

Is Patch Testing with Food Additives Useful in Children with Atopic Eczema?

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Abstract

Background: Atopy patch testing is a useful way to determine delayed-type hypersensitivity reactions to foods and aeroallergens. Although food additives have been accused of worsening atopic eczema symptoms, according to recent studies the role of food additives in atopic eczema remains unclear. The purpose of our study was to investigate food additive hypersensitivity in a group of children with atopic eczema by using standardized atopy patch testing and to determine the role of food additive hypersensitivity in atopic eczema.

Methods: Thirty-four children with atopic eczema and 33 healthy children were enrolled in the study. Children who consumed foods containing additives and did not use either antihistamines or local or systemic corticosteroids for at least 7 days prior to admission were enrolled in the study. All children were subjected to atopy patch testing and after 48 and 72 hours their skin reactions were evaluated by using the guidelines.

Results: Positive atopy patch test results were significantly higher in the atopic eczema group. Forty-one percent of the atopic eczema group ($n = 14$) and 15.2% ($n = 5$) of the control group had positive atopy patch test results with food additives ($p = 0.036$) (estimated relative risk 1.68, case odds 0.7, control odds 0.17). Carmine hypersensitivity and the consumption of foods containing carmine, such as gumdrops, salami, and sausage, were significantly higher in the children with atopic eczema.

Conclusion: This is the first study investigating hypersensitivity to food additives in children with atopic eczema. Our results indicate that carmine may play a role in atopic eczema.

Atopic eczema (AE) is the most frequent chronic skin disease in children and results from the relationship between genetic, immunologic, and environmental

factors such as food and airborne allergens. In the chronic inflammatory process of AE, type I and type IV hypersensitivity reactions to food and inhalant

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allergens may be responsible (1). Food additives, which are extensively used to prolong the shelf life of foods, are generally believed to worsen AE symptoms, but this has not been proven (2). Atopy patch testing is a useful research tool to determine delayed-type hypersensitivity reactions to foods and aeroallergens in AE. There is no study in the literature investigating atopy patch testing for food additives in children with AE. Our aim was to investigate the role of food additives in patients with AE by performing an atopy patch test (APT).

METHODS

Thirty-four of 166 patients with AE admitted to the Pediatric Allergy and Asthma Unit of our institution over 6 months met the inclusion criteria and were sequentially enrolled in the study, along with 33 healthy children. Inclusion criteria for patients with AE were regular consumption of foods containing additives (e.g., fruit juice, fruited yogurt, pudding, jam, syrup, meat products [salami, sausage], gumdrops, candy, biscuit, baked goods, chocolate, chips, ice cream, ketchup, chewing gum), not having used antihistamines or local or systemic corticosteroids for the previous 7 days, and not taking cyclosporine A or undergoing phototherapy on the APT area for the previous 4 weeks. Inclusion criteria for the control group were also the regular consumption of food containing additives; not having any dermatologic disease, AE, allergic rhinitis, or bronchial asthma; and not having used antihistamines or local or systemic corticosteroids for the previous 7 days. Both groups responded to a questionnaire evaluating the frequency of consumption of fruit juice, fruited yogurt, pudding, jam, syrup, meat products (salami, sausages), gumdrops, candy, biscuit, baked goods, chocolate, chips, ice cream, ketchup, and chewing gum. AE was defined according to the criteria of Hanifin and Rajka (3). The severity of AE was evaluated using the Severity Scoring of Atopic Dermatitis (SCORAD) index (1).

All children were evaluated carefully for a history of feeding, including duration of breastfeeding, weaning, and frequency and amount of foods containing additives. Eosinophils and serum total immunoglobulin E (IgE) were measured and the skin prick test (SPT) was performed on the forearm using a panel of standardized allergens (Stallergenes SA, Antony, France), including cow's milk, egg yolk, hazelnut, grass mixture, house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinea*), *Aspergillus* mixture (*fumigatus*, *niger*, *nidulans*),

TABLE 1. Concentrations of Food Additives and Vehicles Used for Allergens

Allergen	Concentration, %	Vehicle
Aspartame	0.1	Coca
Benzoic acid	1	Ethanol or glycerine
Carmine	0.5	Coca or glycerine
Cochineal red (ponceau 4R)	1	Coca or glycerine
Erythrocin	0.25	Ethanol or glycerine
Patent blue	0.25	Coca or glycerine
Pectin	1	Coca
Quinoline yellow	0.1	Ethanol or glycerine
Sorbic acid	2	Ethanol or glycerine
Monosodium glutamate	1	Coca or glycerine
Sodium disulfite	1	Coca or glycerine
Sodium nitrite	2	Water or glycerine
Sodium alginate	1	Coca
Tartrazine	1	Coca or glycerine

cat fur, and cockroach (*Blatella germanica*). The size of any wheal and flare reactions was compared with the size of negative (antigen diluting solution) and positive (histamine hydrochloride 10 mg/mL) controls. An induration ≥ 3 mm wider than the negative control was considered a positive SPT result.

The APT panel was created using the most common foods with additives that patients consumed. Atopy patch testing was performed on the uninvolved skin of the back using 8-mm patch test chambers (AllergEAZE, Smart Practice, Phoenix, AZ) with food additive series (Brial Allergen, Greven, Germany), including aspartame, benzoic acid, cochineal red (ponceau 4R), carmine, erythrocin, patent blue, pectin, quinoline yellow, sorbic acid, monosodium glutamate, sodium disulfite, sodium nitrite, sodium alginate, and tartrazine. The concentrations of food additives and vehicles used for allergens are given in Table 1. Occlusion time was 48 hours, and the patch tests were read at 48 and 72 hours. A pediatric allergy specialist (SO), who was blinded to the study groups evaluated skin reactions according to the guidelines described by the European Task Force on Atopic Dermatitis (ETFAD). These skin reactions were evaluated as negative (–); only erythema, questionable (?); erythema, infiltration (+); erythema, few papules (++); erythema, many or spreading papules (+++); erythema, vesicles (++++). (4).

The Dr. Sami Ulus Research and Training Hospital of Women's and Children's Health and Diseases ethics committee approved the study (reference B1041SM4060017-Per.2123) and all parents signed informed consent before patients were enrolled in the study.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software (version 17.0; SPSS, Chicago, IL). Data are presented as means $\pm$ standard deviations or as medians and ranges for nonnormally distributed variables. Student *t* tests, Pearson chi-square analysis, and Mann–Whitney *U*-tests were used to compare differences between two specific groups, and Spearman rank correlation coefficients were assessed as measures of correlation between variables of interest. For all analyses, $p < 0.05$ was considered statistically significant.

RESULTS

Thirty-four individuals with AE (17 girls; median age 44.5 mos, range 8–165 mos, interquartile range [IQR] 39 mos) and 33 healthy controls (median age 66 mos, range 24–132 mos, IQR 48 mos) were included. Demographic characteristics of the groups were not statistically significantly different. The duration of breastfeeding was significantly shorter in the control group than in the AE group ($p = 0.001$) (Table 2). The prevalence of acute contact urticaria ($p = 0.03$) and history of familial atopy ($p = 0.01$) was higher in the AE group.

Seventeen (50%) patients in the AE group had positive SPT results, 41.2% of which were positive to food allergens, 35.3% to aeroallergens, and 23.5% to both.

According to the SCORAD index, 70.6% of AE patients had mild disease and 29.4% had moderate to severe disease. The age, sex, eosinophil count, and serum total IgE levels the group with mild AE were not statistically significantly different from those of

the group with moderate to severe AE, and no statistically significant difference was found in the prevalence of positive SPTs and APTs between the groups.

When all 77 subjects included in the study were evaluated, it was established that 95.5% of the children consumed an average of four kinds of snacks containing food additives daily. Although the consumption of cookies, chocolate, chips, ice cream, cake, ketchup, fruit juice, fruited yogurt, pudding, jam, chewing gum, and candy were comparable between the AE and control groups, consumption of gumdrops, sausages, and salami was significantly higher in the AE group ($p = 0.005$).

Positive APT results were significantly higher in the AE group; 41.2% of the AE group ($n = 14$) and 15.2% ($n = 5$) of the control group had positive APT results with food additives ($p = 0.04$) (estimated relative risk 1.68, case odds 0.7, control odds 0.17). APT results for food additives are shown in Table 3. In the AE group, 14 of 34 individuals had 39 positive APT results to 15 different food additives; 6 of these positive reactions were 3+, 22 were 2+, and the remaining 11 were 1+. In the control group, 5 of 33 individuals had positive APT results to six different food additives; all of the positive reactions were 1+. Positive APT results for carmine were significantly higher in the AE than the control group, whereas the differences for other food additives were not statistically significant (17.6% and 0.0%, respectively; $p = 0.02$). In the AE group, six patients had positive APT results to carmine ($n = 1, 3+$; $n = 2, 2+$; $n = 3, 1+$). The characteristics of the carmine-positive children with AE are shown in Table 4.

TABLE 2. Demographic Characteristics of the Atopic Eczema and Control Groups

Characteristic	Atopic eczema group, $n = 34$	Control group, $n = 33$	p-Value*
Age, months, mean $\pm$ SD	55.3 $\pm$ 36.6	65.4 $\pm$ 28.4	0.07*
Sex			>0.99†
Male	17	17	
Female	17	16	
Duration of breastfeeding, months, mean $\pm$ SD	16.8 $\pm$ 9	9 $\pm$ 6.6	0.001*
Age when weaned, months, mean $\pm$ SD	6.7 $\pm$ 4.2	7.6 $\pm$ 3.9	0.06*

SD = standard deviation.

*Student *t* test.

†Chi-square test.

TABLE 3. Positive Atopy Patch Test Results for Food Additives

Additive	Atopic eczema group, n (%)	Control group, n (%)	p-Value*
Benzoic acid	3 (8.8)	0 (0)	0.24
Cochineal red (Pontau4R)	8 (23.5)	4 (12.1)	0.34
Carmine	6 (17.6)	0 (0)	0.02
Erythrocine	3 (8.8)	0 (0)	0.24
Patent blue	1 (2.9)	0 (0)	>0.99
Pectin	3 (8.8)	1 (3)	0.61
Quinoline yellow	1 (2.9)	0 (0)	>0.99
Sorbic acid	2 (5.9)	0 (0)	0.49
Sodium glutamate	1 (2.9)	0 (0)	>0.99
Sodium disulfite	2 (5.9)	0 (0)	0.49
Sodium nitrite	4 (11.8)	1 (3)	0.36
Sodium alginate	1 (2.9)	0 (0)	>0.99
Tartrazine	1 (2.9)	0 (0)	>0.99
Aspartame	2 (5.9)	0 (0)	0.49

*Chi-square test.

TABLE 4. *Characteristics of Carmine-Positive Children with Atopic Eczema (n = 6)*

Characteristic	Value
Age, months, mean $\pm$ SD	94 $\pm$ 42.8
Male, n (%)	3 (50)
Duration of breastfeeding, months, mean $\pm$ SD	16.2 $\pm$ 4.2
Age when weaned, months, mean $\pm$ SD	7.5 $\pm$ 5.6
Eosinophil count ($>4\%$), n (%)	1 (16.7)
Serum total immunoglobulin E, IU/mL	597.5 $\pm$ 902.4
Sensitivity in allergy skin prick test, n (%)	
Aeroallergens	4 (66.6)
Food	1 (16.7)
Both	5 (83.3)

SD = standard deviation.

No significant difference in age, duration of breastfeeding, weaning age, SCORAD index, presence of eosinophilia, serum total IgE levels, and SPT positivity was found between the APT-positive and -negative patients with AE, although weaning age was significantly younger in the subjects with positive APT results for the entire study group ($p = 0.01$).

DISCUSSION

Food additives are used for coloring and flavoring purposes and their antimicrobial properties. Although they are blamed for worsening AE symptoms, the role of food additives in AE remains unclear (5,6). In this study we examined the frequency of APT positivity to a number of food additives in children with AE and postulated that this might have a causative or aggravating role in some patients with AE. APT positivity was significantly more common in patients with AE than in healthy controls; 41.2% of the AE group and 15.2% of the control group had positive APT reactions to food additives ($p = 0.04$). In the AE group, 14 of 34 individuals had 39 positive APT reactions to 15 different food additives ($n = 6$, 3+; $n = 22$, 2+; $n = 11$, 1+). In the control group, 5 of 33 individuals had positive APT reactions to six different food additives (all 1+). The use of APTs in clinical practice is limited because of subjective interpretation, intraobserver variation, and differences in the diagnostic accuracy of these tests between studies (7); the clinical relevance of different grades of positive APT reactions is unclear. Moreover, data describing the effect of the severity of skin signs on APTs are scarce (8). Previously, Yang et al (8) reported that erythema and infiltration were not sufficiently indicative of a positive APT result. In the present study, the high ratio (15.2%) of positive reactions in the control group could be due to irritant patch test reactions

since all of the positive reactions were 1+ erythema and infiltration.

Because specific IgE and skin prick tests are correlated only with early reactions, atopy patch testing has been recognized as a useful tool in the diagnosis of delayed-type hypersensitivity reaction in AE. Because of the similarity between the pathophysiological mechanism of the delayed type hypersensitivity reaction and AE lesions, the APT has greater specificity than the skin prick and specific IgE tests (9).

Several food and drug additives have been reported to cause adverse reactions ranging from lethargy to severe asthma and anaphylaxis. Many of them, including synthetic colorants, sulfites, monosodium glutamate (MSG), aspartame, and benzoates, have been extensively studied. In a study with a survey and challenge trial of 1,483 Dutch adults, only 3 individuals had food additive sensitivities, amounting to 0.2% of the population (10). In another large study with a survey of 4,274 Danish school children, open and double-blind challenges were performed and intolerance to food additives was confirmed in 2% of the children with atopic history but only 0.13% of the entire surveyed population (11). In another study, the prevalence of adverse reactions to food additives (a mixture of various colors, preservatives, antioxidants, and flavor enhancers) was calculated at 0.18% (12). In a review of the Cochrane Database, the role of tartrazine in asthma was evaluated, and it was reported that tartrazine does not worsen asthma, nor does avoiding tartrazine improve asthma (13).

In our study, the overall positive reaction to food additives was significantly higher in the AE group than in controls. Because there was no statistically significant difference for other food additives, this significant difference was due to greater carmine sensitivity than in the AE group. In addition, although several food additives provoked reactions in individuals with AE, none (benzoic acid, erythrocinn, pectin, aspartame) resulted in positive reactions in the control group. These findings suggested that atopy patch testing in individuals with AE is more specific than in those without. In addition, food additives other than carmine did not seem to be associated with AE, although these findings could also be related to the small number of study participants.

Carmine, a natural food colorant, and cochineal extract are derived from dried female insects of the species *Dactylopius coccus* Costa, which lives as a parasite on the prickly pear cactus. An aqueous alcoholic extract of the dried insects is made and concentrated by removal of the alcohol to obtain the color additive cochineal extract (6). The intense red

color of carmine (carminic acid) makes it a popular coloring agent for many commercially prepared foods (fruit preparations, jams, syrups, meat products [salami, sausages], candies, and baked goods) (14). Carmine-associated occupational asthma has been found (15), and numerous cases of anaphylaxis have been reported after the ingestion of carmine-containing foods or beverages. Wuthrich et al (16) reported five cases of anaphylaxis associated with carmine and established the likelihood of an IgE-mediated mechanism through demonstrations of positive skin prick tests and radioallergosorbent tests. Several IgE-binding proteins have been identified in carmine using sera from carmine-sensitive patients (17). Ohgiya et al (18) identified a protein, CC38K, that the sera of patients known to have type I hypersensitivity reactions to carmine recognized, indicating that this is a major allergen in carmine reactions. The CC38K sequence showed homology to phospholipases, which are known to be allergens in other insects.

Although there are numerous case reports of type I hypersensitivity reactions to carmine in the literature, only four cases of allergic contact dermatitis have been reported (19,20). There is no study or case report of the relationship between carmine and AE. The allergen in carmine that causes delayed-type hypersensitivity has not been identified. An association between carmine hypersensitivity and house dust mite allergy has been mentioned (21), but a large study evaluating skin prick test results showed that carmine sensitization without sensitization to mites was present in 25% of the patients. The authors emphasized that allergic reactions to carmine were not related to a positive reaction to mites (21). Similarly, in our study, only one of the carmine-sensitive patients (16.7%) had house dust mite sensitivity on the skin prick test, so we conclude that allergic reactions to carmine may occur without concurrent sensitization to dust mites.

The high ratio of positive APT reactions to carmine and consumption of gumdrops, salami, and sausages in patients with AE indicates that carmine may play a role in AE. Although the double-blind placebo-controlled challenge test is the criterion standard method for diagnosis of food allergy, the cost-effectiveness, safety, and practicability of the APT makes it a useful diagnostic tool for detecting delayed hypersensitivity to food additives in AE, especially with regard to late-phase clinical reactions. Standardization and optimization of APTs could help to develop new immunobiologic diagnostic methods and avoid unnecessary dietary restrictions.

Our study has some limitations because of the small number of patients and because it was a pilot

study in which a double-blind placebo-controlled challenge test with food additives could not be used to prove our hypothesis, although the relationship between positive APT reactions to carmine and AE pathogenesis and its clinical importance have not been examined previously. An oral challenge test might reveal patients with relevant clinical symptoms and thus provide more accurate information on the association between carmine exposure and symptoms. Our study showed that it might be useful to perform atopy patch testing with carmine in individuals with AE who report having had symptoms after ingestion of foods containing carmine.

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