In-vivo allergenic potency of Siriraj Mite Allergen Vaccine (SMAV) comparing with standardized vaccine in mite-sensitive patients

Nualanong Visitsunthorn[1], Chaweewan Bunnag[2], Punchama Pacharn[1], Paraya Assanasen[2], Orathai Jirapongsananuruk[1], Torpong Thongngarm[3], Pongsakorn Tantilipikorn[2] and Pakit Vichyanond[1]

Summary

Background: **The prevalence of allergic diseases, particularly asthma and allergic rhinitis, has increased tremendously in Thailand and worldwide. House dust mite (HDM) is the major IgE sensitizer among allergic children and adults. We have developed local standardized mite allergen extracts, Siriraj Mite Allergen Vaccine (SMAV) from *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df) from our source materials which were highly purified (99%).**

Objective: **To compare *in-vivo* allergenic potency of both SMAV Dp and Df with commercial standardized mite allergen vaccine by using skin prick testing in mite-sensitive individuals.**

Methods: **This was a double-blind, randomized, self controlled study comparing SMAV and commercial standardized mite allergen vaccine (Dp and Df) by using skin prick testing in mite-sensitive adult volunteers, 18 – 60 years of age.**

Results: The study was performed in 54 adult volunteers (19 males, mean age 26.6 ± 5.5 years old) who had positive skin test to commercial Dp and Df. Seventeen of them had no allergic disease. The most common allergic disease among the volunteers was allergic rhinitis (21/37). Mean wheal diameter of SMAV Dp and commercial Dp at the concentration of 10,000 and 5, 000 AU/ml were equivalent but at the concentration of 2,500 AU/ml was inequivalent. Mean wheal diameter of SMAV Dp was significantly larger than commercial Dp at concentration of 2,500 AU/ml ($p < 0.05$). Mean wheal diameter of SMAV Df and commercial Df at all 3 concentrations were equivalent. There was no systemic side effect in all subjects.

Conclusion: **The study demonstrated that in mite-sensitive adults, SPT using SMAV Dp (10,000 AU) and Df (10,000 AU) had equivalent allergenic potency to the commercial comparator without any systemic side effect. *(Asian Pac J Allergy Immunol 2011;29:50-6)***

Key words: *Siriraj Mite Allergen Vaccine,* in-vivo *equivalent allergic potency, skin prick testing*

Introduction

The prevalence of allergic diseases, particularly asthma and allergic rhinitis, has increased tremendously worldwide.[1] According to the International Study of Asthma and Allergy in Childhood (ISAAC), the prevalence of these diseases in Thailand is moderately high. The prevalence of childhood asthma has risen 3-fold over the past 15 years from 4% to 15%. Surveys in Thailand have revealed that more than 40 % of Thai children have allergic rhinitis[3] whereas the prevalence was 26 % in adults.[4, 5] The increasing prevalence indicates that allergic diseases have become common among the Thai population and could be a major economic burden to Thai Society. For example, co-morbid conditions of asthma, including allergic rhinitis and sinusitis, could lead to a substantial increase in expenditure on treatment.[6, 7]

IgE-mediated hypersensitivity is a major immuno-pathogenetic basis for allergic diseases. Experience with skin testing (both skin prick test (SPT) and intradermal methods) in Thailand over the past 30 years indicates that house dust mites

From the[1] Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[2] Department of Otorhinolaryngology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[3] Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
Corresponding author: Nualanong Visitsunthorn
Email: nualanongv@yahoo.com
Submitted date: 23/10/2010
Accepted date: 15/2/2011

[HDM; *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df)] are the major IgE sensitizer among allergic patients in Thailand.[8-11] Patients with a higher degree of mite sensitization develop more wheezing and a higher degree of bronchial hyper-responsiveness.[12] The knowledge that patients are allergic to HDM will not only substantiate the diagnosis of allergic diseases but is also an advantage in determining the likely future course of the disease. Furthermore, an environmental control strategy can be developed for patients who are allergic to HDM.

The Siriraj Mite Allergen Vaccines (SMAV) are local standardized mite allergen extracts developed from highly purified (99%) Dp and Df. They are produced by the SMAV Development Project. The SMAV would be useful not only for Thai patients but also for patients in the entire Asia-Pacific region since HDM is also the most common allergen causing sensitization and diseases in Hong Kong, Singapore, Malaysia, Korea and Indonesia.[13-17] The SMAV are produced using a protocol approved by Thai Food and Drug Administration (FDA).[18] Sterility is ensured for all microorganisms with acceptable endotoxin and mycoplasma level test results. The allergenic potency of the SMAV is adjusted using the US FDA reference standards (with both allergen and antibody) to 10,000 AU/ml.[19] Comparison of wheal and flare diameter between the SMAV and standardized commercial mite allergen vaccines in normal Thai adults from our previous report has already shown no significant differences in the rate of false positives and in systemic or large local reactions.[20]

The objective of this study was to compare *in-vivo* allergenic potency of the SMAV both Dp and Df with commercial standardized mite allergen vaccines by using skin prick testing in mite-sensitive individuals.

Methods

This study was a double-blind, randomized, self controlled study comparing SMAV with commercial standardized mite allergen vaccine (Dp and Df) by using skin prick testing in mite-sensitive adult volunteers. The study was approved by Ethics Committee of Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. The study populations were adult volunteers between 18 – 60 years of age who were sensitized to both Dp and Df, according to the results of tests with 10,000 AU/ml of commercial Dp and Df. The subjects did not suffer from systemic diseases, severe anaphylaxis or severe asthma. The female subjects were not pregnant or lactating. The study subjects did not receive any medications which might interfere with skin test results (e.g. antihistamine, oral decongestant, etc.) and were not involve in other studies 30 days before or during this study.

Table 1. The study flow chart.

	Screening 1	Visit 1
Informed Consent	X	
Medical History	X	
Physical Examination	X	
Vital Signs	X	X
Serum specific IgE for Dp and Df CBC, BUN, Creatinine, AST, ALT	X*	
Urine Pregnancy Test for Woman	X	
Inclusion /exclusion criteria	X	
Skin Prick Testing (SPT)	X**	X**
Adverse Event Assessment		X***
Concomitant Drug Assessment		X
Peak Expiratory Flow Rate (PEFR)	X	X

* Blood test would be performed after SPT and urine pregnancy test for women

** At least 30 minutes observation period after skin prick test

*** Telephone calls for delayed adverse events 2 days after skin prick test

The study used the equivalence test for the difference in paired mean. The sample size had to be at least 34 samples to test the null-hypothesis that there was clinical equivalence between the matched pairs, when the difference in paired means was greater than 2.5 mm from the observed mean, assuming that the standard deviation was known to be 5.0 (one-sided alpha 0.05, power = 1-beta = 0.80).

Study interventions

After informed consent had been obtained from each subject, detailed screening procedures, consisting of history taking, physical examination and laboratory testing, including specific IgE measured by ImmunoCAP (Phadia AB, Uppsala, Sweden), were performed. Skin prick testing for Dp and Df commercial vaccines (Dp and Df from ALK Port Washington, New York, 11050, registration no. 1C 3/42(N)) was performed in subjects who were eligible to participate in the study on Visit 1. The study flow chart is shown in Table 1. Subjects had a normal physical examination, laboratory tests and a peak expiratory flow rate (PEFR) which was > 70% of predicted value before the start of the study.

In order to compare the *in-vivo* allergenic potency of SMAV with commercially available mite allergen vaccines, skin prick testing was chosen as an *in-vivo* method in individuals who were sensitized to HDM. The SMAV (Dp and Df produced from Greater Pharma Laboratories in collaboration with Faculty of Medicine Siriraj Hospital, Mahidol University) and standardized commercial mite vaccines were diluted in 0.03 % human serum albumin (HSA) containing normal saline to make 3 dilutions i.e. 10,000, 5,000 and 2,500 AU/ml. All allergen vaccines were stored at 4-8^{o}C until use. The bottles containing both Dp and Df from both sources were randomly coded as bottle A, B, C and D so that the investigator and the research technician who did the skin testing could not identify the type of vaccines in each bottle.

Skin testing was performed on the subject's backs, with the distance between each skin test being 5 cm. Double-blind, four rows of skin tests (10,000, 5,000 and 2,500 AU/ml of Dp and Df of the SMAV and the commercial standardized mite allergen vaccine) were conducted for each experiment. Skin testing with Torrington suturing (peritoneal) needles was performed between 8-12 a.m., to minimize the circadian variation of skin test results.[21] Wheal and flare reactions were recorded at 15 minutes by outlining with a fine ball-point pen and transferred to a hard copy using transparent tape method (Transpore™, 3M, MN 55144-1000, USA). The size of each wheal was measured using a computer programme which determined the means of the longest and diagonal diameters.[22] The positive control was histamine hydrochloride (10 mg/ml) and the negative control was 0.03% HSA diluent. The code was opened after all statistical analysis had been completed.

The skin tests were conducted by a well trained allergy technician in a treatment room fully equipped with resuscitation medication and equipment, such as adrenaline injection, intravenous and oral antihistamine, ambu bag, oxygen, tourniquet, etc. The subjects were observed for 30 minutes after completion of the tests and received telephone calls for possible delayed adverse events 2 days after the tests. All adverse events were recorded in the adverse event record form.

Table 2. Demographic data for 54 mite-sensitive adult volunteers.

Total (N)	**54**
Sex	
Male	19
Female	35
Age (years)	
Mean ± SD	26.6 ± 5.5
Median	25
Minimum	19
Maximum	48
History of allergy	
No allergy	17
Allergy	37
Allergic rhinitis	21
Food allergy	4
Drug allergy	4
Asthma	3
Urticaria	2
Atopic dermatitis	2
Anaphylaxis (no symptoms recently)	1
Skin test reaction at enrollment	
Mild (wheal diameter <5 mm)	25
Moderate (wheal diameter>5-15 mm)	29

Statistical analysis:

The two one-sided test procedure is operationally identical to the procedure of declaring equivalence only if the ordinary 1-2α (not l-α) = 1-2 (0.05) = 90% of the confidence interval for the mean difference is completely within the equivalence interval [-2.5, 2.5] when 2.5 is the equivalence interval. Then equivalence is confirmed if both the lower and upper values of the 90% confidence interval falls within the upper and lower limits. Data were prepared and analyzed using PASW statistics 18.0 (SPSS Inc., Chicago, IL, USA) and Statistic software R version 2.12.0 (R Development Core Team 2010, Austria).

This study was performed according to globally accepted standards of good clinical practice (as defined in the ICH E6 Guideline for Good Clinical Practice, 9 May 1997), in agreement with the latest revision of the Declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, October 2000) and in keeping with local regulations.

Results

Fifty-four adult volunteers who had positive SPT to standard commercial Dp and Df were included in this study. The mean ± SD age was 26.6 ± 5.5 years old (median 25, minimum 19 and

Table 3. Mean wheal diameters, mean wheal diameter difference, 90% confidence intervals of the difference between SMAV and commercial Dp vaccine and equivalence test.

Dp		Wheal diameter (MWD) (mm)		Mean difference of MWD ± SD	90 % CI of the difference (mm)		Equivalence test (*p*-value)
		Mean	SD	(mm)	Lower	Upper	
SMAV	10,000AU	9.78	5.63	0.35 ± 4.24	-1.57	2.26	0.032*
Commercial	10,000AU	9.44	6.34				
SMAV	5,000AU	8.23	4.87	-0.06 ± 3.54	-1.92	1.79	0.016*
Commercial	5,000AU	8.29	6.61				
SMAV	2,500AU	9.52	6.77	2.23 ± 4.78	0.26	4.21	0.412
Commercial	2,500AU	7.28	5.52				

* Significant at 0.05 level.

maximum 48 years old). There were 19 males and 35 females. Seventeen of the volunteers had no allergic disease. The most common allergic disease among the volunteers was allergic rhinitis followed by food and drug allergy. None of them smoked. Demographic data are shown in Table 2. The positive control tests with histamine were positive in all volunteers. There were 2 cases with a $\geq$3 mm diameter wheal in the negative control test with serum albumin. In 17 cases without allergy, only 3 cases had negative specific IgE to both Dp and Df and none of them had negative SPT to both the SMAV Dp and Df. The mean wheal diameters of the SMAV Dp at concentration 10,000, 5,000 and 2,500 AU/ml were 9.78, 8.23 and 9.52 mm while those of the commercial Dp were 9.44, 8.29 and 7.28 mm (Table 3) respectively. The mean wheal diameter for SMAV Dp and commercial Dp at the concentration of 10,000 and 5, 000 AU/ml were equivalent because both the lower and upper values of the 90% confidence intervals were within the upper and lower limits of 2.5 mm However, at the concentration of 2,500 AU/ml, the mean wheal diameter of the SMAV Dp was significant larger than that for the commercial Dp ($p < 0.05$). The mean wheal diameters for the SMAV Df at concentration 10,000, 5,000 and 2,500 AU were 8.92, 8.72 and 7.33 mm while those of the commercial Df were 9.13, 8.85 and 7.95 mm respectively.(Table 4) Mean wheal diameter of SMAV Df and commercial Df at all 3 concentrations were equivalent (p <0.05).

Following testing using SPT with the SMAV Dp 10,000 AU/ml, 4 cases had a negative SPT result. All of them had negative specific IgE for Dp. Following testing with the commercial Dp, 7 of them had a negative result. Only 3 out of 7 cases with negative results to the commercial Dp had negative specific IgE to Dp. SPT using the SMAV Df produced the same result. Two of the volunteers had negative skin test to the SMAV Df. Both of them had negative specific IgE for Df while 4 out of 5 cases with negative skin prick to the commercial Df had negative specific IgE to Df. The negative results for specific IgE to Dp and Df were found in 9 and 16 volunteers respectively.

The concentration of Dp and Df in both the SMAV and the commercial vaccines were checked and it was found that the levels of Dp in the SMAV were higher than those in the commercial extract. The antigen levels of Dp in the SMAV and the commercial extract were 8,267.1 and 7,663.8 AU and those of Df in SMAV and commercial extract were 8,392.6 and 9,574.3 AU, respectively.

The adverse reactions from both Dp and Df of the SMAV and the commercial vaccines were not significantly different. No systemic reaction occured. Only local reactions occured in all positive skin tests and all reactions improved after taking one dose of second-generation antihistamine. All reactions disappeared two days after the study.

Discussion

Dp and Df are the major IgE sensitizers among pediatric and adult allergic patients in Thailand. The sensitization rate of HDM (Dp and Df) is up to 70 % and 50 % in Thai asthmatic children[10] and adults,[9] respectively. A previous study showed that the odds ratios for development of asthma among children sensitized to HDM was clearly related to the level of major HDM allergens in the house.[23] Mite allergen extracts of both Dp and Df are useful for the diagnosis and therapy of mite sensitive allergic patients. The imported commercial mite (Dp and Df) allergen extracts for internationally acceptable and standardized mite

Table 4. Mean wheal diameters, mean wheal diameter difference, 90% confidence intervals of the difference between SMAV and commercial Df vaccine and equivalence test

Df		Wheal diameter (MWD) (mm)		Mean difference of MWD ± SD (mm)	90 % CI of the difference (mm)		Equivalence test (p-value)
		Mean	SD		Lower	Upper	
SMAV	10,000AU	8.92	5.32	-0.21 ± 3.76	-2.10	1.69	0.024*
Commercial	10,000AU	9.13	6.49				
SMAV	5,000AU	8.72	6.30	-0.13 ± 4.69	-2.15	1.89	0.027*
Commercial	5,000AU	8.85	6.36				
SMAV	2,500AU	7.33	4.18	-0.61 ± 4.05	-2.22	1.00	0.027*
Commercial	2,500AU	7.95	5.78				

* Significant at 0.05 level.

SPT are available in Thailand but rather expensive. Due to all of the aforementioned facts about HDM, the SMAV Development Project has been initiated with funding from the Faculty of Medicine Siriraj Hospital to produce allergen vaccines for both diagnostic and therapeutic purposes. The SMAV had been compared to commercially available vaccines using *in-vitro* tests i.e. the ELISA-inhibition/competition as recommended by US-FDA.[24] The present study was the next step to compare the *in-vivo* allergenic potency of the SMAV, both Dp and Df, with the commercial mite allergen vaccines by using skin prick testing in mite-sensitive individuals.

We used SPT to evaluate the *in-vivo* allergenic potency because it had been used since the turn of the century to determine hypersensitivity reactions to many allergens included HDM. In the skin prick test procedure, the epidermis was pricked through a drop of allergen extract (vaccine) to introduce a small amount of allergen to IgE-attached mast cells underneath with only approximately 0.03 ml of reagent being introduced.[21] Through the allergen-IgE/IgE receptor bridging phenomenon, mast cell mediators are released within 15 minutes and wheal and flare reactions develop. Such reactions can be measured to determine the degree of IgE sensitization to that specific allergen. The sizes of skin testing reactions (both wheal and flare) have been shown to correlate well with level of specific IgE to the same allergen.[25] Torrington suturing (peritoneal) needles were used as skin test needles because they had been evaluated in our previous investigation[26] and shown to have a coefficient of variation less than 20%, which is acceptable for skin test research.[22]

We measured level of antigen concentration in both vaccines and found that the SMAV Dp had a higher level than the commercial allergen vaccine while the SMAV Df had a lower level than the commercial allergen vaccine. The levels of Dp and Df from both vaccines were not significantly different. Both Dp and Df vaccine concentrations were within acceptable levels (as indicated) by the CBER, US-FDA.[24]

False positives for the serum albumin negative control were found in 2 adult volunteers. This is probably because of individual skin reactions to pressure with dermographism. Contamination from allergen was not possible since the needles used in negative control were new needles which had not been used for SPTs. This study showed that 17/54 (31.48 %) of adult volunteers who had positive SPT to Dp and Df had no allergic disease. So, a negative history of allergy does not exclude the possibility of mite-sensitivity. Our previous study confirmed that 35.29 % of non atopic adults have positive SPT reaction to Dp and Df vaccines, both from the SMAV and the commercial vaccines.[20] Negative results for specific IgE to Dp and Df were found in 9 and 16 volunteers, while the negative result for SPTs for the SMAV Dp and Df were found in 4 and 2 cases respectively. These findings suggested that SPT had higher sensitivity than specific IgE measured by ImmunoCAP.

The mean wheal diameter of the SMAV Dp was larger than those of the commercial Dp at all 3 concentrations but it was significantly different only at the concentration of 2,500 AU/ml. This might be explained by the slightly higher antigen level of the SMAV Dp (8,267.1 AU), as compared with that of the commercial one (7,663.8 AU). The mean wheal diameter of the SMAV Df was slightly lower than that of the

commercial Df while the antigen level of the SMAV Df (8,392.6 AU) was slightly lower than the commercial one (9,574.3 AU). The 90 % confidence intervals, upper and lower bound of 2.5 mm were used to compare equivalency of the efficacy of both vaccines. The mean wheal diameters of the SMAV Dp and the commercial Dp at the concentration of 10,000 and 5, 000 AU/ml and those of the SMAV Df and the commercial Df at all 3 concentration were equivalent.

All of the volunteers with positive SPTs to Dp and Df at concentration of 10,000 AU also had positive SPTs to Dp and Df at concentration of 5,000 and 2,500 AU. The wheal sizes for Dp SPTs at concentration 10,000AU were larger than those for 5,000 and 2,500 AU. The average wheal size for SPTs, both SMAV and commercial Dp and Df at all concentrations, was more than 7 mm. These findings suggested that the concentration of the extract could be lower than the concentration which had been used.

In this study, only local adverse effects, such as swelling, erythema or itching on the tested areas, were found in all patients who had positive skin test. This is, in fact, the positive reaction of the test, not a side effect. The largest mean wheal diameter was 25 mm which was not categorized as a large local reaction. These reactions were mild. They improved after one dose of second-generation antihistamine and disappeared within 2 days after the procedure. There was no systemic reaction in all subjects.

Skin prick testing is inexpensive, rapid to accomplish and has been shown to carry negligible systemic risk. A National Health and Nutritional Examination Survey in the United States for the period 1976 – 1980 (NHANES II) demonstrated clearly that allergy skin testing in large number of population did not cause any systemic reactions, such as itching of the skin beyond the tested area, eyes, nose and throat, rhinorrhea, nasal congestion, tightness in the chest or throat, difficult breathing, syncope, hypotension, nausea, vomiting or anaphylaxis.[27] Recently, a large survey of skin testing and immunotherapy from the US showed that there was only one patient who died of SPT in a 12-year period.[28] This particular patient had unstable asthma and was tested against a wide range of food allergens (over 90 allergens applied). Such practice is not common in allergy practices. A previous study in Thailand clearly demonstrated that, among 5,879 patients who underwent 82,306 SPTs in the ENT allergy clinic of Siriraj Hospital, there were no systemic reaction observed.[29]

Our results show that the *in-vivo* allergenic potency of the SMAV, in mite-sensitive adult volunteers, was equivalent to commercially available standardized mite allergen vaccine. We propose that the SMAV can be used for diagnosis of allergic patients. This will certainly reduce the cost of importing allergen vaccines and make it more accessible for most patients.

In summary, the study demonstrated that in mite-sensitive adults, SPT using SMAV Dp (10,000 AU) and Df (10,000 AU) had equivalent allergenic potency to the commercial comparator without any systemic side effects.

Acknowledgement

This study was supported by Mahidol University, Bangkok, Thailand.

References

1. The International Study of Asthma and Allergy in Childhood (ISAAC) steering committee. Worldwide variations in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet. 1998; 351: 1225-32.
2. Vichyanond P, Jirapongsananuruk O, Visitsunthorn N, Tuchinda M. Prevalence of asthma, rhinitis and eczema in children from the Bangkok area using the ISAAC (International Study for Asthma and Allergy in Childhood) questionnaires. J Med Assoc Thai. 1998; 81: 175-84.
3. Trakultivakorn M, Sangsupawanich P, Vichyanond P. Time trends of the prevalence of asthma, rhinitis and eczema in Thai children-ISAAC Phase Three. J asthma. 2007; 44; 609-11.
4. Vichyanond P, Sunthornchart S, Singhirannusorn V, Ruangrat S, Kaewsomboon S, Visitsunthorn N. Prevalence of asthma, allergic rhinitis and eczema among university students in Bangkok. Respir Med. 2002; 96: 34-8.
5. Bunnag C, Kongpatanakul S, Jaroencharsri P, Voraprayoon S, Supatchaipisit P. A survey of allergic diseases in university students in Bangkok, Thailand. J Rhinol. 1997; 4: 90-3.
6. Ray NF, Baraniuk JN, Thamer M, Rinehart CS, Gergen PJ, Kaliner M, et al. Direct expenditures for the treatment of allergic rhinoconjunctivitis in 1996, including the contributions of related airway illnesses. J Allergy Clin Immunol. 1999; 103: 401-7.
7. Ray NF, Baraniuk JN, Thamer M, Rinehart CS, Gergen PJ, Kaliner M, et al. Healthcare expenditures for sinusitis in 1996; Contributions of asthma, rhinitis, and other airway disorders. J Allergy Clin Immunol. 1999; 103: 408-14.
8. Wongsathuaythong S. House mites and allergic bronchial asthma. J Med Assoc Thai. 1971; 54: 411-3.
9. Kraisarin C, Direkwattanachai C, Benjaponpitak S, Sasisakulporn C. Aeroallergen Sensitivity in Thai asthmatic children. Siriraj Hosp Gaz. 1997; 49: 449-53.
10. Kongpanichkul A, Vichyanond P, Tuchinda M. Allergen skin test reactivities among asthmatic Thai children. J Med Assoc Thai. 1997; 80: 69-75.
11. Daengsuwan T, Lee BW, Visitsunthorn N, Charoenratanakul S, Ruangruk S, Jirapongsananuruk O, et al. Allergen sensitization to aeroallergens including *Blomia tropicalis* among adult and childhood asthmatics in Thailand. Asian Pac J Allergy Immunol. 2003; 21: 199-204.
12. Peat JK, Salome CM, Woolcock AJ. Longitudinal changes in atopy during a 4-year period: relation to bronchial hyperresponsiveness and

respiratory symptoms in a population sample of Australian school children. J Allergy Clin Immunol. 1990; 85: 65-74.
13. Wong GW, Li ST, Hui DS, Fok TF, Zhong NS, Chen YZ, et al. Individual allergens as risk factors for asthma and bronchial hyperresponsiveness in Chinese children. Eur Respir J. 2002; 19: 288-93.
14. Chew FT, Lim SH, Goh DY, Lee BW. Sensitization to local dust-mite fauna in Singapore. Allergy. 1999; 54: 1150-9.
15. Sam CK, Soon SC, Liam CK, Padmaja K, Cheng HM. An investigation of aeroallergens affecting urban Malaysian asthmatics. Asian Pac J Allergy Immunol. 1998; 16: 17-20.
16. Jeong KY, Jin HS, Oh SH, Hong CS, Lee IY, Ree HI, et al. Monoclonal antibodies to recombinant Der f 2 and development of a two-site ELISA sensitive to major Der f 2 isoallergen in Korea. Allergy. 2002; 57: 29-34.
17. Baratawidjaja IR, Baratawidjaja PP, Darwis A, Soo-Hwee L, Fook-Tim C, Bee-Wah L, et al. Prevalence of allergic sensitization to regional inhalants among allergic patients in Jakarta, Indonesia. Asian Pac J Allergy Immunol. 1999; 17: 9-12.
18. Master formula of the Siriraj Mite Allergen Vaccine. 2004
19. Adinoff AD, Rosloniec DM, McCall LL, Nelson HS. Immediate skin test reactivity to food and drug administration-approved standardized extracts. J Allergy Clin Immunol. 1990; 86: 766-74.
20. Visitsunthorn N, Pacharn P, Jirapongsananuruk O, Weeravejsukit S, Sripramong C, Sookrung N, et al. Comparison between Siriraj mite allergen vaccine and standardized commercial mite vaccine by skin prick testing in normal Thai adults. Asian Pac J Allergy Immunol. 2010; 28: 41-5.
21. Vichyanond P, Nelson HS. Circadian variation of skin reactivity and allergy skin tests. J Allergy Clin Immunol. 1989; 83: 1101-6.
22. Nelson HS. Diagnostic procedures in allergy I. Allergy skin testing. Ann Allergy. 1983; 51: 411-8.
23. Peat JK, Tovey E, Toelle BG, Haby MM, Gray EJ, Mahmic A, et al. House dust mite allergens. A major risk factor for childhood asthma in Australia. Am J Respir Crit Care Med. 1996; 153: 141-6.
24. US Food and Drug Administration. Center for Biologics Evaluation and Research. Allergenic publication. http://www.fda.gov/cber/allergenics/ allpubs.htm. Last accessed May 15, 2007.
25. Witteman AM, Stapel SO, Perdok GJ, Sjamsoedin DH, Jansen HM, Aalberse RC, et al. The relationship between RAST and skin test results in patients with asthma or rhinitis: a quantitative study with purified major allergens.J Allergy Clin Immunol. 1996; 97: 16-25.
26. Viriyataveekul R,Vichyanond P. Comparison of four types of needles used in allergy skin testings. Siriraj Hosp Gaz. 1996; 47: 110-4.
27. Turkeltaub PC, Gergen PJ. The risk of adverse reactions from percutaneous prick-puncture allergen skin testing, venipuncture, and body measurements: data from the second National Health and Nutrition Examination Survey 1976-80 (NHANES II). J Allergy Clin Immunol. 1989; 84: 886-90.
28. Bernstein DI, Wanner M, Borish L, Liss GM. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990-2001. J Allergy Clin Immunol. 2004; 113: 1129-36.
29. Bunnag C, Jaroencharsri P, Tunsuriyawong P, Assanasen P, Voraprayoon S, Dachpunpour P, et al. Adverse reactions to allergen injection: the Siriraj experience. Siriraj Hosp Gaz. 2002; 54: 517-24.