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Prevalence of rat and mouse sensitization in Asian patients with respiratory allergy

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Abstract

Background: Skin prick test (SPT) is useful in identifying rat and mouse sensitization.

Objective: To determine the prevalence of rat and mouse sensitization by using local and commercial allergen extracts.

Methods: Patients with allergic rhinitis or asthma were recruited. SPT of local and commercial rat and mouse allergen extracts were performed. The level of rat and mouse specific IgE (sIgE) was quantified in all patients with positive SPT and randomized patients with negative SPT.

Results: Two hundred and thirty patients, 108 male (47%) and median age 14 years (3.2-63.5 years), were enrolled. Rat sensitization by SPT was 11.7% and mouse sensitization was 17.8%. SPT result to local rat and commercial rat allergen extracts were moderately correlated ($r_s = 0.51$, $p < 0.001$), while SPT result to local mouse and commercial mouse allergen extracts showed low correlation ($r_s = 0.38$, $p < 0.001$). The concordance of SPT results between local rat and commercial rat allergen extracts was 90.4%. Concordance between the local mouse and commercial mouse allergen extracts was 85.2%. When compared with rat and mouse sIgE, the concordance of local rat, commercial rat and commercial mouse allergen extract were > 80% while that of local mouse was 54.4%. No adverse effect was observed in SPT with any allergen or extract.

Conclusions: The prevalence of rat and mouse sensitization was low compared to the study in USA. SPT with local rat and mouse allergen extract was safe and showed good concordance with the SPT result of commercial allergen extracts and rat and mouse sIgE levels.

Key words: rat, mouse, sensitization, skin prick test, specific IgE, asthma, allergic rhinitis

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Abbreviations/Acronyms:
- SPT, skin prick test
- sIgE, specific IgE
- MWD, mean wheal diameter

Introduction

Asthma and related allergic disorders are one of the most common chronic diseases globally. The estimated prevalence of asthma and allergic rhinitis (AR) in the general population is 10-30% worldwide.[1] In Thailand, the International Study of Asthma and Allergies in Childhood (ISAAC) Phase III study (2001) reported the prevalence of asthma among children aged 6-7 years was 15% and aged 13-14 years was 13.9%. The prevalence of AR in children aged 6-7 years was 43.2% and aged 13-14 years was 57.4%.[2] A recent multicenter study in Bangkok reported the prevalence of AR in children aged 6-7 years was 15.0% and in 13-14 years was 17.5%.[3] A study of wheeze prevalence in Bangkok estimated 14.6% of 6–7 year old children and 12.5% of 13–14 year old children age had wheeze.[3]

Previous studies have confirmed that sensitization to indoor allergens increases the risk of development of asthma and allergic rhinitis.[4-6] Most common indoor allergens originate from animals, including house dust mite, cat, dog, mouse, rat

and cockroach. Studies in urban environments have reported the prevalence of rat sensitization to be 19-21%.[7,8] The prevalence of sensitization to rat allergens is directly related to exposure rates.[9,10] Rat allergen sensitization and exposure have been associated with increased asthma morbidity among inner -city residents.[8] The National Cooperative Inner-City Asthma Study (NCICAS) demonstrated that mouse allergens were detected in at least one room in 95% of the houses prevalent in inner-city households.[11] The prevalence of mouse sensitization assessed by skin prick test (SPT) and/or specific IgE (sIgE) response was 18% from NCICAS to 65.7% in other studies.[12-14] Increasing levels of exposure to mice were associated with sensitization.[13] Sensitization to mouse and exposure to rodent environment were associated with increased asthma morbidity in children and adults.[11,14-17]

Identification of rat and mouse allergen sensitization in patients with respiratory allergy is helpful to design avoidance measures that can improve the patients' quality of life. SPT is a useful tool to evaluate aeroallergen sensitization. Unfortunately, rat and mouse allergen extracts are sometimes difficult to procure due to delayed importation of the commercial extracts. In addition, local rat and mouse species may be different than those available in commercial allergen extracts. Rat and mouse sensitization may also be measured by serum sIgE levels. However, this method is expensive, time consuming and invasive and is not suitable as a screening test. Therefore, the rat and mouse allergen extract preparation process was developed in the Siriraj Hospital, Mahidol University laboratory.

This study aimed to assess the prevalence of rat and mouse sensitization using local and commercial allergen extracts and also to compare the efficacy of local and commercial rat and mouse allergen extracts in skin prick testing patients with respiratory allergy.

Methods

Study population

From January 2018 to February 2019, patients aged ≥ 3 years with physician diagnosed allergic rhinitis and/or asthma[18,19] were recruited from the Department of Pediatrics and Department of Internal Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand. Subjects with acute asthma exacerbation (Forced Expiratory Volume 1 < 70%), severe atopic dermatitis (SCORAD > 50), pregnancy or chronic diseases such as autoimmune diseases, immune deficiency, or liver disease were excluded. Antihistamine, systemic corticosteroids ≥ 20 mg/day and topical corticosteroids were discontinued for at least 7 days before testing. The study was registered with Clinical Trials.gov NCT03645161. The Institutional Ethics Committee, Siriraj Hospital, Mahidol University, EC 789/2560 (EC2) approved the study. Written informed consent from parents or guardians and assent from children older than 7 years of age were obtained.

Clinical characteristics, allergic symptoms and environmental exposure were recorded. All subjects received SPT to local rat epithelial, commercial rat epithelial, local mouse epithelial and commercial mouse epithelial extracts. Subjects were also pricked with other common aeroallergens including Bermuda, Acacia, Johnson, Careless weed, *Curvularia*, *Cladosporium*, *Penicillium*, *Aspergillus*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, American cockroach and German cockroach. sIgE to rat and mouse were performed in all patients with a positive SPT (mean wheal diameter, MWD, ≥ 3 mm.) and in 30 controls from randomized SPT negative patients (MWD < 3 mm.) (**Figure 1**)

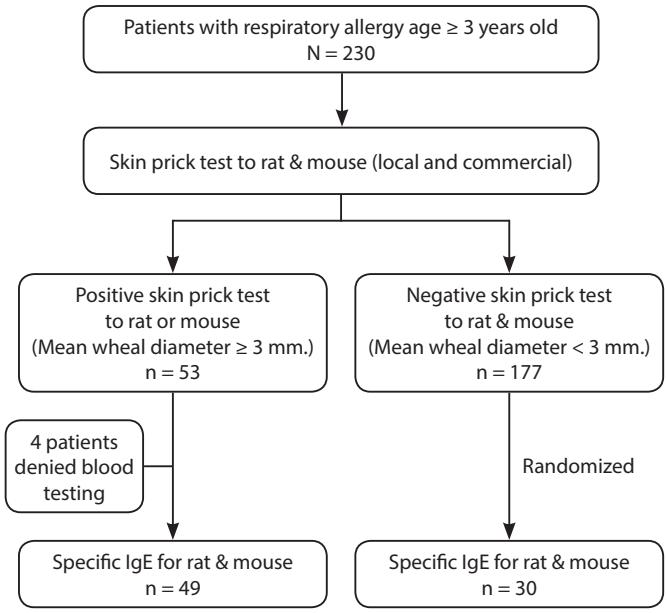

Figure 1. Study flow chart

Rat and mouse local allergen extract preparation

The extraction process developed by researchers at Siriraj Hospital, Mahidol University has been patented by the Thai regulatory authorities. The local rat (*Rattus norvegicus*) and mouse (*Mus musculus*) were raised in the Specified Pathogen Free system (SPF) by M-CLEA Bioresource Co., Ltd. Rat and mouse epithelia were added with phosphate buffered saline, pH 7.4 (PBS), 0.1% Tween-20 (1:20 w/v), and then incubated at 4°C for 30 minutes. The mixture was centrifuged at 8,000 ×g, 4°C for 20 minutes. The supernatant was collected and mixed with glycerol (1:2) and filtrated through 0.2 µm membrane filter. The filtrated solution was kept in a glass bottle at 4°C. Protein concentration, sterility and single dose toxicity were tested before use.

Commercial rat (*Rattus norvegicus*) epithelial allergen extract, 1:20 w/v (Greer Laboratories Lenoir, North Carolina) and mouse (*Mus musculus*) epithelial allergen extract, 1:20 W/V (ALK, Port Washington, New York) were used. Histamine dihydrochloride (10 mg base/ml) and sterile glycerinated saline were used as positive and negative controls, respectively. SPT of local commercial rat and mouse allergen extracts were performed with a blood lancet (Vitrex® steel, Vitrex Medical A/S, Herlev, Denmark) by an experienced technician in a room with full resuscitation equipment. SPT was performed on the patients' skin on the upper back in children and the volar surface of the forearm in adults. The presence of induced wheals and flares induced was recorded 10 minutes after positive control testing and 15 minutes after allergen extract testing. Mean Wheel Diameter (MWD) (the longest diameter plus the perpendicular diameter and divided by 2) was calculated. SPT was considered positive if the MWD was ≥ 3 mm. larger than the SPT of negative controls.

Specific IgE antibodies to rat and mouse allergen

sIgE was measured in all patients who had a positive SPT result to rat or mouse allergen extract. sIgE was also measured in 30 randomized patients from a group of patients with negative SPT results to rat and mouse allergen extracts as negative controls. Randomization was performed using the program from www.randomization.com. sIgE to rat and mouse epithelia, serum and urine allergen were quantified using the ImmunoCap® (Phadia, Uppsala, Sweden). A level of sIgE ≥ 0.35 kAU/L was considered positive.

Statistical analysis

Phipatanakul, et al.[12] reported the prevalence of mouse sensitization in inner-city areas of the United State of America to be 18%. We calculated a target sample size of 227 randomized participants to provide a 95% confidence level and 5% allowable error. The data were analyzed using SPSS software version 18 (SPSS Inc., Chicago, II., USA). Descriptive data are presented as the mean and standard deviation (SD) or median (range) for continuous data or number and percentage for categorical data. Agreement between SPT results of local and commercial allergen extracts was evaluated using kappa and intraclass correlation.[20] Correlation coefficients between the different SPT allergen extract sources and between SPT and sIgE were evaluated using Spearman's rho correlation.[21] The agreement between the SPT result of local and commercial allergen extracts and between SPT and sIgE were presented as percentage of concordance. Factors associated with rat or mouse sensitization were analyzed using the Mann-Whitney *U* test. A p-value < 0.05 was considered statistically significant. Potential predictors of rat and/or mouse sensitivity obtained at the time of enrollment included clinical characteristics, allergic symptoms, environmental exposure, and SPT results from other aeroallergens. Predictive power was estimated using univariate logistic regression analysis and then categorized to facilitate the calculation of odds ratios using multivariable logistic regression.

Results

Two hundred and thirty respiratory allergic patients with the median age of 14 years (range 3.2-63.5) were enrolled. Number (96.5%) of study subjects had allergic rhinitis. One hundred and twenty-five (54.3%) were children, 108 (47%) were male, and more than half (56.5%) resided in Bangkok, the capital city of Thailand. There were no significant differences in any demographic variables between patients with positive or negative SPT results. (**Table 1**) From 230 patients, 15 (6.5%) were positive for local rat epithelial extract SPT and

Table 1. Baseline Patient Characteristics

Character	Skin prick test (SPT) to rat and/or mouse (N = 230)		*p*-value
	Positive SPT to rat or mouse (local and commercial) (n = 53)	Negative SPT to rat and mouse (local and commercial) (n = 177)	
Age (year):			
median (range)	11.4 (3.2-63.5)	15.3 (3.3-62.7)	0.22
< 18 (n = 125)	8.2 (3.2-13.5)	8.8 (3.3-17.4)	0.52
> 18 (n = 105)	26.7 (18.1-63.5)	30.0 (19.4-62.7)	0.23
Gender			
Male	29 (54.7%)	79 (44.6%)	0.20
Urban versus Rural			
Urban (Bangkok)	33 (62.3%)	97 (54.8%)	0.34
Rural	20 (37.7%)	80 (45.2%)	
Atopy			
Atopic dermatitis	7 (13.2%)	11 (6.2%)	0.15
Asthma	8 (15.1%)	19 (10.7%)	0.39
Allergic rhinitis	51 (96.2%)	171 (96.6%)	1.00
Food allergy	11 (20.8%)	25 (14.1%)	0.24
Family incomes (Baht/month)			
≤ 25,000	5 (10.9%)	19 (11.6%)	0.81
25,001–50,000	21 (45.7%)	62 (37.8%)	
50,001–100,000	15 (32.6%)	61 (37.2%)	
> 100,000	5 (10.9%)	22 (13.4%)	

17 (7.4%) were positive for commercial rat epithelial extract. Unexpectedly, more patients had positive responses to commercial mouse epithelium (15.7%) compared to local mouse epithelial extract (5.2%). (**Table 2**) Number (6.5%) of subjects had positive SPT to both rat and mouse allergen extracts. Fifteen of 27 (55%) of patients who had positive SPT results to rat allergen extracts also had positive SPT results to mouse allergen extracts, while 15 of 41 (36%) of patients who had positive SPT results to mouse allergen extracts had positive SPT results to rat allergen. Employing a combination of SPT positive results from local or commercial allergen extracts, the prevalence of rat and mouse sensitization were 11.7% and 17.8%, respectively. (**Figure 2**) Local rat epithelium allergen extracts and commercial rat epithelium allergen extracts showed moderate correlation (correlation coefficient, $r_s = 0.51$, $p < 0.001$), while local and commercial mouse epithelium allergen extracts showed low correlation ($r_s = 0.38$, $p < 0.001$). Fair agreement were found not only between local and commercial rat epithelium allergen extracts (kappa = 0.26) but also between local and commercial mouse epithelium allergen extracts (kappa = 0.23). The concordance of SPT results between local and commercial rat epithelium allergen extracts was 90.4% and between local and commercial mouse epithelium allergen extracts concordance was 85.2%. (**Table 3**)

Serum sIgE was measured in 49 patients with positive SPT to rat or mouse allergen. The median sIgE level of rat was 0.01 kUA/L (max 82.5 kUA/L) and mouse was 0.02 kUA/L (max 48.2 kUA/L). Rat sIgE was positive in 7 of 49 patients (14.3%) and mouse sIgE was positive in 6 of 49 patients (12.2%). In 30 randomized patients with negative SPT to rat and mouse, none had a positive sIgE to rat while one patient (3.3%) had a positive sIgE to mouse allergen.

The correlation coefficient between local rat epithelium and rat sIgE was $r_s = 0.20$ ($p = 0.08$) and between commercial rat epithelium and rat sIgE was $r_s = 0.38$ ($p < 0.001$). The correlation coefficient between local mouse epithelium and mouse sIgE was $r_s = 0.25$ ($p = 0.030$) and between commercial mouse epithelium and mouse sIgE was $r_s = -0.04$, ($p = 0.720$).

The concordance between local rat allergen extract SPT results and rat sIgE level was 84.8%, while commercial rat allergen extract concordance with rat sIgE level was 86.0%. The concordance between local mouse allergen extract SPT results and mouse sIgE level was 54.4%, while commercial mouse allergen extract concordance with mouse sIgE level was 83.5%. Fair agreement was found between level of rat sIgE and MWDs from local rat epithelium allergen extract and MWDs from commercial rat epithelium allergen extract (kappa = 0.46 and 0.38, respectively). Slight agreement was found between level of mouse sIgE and MWDs from local mouse epithelium allergen extract (kappa = 0.15) while poor agreement was found between level of mouse sIgE and MWDs from commercial mouse epithelium extract (kappa = -0.54). (**Table 4**) No local or systemic adverse reactions related to any local or commercial allergen extracts were observed.

Table 2. Patients with positive skin prick test to local or commercial mouse or rat allergen extracts (N = 230)

	Source of allergen extract used	Positive skin prick test No. (%)
Rat	Local epithelium	15 (6.5)
	Commercial epithelium	17 (7.4)
	Local epithelium and commercial epithelium	5 (2.2)
	Local epithelium or commercial epithelium	27 (11.7)
Mouse	Local epithelium	12 (5.2)
	Commercial epithelium	36 (15.7)
	Local epithelium and commercial epithelium	7 (3)
	Local epithelium or commercial epithelium	41 (17.8)
Rat and mouse	Any kinds of allergen extracts	15 (6.5)

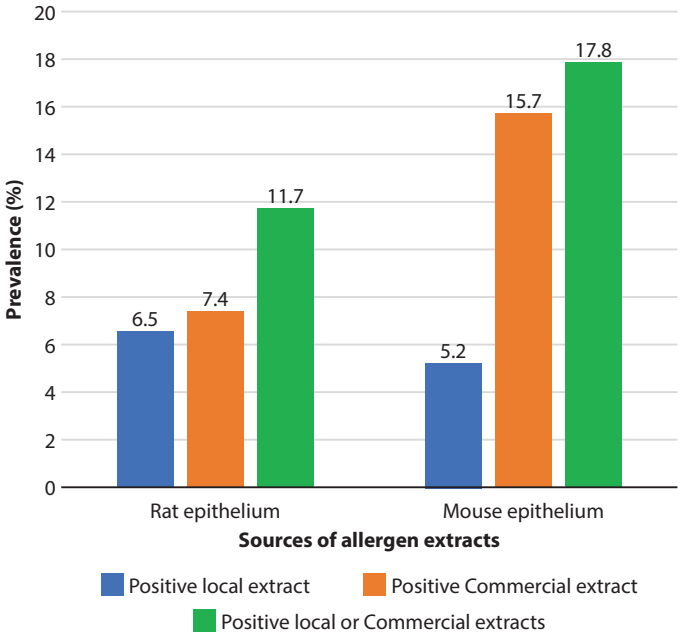

Figure 2. Prevalence of rat and mouse sensitization determined by skin prick testing

Table 3. Concordance and agreement of skin prick test (SPT) results of local and commercial allergen extracts of rat and mouse (N = 230)

SPT	Positive local and Positive commercial	Positive local and Negative commercial	Negative local and Positive commercial	Negative local and Negative commercial	Concordance (%)	Kappa Coefficient
Rat	5	10	12	203	90.4	0.26 (p = 0.09)
Mouse	7	5	29	189	85.2	0.23 (p = 0.11)

Table 4. Concordance and agreement of specific IgE (sIgE) and skin prick test (SPT) results of local and commercial rat and mouse allergen extracts (n = 79)

	Positive SPT and Positive sIgE	Positive SPT and Negative sIgE	Negative SPT and Positive sIgE	Negative SPT and Negative sIgE	Concordance (%)	Kappa Coefficient
Rat						
Local	5	10	2	62	84.8	0.46 (p = 0.14)
Commercial	5	9	2	63	86	0.38 (p = 0.14)
Mouse						
Local	2	31	5	41	54.4	0.15 (p = 0.15)
Commercial	2	8	5	64	83.5	-0.54 (p = 0.07)

Discussion

We observed the prevalence of rat and mouse sensitization in children and adults with respiratory allergy (allergic rhinitis and/or asthma) to be 11.7% and 17.4%, respectively. Previous studies among children with asthma reported 2-4 times higher prevalence of rat (19-21%)[7,8] and mouse sensitization (18-65.7%).[12-14] Important differences in these studies include the study location, the age of the studied population and the underlying allergic diseases of the patients. Our study in Thailand included both children and adults while the other studies were performed in United States of America and included mostly children. Ninety six percent of our study subjects had allergic rhinitis, while the previous studies focused on patients with asthma.[7,8,12-14]

The prevalence of patients who had positive SPT results to the local rat epithelial extracts (6.5%) was close to that of commercial rat epithelial extracts (7.4%). Moderate correlation (correlation coefficient, $r_s = 0.51$, $p < 0.001$) and high concordance (90.4%) were found between local and commercial rat allergen extracts. These results suggest that our local rat epithelial extract is suitable for screening of rat sensitization in Thailand.

In contrast, the percentage of patients with a positive SPT to mouse allergen extract was higher using commercial mouse epithelial extract (15.7%) than with local extract (5.2%). The correlation between local and commercial mouse allergen extracts was low ($r_s = 0.38$, $p < 0.001$). This may be due to the difference in preparation methods, lack of standardization of local allergen extracts and differences of their allergenicity. We also found that the positivity of SPT was increased to 17.8% when we combined the results of local and commercial allergen extracts. However, we could not conclude that either extract was superior since the percentage of sensitization may not reliably indicate the presence of clinical allergy. To determine this, the gold standard respiratory provocation test for clinical sensitivity[22] should be performed.

Rat and mouse allergens can be obtained from epithelium, serum and urine. Urine is believed to be the major source of rat and mouse allergenic protein[23] because the major allergen of rat (Rat n 1) and mouse (Mus m 1) is excreted in large amounts in the urine.[24] However, the preparation of urine allergen extract is challenging. A pilot study was performed with local rat and mouse urine allergen extracts prepared by our researchers but this yielded sufficient extract to skin test only 70 patients. The low rates of positivity of local rat urine extract 8.6% (6/70) and local mouse urine extract 7.1% (5/70) prevented us from reliably estimating the correlation between SPT with local urine extracts and commercial extracts.

This is the first study to compare the prevalence of rat and mouse sensitization by SPT to local and commercial allergen extracts and sIgE in both pediatric and adult populations with respiratory allergy. This was also the first time that local rat and mouse antigen extracts were prepared for SPT in Thailand. Qualified personnel and standard laboratory were used in performing SPT and sIgE. We did not confirm rat or mouse allergy by respiratory provocation test so we report only the sensitization to rat and mouse allergens. Future studies should also include the respiratory provocation test in addition to SPT with rat and mouse urine allergen extracts. The preparation of local mouse epithelial allergen extracts also merits further study.

Conclusion

SPT with local rat and mouse allergen extract was safe and showed good concordance with the SPT result of commercial allergen extracts and rat and mouse sIgE levels.

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Conflict of interest declaration

All authors declare that they have no personal or professional conflicts of interest, and have not received any financial support from the companies that produce or distribute the drugs, devices, or materials described in this report.

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