a dysphagia score.[19] Esophageal wall thickness was measured in a contracted state to avoid distortion caused by longitudinal furrows and ensure constant distensibility of the esophagus.[19] A non-validated, modified version of

the dysphagia score used by Straumann *et al.* in a randomized placebo-controlled trial of oral viscous budesonide in adult EoE patients was used in the study.[20] This score assessed the frequency (none [0] to several times per day [5]) and intensity of dysphagia (unhindered swallowing [0] to obstruction requiring endoscopic intervention [5]) with total scores ranging from 0 to 10.[19] These patients were then invited to return for a follow-up endoscopy with EUS and mucosal biopsies along with completion of a dysphagia score ≥1 year after their initial assessment.

All endoscopies and USs were performed by either of the two gastroenterology investigators (SW and NN).

Statistical analysis

Descriptive statistics were used to describe the results, with normality assessed using the Kolmogorov–Smirnov test. Paired *t*-test was used to evaluate the parameters between the two assessments. Spearman's rank correlation coefficient (Spearman *r*) was used to detect any significant correlation between variables in each study group. Statistical significance was determined by a *P*-value of <0.05. Analyses and graph construction were performed using IBM SPSS software, version 28, and GraphPad Prism software, version 9.

Table 1 Demographics and clinical characteristics

	Initial assessment	Follow-up assessment	*P*-value
Age, median (IQR) years	43.5 (28)	45.4 (60.5)	
Gender (Male: Female)	14 M:2F		
Duration of disease, (median) IQR years	2.5 (7)		
Mediations, *n* (%)	6 (38%)	5 (31%)	0.7505
PPI, *n*	6	5	
Steroids, *n*	1	2	
Refractory to therapy, *n* (%)	5 (83%)	2 (40%)	
Dysphagia score, median (IQR)	4 (3.75)	3 (5)	0.8945
EREFS total, median (IQR)	2.5 (2.75)	3 (2.75)	0.5805
Fixed rings, *n* (%)	12 (75%)	14 (88%)	
White plaques, *n* (%)	7 (44%)	8 (50%)	
Longitudinal furrows, *n* (%)	15 (94%)	13 81%)	
Peak eosinophil count/hpf, median (IQR)			
Proximal	18 (25.5)	22.5 (25)	0.9248
Mid	25 (19.5)	25.5 (24)	0.3636
Distal	23.5 (16)	30 (37.5)	0.8168
Other histological findings, *n*			
Eosinophil abscess	2	3	
Basal zone hyperplasia	13	13	
Dilated intracellular spaces	4	2	
Lamina propria fibrosis	1	1	
Eosinophil surface layering	6	6	

Abbreviation: EREFS, endoscopic reference score.

Results

Demographics and clinical characteristics. A total of 16 EoE patients were included in the study with a mean follow-up duration of 2.2 ± 1.2 years. The demographics and clinical characteristics of our patient cohort are summarized in Table 1.

Progression of esophageal wall thickness. Esophageal wall thickness measurements obtained via EUS are summarized in Table 2. In 14/16 (88%) patients, the total thickness of the distal esophageal wall significantly increased over the 2.2 ± 1.2 years ($P = 0.0012$) as a result of thickening of the muscularis propria layer ($P = 0.0218$). Of these, the majority had persistent dysphagia (9/14 [64%]), with only one of these patients having an increase in dysphagia score. Only five patients (36%) who had an increase in wall thickness experienced a reduction in dysphagia score (from 4 [3] to 0 [3]) (Fig. 1). In two patients who did not have thickening of the esophageal wall, the dysphagia scores remained stable at 5 and 8, respectively.

The muscularis propria layer of the mid esophagus was thicker ($P = 0.0259$) on follow-up assessment, but the total thickness alone showed a trend towards statistical significance ($P = 0.0542$).

No correlation was found between the dysphagia score and proximal ($P = 0.78$, $r = -0.08$), mid ($P = 0.58$, $r = 0.15$), and distal ($P = 0.14$, $r = 0.39$) esophageal wall thickness at follow-up assessment. There was also no significant correlation between the change in dysphagia score and distal esophageal wall thickness at follow-up assessment ($P = 0.41$, $r = -0.22$).

Discussion

This is the first longitudinal study to assess esophageal wall thickness, endoscopic appearance, histology, and dysphagia score in adult EoE patients over a mean duration of 2.2 years. Our data show that the total distal esophageal wall thickness significantly increased over time as a result of thickening of the muscularis

TABLE 2 Esophageal wall thickness

Esophageal wall thickness	Initial assessment	Follow-up assessment	*P*-value
Proximal, median (IQR), mm			
Total	2.6 (1.55)	3.15 (1.7)	0.0555
Muscularis propria	2 (0.3)	2.1 (1.6)	0.8955
Submucosa	1.55 (0.55)	1.3 (0.825)	0.5567
Mucosa	0.8 (0.9)	1.2 (0.6)	0.6452
Mid, median (IQR), mm			
Total	2.85 (1.65)	3.65 (1.1)	0.0542
Muscularis propria	1.3 (1.2)	1.7 (1.1)	**0.0259**
Submucosa	1.85 (1.45)	1.45 (0.7)	0.4888
Mucosa	1.1 (0.6)	1 (0.4)	0.9510
Distal, median (IQR), mm			
Total	3.9 (2.3)	5.6 (2)	**0.0012**
Muscularis propria	1.3 (1.3)	2.4 (0.8)	**0.0218**
Submucosa	2.4 (1.33)	2.7 (1.85)	0.5711
Mucosa	1.3 (1.1)	1.3 (0.35)	0.6470

Note: Bold indicates values that are statistically significant.

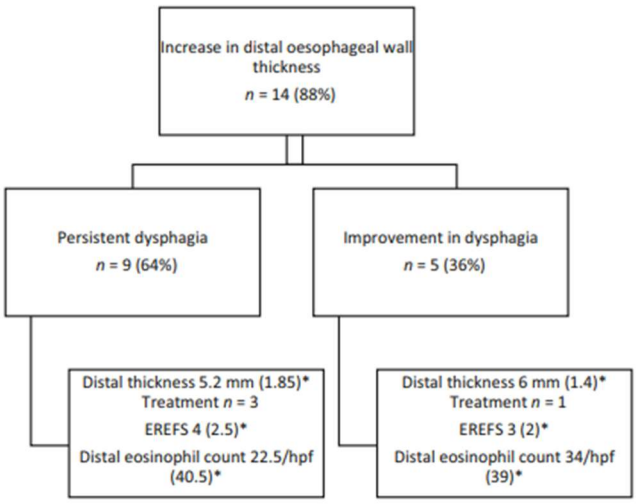

Figure 1 Characteristics of patients showing an increase in distal esophageal wall thickness at follow-up assessment based on the dysphagia score. *Median (IQR).

propria layer in 88% of patients and was independent of the dysphagia score. No significant difference was found in the endoscopic reference score (EREFS), eosinophil count, and total wall thickness in the mid and proximal esophagus between the initial and follow-up assessment.

Previous studies assessing the esophageal wall in EoE patients have shown significant thickening involving the mucosa, submucosa, and muscularis propria on EUS, supporting the occurrence of a chronic remodeling process.[14–16,18,21] Additionally, an assessment of full-thickness esophageal samples in 12 EoE patients showed that histological changes and mediators of EoE pathogenesis were present in both the submucosa and muscularis propria.[22] Our study shows that remodeling and development of esophageal wall thickening appears to be progressive with disease duration but is limited to the muscularis propria layer. This layer may thus be the source of esophageal non-compliance and stiffness seen in studies using the endoluminal functional imaging system (FLIP).[23,24]

The precise mechanism of dysphagia in EoE patients without strictures is unclear.[7,25] It is hypothesized that dysphagia is due to the remodeling process as described in the previous paragraph, which leads to irreversible structural changes and subsequent loss of function.[9,25,26] The correlation between severity of symptoms and histological and endoscopic findings is unclear given conflicting study results.[1,7,9,25,27–29] Relying on symptoms alone is therefore inadequate to allow for either a diagnosis or assessment of efficacy of therapy.[1] Standard esophageal biopsies may also be an insufficient way of assessing overall disease severity given that the changes of EoE involve the sub epithelium of the esophagus.[30] Our finding that esophageal wall thickening progresses independent of the dysphagia score and eosinophil count is thus not unexpected given this poor correlation and the lack of a readily available, validated symptom score for EoE.

The strength of our study is that it is a comprehensive longitudinal study assessing esophageal wall thickness in adult EoE patients. The main limitation of our study is the small sample size where type II errors may occur. We did not have a standardized treatment protocol; however, most of our patients (approximately 70%) were not on treatment due to noncompliance. The duration of our study may not have been adequate to allow changes in the esophageal wall thickness to have an impact on the symptom of dysphagia. Additionally, biopsies of the submucosa and muscularis propria were unobtainable and thus we were unable to depict the histological findings of this layer.

Conclusion

Distal esophageal wall thickness increases with time in EoE patients, independent of the dysphagia score and eosinophil count. Larger studies are required to confirm this finding and assess its impact on clinical management of these patients.

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