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Sensitivity Comparison of the Skin Prick Test and Serum and Fecal Radio Allergosorbent Test (RAST) in Diagnosis of Food Allergy in Children

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Abstract

Background: Diagnosis of food allergy is difficult in children. Food allergies are diagnosed using several methods that include medical histories, clinical examinations, skin prick and serum-specific immunoglobulin E (IgE) tests, radio-allergosorbent test (RAST), food challenge, and supervised elimination diets. In this study we evaluated allergies to cow's milk, egg, peanut, and fish in children with suspected food allergies with skin prick tests and serum and feces RAST.
Methods: Forty-one children with clinical symptoms of food allergies were enrolled in the study. Skin prick tests and serum and fecal RAST were performed and compared with challenge tests.
Results: The most common sites of food allergy symptoms were gastrointestinal (82.9%) and skin (48.8%). 100% of the patients responded to the challenge tests with cow's milk, egg, peanut, and fish. 65% of the patients tested positive with the skin prick test, 12.1% tested positive with serum RAST, and 29.2% tested positive with fecal RAST.
Conclusions: The skin prick test was more sensitive than serum or fecal RAST, and fecal RAST was more than twice as sensitive as serum RAST.

Keywords: Food Hypersensitivity, Radioallergosorbent test, Sensitivity, Specificity

Introduction

A food allergy is an adverse immune response to a food protein. Children with atopic diseases are more likely to have food allergies than children without them. Approximately 30% of atopic children have this problem with moderate to severe scores (1, 2).

Food allergies are highly prevalent in preschool children (3). About 4% to 5% of children between 5 and 17 have at least one food allergy.

Food allergies are usually diagnosed using methods that include medical histories, clinical examinations (4), skin prick tests (SPTs) (5, 6), serum-specific immunoglobulin E (IgE) tests using the radioallergosorbent test (RAST), oral food challenge, and elimination diets (7-10).

An alternative method for food allergy diagnosis was established by Kolmannskog and Haneberg in 1985 (11). They detected specific IgE by a double-antibody radioimmunoassay technique (PRIST) in the feces of children with gastrointestinal allergy, atopic dermatitis, hay fever, and/or bronchial asthma. They concluded that IgE may be produced in the gut in response to food allergens. Therefore, a positive correlation may exist between specific fecal and serum IgE levels (7). In present study we aimed to

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measure the sensitivity of the SPT, and serum and fecal RAST in pediatric patients with food allergies. Due to the importance of cow's milk, egg, peanut, and fish in pediatric allergy (2-6), we used these four as allergenic foods in our study.

Materials and Methods

Study participants

This cross-sectional analytical study included 41 children who were referred to the pediatric gastrointestinal clinic for evaluation of suspected IgE-mediated food hypersensitivities. If a patient's history and SPT indicated food hypersensitivity, the patient was referred to the Allergy Research Center of Mashhad University of Medical Sciences for blinded oral food challenges to verify the diagnosis. Patient sera were obtained at the initial visit to quantify food specific IgE antibodies. Our inclusion criteria were age greater than three months, and all the patients had at least one manifestation of food-allergic IgE-mediated hypersensitivity such as gastrointestinal symptoms, urticaria, eczema, or wheezing. Patients receiving antibiotics and/or corticosteroids were excluded from the study.

Informed consent was obtained from all the parents. All clinical symptoms including urticaria, eczema, wheezing, diarrhea, growth disorders, and histories of allergic diseases, including allergic rhinitis, asthma, and drug reactions were assessed. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences.

Laboratory studies

We performed oral food challenge, and serum and fecal RAST, for all 41 patients to assess the sensitivity of these diagnostic tests and compare them to each other. The SPT was performed on 36 patients; five patients were excluded from the SPT study due to not referring to the clinic and/or using antihistamines.

Skin Prick Test

The SPT was performed as described in the European Academy of Allergy and Clinical Immunology manual (12). Food extracts of cow's milk, egg, peanut, and fish (1:10 or 1:20, kindly provided by Greer Laboratories, Lenoir, NC, USA) were applied by means of the prick technique, along with positive histamine controls. Food allergens with mean wheal diameters at least 3 mm greater than the positive controls were considered positive.

Oral food challenges

All the children in the study were orally challenged with the four food allergens and a placebo (13). Children taking antihistamine (predominantly Cetirizine) were advised to avoid it for 72 h before provocation. Topical glucocorticosteroids were allowed twice a day at maximum concentrations of 1% for hydrocortisone and 0.01% for betamethasone. Samples were ingested in stepwise increments equaling 1/16, 1/16, 2/16, 4/16, and 8/16 of the final amount every 15 minutes so the total amount would be ingested in 60 min. Our controlled oral food challenges were performed on 41 patients under single blinded, placebo-controlled conditions. The time interval between doses was 15 minutes. The oral food challenge was carried out under the supervision of an allergist and a nurse with emergency equipment available. The provocation was stopped if clinical symptoms occurred or the when highest dose was reached. The patients were observed for 48 h after each challenge on an in-patient basis to detect late clinical reactions. The food challenges were considered positive if respiratory (wheezing), gastrointestinal (vomiting, diarrhea, abdominal pain), or cutaneous (skin eruptions, pruritus, urticaria, worsening of eczema) symptoms, or shock, developed after food ingestion. We evaluated the early reactions with symptoms occurring within two hours after food ingestion and late reactions with symptoms occurring more than two hours after ingesting the highest dose.

Radioallergosorbent Test (RAST)

We performed RAST to assess allergen-specific IgE for the four foods using a Pharmacia ImmunoCAP 250 analyzer (Phadia, Uppsala, Sweden) according to the manufacturer's recommendations. Briefly, 50 cc of serum were dispensed in cups containing allergen covalently coupled to ImmunoCAP, which is a cellulose derivative. After 30 minutes of incubation, the excess sample was removed. Enzyme-labeled anti-IgE antibodies were added and the contents allowed reacting for 30 minutes at 37 °C. After incubation, unbound enzyme-linked anti-IgE was washed away and bound complexes were incubated with developing agent (4-

methylumbelliferyl-beta-D-galactoside) for 10 minutes. During this time the substrate was cleaved and the fluorescing product, 4-methylumbelliferon, was released. Test response was detected by measuring the fluorescence activity. Fecal RAST was also performed on samples from all the subjects. Specific IgE for the four food allergens was measured by immunoblotting. Due to the high concentrations of enzymes and gastric acids in the stool samples, the pH of reaction container was set on 6.5 to 7 at first and then all samples were processed identically to the serum samples.

Statistical analysis

SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA) was used in all statistical procedures. Numerical data are expressed as means ± SDs or as proportions of the sample size. Differences in proportions were judged by the $\chi 2$ test. P-values < 0.05 were considered statistically significant. Two-by-two tables were used to calculate the sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs). Test sensitivity was defined as the proportion of true positives detected, and specificity as the proportion of true negatives detected. The PPV describes the proportion of symptomatic individuals among test positives, and the NPV describes the proportion of non-symptomatic individuals among test negatives.

Results

The present study included 41 patients. Twenty were males and 21 were females. Their mean age was 18.48±14.54 months and their ages ranged from 3 months to 5 years. Onset of allergy symptoms varied from birth to 30 months of age. Among our study participants, 29 children (70.7%) had positive family histories of food allergies and 11 (26.8%) had history of other allergic diseases including asthma, allergic rhinitis, and drug allergies. The clinical allergy symptoms included gastrointestinal symptoms, containing diarrhea, abdominal pain, and vomiting, in 34 children (82.9%), cutaneous symptoms, including urticaria, in 20 children (48.8%), growth disorders in 13 children (31.7%), and respiratory symptoms, including wheezing, in 9 children (22%). No gender difference in food allergy prevalence was observed (*P*=0.45).

Table 1. Number and percent of patients who tested positive for each food with the diagnostic tests.

Test	Milk (%)	Egg (%)	Peanut (%)	Fish (%)	Total (%)
Skin Prick Test	9 (22)	7 (17.1)	8 (19.5)	3 (7.3)	*27 (75)
RAST	1 (2.4)	4 (9.7)	0	0	5 (12.1)
Fecal RAST	1 (2.4)	6 (14.6)	5 (12.1)	0	12 (29.2)
Challenge test**	14 (34.1)	1 (2.4)	5 (12.8)	0	41 (100)

* Skin Prick Test performed for 36 patients. ** Challenge test: Milk +peanut = 11 (26.8), Milk + fish = 1 (2.4), Milk + egg = 6 (14.6), Peanut + Fish = 1 (2.4), Peanut + Egg = 1 (2.4).

Table 2. Diagnostic capacity of skin prick test, RAST, and Fecal RAST for oral food challenges

Tests		Milk (%)	Egg (%)	Peanut (%)	Fish (%)
SPT*	Sensitivity	21 (6.9-35.1)	55 (22.5-87.5)	26 (6.3-45.7)	100 (1-1)
	Specificity	88 (66.8-1)	81 (64.7-94.6)	68 (48.5-87.5)	86 (46.7-1)
	PPV	87 (66.7-1)	71 (37.4-1)	55 (22.5-87.5)	100 (1-1)
	NPV	28 (11.4-46.7)	83 (70.9-95.1)	55 (36.2-73.8)	100 (1-1)
RAST	Sensitivity	3 (2.9-2.9)	33 (2.3-64)	----	----
	Specificity	100 (1-1)	93 (84.3-1)	53 (37.7-68.2)	100 (1-1)
	PPV	100 (1-1)	75 (32.5-1)	----	----
	NPV	28 (11.4-46.7)	83 (70.9-95.1)	53 (37.7-68.2)	92 (83.7-1)
Fecal RAST	Sensitivity	3 (2.9-2.9)	55 (23.1-88.1)	26 (6.3-45.7)	----
	Specificity	100 (1-1)	96.8 (90.7-1)	100 (1-1)	100 (1-1)
	PPV	100 (1-1)	83.3 (53.4-1)	100 (1-1)	----
	NPV	28 (11.4-46.7)	88.6 (79-99.1)	61 (45.1-79.8)	92 (83.7-1)

* SPT; Skin Prick Test, Number in the parenthesis shows the confidence interval (CI), PPV, positive predictive value; NPV, negative predictive value.

The number and percent of patients who tested positive with each test for the various food samples is shown in Table 1. Table 2 shows the diagnostic capacity of each test.Of the four allergens tested, we found that the oral food challenge was most sensitive for cow's milk (22%) and peanut (19.5%). The oral food challenge test was positive for all four allergens, so the specificity and predictive value of this test was not measurable by statistical means. Of the 36 children tested with the SPT, 27 (75%) were positive to at least one of the four foods. Skin prick test results showed that this test was most sensitive to fish, followed by egg, peanut, and cow's milk.

Of the 41 cases, serum RAST was positive for five (12.1%) and fecal RAST was positive for 12 (29.2%). Serum RAST sensitivity was greater for egg than milk. The sensitivity order for fecal RAST was egg, peanut, and then milk. Neither test was sensitive to fish allergen.

We found that the specificities of SPT, serum RAST, and fecal RAST for the four allergens were 65, 12.1, and 29.7%, respectively.

Discussion

Simple, straightforward diagnostic tests for suspected food allergies in children are in great demand. Similar to previous studies (14, 15), we found the most common clinical symptoms in pediatric food allergies to be gastrointestinal and cutaneous. In addition, as in previous reports (14-16), the prevalence of food allergy did not differ between genders.

According to previous reports, a positive SPT does not necessarily prove a food allergy is clinically relevant (16). Thus, the specificity of the SPT for the outcome of oral food challenges is limited. Previous studies concluded that both serum IgE tests and SPT are sensitive and have similar diagnostic properties (17-20). The immediate results visible to the patient and family and low cost compared with serum IgE tests are the valuable advantages of the SPT. However, the need to withhold medications with antihistamine properties, having rash-free skin available for the test, and restlessness in children during the SPT are disadvantages. Advantages of serologic tests include their availability, lack of interference from antihistamines, and lack of extensive dermatitis. Disadvantages include the need to obtain blood samples, delayed results, and cost (16, 21-23).

In our study the food challenge test was most sensitive to cow's milk and the SPT was most sensitive to fish. Oral food challenge, serum RAST, and fecal RAST were negative for fish allergy. However the negative response of the RAST tests to fish allergen might be due to application of fish extract of brine water, while for SPT we used fish extract of fresh water.

In the present study, we found 100 and 75% of patients were positive by food challenge and SPT respectively. The order of SPT sensitivity was cow's milk (22%) and then peanut (19.5%) contrary to previous results, which found SPT sensitivity was highest to cow's milk and egg (6, 24, 25). Although reported results varied, ranging from 50 to 90%, with regard to the sensitivity and specificity of SPT (6), we found the sensitivity of SPT to be 65%, with 100% positive predictive value.

Verstege et al found that 43% of 385 children were positive to oral food challenges (24). Regarding the efficacy of the SPT, they concluded that the predictive decision points for a positive outcome of food challenges can be calculated for egg and cow's milk using weal size; therefore, oral food challenges may be avoided. However, Imai et al found that the diagnostic accuracy of the SPT had not been satisfactory to judge the acquisition of tolerance in allergic children for eggs, milk, and wheat. They concluded that the evidence is not sufficiently strong to test the tolerance of the immediate type food allergy (25).

We found serum and fecal RAST sensitivities to be 12.1 and 29.7%, respectively. Until now, sensitivity of fecal RAST had not been investigated. Kolmannskog et al. reported that IgE was increased in stool samples of food-allergic cases with gastrointestinal symptoms (11). In our study of 41 cases, fecal RAST was positive for 12 (29.2%) for all allergens, and egg (50%) and peanut (41.6%) were the highest individual allergens. Serum RAST and SPT had the highest specificities for cow's milk, followed peanut and egg. The sensitivity of serum RAST was lower than in previous studies, which reported 62 and 50%, respectively for cow's milk and egg (26, 27). This result might be due to the

small sample size and small amount of allergen used in this study.

Due to positives of oral food challenge test for all cases, we could not measure its sensitivity or negative predictive value. Although fecal RAST sensitivity was lower than that of SPT, the authors recommend using fecal RAST due to its greater sensitivity than serum RAST and its simple sampling compared to SPT. We recommend further studies with larger sample sizes and double-blinded placebo-controlled oral food challenge tests to detect the real specificity and negative predictive value of fecal RAST compared to serum RAST, oral food challenge, and the SPT.

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References

1. Burks A, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: clinical relevance of food hypersensitivity reactions. The Journal of pediatrics. 1988; 113(3):447-51.
2. Novembre E, Martino Md, Vierucci A. Foods and respiratory allergy. Journal of Allergy and Clinical Immunology. 1988; 81(5):1059-65.
3. Mahoney EJ, Veling MC, Mims JW. Food allergy in adults and children. Otolaryngologic Clinics of North America. 2011; 44(3):815-33.
4. Ives AJ, Hourihane JOB. Evidence-based diagnosis of food allergy. Current Paediatrics. 2002; 12 (5):357-64.
5. Sampson HA. Food allergy. JAMA. 1997; 278 (22):1888-94.
6. Hill DJ, Heine RG, Hosking CS. The diagnostic value of skin prick testing in children with food allergy. Pediatric allergy and immunology. 2004; 15 (5):435-41.
7. Sampson HA. Update on food allergy. Journal of Allergy and Clinical Immunology. 2004; 113(5):805-19.
8. Moneret-Vautrin DA, Kanny G, Fremont S. Laboratory tests for diagnosis of food allergy: advantages, disadvantages and future perspectives. European annals of allergy and clinical immunology. 2003; 35(4):113-9.
9. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. Journal of Allergy and Clinical Immunology. 2001; 107(5):891-6.
10. Baral V, Hourihane JOB. Food allergy in children. Postgraduate Medical Journal. 2005; 81(961):693-701.
11. Kolmannskog S, Haneberg B. Immunoglobulin E in feces from children with allergy. International Archives of Allergy and Immunology. 1985; 76(2):133-7.
12. Sampson HA. Comparative study of commercial food antigen extracts for the diagnosis of food hypersensitivity. Journal of Allergy and Clinical Immunology. 1988;82(5):718-26.
13. Niggemann B, Wahn U, Sampson H. Proposals for standardization of oral food challenge tests in infants and children. Pediatric Allergy and Immunology. 1994;5(1):11-3.
14. Chiang WC, Pons L, Kidon MI, Liew WK, Goh A, Wesley Burks A. Serological and clinical characteristics of children with peanut sensitization in an Asian community. Pediatric Allergy and Immunology. 2010;21(2p2):e429-e38.
15. Priftis KN, Mermiri D, Papadopoulou A, Papadopoulos M, Fretzayas A, Lagona E. Asthma symptoms and bronchial reactivity in school children sensitized to food allergens in infancy. The Journal of asthma: official journal of the Association for the Care of Asthma. 2008;45(7):590-5.
16. Niggemann B, Beyer K. Diagnostic pitfalls in food allergy in children. Allergy. 2005;60(1):104-7.
17. Bernstein I, Li J, Bernstein D, Hamilton R, Spector S, Tan R, et al. American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology. Allergy diagnostic testing: an updated practice parameter. Ann Allergy Asthma Immunol. 2008;100(3 suppl 3):S1-S148.
18. Chafen JJS, Newberry SJ, Riedl MA, Bravata DM, Maglione M, Suttorp MJ, et al. Diagnosing and managing common food allergies: a systematic review. JAMA. 2010;303(18):1848-56.

19. Sporik R, Hill D, Hosking C. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. Clinical & Experimental Allergy. 2000;30(11):1541-6.
20. Boyano Martinez T, García-Ara C, DÍaz-Pena J, Muñoz FM, Garcia Sanchez G, Esteban MM. Validity of specific IgE antibodies in children with egg allergy. Clinical & Experimental Allergy. 2001; 31(9):1464-9.
21. Lieberman P, Nicklas RA, Oppenheimer J, Kemp SF, Lang DM, Bernstein DI, et al. The diagnosis and management of anaphylaxis practice parameter: 2010 update. Journal of Allergy and Clinical immunology. 2010;126(3):477-80. e42.
22. Moffitt JE, Golden DB, Reisman RE, Lee R, Nicklas R, Freeman T, et al. Stinging insect hypersensitivity: a practice parameter update. Journal of Allergy and Clinical Immunology. 2004; 114 (4):869-86.
23. Cox L, Williams B, Sicherer S, Oppenheimer J, Sher L, Hamilton R, et al. Pearls and pitfalls of allergy diagnostic testing: report from the American college of allergy, asthma and immunology/American academy of allergy, asthma and immunology specific IgE test task force. Annals of Allergy, Asthma & Immunology. 2008; 101(6):580-92.
24. Verstege A, Mehl A, Rolinck-Werninghaus C, Staden U, Nocon M, Beyer K, et al. The predictive value of the skin prick test weal size for the outcome of oral food challenges. Clinical & Experimental Allergy. 2005; 35(9):1220-6.
25. Imai T, Yanagida N, Ogata M, Komata T, Tomikawa M, Ebisawa M. The Skin Prick Test is Not Useful in the Diagnosis of the Immediate Type Food Allergy Tolerance Acquisition. Allergology International. 2014; 63:205-10.
26. Cudowska B, Kaczmarski M. Atopy patch test in the diagnosis of food allergy in children with atopic eczema dermatitis syndrome. Roczniki Akademii Medycznej w Bialymstoku. 2005; 50:261-7.
27. Jarvinen KM, Turpeinen M, Suomalainen H. Concurrent cereal allergy in children with cow's milk allergy manifested with atopic dermatitis. Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology. 2003; 33(8):1060-6.