

The Influence of Atopic Status and Potential Risk Factors for Sensitization on Histamine Skin Reactivity in Unselected Belgian Children

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Abstract: The histamine skin response is widely used as a standardized positive control in the performance of skin prick testing. As a part of the Aalst Allergy Study, we investigated whether histamine skin reactivity, using histamine 10 mg/mL, was influenced by age, gender, ethnic origin, environmental exposure, specific sensitization patterns, or other factors. The parents of 2021 nonselected children aged 3.4–14.8 years completed a baseline questionnaire and gave written informed consent for skin prick testing with seven common aeroallergens on their child. Sensitized children had significantly larger histamine wheal sizes than nonsensitized children (mean 4.3 vs 4.0 mm, $p < 0.05$). A significant difference was found in histamine wheal size between nonsensitized versus multiply sensitized children ($p < 0.001$), but not between mono-sensitized versus multiply sensitized children ($p = 0.105$). The only other factors associated with significantly larger histamine wheal sizes were a history of eczema and a history suggestive for asthma on the Brief Pediatric Asthma Screen Plus. The histamine wheal was absent in 8.9% of children. In conclusion, sensitization and some other clinical indicators of atopy were associated with a larger histamine skin wheal. Other conditions including gender and environmental exposure did not have an influence on histamine skin reactivity, which strengthens the use of histamine as a reference in skin prick testing in this age group.

Allergen exposure and sensitization is an important risk factor for allergic disease. Allergy tests are used to define the subset of subjects who, because of sensitivity to allergens, have symptoms caused by one or more allergens, and to identify the involved allergens.

Skin prick testing represents an inexpensive, quick, and safe diagnostic procedure to detect immediate (antibody-mediated) sensitivity. Efforts were made to standardize this test (1–3). The results depend nonetheless on many factors (4). Some are linked to the conditions chosen for the test (1,5,6), others relate to

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individual characteristics of the subject such as age (7), and gender (8), although different studies sometimes have given conflicting results (9,10); the results of skin prick tests (SPT) also depend on total serum immunoglobulin E (IgE) and histamine skin reactivity (4,11–14).

As variations in histamine skin reactivity may influence the prevalence of positive specific allergen SPT, we investigated in a nonbiased sample of 2021 Belgian school children between 3.4 and 14.8 years of age, as part of the Aalst Allergy Study, whether histamine skin reactivity was influenced by age, gender, ethnic origin, environmental exposure, specific sensitization patterns, or other factors.

PATIENTS AND METHODS

Study Population

The study was performed in a nonselected sample of children (3.4–14.8 years, mean age 9.3 years) attending randomly selected nursery, primary and secondary schools in the city of Aalst and surroundings. Aalst is a Flemish-speaking Belgian municipality, 19 miles northwest of Brussels. Aalst has a total population of 76,852, living in a total area of 78.12 km², which gives a population density of 983.83 inhabitants per square kilometer. All 2674 children of the second grade of nursery school, the first, third, and fifth grade of primary school and the first grade of secondary school were contacted, received a questionnaire (see below) and were invited to participate in this study.

Questionnaire

Parental questionnaires were distributed through the medical school doctors of the involved schools in Aalst and surroundings. The questions on respiratory and allergic disorders, i.e., rhinoconjunctivitis, asthma, and eczema, were adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (15) and covered also the items of the Brief Pediatric Asthma Screen Plus (BPAS+) (16) (the scoring for the asthma component of the BPAS+ is a positive response to any one of four items: wheeze, persistent cough, night cough, and response to change in air temperature). The questionnaire further included questions on demographic characteristics (age, gender, nationality, maternal, and paternal profession) and on potential risk factors for sensitization, such as prematurity, feeding practices in the neonatal period, family history of allergy, number of siblings, frequency of childhood infections, vaccinations, place of residence (urban or rural), housing characteris-

tics (age of building, heating, dampness, bedroom conditions), animal contacts, and tobacco smoke exposure (pre- and postnatal). Ethnicity was defined according to the ethnicity of both parents, i.e., the country of origin of the parents.

Skin Prick Tests

Skin prick testing with the most common aeroallergens was performed by the same two trained pediatricians to ensure uniformity in testing technique and interpretation. The allergen panel consisted of *Dermatophagoides pteronyssinus*, *Alternaria tenuis*, cat, dog, mixed grass pollens, tree pollens, and *Blattella germanica* (cockroach). The concentration of allergen extracts was 100 IR/mL (Stallergenes, Belgium). Histamine solution in distilled water, 10 mg/mL, was used as a positive control and saline as negative control. Each child was tested on the volar surface of the forearm using 1 mm prick lancets (Stallergenes, Belgium) (17). The skin reaction was recorded after 15 min by evaluating the skin response rate to inoculation of each allergen extract, in comparison with the wheal given by the positive and the negative control. The size of each wheal was documented as the mean of the longest and the midpoint orthogonal diameter. A positive SPT was defined as a mean wheal size, after subtraction of the negative control, of at least 3 mm, and a ratio of wheal size to allergen over wheal size to positive control of at least 0.4. Histamine skin reactivity was defined by the diameter of the skin wheal elicited by histamine 10 mg/mL. Any H1 antihistaminic drugs were stopped at least 7 days before skin prick testing.

Statistical Analysis

Data are presented as mean $\pm$ standard error (SE) for continuous data and as percentages for categorical variables. Normal distribution of the data was tested by means of the Kolmogorov–Smirnov test (18) both for the nontransformed and log-transformed data. The differences in histamine wheal diameter were tested by means of an unpaired *t*-test or nonparametric Mann–Whitney *U*-test. In case of more than two groups, an analysis of variance (ANOVA) with post-hoc Tamhane's test or the nonparametric Kruskal–Wallis test was used to study the difference in histamine wheal diameter size. A linear regression analysis was used to study the association between age and the histamine wheal diameter size. For all analyses, a *p*-value of <0.05 (two-tailed) was considered significant. All analyses were performed using SPSS/PC 13.0 software (SPSS Inc., Chicago, IL).

TABLE 1. Baseline Characteristics of the Study Population

	Study population n (2021)
Age [years]	3.4–14.8 [mean 9.3 ± 0.1]
3.4–< 6	358 (17.7)
6–< 8	278 (13.8)
8–< 10	302 (14.9)
10–< 12	666 (33.0)
12–14.8	417 (20.6)
Sensitization	
Non-sensitized	1538 (76.1)
Mono-sensitized	301 (14.9)
Multiple sensitized	182 (9.0)
Gender	
Male	990 (49)
Female	1031 (51)
Ethnic origin	
Autochthonous	1842 (91.1)
Allochthonous	179 (8.9)
Current animals	
No animals	627 (31.0)
Cat	395 (19.5)
Dog	494 (24.4)
Cat and dog	500 (24.7)
Missing	5 (0.2)
Animals at birth	
None	1103 (54.6)
Cat	217 (10.7)
Dog	486 (24.0)
Cat and dog	179 (8.9)
Missing	36 (1.8)
Passive smoke exposure	
No smoking	1160 (57.4)
Smoking mother	211 (10.4)
Smoking father	337 (16.7)
Smoking mother and father	287 (14.2)
Missing	26 (1.3)
Maternal smoking during pregnancy	
No	1657 (82.0)
Yes	307 (15.2)
Missing	57 (2.8)
Prematurity	
No	1867 (92.4)
Yes	122 (6.0)
Missing	32 (1.6)
Breastfeeding	
No	1128 (55.08)
0–3 months	506 (25)
3–6 months	222 (11)
> 6 months	108 (5.3)
Missing	57 (2.8)
Eczema	
No eczema	1529 (75.7)
Eczema in infancy only	227 (11.2)
Current eczema only	92 (4.6)
Eczema both in infancy and present	140 (6.9)
Missing	33 (1.6)
Respiratory symptoms	
BPAS+: Negative	1159 (57.3)
Positive	862 (42.7)
Rhinoconjunctivitis	
No rhinoconjunctivitis	1492 (73.8)
Conjunctivitis	52 (2.6)
Rhinitis	262 (13)
Rhinoconjunctivitis	153 (7.6)
Missing	62 (3.1)
Family history of allergy	
No allergy	433 (21.4)

TABLE 1. Continued

	Study population n (2021)
Parental allergy only	312 (15.4)
Sibling allergy only	171 (8.5)
Parental and sibling allergy	315 (15.6)
Missing	790 (39.1)
Day-care center attendance	
No	1190 (58.9)
Yes	773 (38.2)
Missing	58 (2.9)

Values are expressed as n (%).

RESULTS

The parents of 2021 children (75 % of all contacted children) returned a completed questionnaire and also gave permission, by written consent, for skin prick testing. Table 1 shows the baseline characteristics of the study population. Skin prick testing was performed from January 2004 to June 2005 and was well tolerated. Only six children had a minor transient vagal reaction; no other complications were observed.

Figure 1 shows the distribution of the histamine wheal size for the whole study group. The mean size is 4.1 mm (median: 4.5 and SE: 0.0). We found a very similar distribution for boys (mean 4.0, median 4.0 and SE: 0.1) and girls (mean 4.1, median 4.5 and SE: 0.1).

The influence of several factors on histamine skin reactivity was checked (Table 2). Sensitized children had significantly larger histamine wheal sizes than nonsensitized children [mean 4.3 mm (± 0.1) vs 4.0 mm (± 0.0)]. This was found for the whole group ($p < 0.001$), as well as for the group of boys ($p = 0.017$) and girls ($p = 0.003$) separately.

A statistically significant difference in histamine wheal size was found between nonsensitized versus multiply sensitized children [mean 4.0 mm (± 0.0) vs 4.5 mm (± 0.1), $p < 0.001$], but not between mono-sensitized versus multiply sensitized children [mean 4.2 (± 0.1) vs 4.5 mm (± 0.1), $p = 0.105$].

Other factors associated with larger mean histamine wheal size, were ever eczema [mean 4.2 mm (± 0.1) vs 4.0 mm (± 0.0), $p = 0.002$], current eczema only versus never eczema [mean 4.4 mm (± 0.1) vs 4.0 mm (± 0.0), $p = 0.02$] and a history suggestive for asthma on BPAS+ [mean 4.1 mm (± 0.1) vs 4.0 mm (± 0.1), $p = 0.01$] (Table 2).

On repeating the analyses, including the missing variables as a separate category, we still found a significant association between sensitization, ever eczema, current eczema, a history suggestive for asthma on BPAS+ and histamine wheal size. Age was

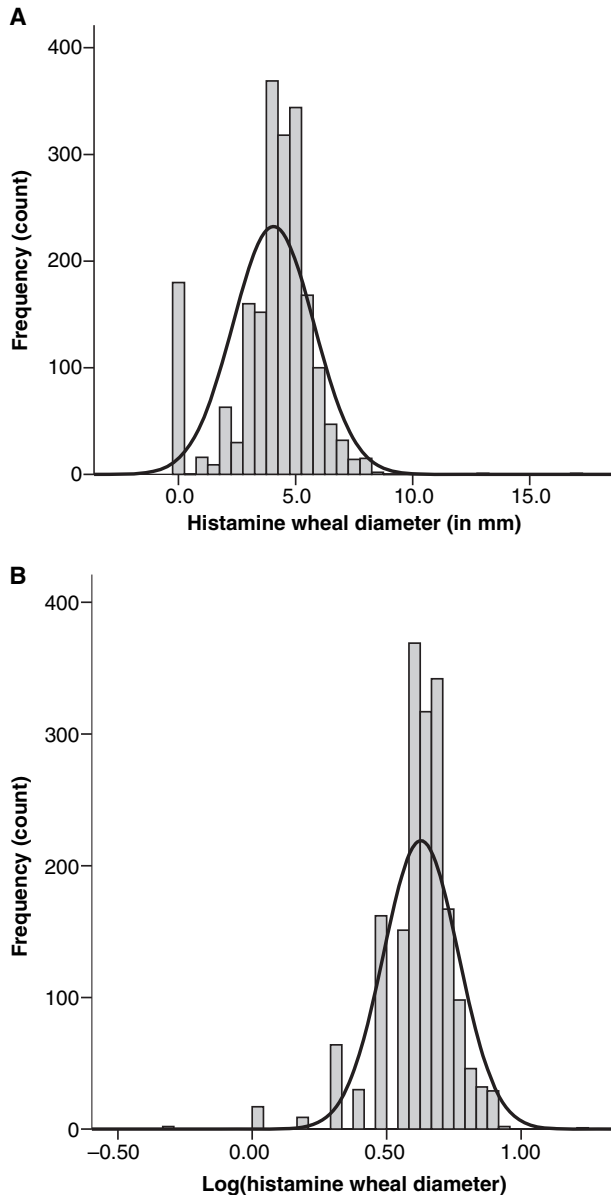


Figure 1. (A) The distribution of histamine wheal size for the study group. (B) The distribution of the log-transformed histamine wheal size data for the study group.

associated with histamine wheal size although the relationship was not linear. In reference to the youngest age category, the histamine wheal size was smaller for the age groups 6–8 and 8–10 years; from the age of 10 years on, the histamine wheal size increased again (Table 2). The same results were observed when stratifying between boys and girls.

All other analyzed factors such as gender, ethnicity, breastfeeding, a personal history of rhinoconjunctivitis, a family history of allergy, exposure to animals at birth

and at present, passive smoke exposure pre- and post-natally, prematurity, and day-care attendance did not influence mean histamine wheal size.

A histamine wheal was absent in 180 children (8.9%), 86 boys (8.7% of the boys) and 94 girls (9.1% of the girls). The absence of histamine wheal was equally distributed in the different age groups. Only two of the 180 children had a history of antihistamine use during the last months prior to SPT.

DISCUSSION

Skin prick testing is the method of choice for the *in vivo* diagnosis of atopy (19). The histamine skin response is widely used as a standardized positive control in the performance of SPT. As variations in histamine skin reactivity may influence the prevalence of positive specific allergen SPT we decided to investigate, as a part of the Aalst Allergy Study, whether histamine skin reactivity was influenced by age, gender, ethnic origin, environmental exposure, specific sensitization patterns, or other factors.

We did find significantly larger histamine wheals in the atopic versus the nonatopic individuals. This significant difference was also observed in several children with different allergic characteristics: eczema and a history suggestive for asthma on BPAS+. These data confirm the work of Stuckey et al (12), who demonstrated that allergen responsiveness is not only associated with IgE, but also with sensitivity to histamine, independent of IgE.

Intracutaneously applied histamine elicits the characteristic ‘triple response of Lewis’ (red spot, flare and wheal) (20) that derives from stimulation of four histamine receptors. Of these, H1 receptors are the most important and their stimulation causes the production of potent vasodilator substances: increased sensitivity of H1 histamine receptors in the atopic children could explain our results (21). In atopic children, the histamine reaction together with the simultaneous influence of other mediators such as leucotriens (22), may enhance the final skin reaction. All these mechanisms alone or together could explain the higher histamine skin response in atopic children, which is induced by a polarization of the Th₂ response and an increase in the production of type 2 cytokines.

In contrast with the cross-sectional study of Skassa-Brociek et al (7), we did not observe that the histamine wheal size increased with increasing age. In our population, the histamine wheal size was the largest for the youngest and the eldest age categories. One of the reasons which might explain this difference could be that those authors’ cross-sectional study only included 365 people,

TABLE 2. Influence of personal, familial and environmental characteristics on histamine wheal size (all cited *p*-values result from nonparametric tests)

	Histamine wheal diameter size [mean (in mm) $\pm$ SE]	<i>p</i> -value
<i>Age (continuous variable)</i>		0.54
<i>Age (categories)</i>		
3.4–< 6	4.2 $\pm$ 0.1	0.01
6–< 8	3.9 $\pm$ 0.1	
8–< 10	3.8 $\pm$ 0.1	
10–< 12	4.1 $\pm$ 0.1	
12–14.8	4.2 $\pm$ 0.1	
<i>Gender</i>		
Boys	4.0 $\pm$ 0.1	0.49
Girls	4.1 $\pm$ 0.1	
<i>Sensitization</i>		
Non-sensitized	4.0 $\pm$ 0.0	0.001
Sensitized	4.3 $\pm$ 0.1	
<i>Sensitization</i>		
Non-sensitized	4.0 $\pm$ 0.0	0.001
Mono-sensitized	4.2 $\pm$ 0.1	
Multiple sensitized	4.5 $\pm$ 0.1	
<i>Ethnic origin</i>		
Autochthonous	4.0 $\pm$ 0.0	0.34
Allochthonous	4.1 $\pm$ 0.1	
<i>Current animals</i>		
No animals	4.0 $\pm$ 0.1	0.59
Cat	4.1 $\pm$ 0.1	
Dog	4.0 $\pm$ 0.1	
Cat and dog	4.1 $\pm$ 0.1	
<i>Animals at birth</i>		
None	4.0 $\pm$ 0.1	0.88
Cat	4.1 $\pm$ 0.1	
Dog	4.0 $\pm$ 0.1	
Cat and dog	4.1 $\pm$ 0.1	
<i>Passive smoke exposure</i>		
No smoking	4.1 $\pm$ 0.1	0.53
Smoking mother	4.1 $\pm$ 0.1	
Smoking father	4.1 $\pm$ 0.1	
Smoking mother and father	3.9 $\pm$ 0.1	
<i>Maternal smoking during pregnancy</i>		
No	4.1 $\pm$ 0.0	0.53
Yes	4.0 $\pm$ 0.1	
<i>Prematurity</i>		
No	4.1 $\pm$ 0.0	0.69
Yes	4.0 $\pm$ 0.1	
<i>Breastfeeding</i>		
No	4.0 $\pm$ 0.1	0.12
0–3 months	4.1 $\pm$ 0.1	
3–6 months	4.1 $\pm$ 0.1	
> 6 months	4.4 $\pm$ 0.1	
<i>Eczema</i>		
No	4.0 $\pm$ 0.0	0.002
Yes	4.2 $\pm$ 0.1	
<i>Eczema</i>		
No eczema	4.0 $\pm$ 0.0	0.02
Eczema in infancy only	4.2 $\pm$ 0.1	
Current eczema only	4.4 $\pm$ 0.1	
Eczema both in infancy and present	4.2 $\pm$ 0.1	
<i>Respiratory symptoms</i>		
BPAS+: Negative	4.0 $\pm$ 0.1	0.01
positive	4.1 $\pm$ 0.1	

TABLE 2. Continued

	Histamine wheal diameter size [mean (in mm) $\pm$ SE]	<i>p</i> -value
<i>Rhinoconjunctivitis</i>		
No rhinoconjunctivitis	4.0 $\pm$ 0.0	0.53
Conjunctivitis	4.3 $\pm$ 0.2	
Rhinitis	4.1 $\pm$ 0.1	
Rhinoconjunctivitis	4.1 $\pm$ 0.1	
<i>Family history of allergy</i>		
No family allergy	4.0 $\pm$ 0.1	1.00
Parental allergy only	4.2 $\pm$ 0.1	
Sibling allergy only	4.0 $\pm$ 0.1	
Parental and sibling allergy	4.0 $\pm$ 0.1	
<i>Day-care center attendance</i>		
No	4.0 $\pm$ 0.0	0.96
Yes	4.1 $\pm$ 0.1	

with an age range from 1 to 85 years, whereas our population consisted of a much larger pediatric population (2021 children, aged 3.4–4.8).

In contrast with Bordignon et al (23) who studied 620 consecutive Caucasian individuals requiring allergologic examination (mean age: 23.89, SD: 15.38) we observed no effect of gender on skin histamine reactivity in our study population. This held true when separately analyzing allergic subjects.

As shown in Table 2, our data do not confirm the results of Kidon et al (24), who studied a population of atopic children in Singapore and found that patients with passive smoke exposure tend to have a decreased histamine skin response.

In conclusion, histamine wheals in the atopic children of our study were statistically significantly larger than in the nonatopic, but the difference in mean histamine skin reactivity was relatively small. As all other analyzed factors did not influence the mean histamine wheal size, the histamine skin response can be considered as a useful standardized positive control in the performance of SPT in children between 3.4 and 14.8 years.

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