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Sensitization to inhalant and food allergens in Brazilian atopic children by *in vitro* total and specific IgE assay. Allergy Project – PROAL

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Abstract

Objective: To determine the frequency of sensitization to inhalant and food allergens in children seen at Brazilian allergy services.

Patients and methods: Total and specific IgE serum levels to inhalant and food allergens (RAST, UniCAP® – Pharmacia) were measured in 457 children accompanied in pediatric allergy services and in 62 control children age matched. RAST equal or higher than class 1 was considered as positive (R+).

Results: Frequency of R+ was significantly higher among atopics (361/457, 79%) when compared to controls (16/62, 25.8%). There were no differences according to gender. The frequency of R+ to all allergens evaluated were higher among atopics when compared to controls. Significantly higher total IgE serum levels were observed among the atopics with R+ in comparison to those with R-. The frequency of R+ to main inhalant allergens were: *D. pteronyssinus* = 66.7% x 14.5% ($p < 0.05$), *D. farinae* = 64.5% x 17.8% ($p < 0.05$), *B. tropicalis* = 55.2% x 19.4% ($p < 0.05$), cockroach = 32.8% x 9.7% ($p < 0.05$), and cat = 12% x 8.1%. In relation to food allergens we observed: fish = 29.5% x 11.3% ($p < 0.05$), egg = 24.4% x 4.8% ($p < 0.05$), cow's milk = 23.1% x 3.2% ($p < 0.05$), wheat = 20% x 8.1% ($p < 0.05$), peanuts = 14% x 4.8% ($p < 0.05$), soy = 11.8% x 4.8% ($p < 0.05$), and corn = 10.6% x 4.8% ($p < 0.05$). With respect of age, food allergen sensitization predominates in young children whereas the inverse occurs with inhalant allergens.

Conclusions: There was a predominant frequency of sensitization to inhalant allergens, mainly house dust mites in the evaluated patients. Food allergens were also responsible for a significant proportion of sensitization, mainly in infants.

J Pediatr (Rio J). 2004;80(3):203-10: Allergy, allergen, IgE, mite, food.

Introduction

Recent studies have documented an increase in the prevalence of atopic diseases in several different parts of the world. In Brazil the prevalence of asthma, allergic rhinitis and atopic eczema were recorded for the first time as part of an international study and were found to be, on average, 21%, 39% and 8% respectively.[1-3] These diseases often have onset in early childhood and etiologic diagnosis is not always easy to perform. The presence of allergen-specific IgE antibodies in serum characterizes allergic

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etiology. These antibodies can be detected by *in vivo* testing or by biological *in vitro* tests.

Immediate hypersensitivity skin tests are the weapon that has been most often used to identify specific IgE *in vivo* in serum. The choice of allergens to be tested for should be guided by anamnesis and those that are most relevant to a given region should make up a standard battery. Previous studies, performed in varying locations around Brazil, point to the domestic mites *Dermatophagoides pteronyssinus, Dermatophagoides farinae* and *Blomia tropicalis* as the principal etiologic agents of respiratory allergies.[4-7] In the south of the country, pollen has also been identified as significant.[8,9]

While they are easy to perform, immediate hypersensitivity skin tests are not risk-free and their results can suffer interference from a number of different factors. In infants, cutaneous allergic inflammatory responses are reduced,[10] making a larger proportion of false-negative results possible.

Tests performed *in vitro* attempt to identify specific IgE in patients' serum and as such require a substrate in which this immunoglobulin can be fixed for quantifying. Questions could also be raised as to whether, produced abroad, they are relevant in our country. Nevertheless, the allergen epitopes employed are universal and therefore used in all countries. This study assessed the presence of serum IgE specific to inhalant and food allergens in a population of Brazilian children treated at allergy centers in different parts of the country.

Patients and methods

Four hundred and fifty-seven children participated in the study (177 girls [38.7%] and 280 boys [61.3%]) aged between 12 and 144 months, treated at allergy centers in all five of Brazil's regions. The children were classed into five age groups as follows: 1 to 2 years, 2 to 3 years, 3 to 4 years, 4 to 5 years and 5 to 12 years. Children were classed as atopic if they presented at least one positive immediate hypersensitivity skin test (mean wheal diameter greater than or equal to 3 mm)[10,11] to at least one inhalant or food allergen, tested randomly at the patient's allergy center of origin. The control group was made up of 62 other children recruited from the investigation centers in the Northeast, Southeast and South regions with no history of allergic disease and negative immediate hypersensitivity skin tests results for the same allergens used at the centers they came from and who had required blood testing for other reasons such as preoperative assessments for elective surgery.[10,11]

Depending on the reason for referral, patients were classed as: wheezing infants (n = 20), food allergy (n = 16), atopic dermatitis (n = 56), and respiratory allergy (n = 348). Babies were defined as wheezing if they were less than two years old and presented recurrent episodes of wheezing and other possible causes had been ruled out (aspiration syndromes, fibrocystic disease, airway malformations among others). Patients with proven asthma and/or rhinitis were defined as having respiratory allergies.

Peripheral blood samples were taken from both allergic and control patients so that IgE serum levels specific to inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia tropicalis*, cat, dog, molds, cow's epithelium, horse, grasses and cockroach) and food allergens (cow's milk, egg, peanut, wheat and seafood panel) and total IgE serum levels (UNiCAP®-Pharmacia) could be assayed.[12,13] Specific IgE levels (RAST) greater than or equal to 0.35 UI/ml (class 1) were defined as positive[13,14] The study was approved by the relevant Ethics Committees and free and informed consent was obtained.

Non-parametric tests were employed to analyze variables and in all cases the cut off for null hypothesis rejection was set at 5%.

Results

Observing Table 1 we find the distribution of allergic patients. As will be noted, distribution was even in terms of age groups and region of origin. The same was true of sex (data not shown). Controls were limited to the Northeast, Southeast and South regions because there were less of them and also exhibited no differences in terms of age group: less than 2 years old = 19.4%; 2 to 3 years = 20.9%; between 3 and 4 years = 14.5%; between 4 and 5 years =21.0% and between 5 and 12 years = 24.2%.

The presence of specific IgE to inhalant and/or food allergens was variable, being significantly less common among younger allergic patients (Table 2). There were no differences observed between patients with negative RAST results and non-allergic controls (Table 2).

Total IgE serum levels vary greatly and because of this they are expressed as geometric means. Levels were significantly more elevated among atopic subjects when compared to controls and increased with age (Table 3 and Figure 1). When the presence or absence of IgE specific to any of the tested allergens was analyzed among the atopic patients, it was found that, for all age groups, those that were RAST positive (R+) also had significantly more elevated total IgE values than those who were RAST negative. The same was true when R+ subjects were compared with controls (Table 3 and Figure 1). Comparative analysis of the controls and the RAST negative atopic children did not reveal any significant differences with the exception of the 4-5 year age group (Table 3 e Figure 1).

Serum IgE specific to inhalant and/or food allergens tests were positive for 361/457 (79%) of the patients and 16/62 (25.8%) of the controls. Table 4 contains the frequency of each result according to allergen tested. It shows that inhalant allergens, in particular mites, were most prevalent. In the food category fish, egg, cow's milk and wheat stand out (Table 4). Table 5 shows the frequency of positive results for each of the allergens tested by age group and taking the total number of positive results for each particular allergen as a reference. Significantly lower frequencies will be observed among younger children. There were no differences in terms of IgE specific to cat, cow's epithelium, dog, horse or molds (Table 5). Of all the foods, sensitization

Table 1 - Distribution of patients by age group and region

Age group	Total		N/NE		CW		SE		S	
(years)	n	%	n	%	n	%	n	%	n	%
< 2	79	17.3	18	13.9	12	23.1	38	16.9	11	21.6
2-3	83	18.2	25	19.3	10	19.2	40	17.8	8	15.7
3-4	102	22.3	27	21.0	13	25.0	53	23.5	9	17.6
4-5	81	17.7	30	23.3	9	17.3	33	14.7	9	17.6
5-12	112	24.5	29	22.5	8	15.4	61	27.1	14	27.5
Total	457	100.0	129	100.0	52	100.0	225	100.0	51	100.0

N/NE: North and Northeast; CW:Central West; SE: Southeast; S: South.

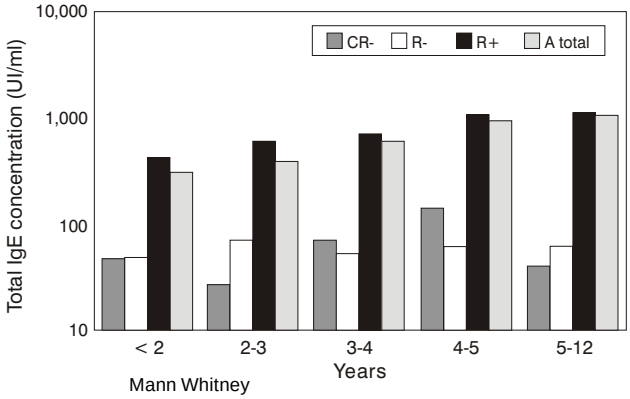
Table 2 - Patients with specific IgE and inhalant and/or food allergens in different age groups

Age group	Allergic			Controls		
	n	Positive specific IgE		n	Positive specific IgE	
(years)	total	n	%	total	n	%
< 2 (a)	79	50	63.29	12	2	16.7
2-3 (b)	83	59	71.08	13	3	23.1
3-4 (c)	102	74	72.5	9	3	33.3
4-5 (d)	81	68	83.9	13	4	30.7
5-12 (e)	112	110	98.2	15	4	26.7
Total	457	361	79.0	62	16	25.8

Chi-square test. Significant differences: allergic patients: a < c, d, e; b < d, e; c < e.

to the seafood panel was most frequent, being lower among the younger subjects. There was a high frequency of sensitization to cow's milk across all age groups (Table 5). Ninety-six patients were found to be exclusively sensitized to either food or inhalant allergens (Table 6). Comparing the two groups, we found that sensitization to foods predominated for the first few years and sensitization to inhalants in later age groups.

We found different levels of positivity to tested allergens according to the primary complaint leading to observation (Table 7). Children with respiratory allergies and atopic dermatitis had a greater frequency of positivity to inhalant allergens. Nevertheless, 38.8% of those with respiratory allergies were sensitized to fish. A lower proportion was observed among those with atopic dermatitis. Children with food allergies were more frequently sensitized to cow's milk, egg, wheat, fish and soya. Despite this, the group also recorded a significant level of sensitization to domestic dust mites and, in particular, cow's epithelium (Table 7). A lower prevalence of sensitization was observed among the wheezing infants with a predominance of inhalant allergens (mites and cow's epithelium).



Mann Whitney
Controls *versus* Allergic patients
CR- *versus* R-: no difference, except for 4-5 years: CR- > R-
CR- *versus* R+: in all CR- < R+
Allergic patients: R- *versus* R+: all ages - R+ > R-
Kruskal Wallis: < 02 x 2-3 x 3-4 x 4-5 x 5-12
CR-: 4-5 > all others
R+: 02, 2-3 < 3-4, 4-5, 5-12; 3-4 < 4-5, 5-12

Figure 1 - Mean total IgE serum levels (Log concentration UI/ml) of patients in the control group with specific IgE serum (RAST) negative (CR-) or in the allergic group with RAST negative (R-), positive (R+) and total (Atotal)

Table 3 - Total IgE serum levels (geometric mean) of patients according to the RAST results in each age group

Age (years)	Controls RAST negative		Allergic group RAST negative		RAST positive		Total	
	n	UI/ml	n	UI/ml	n	UI/ml	n	UI/ml
< 2 (a)	10	46.4	24	47.0	53	417.7	77	302.1
2-3 (b)	10	26.4	31	69.2	47	586.5	78	380.9
3-4 (c)	6	69.3	16	51.9	86	692.4	102	591.9
4-5 (d)	9	138.3	10	60.1	69	1,036.7	79	913.1
5-12 (e)	9	39.4	5	60.7	106	1,089.2	111	1,043.2

Mann-Whitey; controls x allergic patients: controls x negative RAST, no difference, except for d (controls > allergic patients); controls x positive RAST, in all controls < positive RAST; allergic patients: negative RAST x positive, all age groups positive > negative; Kruskal Wallis: a x b x c x d x e; controls: d > all the others; allergic patients: positive RAST: a, b < c, d, e; c < d, e.

Sensitization to more than one allergen was observed in 85% of the patients, particularly among the oldest. The most frequently encountered combinations were the three types of mite, cow's milk with cow's epithelium and mites, cockroaches and fish all three concomitantly. There were no differences in the pattern of sensitization distribution by patient region of origin (data not shown).

Discussion

Elevated IgE serum levels have been hailed as markers for atopic disease. However, a number of different clinical circumstances, in addition to allergic diseases, may progress with elevated total IgE serum levels, for example intestinal helminthiasis and an active smoking habit. Prospective studies in which total IgE was measured in cord blood

Table 4 - Distribution of patients according to positivity to the different allergens comparing the total (%) and the positive exams (%). Data of non-allergic controls

Allergen	Atopic (n = 457)		Controls (n = 62)	
	Positive RAST	% total	Positive RAST	% total
D. pteronyssinus	310	67.8*	9	14.5
D. farinae	304	66.5*	11	17.8
B. tropicalis	261	57.1*	12	19.4
Cockroach	157	34.4*	6	9.7
Cat	56	12.2	5	8.1
Grasses	49	10.7*	3	4.8
Cow's epithelium	52	11.4*	4	6.5
Dog	37	8.1*	2	3.2
Horse	21	4.6	0	0.0
Fungus	14	3.1	0	0.0
Fish	138	30.2*	7	11.3
Egg	112	24.5*	3	4.8
Cow's milk	93	20.3*	2	3.2
Wheat	92	20.1*	5	8.1
Peanut	67	14.7*	3	4.8
Soy	56	12.3*	3	4.8
Corn	50	10.9*	3	4.8

Chi-square; controls < allergic patients.
* $p < 0.05$.

Table 5 - Patients according to specific IgE (RAST) to different allergens. Data (%) shown regarding the total number of positive patients in each category

Allergen	< 2 years (a)		2-3 years (b)		3-4 years (c)		4-5 years (d)		5-12 years (e)		Total		Chi-square/ Fisher
	n	%	n	%	n	%	n	%	n	%	n	%	
D. pteronyssinus	23	7.6	44	14.6	67	22.3	59	19.6	108	35.9	301	66.7	a < b < c = d < e
D. farinae	17	5.8	45	15.5	66	22.7	56	19.2	107	36.7	291	64.5	a < b < c = d < e
B. tropicalis	11	4.4	34	13.7	52	20.9	52	20.9	100	40.2	249	55.2	a < b < c = d < e
Cockroach	6	4.1	11	7.4	28	18.9	38	25.7	65	43.9	148	32.8	a = b < c < d < e
Cat	4	7.4	11	20.4	8	14.8	9	16.7	22	40.8	54	12.0	a = b = c = d = e
Cow's epithelium	13	28.9	6	13.3	7	15.6	11	24.4	8	17.8	45	10.0	a = b = c = d = e
Grasses	1	2.2	1	2.2	8	17.8	13	28.9	22	48.9	45	9.9	a = b = c = d < e
Dog	3	8.5	6	17.1	4	11.5	9	25.7	13	37.2	35	7.8	a = b = c = d = e
Horse	1	5.3	2	10.5	4	21.1	5	26.3	7	36.8	19	4.2	a = b = c = d = e
Fungus	1	7.7	1	7.7	4	30.8	5	38.5	2	15.4	13	2.9	a = b = c = d = e
Fish	5	3.8	16	12.0	32	24.1	32	24.1	48	36.1	133	29.5	a < b < c = d = e
Cow's milk	27	26.0	18	17.3	17	16.3	21	20.4	21	20.2	104	23.1	a = b = c = d = e
Wheat	15	16.7	6	6.7	13	14.4	23	25.6	33	36.7	90	20.0	b < a = c = d = e
Peanut	8	12.7	4	6.3	10	15.9	19	30.2	22	35.0	63	14.0	a = b = c < d = e
Soy	7	13.2	4	7.5	8	15.1	14	26.4	20	37.8	53	11.8	a = b = c < d = e
Corn	8	16.7	3	6.3	8	16.7	15	31.3	14	29.2	48	10.6	b < d
Egg	29	26.4	21	19.1	17	15.5	19	17.3	24	21.8	110	10.0	a = b = c = d = e

Table 6 - Patients with specific IgE exclusively sensitized to food or inhalant allergen according to age group

Group (years)	Inhalant (I)		Food (F)		Qui-square I x F
	n	%	n	%	
< 2	7	9.9	14	56.0	I < F
2-3	13	18.3	6	24.0	I = F
3-4	20	28.2	3	12.0	I = F
4-5	11	15.5	1	4.0	I = F
5-12	20	28.1	1	4.0	I > F
Total	71	100.0	25	100.0	

allowed increased risk for the development of asthma and allergic diseases to be detected in newborn babies with levels above 0.9UI/ml.[15] In a previous study that attempted to establish normal total IgE serum values, Mancini et al. recorded total IgE values below 1.0 UI/ml, in cord blood. However, in older children levels were observed to increase with age and become greatly divergent which meant that the production of a national standard for IgE serum levels was not viable.[16]

Lopez et al. studied the relationship between total IgE serum levels and early wheezing in infants followed for the first year of life.[17] They found higher levels of total IgE in the cord blood of those that would later wheeze, but the difference was not significant. The newborn babies were monitored and there was a significant increase in IgE serum levels as they got older. At 12 months there was also specific sensitization to egg white (43%), cow's milk (60%) and, to a lesser degree, to *D.pteronyssinus* (30%) and *D. farinae* (33%).[17]

In the current study total IgE serum levels in the control group exhibited a tendency towards more elevated values in older children. Values in the atopic group were significantly higher for all age groups, with significant increases with age (Table 3 and Figure 1). One interesting fact was that when the atopic group was divided by the presence of at least one R+, we found that children with negative results presented values significantly lower than those with R+ and a little higher than the controls.

In addition to genetic potential, environmental exposure to allergens is fundamental to atopic sensitization and disease expression. Longitudinal studies of wheezing infants point to the presence of serum IgE specific to inhalant allergens as an indicator of high risk for the development of asthma when school age.[18] In children less than two years old hospitalized for wheezing, the presence of R+ for wheat, egg white and domestic dust mites was associated with an increased frequency of asthma later in life.[19] Despite this, specific IgE serum assay is not an available test at the majority of health care services, whether governmental or private.

Table 7 - Patients with positivity to tested allergens according to the primary complaint and the specific IgE serum

Allergen	Wheezing baby (n = 20)	Food allergy (n = 16)	Atopic dermatitis (n = 56)	Respiratory allergy (n = 348)
D. pteronyssinus	40.0	62.5	80.4	76.7
D. farinae	35.0	50.0	78.6	73.6
B. tropicalis	20.0	43.8	64.3	69.8
Cockroach	15.0	18.8	37.5	43.9
Cat	5.0	12.5	21.4	12.1
Grasses	0	6.3	10.7	12.9
Cow's epithelium	25.0	62.5	14.3	9.5
Dog	0	6.3	12.5	8.9
Horse	0	6.3	10.7	4.6
Fungus	5.0	6.3	10.7	2.0
Fish	15.0	18.8	32.1	38.8
Egg	15.0	50.0	30.4	21.8
Cow's milk	10.0	68.8	28.6	16.1
Wheat	10.0	31.3	30.4	22.1
Peanut	10.0	12.5	17.9	16.9
Soy	10.0	18.8	16.1	10.6
Corn	0	12.5	12.5	10.0

When assessing allergic patients it is important to bear the concept of the allergic march in mind. It is known that allergic manifestations progress from atopic eczema and food allergies in younger children to respiratory allergy later on, manifesting as asthma and rhinoconjunctivitis.[20] This fact was taken into account for the present study. Despite a predominance of children from the Southeast region of Brazil, the distribution according to age group was comparable for all regions

Taking the sample as a whole, we observed an accentuated predominance by inhalant allergens, in particular the mites *D. pteronyssinus* (67.8%), *D. farinae* (66.5%) and *Blomia tropicalis* (57.1%) and cockroach allergens (34.4%). This data is explained by the fact that 348/457 (76.1%) of the patients were being monitored because of respiratory allergy. Previous studies in our country have already isolated these mites as having the greatest sensitization prevalence.[4-7]

One further fact that attracted our attention was the elevated rate of sensitization to domestic mites observed among patients suffering from atopic dermatitis (AD). A longitudinal study on secondary prophylaxis for asthma found that 17% of the European children with AD being studied were sensitized to domestic dust mites and 10% to pollen.[21] Furthermore, the reduction in DA symptom scores after measures had been taken to reduce exposure to domestic dust mites, in particular from mattresses, together with the development of asthma by around 50% of DA patients[22] reinforces the evidence for the participation of mites as important etiologic agents of DA.

Among patients with clinical history of food allergies there was a predominance of sensitization to aeroallergens, albeit in smaller proportions. Cow's milk was the most often identified food allergen followed by egg white, wheat, fish and soya (Table 7). In general cow's milk proteins are the first heterologous dietary proteins with which infants come into contact, which explains early sensitization.[23] Within this population we observed a predominance of sensitization to cow's milk among children less than two years old and sensitization to fish occurred among older children, bearing in mind that the introduction of this element into the diet generally occurs after one year of life. We did not detect any differences in sensitization prevalence to the different allergens according to patient region of origin.

Around 15% of the patients assessed were mono-sensitized. Food allergen sensitization was more prevalence among the younger subjects. Aeroallergens predominated among older children (Table 6). Insidious exposure to small quantities of aeroallergens may be the explanation for the delay in aeroallergen sensitization.[20] A prospective study of children, from birth to six years old, recorded a fall in sensitization to food allergies from 10% during the first year to 3% at six. The opposite behavior was observed in relation to inhalant allergens which, from 1.5%, reached 8% at six years.[24] The current study was not prospective and as such it was not possible to study this type of behavior.

The presence of serum IgE specific to inhalant and/or food allergens was significantly greater among the atopic subjects and was related to age, oscillating between 63.6% for those less than 24 months old to 98.2% for those over 60 months old. While there were positive results in the control group, IgE levels were lower and reached a maximum of 37.5% of positive exams. Table 4 lists the frequency of positivity to the allergens tested. There was a predominance of inhalant allergens (*D. pteronyssinus*, *D. farinae*, *Blomia tropicalis* and cockroach) over food allergens (fish, egg, cow's milk and wheat). The same occurred with the control group although in less significant proportions (Tables 4 and 5).

The battery of allergens employed for the immediate hypersensitivity skin tests were not standardized in terms of their origin. This being the case it was not possible to study agreement between the skin tests and the specific IgE tests.

Within the population under study, only 15% exhibited sensitization to just a single allergen. Among the remaining patients we observed frequent concomitant sensitization combinations of all three mites or all three mites and cockroach, to the preceding four and the seafood panel. The existence of cross-reactivity between these allergens is the most plausible explanation. The all have tropomyosin in common, which is a contractile protein present in the mites, cockroaches and seafood (shrimps).[25] Another combination of positive results was cow's milk with cow's epithelium. Could the presence of bovine serum albumin the main cause of their cross-reactivity? This question should be researched.

In conclusion, this is the first national Brazilian study that has assessed the pattern of sensitization to inhalant and food allergens in atopic children. It has identified a similar pattern of sensitization in all regions of the country. For patients with respiratory symptoms a panel composed of domestic dust mites, possibly with cat or dog allergens added can aid in the identification of sensitized individuals. When patients have food allergies, a panel made up of cow's milk, wheat, fish, egg white and soy allergens can be of assistance.

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