

Original Article

Title: Food allergy among children with refractory gastroesophageal reflux disease

Running title: Food allergy and GERD

Authors: Yukselen A¹, Celtik C².

¹ Ayfer Yukselen, MD., Associate Professor, Clinic of Pediatric Allergy and Immunology, Gaziantep Children Hospital, Gaziantep, Turkey

² Coskun Celtik, MD., Associate Professor, Clinic of Pediatric Gastroenterology, University of Sifa, İzmir, Turkey

Correspondence to:

Ayfer Yukselen, MD.

E-mail: ayfyukselen@gmail.com

Fax: 0090 342 2200072

Address: Incilipinar mah. 36006 cad.

Kepkep Plaza K.5 No: 501

Sehitkamil, Gaziantep / Turkey

Text pages: 18

Word count: 3028

Tables: 2

Figure: No

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ped.12779

Abstract

Background: Gastroesophageal reflux disease (GERD) and food allergy are frequent disorders of childhood. The purpose of this study was to determine the frequency of food allergy in children with refractory GERD.

Methods: 151 children resistant to pharmacologic treatment of GERD were underwent diagnostic procedures as skin prick test (SPT), specific immunoglobulin E (sIgE), eosinophil count, atopy patch test (APT), oral food challenge and then divided into three groups according to the results of oral milk -challenge and allergy work-up: Group A1 – children with positive results for both oral milk challenges and IgE-mediated allergy tests; Group A2- children with positive milk challenges but negative IgE-mediated allergy tests; Group B – children with negative results for both oral milk challenges and allergy tests.

Results: There were 35, 30 and 86 patients in Group A1, Group A2 and Group B, respectively. 28 of 35 patients in Group A1 had cow's milk allergy and the other seven patients had egg allergy. APT positivity was more common in patients belonging to Group A2. Endoscopic esophagitis was observed in 6 patients of Group A1 and in 4 patients of Group A2. Bloody stools, atopic dermatitis and recurrent wheezing episodes are significantly more common in Group A1 than Group A2 and Group B ($p < 0.001$, for both).

Conclusion: Cow's milk allergy was observed frequently in children with resistant to pharmacologic treatment of GERD. Combined skin prick and specific IgE tests, atopy patch test and oral food challenge is essential for avoidance of unnecessary elimination diet.

Key words: children, food allergy, gastroesophageal reflux disease.

Introduction

Approximately 7% to 8% of children are affected by food allergies, the most common being cow's milk allergy (CMA), and egg and peanut allergies.¹⁻² The mechanisms involved include both immunoglobulin E (IgE)- and non-IgE-mediated reactions.³⁻⁴ IgE-mediated allergic reactions can occur within seconds to minutes; allergies that take days to weeks to appear are more often non-IgE-mediated. GI manifestations of CMA can occur in all parts of the GI tract such as the esophagus, stomach, small intestine, and/or colon and rectum.⁵

Many authors emphasize the more frequent coexistence of pathological gastroesophageal reflux (GERD) with food allergy in children younger than three years old.⁶⁻¹⁰ In infancy, this frequency can reach 40 %.⁶ There is a pathogenetic cause-and-effect relationship between these conditions. The main allergen responsible for the observed symptoms is cow's milk protein.¹¹

Among the clinical symptoms of GERD in small children are regurgitation, vomiting, lack of thriving, disturbances in growth, recurrent infections of the upper and lower respiratory tract, anxiety, irritability, disturbed sleep. These symptoms and findings are frequently similar to those of food allergy. Therefore it is difficult to distinguish between primary gastroesophageal reflux and reflux secondary to food allergy when taking into account only the clinical picture. Chronic rhinitis, atopic dermatitis, and diarrhea are more frequently observed in children with coexisting food allergy and gastroesophageal reflux.⁷⁻⁹

The diagnosis of gastroesophageal reflux secondary to food allergy is particularly important in the choice of treatment method. The basic method in the treatment of gastroesophageal reflux secondary to food allergy is the introduction of elimination diets: formulas based on casein hydrolysates and whey proteins, hypoallergic diets, and anti-allergic drugs.¹²

The objective of the present study was to estimate the frequency of food allergy in children under five years of age with inadequate response to pharmacologic treatment of gastroesophageal reflux disease.

Patients and Methods

A total of 151 children aged between 3-60 months with persistent vomiting and refractory gastroesophageal reflux disease were included in the study. All patients underwent a complete medical history that included demographic data, history of environmental, food, and drug allergies, clinical symptoms suggestive of GERD, diagnostic testing for GERD, length of symptoms, type and duration of GERD treatment, adherence to treatment, and associated comorbid states.

The medical history of 96 patients revealed prescription of milk-elimination diet by practitioners or pediatricians without performing allergy work-up tests.

Upper endoscopy was performed in all patients; additionally, scintigraphic evaluation of gastroesophageal system or ambulatory monitoring of 24-h esophageal pH were performed for establishing the diagnosis. Patients having organic causes for persistent vomiting were excluded.

All patients received a once-daily treatment with standard-dose proton pump inhibitors (PPI's) or a combined treatment with PPI's and H₂-receptor antagonists (anti-H₂) for at least 8 weeks without interruption. Refractory GERD was defined as the persistence of symptoms after treatment.

Allergy was diagnosed on the basis of the patient's medical history, family history, physical examination, laboratory tests, i.e. eosinophil count, total serum IgE, skin prick and patch test with food allergens.

Patients provided written informed consent before being enrolled in the study.

At the beginning of the study, all patients diagnosed as refractory GERD were challenged orally with cow's milk after two-weeks elimination diet. We designed this procedure in order to make clinical and statistical analysis.

According the results of oral cow's milk challenges, examined children were divided into these groups:

Group A, children with cow's milk allergy and refractory GERD,

Group B, children with refractory GERD but without allergy to cow's milk.

Skin-prick test

The skin-prick tests with cow's milk and other food allergens were performed in all examined children, by the same person on the volar side of the forearm in accordance with the instructions of the European Academy of Allergy and Clinical Immunology.¹³ All patients were advised to stop medications such as antihistamines and montelukast that interfere with the results before skin testing. Food allergens (cow's milk, hen's egg, wheat, peanut, fish, cacao, soya) were tested by means of prick to prick and/ or skin prick technique. To perform tests with aeroallergens (mites, tree, grass and weed pollen, dog and cat epithelia, moulds) commercial extracts from Allergopharma Company were used - a 1 mm, single-peak lancet on the forearm and negative (NaCl 0.9%) and positive (Histamine dihydrochloride, 10 mg/ml) as control. Reactions were read after 15 minutes by the author. The mean wheal diameter was calculated and a reaction of at least 3 mm was considered as positive without reaction of negative control.

Atopy patch test

Atopy patch tests were applied on uninvolved skin of the child's back, according to the method described by Isolauri and Turjanmaa.^{14,15} We used 8-mm diameter aluminium cups for children younger than 3 years and 12-mm aluminium cups for children older than 3 years (Finn Chamber, Epitest Ltd., Finland). Microcrystalline cellulose was used as negative

control. Application sites were checked after 20 minutes for immediate reactions. The occlusion time of patch test was 48 hours. The results were read for the first time 15 min after removal of the cups. If irritative redness was found in the test area, the results were read after 30 min. The second evaluation was done 72 hours after attaching the patch tests. Reactions were classified according to standards and considered as: 0 - negative – no reaction, either visible or palpable; + - redness – negative or doubtful reaction; ++ - redness and palpable infiltration with papules – positive reaction; +++ - redness, palpable infiltration with many papules and eczema – strong positive reaction. If the reaction was very intensive as: redness, edema, palpable infiltration and vesicles, the result was marked as +++++. Reactions 0 and + (redness without infiltration) were regarded as negative. Occurrence of alone redness can be the results of local irritation.

Determination of total IgE and specific IgE antibodies

Serum samples (2 ml) were analyzed for concentration of total IgE and specific IgE antibodies to food allergens as: cow's milk, egg white, soy, wheat (Pharmacia, Uppsala, Sweden) with a fluoroimmunoenzymatic assay (UniCAP) according to the manufacture's instruction. The detection limit of the CAP system is 0.35 kU/L IgE.

Oral food challenge

Standardized oral milk challenge was performed according to guidelines^{16,17}, with the increasing amounts of milk at 30 min intervals until intake appropriate for their age was reached. A challenge was taken on all children after 2 week on an elimination milk-free diet. Cow milk challenge was performed as the open challenge being a reliable method for infants and young children (<2 year) and as a blinded test for older children (>2 year). The foodstuff was blinded in apple pulp or rice if there was no sensitization (SPT, negative history of consumption) to these allergens. The same doses of milk formula were given in open and blinded challenges. Immediate reactions were defined as reactions within 2 hours after the

last dose of milk was administered. The challenge was started in the hospital when rising doses of the infant formula were given and continued after 24 hours of observation in the patient's home, where the parents recorded the symptoms of the child being with telephone contact with the author. The challenge was discontinued when a clinical reaction was noticed. If one of the following symptoms such as skin eruptions, pruritus, edema, urticaria, vomiting, diarrhea, irritability, rhinitis, wheezing, anaphylactic shock, was noticed, the challenge was regarded as positive. The evaluation of symptoms and decision to stop a challenge was made by the investigator. All children were examined once a week to verify reported symptoms and seen 1 month after the beginning of challenge to confirm the diagnosis.

Endoscopy

Upper endoscopy was performed under general anesthesia using an Olympus Gif N230 videoscope (Ballerup, Denmark). Endoscopic esophagitis was classified according the Los Angeles classification, Grade A–D.¹⁸ Findings that might indicate food hypersensitivity, such as duodenal nodules, were noted. Biopsies were performed in conjunction with the endoscopy. All biopsies were reviewed by an experienced team of pathologists in the laboratory department. Histological diagnosis of eosinophilic esophagitis was defined as the presence of more than 20 eosinophils/HPF discovered on biopsies taken at endoscopy.

Statistical analysis

All the analyses were performed using computer software (SPSS version 11.0; SPSS; Chicago, Illinois, USA). Data were presented as min-max, mean $\pm$ SD or median, as indicated. Mann-Whitney-U test for between group analysis were performed.

Results

A positive challenge response to cow's milk was found in 58/151 (38.4 %) of children with refractory GERD. According to these results children firstly were divided into two groups:

Group A – with positive challenge to cow's milk, Group B- with negative challenge to cow's milk.

Seven patients showed positive results only to egg in skin prick tests and/or specific IgE tests.

After confirmation of egg allergy with oral egg challenges, these 7 patients also included in the Group A1 (n= 35 children: 28 patients with only cow's milk allergy and 7 patients with only egg allergy). Therefore, patients belonging to Group A1 had IgE-mediated food allergy.

After this evaluation, Group A was divided into two groups according to results of allergy work-up:

Group A1: Patients showed positive skin prick and/or specific IgE results to foods (cow's milk or egg) (n= 35) (As stated below, Group A1 included patients with IgE-mediated food allergy)

Group A2: Patients with negative skin prick and/or specific IgE results, but with positive challenge results to cow's milk (n=30)

Allergy workup

The demographic characteristics and results of allergy tests of study groups were shown in Table 1.

There is no statistical difference in terms of age and gender between three groups ($p=0.86$ and $p=0.77$, respectively).

Both total eosinophil counts and total IgE levels are found significantly higher in Group A1 in comparison to Group A2 ($p<0.001$) and Group B ($p<0.001$).

In Group A1, 23 of 28 milk –challenge positive children had positive skin prick test with cow's milk. Four patients who respond positively to egg challenge were found also to have positive skin prick test to egg.

Specific IgE levels were positive in 32 of 35 children in Group A1 (26 of them had positive specific IgE to cow's milk and six patients had positive skin prick test to egg).

Positive APT to cows milk was noticed in 4 of 35 children belonging to Group A1 and 12 of 30 children belonging to Group A2.

Additionally, five patients classified in Group A1 had also skin prick test positivity to inhalant allergens (grass pollens and moulds).

Endoscopic findings

Histological diagnosis of eosinophilic esophagitis defined as the presence of more than 20 eosinophils/HPF discovered on biopsies taken at endoscopy was observed in 6 patients of Group A1 and in 4 patients of Group A2. Nodular duodenitis was noted in 2 patients belonging to Group A1.

Associated clinical findings

Some clinical features associated with study groups are seen in Table 2.

Vomiting is significantly more common observed in patients belonging to Group A2 ($p<0.001$).

Recurrent or chronic diarrhea is significantly more common in patients belonging to Group A2 ($p<0.001$).

Bloody stools, atopic dermatitis and recurrent wheezing episodes are significantly more common in Group A1 than Group A2 and Group B ($p<0.001$, for both).

Positive family history of food allergy is observed in 40 % and 30 % of patients belonging to Group A1 and Group A2, respectively.

Discussion

The coexistence of GERD with food allergy in children under 5 years old is a frequent event (6-10). As shown in the present study, food allergy is observed in (65/151) 43 % of children with gastroesophageal reflux disease. 58 of 151 (38.4 %) children were found allergic to only cow's milk while 7 (4.6 %) patients were allergic to only egg. Additionally, we showed that 35 patients (23.2 %) had both positive results related to skin prick and/or specific IgE levels and oral food challenges; however, 30 patients (19.9 %) who had negative skin prick and/or specific IgE levels were also cow's milk allergic which can be diagnosed with oral milk challenges.

GERD is a disease that commonly occurs during the first year of life, and some forms of severe GERD have been shown to be associated with food allergy. In the study of Nielsen et al.¹⁹, the GERD-cow milk hypersensitivity association was demonstrated in 10 of 17 patients with severe GERD using strict criteria for both GERD and food hypersensitivity. Iacono et al.⁶ reported that food allergy was diagnosed in 41.8 % of children with pathological gastroesophageal reflux. Semeniuk et al.²⁰ found cow's milk allergy in 43% of children aged 2–15 months with gastroesophageal reflux. Janiszewska and Czerwionka-Szaflarska²¹ studied a large group of children aged 1–16 years with diagnosed acidic gastroesophageal reflux based on 24-hour esophageal pH-metry and in 48 % of the cases found IgE-dependent food allergy. In the present study, we included a large group of patients with GERD to evaluate the prevalence of food allergy and, as reported in the previous studies, we demonstrated that 43 % of children with GERD have also food allergy.

Recently, attention has been drawn to an altered clinical spectrum of food allergy in the foregut. The data from Kokkonen et al.²² suggest that duodenal lymphoid nodules is a distinct endoscopic feature associated with food allergy, as lymphoid nodules in the duodenal bulb were seen frequently among infants and children with this condition. In the present study

lymphoid nodules in the duodenum were observed in two patients allergic to cow's milk in the Group A1.

Eosinophilic oesophagitis is a member of the family of eosinophilic gastroenteropathies. It occurs mainly in children 5 to 15 years old, and appears to be related to food allergy and, to a lesser extent, aeroallergens, in the majority of cases.²³⁻²⁶ Data from the United States indicate that there is an increasing prevalence of new cases of eosinophilic oesophagitis in children.²⁴ Endoscopic findings of esophagitis in the upper esophagus associated with esophageal rings and webs are characteristic findings of eosinophilic esophagitis, which may be primary or secondary to food allergy. We demonstrated that 10 of 151 (6.6 %) children (6 and 4 patients in Group A1 and Group A2, respectively) showed endoscopic findings of eosinophilic oesophagitis.

It has been known that food allergy can be classified as IgE-mediated, non-IgE mediated or mixed (IgE-mediated plus cellular-mediated) reactions.^{3,27} In the present study, we detected that 35 patients with GERD had positive reactions with skin prick and/or specific IgE tests. However, 30 patients allergic to cow's milk identified by elimination diet followed by milk challenges. Reactions at challenge were mainly restricted to the gastrointestinal tract.

None of these patients had positive results of skin prick tests, and tests for specific IgE and thus showed no evidence of a general IgE-mediated immune reactivity. Skin patch tests could be a potentially useful diagnostic test in patients with non-IgE mediated reactions, as the reactivity in the skin patch test presumably depend on T-cell mediated reactions. Several studies suggest a clinical relevance of patch tests in skin prick negative infants/children with challenge proven cow's milk allergy^{28,29} and in children with mainly gastrointestinal/GERD manifestations of cow's milk allergy.³⁰ In the present study twelve of 151 children with GERD (7.9 %) reacted with grade one or two on patch test for cow's milk.

We found that 18 patients with GERD had positive milk challenges in spite of the negative results in skin prick, patch and specific IgE tests. There are many studies which suggest that the possible allergic mechanisms involved might be of local gastrointestinal nature. Local immunologic mechanisms within adverse reactions to foods have been indicated to occur in challenge-proven but skin prick test and serum IgE antibody negative patients. In adults food challenge in a segment of the jejunum as evaluated by a double balloon technique induced an increase of luminal histamine and eosinophilic cationic protein levels.³¹ Other studies have reported increased numbers of cytotoxic TIA-1 positive T-cells³² and activated eosinophils³³ in the duodenum. Taken together these studies suggest that the further categorization of food hypersensitive children with mainly gastrointestinal symptoms and negative tests for general IgE sensitization depend on the development of local organ-based diagnostic tests.

We have not observed a significant difference in terms of growth between three study groups. However, allergic rhinitis, recurrent wheezing attacks and atopic dermatitis were more frequently seen in patients having positive IgE-mediated tests as expected.

As some previous studies, we also observed that diarrhea was significantly more frequent in children with coexisting food allergy and GERD than in isolated gastroesophageal reflux.³⁴⁻³⁵

In the present study, we found that 65 of 151 patients had positive oral food challenges; however, 96 of 151 patients had history of milk-elimination diet before including in this study because of prescription by practitioners or pediatricians. This suggests that these diets are overprescribed in our region, often without adequate allergologic evaluation. Even if a food elimination diet is still considered a pivotal decision in the treatment of children with food allergy, it must be remembered that a food allergy diagnosis can have a strong social and economic impact and affect the psycho-physical development of a child. The exclusion of one fundamental food must be decided on only after careful evaluation of allergy tests and

only after considering the child's clinical history in order to prevent growth problems or inadequate nutrient intake.

There are some limitations of the study. Firstly, although reflux symptoms of all patients were resistant to pharmacologic treatment, the reflux diagnosis was confirmed with different methods, eg. scintigraphic evaluation or ambulatory monitoring of 24-h esophageal pH.

Secondly, we did not evaluate the difference in reflux pattern of children with coexisting food allergy.

In conclusion, an association between food allergy and GERD was observed in an important part of infants and children. The investigation and detection of the food allergy especially in patients with inadequate response to pharmacologic treatment of GERD provide effective therapy and follow-up of these patients. Besides skin prick and specific IgE tests, atopy patch test and oral food challenges have great diagnostic value. Additionally, clinicians should keep in mind the possibility of food allergy and should make further investigation to exclude this possibility in all children with chronic or recurrent diarrhea, or with allergic respiratory, skin or nasal symptoms. Further studies are needed to characterize the association of GERD and food allergy, especially the interaction of a possible allergic inflammatory process.

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Table 1. The demographic characteristics and allergy test results of study groups.

	Group A1 Positive results for both milk challenges and allergy tests (n=35)	Group A2 Negative IgE-mediated - allergy tests but positive milk challenges (n=30)	Group B Negative results for both milk challenges and allergy tests (n= 86)
Male /Female	19 / 16	20 / 10	42 / 44
Age (months), Median	21.4	17.8	18.1
Min-max	(3-48)	(6-30)	(4-32)
Length (cm), Mean $\pm$ SD	80.5 $\pm$ 10.2	77.8 $\pm$ 7.7	75.5 $\pm$ 8.4
Min-max	(56-104)	(65-96)	(60-95)
Weight (kg), Mean $\pm$ SD	10.7 $\pm$ 2.4	10.0 $\pm$ 2.1	9.4 $\pm$ 2.1
Min-max	(5.5-16.5)	(6-16)	(5.8-15.8)
Total IgE (IU/l), Median	276.3	15.7	22.9
Min-max	(5-1000)	(5-48)	(4.3-74)
Total eosinophil (/mm ³) Median	760.8	251.2	244.1
Min-max	(12-1824)	(32-480)	(11-2071)
SPT positivity, n (%)	27/35 (77.1)	0 (0)	0 (0)
sIgE positivity, n (%)	32/35 (91.4)	0 (0)	0 (0)
sIgE level (kU/L) Median	22.3	<0.35	<0.35
Min-max	(5-60)	<0.35	<0.35
APT positivity, n (%)	4/35 (11.4)	12/ 30 (40)	0 (0)
Inhalant allergy, n (%)	5 /35 (14.2)	0 (0)	0 (0)
Symptoms induced by food challenge, n (%)			
Urticaria	10 / 35 (28.5)	0 (0)	
Eczema	6 / 35 (17.1)	0 (0)	
Vomiting	4 / 35 (11.4)	25 / 30 (83.3)	
Diarrhea	0 (0)	15 / 30 (50)	
Rhinitis	12 / 35 (34.2)	0 (0)	
Wheezing	14 / 35 (40)	0 (0)	
Anaphylactic shock	1 / 35 (2.8)	0 (0)	
Endoscopic findings, n (%)			
Endoscopic esophagitis	6 / 35 (17.1)	4 / 30 (13.3)	
Nodular duodenitis	2 / 35 (5.7)	0 (0)	

Table 2. Associated clinical features of study groups.

	Group A1 Positive results for both milk challenges and allergy tests (n=35)	Group A2 Negative IgE- mediated- allergy tests but positive milk challenges (n=30)	Group B Negative results for both milk challenges and allergy tests (n= 86)	p*
Vomiting, n (%)	3 (8.5)	22 (73.3)	2 (2.3)	<0.001
Constipation, n (%)	7 (22.1)	5 (16.7)	19 (20.0)	NS
Diarrhea, n (%)	18 (51.4)	26 (86.6)	6 (7.1)	< 0.001
Bloody stools, n (%)	5 (14.3)	0 (0)	1 (1.2)	< 0.001
Recurrent wheezing, n (%)	13 (37.1)	4 (13.3)	6 (6.97)	< 0.01
Allergic rhinitis, n (%)	2 (5.7)	0 (0)	1 (1.2)	NS
Atopic dermatitis, n (%)	8 (22.9)	0 (0)	3 (3.5)	< 0.01

NS; not significant

*p value between Group A1 and Group A2.