



Elevated Tryptase in EoE Is an Independent Phenomenon Associated with Extra-Esophageal Symptoms

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Abstract

Background Eosinophilic esophagitis (EoE) is a chronic disease characterized histologically by > 15 eosinophils per high-power field (eos/hpf). Esophageal mucosal mast cells have been implicated in EoE pathogenesis. The association of atopy with EoE has been established but has not been correlated with levels of serum tryptase. The lack of concurrent atopy in some patients suggests the possibility that atopy may either be the related subtype of EoE or may be a sign of comorbidities. No study has looked at whether patients present with different phenotypes/comorbid disease when they have evidence of elevated serum tryptase. We hypothesized that these patients differ with respect to presentation and comorbidities with more refractory GI disease.

Aims To examine whether elevations of serum tryptase associate with different, more severe clinical presentations in EoE patients which may be explained via mast cell activation.

Materials and Methods Retrospective chart review identified 72 patients with EoE with results for serum tryptase between 2015 and 2016. Patients were classified as Tryptase^{HI} (tryptase > 10.9 µg/l) and Tryptase^{LO} (< 10.9 µg/l). Clinical characteristics and treatment response were compared using univariate analysis and multivariate regression between the groups.

Results Out of 72 patients, 12 were tested as Tryptase^{HI} (16.7%, 95% CI 8.1–25.3%). Tryptase^{HI} was associated frequently with asthma ($P=0.0003$), urticaria ($P=0.002$), arthralgia ($P=0.005$), sinusitis ($P=0.03$), nausea/vomiting ($P=0.046$), and eosinophilic gastrointestinal disease ($P=0.001$). Asthma and arthralgia were found to be significantly associated with Tryptase^{HI} ($P=0.0013$, $P=0.0098$, respectively). Mucosal eosinophil counts and tryptase levels were not correlated (R^2 0.095, $P=0.77$). Tryptase did not resolve with resolution of esophageal eosinophilia.

Conclusions We found that EoE patients with elevated tryptase levels more commonly presented with asthma, urticaria, arthralgia, nausea/vomiting, sinusitis, and more distal eosinophilia. This indicates that atopy in EoE patients warrants further exploration. The lack of correlation between histologic remission and reduction of serum tryptase levels post-treatment suggests that mast cell activation may be an independent, yet associated disease. More study into this unique association is warranted.

Keywords Eosinophilic esophagitis · EoE · Eosinophils · Mast cells · Atopy · Serum tryptase · Asthma · Urticaria · Arthralgia · Sinusitis · Nausea · Vomiting · Eosinophilic gastrointestinal disease · EGID · Mast cell activation

Introduction

Eosinophilic esophagitis (EoE) is a chronic, allergen/immune-mediated disease defined by symptoms of esophageal dysfunction and an esophageal eosinophilic infiltrate of ≥ 15 eosinophils per high-power field (eos/hpf), in the absence of competing causes [1–3]. Males are more commonly affected than females [4–7]. In addition to eosinophils, IgE-bearing mast cells have been found in EoE [1, 8]. Several recent studies demonstrate the occurrence of esophageal mastocytosis in patients with EoE. Chehade et al. noted

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mast cells in esophageal mucosa correlating with basal cell hyperplasia, eotaxin-3, and KIT ligand expression in EoE patients [9–11]. Wang et al. [12] demonstrated in the study that an increased esophageal mast cell number correlated with the severity of symptoms of patients while there was no correlation with eosinophil count, indicating that mast cell involvement could be an independent phenomenon. Although elevated peripheral eosinophil common findings in EoE patients, evidence of peripheral mast cell activation, and its relationship to eosinophilic disease, has not been explored [13]. EoE patients often present with concurrent atopy and additional complaints unrelated to the esophagus. Yet, these associations are not uniform, suggesting that EoE may be associated with comorbidities resulting in symptoms independent of esophageal inflammation [19]. Mast cell activation syndrome (MCAS) is a mast cell-mediated disease that has been associated with atopic diseases such as allergic rhinitis, asthma, atopic dermatitis, IgE-mediated anaphylaxis, dysphagia, chronic fatigue. The diagnostic criterion consists of major criterion that includes the presence of constitutional symptomatology, elevated laboratory markers with symptom improvement with the use of medications that block or treat elevations in these mediators. The most specific laboratory evidence of mast cell burden and activation is the elevated serum tryptase.

We hypothesized that tryptase levels may correlate with larger burden of symptoms in EoE patients indicating that some EoE patients with concurrent atopic and gastrointestinal symptoms may suffer from mast cell activation. We further hypothesized that this activation was related to EoE and tryptase levels would resolve in parallel with resolution of esophageal eosinophilia.

Methods

This study was approved through the Utah Institutional Review Board (IRB #00061947). Chart review was performed on all patients seen at the Esophageal Clinics and Allergy clinics at the University of Utah from 2015 to 2016. Routine testing for tryptase was performed in allergy clinics. Patients were included for evaluation if they had the following: (1) histologic evidence of isolated EoE defined as a peak esophageal eosinophil count of 15 or more eos/hpf at $\times 400$ light microscopy after a minimum of 8-week high-dose proton pump inhibitor therapy and (2) serum tryptase levels. Patients were classified based on serum tryptase levels obtained prior to any treatment for EoE as Tryptase^{HI} (tryptase > 10.9 $\mu\text{g/l}$) and Tryptase^{LO} (< 10.9 $\mu\text{g/l}$) based on the reference value given by the local laboratory (ARUP Laboratories: A National Reference Laboratory with Reference Interval for serum tryptase of less than 11.0 $\mu\text{g/l}$). Patients were identified initially based upon whether tryptase levels were checked. Tryptase levels

were recorded. Subsequently, charts were further reviewed by two investigator blinded to tryptase levels (G. Kutty and H. Crispin) for patient characteristics including age, gender and presenting symptoms, dysphagia, food impactions, bloating, abdominal pain, diarrhea, nausea/vomiting and arthralgia, subsequent or current diagnoses of eosinophilic gastrointestinal disease (EGID) as reported via histopathologic interpretation via the findings of sheets or eosinophils in the lamina propria, associated comorbidities such as asthma, esophageal stricture, urticaria, sinusitis, nausea/vomiting seasonal allergies, eczema, rhinitis, allergic conjunctivitis, history of anaphylaxis, flushing, IgE-mediated food allergies, and eosinophilic gastrointestinal disease (EGID) as diagnosed by pathologic interpretation (i.e., comments of high numbers of eosinophils in the lamina propria consistent with eosinophilic gastrointestinal disease). Due to the controversy in the diagnosis of eosinophilic gastrointestinal disease (EGID), EGID was defined by excess eosinophils identified on biopsy specimens in the stomach and small bowel in which no causative agents were identified. We did not demand eosinophil counts but merely defined the disease based upon histopathologic interpretation. The presenting symptomatology and clinical characteristic were divided into esophageal (i.e., dysphagia, chest pain, heartburn) and extra-esophageal symptomatology as determined a priori as symptoms directly attributable to EoE and those which were not routinely associated with EoE. The groups were evaluated for response to treatment looking at esophageal symptoms (i.e., dysphagia), histology (i.e., < 15 eos/hpf), and serum tryptase.

Statistical Analysis

Patient characteristics and response to treatment were stratified by the Tryptase^{HI} and Tryptase^{LO} groups. Univariate analysis and simple logistic regression were used to compare characteristics between the two groups that were calculated using analytical software program using Fisher exact test or the Kruskal–Wallis test as appropriate. Variables that were significant at or less than the P value of 0.05 level in the univariate analysis were also included in the multiple logistic regression models by estimating odds ratios (ORs) with 95% confidence intervals (CIs). The groups were also compared against each other to look for response to treatment looking at both the esophageal and extra-esophageal symptoms using univariate analysis. Statistical significance was set at the 0.05 level.

Results

A total of 72 patients seen in the Eosinophil clinic at the University of Utah satisfied the inclusion criteria. Demographic characteristics of the subjects are shown (Table 1). The distribution of male and female in the

patients was equal with females (48.6%; $n = 35$). The median age was 33.6 years (range 16.2–51 years). Patients were similar in age between tryptase groups ($P = 0.668$). All patients presented for initial evaluation with at least 1 esophageal symptom such as dysphagia, food impactions, or esophageal stricture. Twelve out of the seventy-two (16.7%, 95% CI 8.1–25.3%) patients were Tryptase^{HI}, and sixty were Tryptase^{LO} (Table 1). The tryptase levels were > 10.9 and < 20 at the time of diagnosis. All of the 12 patients in the Tryptase^{HI} group (12) had tryptase levels rechecked post-treatment, of which 2 out of the 12 had tryptase levels > 20 and 1 had tryptase level > 40 .

Esophageal Symptoms

Elevated tryptase did not appear to affect esophageal symptoms. Dysphagia was present equally in both Tryptase^{HI} and Tryptase^{LO} groups. The presence of history of food impaction was noted in about half of the patients of both groups (41.7% in Tryptase^{HI} group and 48.3% in Tryptase^{LO} group). No difference in the presence of esophageal stricture was noted between groups (Table 1).

Extra-Esophageal Symptoms

On univariate analysis, asthma ($P = 0.0003$), urticaria ($P = 0.002$), arthralgia ($P = 0.005$), sinusitis ($P = 0.03$), and nausea and vomiting ($P = 0.046$) were documented more frequently in the EoE Tryptase^{HI} group (Table 2). Elevated tryptase was associated with numerically greater symptoms of abdominal pain (though not statistically significant) ($P = 0.057$). Simple logarithmic regression analysis of all associated variables revealed that asthma and arthralgia were significantly associated with elevated tryptase ($P = 0.0013$, $P = 0.0098$). More extensive eosinophilic gastrointestinal disease (EGID) was also diagnosed more frequently in patients with elevated tryptase ($P = 0.001$) (Table 2).

Response to Treatment

Different treatments were given to all subjects in both groups. The patient charts were reviewed for documentation of resolution of symptoms by clinical assessment during clinic visits. Patients underwent food elimination trial as well as topical steroids (Table 3). Resolution of esophageal dysphagia post-treatment did not differ significantly between the groups ($P = 0.3$) (Tables 3 and 4).

Table 1 Baseline characteristics and esophageal symptoms of the study population and Tryptase^{HI}/Tryptase^{LO}

Variables, n (%)	Patients	Tryptase ^{HI} (%)	Tryptase ^{LO} (%)	P value
Number of patients	72 (100)	12 (16.7)	60 (83.3)	NA
Age, mean years $\pm$ SD	33.6 $\pm$ 11.6	28.0 $\pm$ 18.9	24.9 $\pm$ 17.0	0.668
Female	35 (48.6)	7 (58.3)	28 (46.7)	0.537
Dysphagia	66 (91.7)	11 (91.7)	55 (91.7)	1
Food impactions	34 (47.2)	5 (41.7)	29 (48.3)	0.758
Stricture	17 (23.6)	2 (16.7)	15 (25)	0.719

Table 2 Documented comorbidities and extra-esophageal symptoms of the study population and Tryptase^{HI}/Tryptase^{LO}

Variables	Patients (%)	Tryptase ^{HI} (%)	Tryptase ^{LO} (%)	OR (Tryptase ^{HI}) (95% CI)	P value
Asthma	20 (27.8)	9 (75)	11 (18.3)	13.4 (3.1–57.6)	0.00025
Arthralgia	9 (12.5)	5 (41.7)	4 (6.7)	10.0 (2.2–46.3)	0.00493
Urticaria	15 (20.8)	7 (58.3)	8 (13.3)	9.1 (2.3–35.7)	0.00193
Sinusitis	21 (29.2)	7 (58.3)	14 (23.3)	4.6 (1.3–16.8)	0.0319
Nausea/vomiting	27 (37.5)	8 (66.7)	19 (31.7)	4.3 (1.2–16.1)	0.0462
Bloating	26 (36.1)	7 (58.3)	19 (31.7)	3.0 (0.9–10.8)	0.104
Abdominal pain	41 (56.9)	10 (83.3)	31 (51.7)	4.7 (0.94–23.2)	0.0574
Diarrhea	26 (36.1)	7 (58.3)	19 (31.7)	3.0 (0.9–10.8)	0.104
History of anaphylaxis	9 (12.5)	3 (25)	6 (10)	3.0 (0.6–14.2)	0.166
Flushing	6 (8.3)	2 (16.7)	4 (6.7)	2.8 (0.5–17.4)	0.26
Food allergies (IgE)	44 (61.1)	9 (75)	35 (58.3)	2.1 (0.5–8.7)	0.346
EGID	19 (26.4)	8 (66.7)	11 (18.3)	8.9 (2.3–35.0)	0.00159

All the associated variables (asthma, arthralgia, urticaria, sinusitis, nausea/vomiting and EGID) that were found to be statistically significant which is associated with elevated tryptase are given in bold

Correlation Between Tryptase Levels and Esophageal Eosinophilia

There was no correlation between eosinophil counts in the esophagus and tryptase levels before or after treatment in the EoE Tryptase^{HI} group (R^2 0.095, $P=0.77$). Serum tryptase did not normalize when esophageal eosinophilia resolved.

Discussion

EoE is considered a classic chronic atopic disorder that is antigen driven resulting in a mixed inflammatory infiltrate in the esophagus including eosinophils, mast cells, basophils, and Th2 cells [7, 14–18]. Although, by definition, eosinophilic esophagitis (EoE) is associated with eosinophils, there is emerging evidence from several recent studies demonstrating the presence of esophageal mast cells in patients with EoE.

We report for the first time that serum tryptase may be elevated in patients with EoE resulting in presentations with distinct clinical symptomatology. These EoE patients

have clinical symptoms of mast cell activation syndrome (MCAS), likely contributing to asthma, urticaria, arthralgia, sinusitis/rhinitis, nausea and vomiting. Elevated tryptase was found more often in patients with eosinophilic gastrointestinal disease (EGID). When comparing MCAS to EOE patients with elevated tryptase, we note similarities in terms of clinical presentation, associated comorbidities, and response to treatment. Our findings suggest that patients with EoE with elevated serum tryptase levels are more likely to present with comorbidities including asthma, urticaria, arthralgia, nausea, vomiting and sinusitis characteristic of MCAS. Elevated tryptase increased the odds of being diagnosed with distal gastrointestinal eosinophilic disease. Interestingly, our cohort demonstrated no reduction of tryptase levels when eosinophil disease resolved—arguing that these entities may be associated but not dependent upon one another.

These possibilities raise the question of whether eosinophilic esophagitis with significant extra-esophageal symptoms may be mediated more prominently via the effects of tryptase on smooth muscle function and epithelial responsiveness. EoE is commonly associated with concurrent atopic disease, but it appears to occur more frequently when the tryptase is elevated. Additionally, abdominal pain, and nausea and vomiting also appear to occur more often in this subset of patients. These symptoms, if directly related to mast cells, may respond to antihistamines. Due to the over-the-counter nature of antihistamines, we were unable to confirm improvement of such extra-esophageal symptoms with their use. Thus, we cannot definitely diagnose such patients with mast cell activation syndrome. Looking into associated comorbidities can help clinically differentiate them from patients with EoE with normal tryptase levels and will aid clinicians in counseling patients regarding additional therapies (i.e., histamine antagonists and mast cell stabilizers) that may alleviate the extra-esophageal symptoms.

The main limitation of the present study is the retrospective design. The decision to measure tryptase could have been influenced by the presentation of patients who appeared to have symptoms not well explained by their

Table 3 Types of therapies and patient distribution to each type

Treatments	Total number (Tryptase ^{HI} , Tryptase ^{LO})
PPI	72 (12, 60)
Six-food elimination diet (SFED)	34 (3, 14)
Topical steroids only	13 (0, 13)
Sequential therapy	17 (6, 11)
1. SFED → topical steroids	11 (3, 8)
2. SFED → elemental diet	6 (2, 0)
3. SFED → topical steroids → systemic steroids	1 (1, 0)
4. SFED → elemental diet → systemic steroids	1 (1, 0)
Systemic steroids	2 (2, 0)

All patients underwent high-dose PPI trial. Additionally, multiple different therapies consisting of differing forms of diet and topical steroids were used on both cohorts

Table 4 Post-treatment histologic and esophageal symptom response in Tryptase^{HI} and Tryptase^{LO} cohorts

Cohort (N)	Tryptase ^{HI} (12)	Tryptase ^{LO} (60)
Patients that underwent treatment	12	60
Patients with post-treatment follow-up esophageal biopsies performed	9	50
1. Esophageal eosinophil < 15 eos/hpf (Group A)	8	34
Resolution of esophageal symptoms	8 (100%)	31 (91%)
2. Esophageal eosinophil > 15 eos/hpf (Group B)	1	16
Resolution of esophageal symptoms	0 (0%)	15 (93.8%)

Most patients in each group underwent treatment for EoE. Interestingly independent of Tryptase, esophageal symptoms improved in most patients regardless of improvement in esophageal eosinophilia

esophageal disease. Due to the retrospective nature, we cannot control for the reason why tryptase was checked. The tryptase levels could have been checked due to suspicion of atopy that seemed excessive from that usually seen in EoE. However, this bias would have made it less likely to differentiate atopy symptoms and extra-esophageal symptoms in the tryptase groups. From our review, tryptase was checked at the time that EoE patients presented to clinic. The reviewed records were only from the University, and thus, the temporal association of tryptase with the onset of EoE cannot be described. Longitudinal assessment of symptom response to treatment was limited secondary to patient treatment and clinic follow-up visit compliance. Due to the multiple treatment methods, the lack of correlation of tryptase with resolution of eosinophils suffered from the lack of homogeneity. However, chart review demonstrated that the tryptase did not resolve in any patient. This will be important to evaluate prospectively. Serum tryptase was the only enzyme that was studied, and other enzymes that are secreted by mast cells such as histamine (*N*-methyl histamine) were examined as they are not easily assessed in routine labs.

To date, this is the only known study looking closely at the presentation of EoE patients with elevated tryptase. From our analysis, elevated tryptase did not influence symptomatic response of esophageal symptoms in EoE patients. However, patients with EoE with elevated tryptase were more likely to present with multiple comorbid conditions than with EoE alone. Thus, further relationships between mast cell activation and EoE are warranted to determine whether such conditions are associated with one another.

Conclusion

Eosinophilic esophagitis continues to be an enigmatic disease with new evidence supporting its association with mast cells. Atopic disease has been reported to occur more commonly in EoE patients. We have found that EoE patients with associated comorbidities may have laboratory evidence of mast cell activation. Measurement of serum tryptase in EoE may identify a subset of patients who suffer from mast cell activation. Such patients may continue to suffer from substantial comorbid conditions even after resolution of EoE. Furthermore, investigation into mast cell-mediated manifestations and the presence of distal eosinophilia with gastric, small bowel, and colon biopsies should be pursued in all EoE patients with elevated tryptase. Our study opens the discussion of targeting activated mast cells in this subset of EoE patients with persistent extra-esophageal symptoms. Mast cells represent a unique and exciting pharmacologic target with the potential to modulate varied features of EoE.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors confirm that due consideration was given to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In doing so, the authors have followed the regulations of our institutions concerning intellectual property. The authors confirm that no aspect of the work covered in this manuscript has involved either experimental animals or human patients or required use of informed consent. This study was approved through the Utah Institutional Review Board (IRB #00061947).

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