

Bimodal skin reactivity to histamine in atopic children in Singapore: influence of specific sensitizations

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Histamine skin prick test (SPT) is used as the 'golden standard' for positive control in *in vivo* immediate type hypersensitivity testing. The skin reactivity to histamine can, however, be modulated by a bevy of extraneous factors. We aimed to define whether histamine skin reactivity in atopic children in Singapore is influenced by age, ethnic origin, gender, environmental exposure or specific sensitization patterns. A retrospective analysis of children, with specific aeroallergen sensitization (as measured by at least one allergen-specific SPT with a wheal size > 3 mm compared with the negative control) from the outpatient speciality clinic of the KK Children's Hospital, during 06/2002–06/2003. A total of 315 patients were included, 235 (75%) were males, 252 (80%) were Chinese, age mean was 7.7 yr (range: 2–15). Patients were referred to the SPT with a diagnosis of one or more of: allergic rhinitis 287 (91%), asthma 112 (36%) or atopic dermatitis 60 (19%). The mean histamine response showed a bimodal distribution, independent of age, ethnic origin, gender or phenotypical expression of allergic disease. Histamine skin reactivity was higher in atopic patients with polysensitization (mean 5.0 mm vs. 2.9 mm in monosensitized patients, $p < 0.001$), and in patients with mould sensitization (mean 5.1 mm vs. 3.3 mm in patient not sensitized to moulds, $p < 0.001$). The presence of passive smoking increased the likelihood of a diminished histamine skin response. Histamine skin response data strongly suggested the presence of two heterogeneous subpopulations. Children with polysensitization and mould sensitization were more likely to show a large significant histamine response, whereas children with passive smoke exposure, showed a diminished skin reactivity to histamine.

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The histamine skin response is widely used as a standardized positive control in the performance of allergy skin tests. Nevertheless, many variables are known to affect this 'standardized' test.

The immunoglobulin E (IgE)-mediated sensitivity increases throughout childhood (1). In parallel, the diameter of the skin reaction to a fixed concentration of histamine peaks in the third decade of life and declines progressively thereafter (2).

Seasonal variation in histamine skin response as well as allergens, have been documented in patients living in temperate climatic areas (3).

Different application techniques, such as body area tested (4), pricking device chosen (5–8), histamine concentration used (9–11) and operator differences, all combine to the variability of this seemingly simple test.

Recent studies have shown significant differences in skin histamine reactivity between different paediatric populations (12).

As variations in histamine reactivity may influence the prevalence of positive specific allergen skin tests (13), and therefore influence therapeutic decisions in the patient's care, we decided that a better understanding of the characteristics of the histamine skin reactivity in our own paediatric population is needed.

Objective

We aimed to define whether histamine skin reactivity in atopic children in Singapore is influenced by age, ethnic origin, gender, environmental exposure or specific sensitization patterns.

Methods

A retrospective analysis, through the medical records of patients undergoing aeroallergen skin prick testing (SPT) in the outpatient speciality clinic of the KK Children's Hospital, from 06/2002 to 06/2003.

Patients were included in the analysis, if they had clear evidence of environmental sensitization (ascertained by SPT with a standard panel of aeroallergens), and a clinical diagnosis of allergic rhinitis, atopic dermatitis and/or asthma.

Data acquisition

The full hospital medical record of all patients was reviewed. Whenever possible, environmental exposure data was confirmed through a direct telephone survey with a standard risk assessment questionnaire including information on exposure to house pets, passive smoking and use of air conditioning in households and in the children's bedroom.

SPT tests

All tests were performed in the respiratory laboratory by an experienced technician using commercial allergen extracts and the GreerPick SPT device (Greer Laboratories, Lenoir, NC, USA) and evaluated by an experienced paediatric allergist. All tests were applied to the forearm, at least 2 cm apart and performed by pressing the tip of the 1 mm serrated plastic device firmly at 90° to the skin through a drop of the allergenic extract. A separate pick was used for each test. Tests were read within 15–20 min of application, the measured maximal wheal diameter was recorded. For aeroallergen

sensitization, a wheal diameter of 3 mm or more in excess of the negative control was considered a positive test result. For all positive SPTs and the positive histamine control, a significant wheal size was calculated by subtracting the result of the diluent control (the background noise) from the observed wheal response. The allergens included in our standard panel are: house dust mite (HDM) mix (*Dermatophagoides farinae*, *D. pteronyssinus* 5000 AU/ml each, standardized), *Blomia tropicalis*, mixed feathers, Canary feathers, Kapok seeds, *Alternaria tenuis*, *Curvularia spicifera*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Candida* (*Monilia*) *albicans*, cat hair (10,000 BAU/ml, standardized), dog epithelia, grass mix (9), *Acacia auriculoformis*, *Melaleuca quinquenervia*, Beefwood (Australian *Pine/Casuarina*), Oil Palm Elaeis (10,000 AU/ml), sage mix, weed mix. Skin response to histamine was evaluated by using a standardized histamine solution of glycerinated histamine phosphate 5 mg/ml (1.8 mg/ml histamine base).

Statistical analysis

Normal mixture models with one to three sub-distributions were fitted to the significant histamine size data using a maximum likelihood method (14). The Akaike's Information Criteria (AIC), which penalizes the models' likelihood for the models' complexity, was used to select the best fitting model. The model with a smaller AIC was considered the better fitting model (15).

From the fitted probability distribution a cut-point was found to classify the subjects as having large vs. small significant histamine size. Logistic regression was used to analyse the relation between various potential factors and large histamine size, both individually and in a multiple logistic regression analysis. In a supplemental analysis, we used the weighted regression method of Reilly and Pepe (16) to incorporate cases with missing values into the logistic regression model. The essence of this analysis is that within each stratum of combinations of completely observed outcome and significant independent variables, each of the n_c subjects with completely observed data is weighted as $1 + n_m/n_c$ subjects, where n_m is the number of subject with missing values in that stratum. This approach uses as much data as possible while acknowledging the potential confounding and uncertainty because of the missing values.

Results

315 patients were included, 235 (75%) were males, 252 (80%) were Chinese, age mean was 7.7 yr (range: 2–15). Patients were referred to the SPT with a diagnosis of one or more of: allergic rhinitis 287 (91%), asthma 112 (36%) or atopic dermatitis 60 (19%). All patients were sensitized to at least one aeroallergen tested, 94.6% to dust mites, 18.6% to moulds, 18% to a pet, 12.3% to a tree pollen and 9.3% to grass or weed pollen. Monosensitized were 68.8%, 22.2% were sensitized to three or more allergens (polysensitization).

Fig. 1 shows the distribution of the significant histamine size. The distribution suggests the sample may reflect a mixture of two or three subpopulations, roughly centering at about 2.5, 6 and 8.5. Normal mixture models with one to three components had AIC 1348.86, 1246.90 and

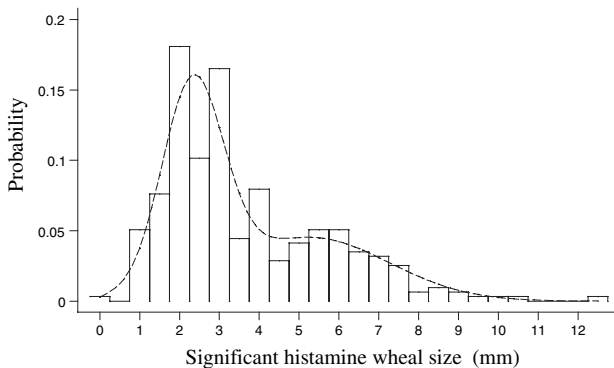


Fig. 1. Probability distribution of significant histamine size ($n = 315$). Bars show observed distribution; dashed line denotes distribution fitted by a two-component normal mixture model. The lowest point of the estimated probability curve was at about 4.6. Hence, we classified significant histamine size of at least 4.6 as large size and otherwise as small.

1248.05, respectively. The model with two subpopulations was chosen as it has the best (smallest) AIC. The fitted probability distribution of the two subpopulation combined is superimposed on the observed distribution. The lowest point of the estimated probability curve was at about 4.6. Hence, we classified significant histamine size of at least 4.6 as large size and otherwise as small. A quarter of our patients (27.6%) showed a large histamine response according to these criteria.

Table 1 presents the distribution of patient characteristics for the whole group and for subjects with large and small histamine wheal size. Significantly, patients with large histamine responses had a higher incidence of mould sensitization and polysensitization (42% and 48% respectively) when compared with the entire group (19% and 21% respectively, $p < 0.001$). These patients also showed a trend towards a lower incidence of passive smoke exposure (13% vs. 21% in the general group, $p = 0.07$). When compared with each other, the groups with large and small histamine responses showed the same pattern except that because of diminished number of cases, the differences in passive smoke exposure, 13% vs. 24%, did not show statistical significance.

Patients with mould sensitization had a significantly increased mean histamine wheal size, 5.1 mm vs. 3.3 mm in patient without mould sensitization, $p < 0.0001$. Patients with polysensitization had a significantly increased mean histamine wheal size, 5.0 mm compared with 2.9 mm in monosensitized patients, $p < 0.001$, illustrated in Fig. 2.

Table 2 presents odds ratios (OR) for the relation between each variable with the odds of having a large histamine wheal size, calculated in a multiple logistic regression model. Polysensiti-

Table 1. Distribution of patient characteristics for the whole group ($N = 315$) and for subjects with large ($N_L = 87$) and small ($N_S = 228$) histamine wheal size

	Mean/proportion in the whole group (%)	Mean/proportion in small histamine response (%)	Mean/proportion in large histamine response (%)	p-Value
Age (yr)	7.68	7.72	7.55	NS
Gender	75 male	76 male	71 male	NS
Race	80 Chinese	81 Chinese	77 Chinese	NS
Atopic dermatitis	19	19	19	NS
Allergic rhinitis	91	93	86	NS
Asthma	36	36	35	NS
Monosensitization	69	77	22	0.001
Polysensitization	21	11	48	0.001
Mould sensitization	19	10	42	0.001
Family history of atopy	81	82	78	NS
Air conditioning at home	76	78	71	NS
Passive smoke exposure	21	24	13	0.07

p-Values are calculated for the differences between subjects with large and small histamine wheal size.

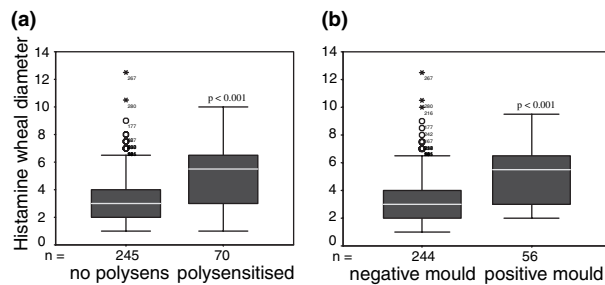


Fig. 2. Comparison of mean significant wheal size between groups of patients. (a) With and without polysensitization. (b) With and without mould sensitization.

Table 2. Multiple logistic regression, comparing odds ratio for a large histamine wheal size according to population characteristics, associated clinical diagnosis, specific sensitization pattern and environmental exposures

Large histamine size	Odds ratio	95% confidence interval	p-Value*
Age	1.00	0.87–1.15	0.994
Gender (male)	0.76	0.31–1.86	0.547
Race (Chinese)	0.61	0.23–1.57	0.303
Atopic dermatitis	1.43	0.57–3.62	0.449
Allergic rhinitis	0.45	0.15–1.38	0.161
Asthma	0.68	0.29–1.57	0.366
Family history	0.77	0.27–2.16	0.613
Polysensitization	6.34	2.48–16.22	<0.001
Air conditioning	0.86	0.31–2.38	0.765
Passive smoke	0.29	0.09–0.98	0.047

*p < 0.05.

zation (OR = 6.34; $p < 0.001$) was significantly related to large histamine size. Passive smoking significantly reduced the odds of a large histamine size (OR = 0.29; $p < 0.05$). In this model, mould sensitization did not add significantly to the risk of increased skin reactivity to histamine.

On re-evaluation of the data, three variables, namely family history of allergy, air conditioning at home and passive smoking, had significantly large proportions of missing values (more than 30%). This led to a reduction of sample size by about half in the multiple logistic regression analysis. In a supplementary analysis, we used the weighted regression method of Reilly and Pepe (16) to incorporate the cases with missing values in these variables into the logistic regression model. In this analysis, polysensitization remained highly significant (OR = 7.63; $p < 0.01$). The OR for passive smoking (OR = 0.33; $p = 0.077$) was significant at the 0.10 level but as not at the conventional 0.05 level. This effect was not seen on the analysis of skin reactivity to HDM. Mean HDM wheal size was 7.7 mm in patients exposed to passive smoke vs. 7.4 mm in patients without smoke exposure, $p > 0.7$.

In a subgroup of children (30 patients), a histamine skin titration was performed by using both a 1.8 mg/ml and a 10 mg/ml solutions as positive controls. The mean wheal size for histamine at a concentration of 1.8 mg/ml was 4.3 mm vs. 5.5 mm with 10 mg/ml ($p = 0.01$). The difference between the skin wheal responses in individual patients was on average 1.2 mm (range: 0–4). The higher concentration of histamine produced wheals at least 3 mm in excess of the negative control in 87% (26 of 30) of patients whereas the 1.8 mg/ml concentration produced such wheals only in 73% (22 of 30). Nevertheless since our cutoff for a positive test is solely dependent on the comparison of the specific allergen wheal size to the negative control, the individual patient's-specific sensitization patterns were not changed.

Discussion

Based on the published data showing increased histamine reactivity in paediatric population with increased incidence of atopic disease (12), we expected a uniformly high histamine skin response in our population. This mainly because we tested patients with symptomatic atopic disease from a population where documented incidence of atopy and atopic disease in children is high (17–19). The significant histamine wheal size data strongly suggests that our population incorporates some heterogeneous subpopulations. A two-component normal mixture model fitted better than a one-component or a three-component model, arguing for at least two distinct subpopulations. As postulated by Stuckey et al. (13) in our series as well, children with polysensitization were much more likely to show a large significant histamine size. As previously shown (20–22), multiple aeroallergen sensitization in our area is primarily because of mould sensitization.

Our data suggests that passive smoking may reduce the odds of having a large significant histamine size. In the supplemental analysis using a weighted regression model incorporating subjects with missing values, the OR for passive smoking (OR = 0.33; $p = 0.077$) while similar to that obtained in the conventional multiple regression analysis, was significant only at the 0.10 level. This finding may be due to the secondary effect of smoking on the severity of symptoms of allergic disease and therefore to a referral bias or to a direct effect of passive smoke exposure.

In the last few years, the accepted standard in Europe has been to use a 10 mg/ml histamine

solution as positive control in allergy SPTs (9, 23). Less standardization is prevalent in other areas of the world, although a concentration higher than 1 mg/ml of histamine base, is recommended by most authorities. Dreborg et al. (10) found a dose-response relationship between the skin wheal response and the concentration of histamine, best fitted to a log-log model with a slope of 0.2 for wheal diameters. In the subgroup of children where both a 1.8 mg/ml and a 10 mg/ml solutions were applied as positive controls the mean wheal size for histamine 1.8 mg/ml was 4.3 mm vs. 5.5 mm with 10 mg/ml ($p = 0.01$), consistent with the Dreborg model. The difference between the skin wheal responses in individual patients was on average 1.2 mm (range: 0–4) a difference that will most probably not have a significant effect on our data analysis.

The use of significant (i.e. absolute wheal size – diluent wheal size) as opposed to absolute wheal size initially stemmed from the high proportion of children in our population with >2 mm wheal response to the diluent-negative control. This high proportion (58.4%) did not show significant week-to-week or interoperator variability, was increased if 'press and twist' was used instead of our usual 'press only' technique, or when using an alternative prick device. The same correlation with increased wheal size (positive with polysensitization and negative with passive smoke exposure) were also seen when analysing the absolute histamine size, but the data had a significantly higher 'noise ratio' making the bimodal distribution harder to discern.

Conclusion

Histamine response in atopic children in Singapore strongly suggests the presence of two heterogeneous subpopulations. Children with polysensitization and mould sensitization were more likely to show a large significant histamine size thus confirming the seminal work of Stuckey et al. (13) from 'the other side of the world'.

In our population, patients with passive smoke exposure tend to have a decreased histamine skin response. Whether this represents a true 'protective effect' or is a result of increased symptoms in 'less atopic' children with passive smoke exposure and therefore a referral bias needs further elucidation.

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