
Sensitization to foods in gastroesophageal reflux disease and its relation to eosinophils in the esophagus: is it of clinical importance?

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Background: Refractory gastroesophageal reflux disease (GERD) can be related to greater sensitization to foods.

Objective: To evaluate sensitization to foods in patients with refractory GERD.

Methods: Patients with refractory GERD after using at least 40 mg of a proton pump inhibitor were given a restriction diet based on the results of skin prick testing and atopy patch testing with foods. The characteristics of sensitized patients were compared with those of nonsensitized patients in relation to atopy and number of eosinophils in the esophageal mucosa.

Results: The prevalence of sensitization to foods was 27.7%. Asthmatic patients showed higher sensitization to foods ($P = .008$). Eosinophils were determined to be present in the esophageal mucosa in 15.8% of patients, and this correlated with greater sensitization to foods ($P = .01$). One case of eosinophilic esophagitis was confirmed. A diet excluding identified sensitizing foods led to clinical improvement regarding GERD symptoms ($P = .004$).

Conclusion: The presence of eosinophils in esophageal mucosa associated with greater sensitization to foods and the response to a restriction diet in patients with positive test results suggest that refractory GERD can represent an initial stage of eosinophilic esophagitis.

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INTRODUCTION

Approximately 20% of patients with gastroesophageal reflux disease (GERD) are refractory to treatment with a standard dose of proton pump inhibitors (PPIs).¹ This lack of response to treatment has been attributed to, among other causes, eosinophilic esophagitis (EE).² The symptoms of EE may be similar to those of GERD, but these patients are generally atopic, show histologic findings in the esophagus with more than 20 eosinophils per high-power field, have a high prevalence of food sensitization, and improve with restriction of foods identified in allergy tests.³ Rothenberg⁴ suggested that there could be an intermediate group between patients with GERD and patients with EE who could benefit from limiting exposure to allergenic foods.

Refractory GERD can be related to greater sensitization to foods through two mechanisms: (1) peptic acid damage to intercellular junctions of epithelial cells that results in an

increase in permeability and dilatation of the intercellular space, exposing the squamous epithelium of the esophagus to antigens that normally do not penetrate the mucosa⁵ and (2) increased gastric pH by PPIs, hindering peptic digestion and, thereby, preserving the structure of food proteins and their epitopes.⁶

Because adults use more PPIs than do children and because no studies have evaluated the association of refractory GERD with food sensitization in adults, we chose to study this age group. Thus, the principal objective of this study was to determine sensitization to foods in adults with refractory GERD. The secondary objectives were to compare the characteristics of sensitized patients with those of individuals nonsensitized to foods in relation to atopy and the number of eosinophils in the esophagus and to determine whether a restriction diet excluding foods to which the patient was sensitized leads to an improvement in GERD symptoms.

METHODS

After receiving approval from the commission of ethics and research of the Hospital das Clínicas of São Paulo, a cross-sectional study was conducted in which patients refractory to treatment for GERD followed up in the Gastroenterology Outpatient Clinic between April 1, 2006, and December 31, 2007, were asked to participate.

The study included 65 adults who signed an informed consent form and who presented with symptoms of pyrosis or regurgitation that persisted with a frequency greater than 2 times a week and were taking at least 40 mg of PPI for at least 6 consecutive weeks regardless of previous investigation.^{1,7}

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The criteria for exclusion were use of medications with xanthines, use of antihistamines that could not be interrupted to undergo skin tests, serious psychiatric disease, autoimmune disease, pregnancy, immunodeficiency or neoplasia, and use of an oral corticosteroid medication at doses greater than 1 mg/kg/d.

Patients were asked about family and personal history, time of use and dose of PPIs, and use of oral or inhalation corticosteroids. Patients were evaluated by means of eosinophil counts in peripheral blood, total serum IgE levels, analysis of serum IgE antibodies to airborne allergens, endoscopic findings, histologic features of esophageal mucosa, and skin prick testing (SPT) and atopy patch testing (APT) with 24 foods. Oral food challenge was not performed, only food sensitization was evaluated, and patients with positive test results answered a validated questionnaire for the evaluation of GERD symptoms before and after following a 6-week restriction diet. In the questionnaire, symptoms of GERD were evaluated using a point system, where 0 is the best score and 50 is the worst score.⁸

Serum IgE antibodies to *Dermatophagoides pteronyssinus*, *Blomia tropicalis*, animal epithelia, fungi, and grasses were measured using the ImmunoCAP 100^E system (Phadia, Brazil). ImmunoCAP was used instead of the SPT for environmental allergens because SPT for 24 foods in duplicate required both of the patient's arms.

The SPTs for foods were performed using commercial extracts (IPI-ASAC, Brazil), and for fruits and vegetables, prick to prick testing was also used. A positive SPT reaction was defined as the presence of a wheal with a mean diameter at least 3 mm greater than the negative control, accompanied by erythema, 15 minutes after application of the extract. The APTs were performed with lyophilized food in the following manner. On the first day of the test, 2 g of each lyophilized food was mixed with 2 mL of isotonic saline solution. The mixtures were placed in plastic chambers measuring 12 mm (IPI-ASAC, Brazil) and were adhered to the back of the patient in accordance with previously described methods.³ The APT was removed at 48 hours, and the first reading was taken. The second reading was recorded at 72 hours. The reactions were classified in the following manner: +, discrete erythema with some isolated papules; ++, erythema and papules; and +++, intense erythema, papules, and vesicles.³ The following foods were separately tested: milk, soy, egg, peanut, fish, shrimp, beef, chicken, pork, rice, wheat, oats, corn, barley, manioc, beans, peas, carrot, pumpkin, potato, apple, banana, papaya, and orange.

The use of PPIs was not discontinued for endoscopy. Endoscopy showed the presence of erosive esophagitis, hiatal hernia, or changes suggestive of EE. Six biopsies were performed by a single pathologist, four of the esophagus using 2 fragments from 2 different sites in the esophagus (distal and middle esophagus). An intraepithelial eosinophil count of at least 20 eosinophils per high-power field (magnification $\times 400$) was considered diagnostic of EE.⁹ Figure 1 shows the

distribution of the patients studied in relation to the procedures and interventions performed.

Initially, a descriptive analysis of the skin tests was performed, and the prevalences and respective confidence intervals of sensitization to foods were calculated. Afterward, patients sensitized and nonsensitized to foods were compared by means of bivariate analyses using the Fisher exact test. The distributions of the scores before and after the restriction diet in relation to each symptom were compared using the Wilcoxon test. The level of statistical significance was set at 5% ($P < .05$). All statistical analyses were performed using a software program (SPSS version 13.0; SPSS Inc, Chicago, Illinois).

RESULTS

A ratio of 3.3:1 was observed between women and men, who together had a mean age of 47 years. Of the 65 patients evaluated, 74% were taking 80 mg or more of PPI, and 63% were taking PPI for more than 6 months. The prevalence of sensitization to foods was 27.7% (Table 1). No patient showed positivity in both skin tests (SPT and APT). The prevalence of sensitization to aeroallergens was 30.8%, especially for mites. Sex, age, and time of use or dose of PPIs had no effect on sensitization to foods. We found more asthmatic patients sensitized to foods than not sensitized (Table 2).

Upper digestive endoscopy was performed in 57 patients (88%). Nine of these patients (16%) showed eosinophils in the esophagus: 8 (14%) had fewer than 5 eosinophils per field and 1 (2%) had 32 eosinophils per field; the latter patient had a confirmed diagnosis of EE. The mean (SD) number of eosinophils in the esophageal mucosa was 0.79 (4.28). Of the 57 patients who had a histologic assessment of the esophagus, 12 (21%) were receiving inhalation corticosteroids for asthma treatment and 1 was taking 5 mg of oral corticosteroids. In addition, the presence of eosinophils in the esophageal mucosa correlated with sensitization to foods (Table 3). Except for 1 patient diagnosed as having EE, the other patients showed no endoscopic changes suggestive of EE. The presence of erosive esophagitis or hiatal hernia was not associated with sensitization to food (Table 3).

The patient with EE was not included in the restriction diet because the objective was to determine the role of restriction diet in clinical improvement of GERD. Of the 15 patients who followed the diet, 12 (80%) showed a lower score on the questionnaire for the evaluation of symptoms of GERD, corresponding to clinical improvement (Table 4).

DISCUSSION

Although the underlying causes of eosinophilic gastrointestinal disorders are not yet understood, several investigations^{10,11} have demonstrated an association between atopy and gastrointestinal disorders. The etiologic factors involved in EE development have shown advances regarding the demonstration of complex genetic backgrounds combined with exposures to foods and aeroallergens.¹²

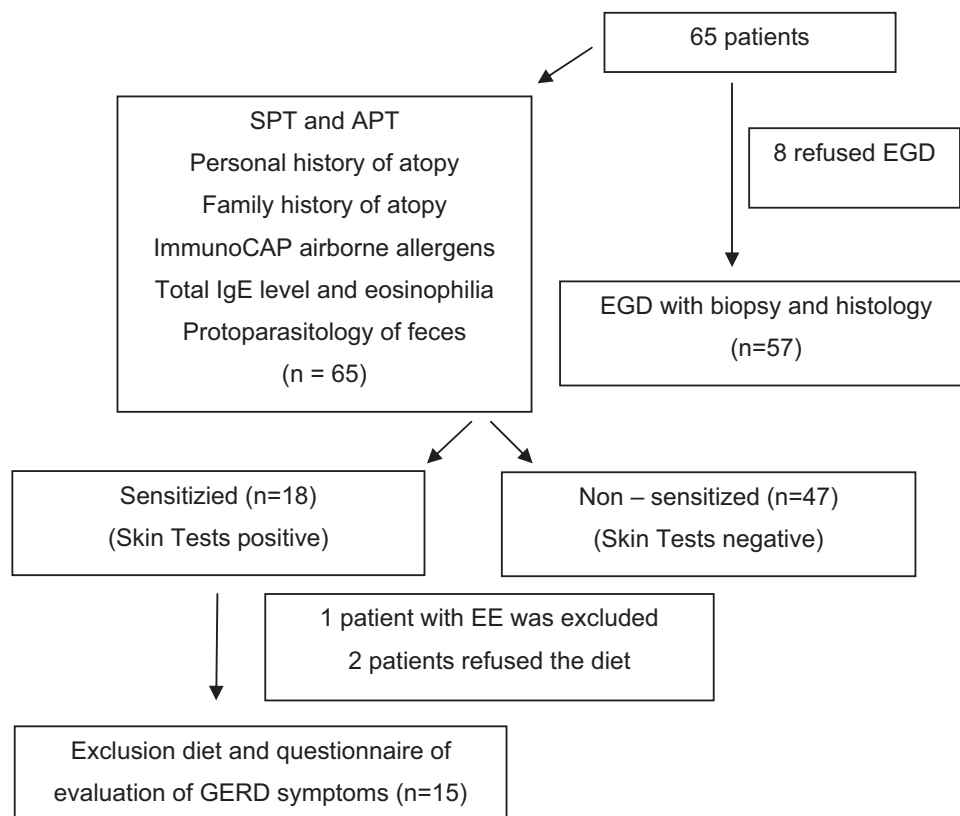


Figure 1. Distribution of the patients studied in relation to the procedures and interventions performed. APT indicates atopy patch test; EGD, esophagogastrroduodenoscopy; EE, eosinophilic esophagitis; GERD, gastroesophageal reflux disease; and SPT, skin prick test.

This is the first study, to our knowledge, to evaluate food sensitization in patients with refractory GERD and its relationship with the presence of eosinophils in the esophagus. The prevalence of sensitization in patients with EE reported in the literature is considerably greater, that is, 76% by SPT and 85% by APT in which 24 foods were tested in each kind of test, similar to the present study.³

Table 1. Prevalence of Sensitization to Foods in 65 Patients With GERD Refractory to Treatment

	Sensitized patients, No. (%) [95% CI]
Skin test positive	18 (27.7) [16.8–38.6]
Skin prick test	10 (15.4) [6.6–24.2]
Shrimp	4 (6.2) [0.3–12.0]
Barley	3 (4.6) [0.0–9.7]
Milk	1 (1.5) [0.0–4.5]
Atopy patch test	8 (12.3) [4.3–20.3]
Corn	3 (4.6) [0.0–9.7]
Potato	2 (3.1) [0.0–7.3]
Fish	2 (3.1) [0.0–7.3]
Manioc	2 (3.1) [0.0–7.3]

Abbreviations: CI, confidence interval; GERD, gastroesophageal reflux disease.

When we compare the prevalence of sensitization to particular foods using SPT with the prevalence in the general population, we find a prevalence of 6.2% for shrimp and 1.5% for milk, whereas the values reported in the literature for the general population are 2% and 0.3%, respectively.¹³ The APT is still not standardized, but its utility has a growing basis in the literature, especially when food allergy is associated with gastrointestinal symptoms or atopic dermatitis.¹⁴ The fact that no patient showed sensitization by both tests can be explained by the difference in mechanisms being examined. The skin biopsies of APT demonstrated a type IV hypersensitivity mechanism, and in this type of hypersensitivity, there is sensitization to foods different from those most prevalent in type I reactions.¹⁵

Based on the proposed escalation of the PPI dose,⁷ the doses used by the present patients were higher than routine doses. We found that the time of use of PPIs had no effect on sensitization to foods, which can be explained by the fact that PPIs increase gastric pH to greater than 5.0 within a few days of treatment.¹⁶

Although many of the present patients reported a personal history of atopy, only 30% were sensitized to aeroallergens, and, thus, the number of patients who reported having allergic rhinitis could be overestimated because nasal/sinus symptoms

Table 2. Comparison of Patients Sensitized to Foods With Nonsensitized Patients in Relation to History and Laboratory Test Results Suggestive of Atopy

	Patients, No. (%)			P value ^a
	Sensitized (n = 18)	Nonsensitized (n = 47)	Total (N = 65)	
Personal atopy history	15 (83.3)	35 (74.5)	50 (76.9)	.53
Rhinitis	14 (77.8)	34 (72.3)	48 (73.8)	.76
Asthma	9 (50.0)	7 (14.9)	16 (24.6)	.008
ImmunoCAP aeroallergens	7 (38.9)	13 (27.6)	20 (30.8)	.39
Family history of atopy	8 (44.4)	16 (34.0)	24 (36.9)	.57
Elevated total IgE level	6 (33.3)	15 (31.9)	21 (32.3)	>.99
Eosinophilia	4 (22.2)	7 (14.9)	11 (16.9)	.48

^a Fisher exact test.

and retropharyngeal secretion may not be truly due to allergic rhinitis but can represent an atypical manifestation of GERD itself. Furthermore, there was no significant difference in sensitization to foods for other variables suggestive of atopy, except for asthma.

The statistical association between asthma and greater prevalence of sensitization to foods can be attributed to the fact that asthma and food allergy are atopic diseases or can reflect a physiopathologic causal interrelation with clinical implications. Some possibilities to explain this would be that food particles could be inhaled during food reflux, directly stimulating airway mastocytes, or could even reach the lung via blood circulation after having been absorbed intact by the intestine. Another possibility is that food allergens can act indirectly in the lower respiratory airways through inflammatory mediators released by the skin, gastrointestinal tract, and circulation.¹⁷ These mechanisms require further study.

The esophagus is normally devoid of eosinophils, and their detection denotes disease.¹⁸ Some authors suggest that GERD can be associated with the recruitment of eosinophils to the esophageal mucosa. The possible explanations would be that the damage caused by acidity recruits eosinophils to the esophageal epithelium or increases permeability to antigens, allowing them to penetrate the squamous epithelium of the esophagus.¹⁹ However, the basal layer expansion observed in patients with EE is more extensive than that in patients with GERD,²⁰ suggesting that other etiologic factors affect EE disease pathogenesis. It was demonstrated that lymphocytes, eosinophils, and mast cells infiltrating the esophagus may release soluble mediators able to activate the transcription of

epithelial cell genes; for example, T_H2 cell–derived interleukin 13 may act directly on epithelial cells to induce the release of eotaxin-3.¹² It would be interesting to study markers of patients with refractory GERD sensitized to food to help us differentiate EE from GERD.

We found a prevalence of EE of 2% in patients with refractory GERD, whereas another study²¹ with a similar number of refractory patients found a prevalence of 8.8%. There was no evidence of any patient with an intermediate number of eosinophils, which could have been associated with food allergy and asthma, according to Rothenberg.⁴ The use of inhaled corticosteroids in 21% of patients may have contributed to the low number of eosinophils found in the esophagus of the total patients studied, although a difference between those sensitized and not sensitized to food has not been shown.

Unlike EE, where dysphagia is the main complaint and it subsides with a restriction diet,²² there was no evidence of dysphagia symptoms improving when evaluated separately but, rather, an improvement in heartburn and regurgitation, which are the most frequent symptoms of GERD. However, similar to that reported in the literature on patients with EE,³ patients with refractory GERD also showed improvement with the restriction diet, and the presence of eosinophils in the esophageal mucosa correlated with greater allergic food sensitization, corroborating the hypothesis that refractory GERD can represent an initial stage of EE. Food sensitization and the statistically significant difference after the diet for the total scores of GERD symptoms suggests the diagnosis of food allergy, but oral food challenge, which is the gold

Table 3. Comparison of Patients Sensitized to Foods With Nonsensitized Patients in Relation to Esophageal Histologic Findings, Endoscopic Findings, and Use of Inhalation Corticosteroids

	Patients, No. (%)			P value ^a
	Sensitized (n = 16)	Nonsensitized (n = 41)	Total (N = 57)	
Presence of eosinophils in esophageal mucosa	6 (37.5)	3 (7.3)	9 (15.8)	.01
Erosive esophagitis	2 (12.5)	9 (22.0)	11 (19.3)	.71
Hiatal hernia	4 (25.0)	10 (24.4)	14 (24.6)	>.99
Inhalation corticosteroids	5 (31.3)	7 (17.1)	12 (21.1)	.29

^a Fisher exact test.

Table 4. Comparison of the Distribution of Questionnaire Scores Before and After Following a Restriction Diet in Relation to Each Symptom (n = 15)

	Scores		P value ^a
	Before diet	After diet	
Heartburn	8.867	3.800	.005
Dysphagia	2.800	2.333	.14
Use of medication	0.667	0.933	.50
Regurgitation	2.333	1.333	.007
Satisfaction	2.867	2.533	.41
Total	14.667	8.400	.004

^a Wilcoxon test.

standard for diagnosis,²³ was not performed. Thus, we cannot affirm that these patients have a food allergy associated with GERD, and there may be a plausible mechanism that supports the observed association. The assessment of a cause and effect was not the aim of this study, but it does open the way for further investigations.

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