

# Comparison of the efficacy and safety of pollen allergen extracts using skin prick testing and serum specific IgE as references.

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## Abstract

**Background:** Allergen extracts may be different due to the difference in dissemination of allergen-containing species in various geographical areas. Therefore, we wish to develop our own extracts to ensure the precision and quality of diagnosis.

**Objectives:** To compare the efficacy and safety of our locally prepared pollen allergen extracts to imported ones, using skin prick testing (SPT) and serum specific IgE (sIgE) as references.

**Methods:** This prospective, randomized, double-blinded, self-controlled study was performed in respiratory allergic adult volunteers who are sensitized to at least one kind of pollen. Each subject was pricked with our Bermuda grass, Johnson grass and careless weed pollen allergen extracts, and also with the imported ones. sIgE levels were measured by using ImmunoCAP®.

**Results:** In 68 volunteers, our Bermuda, Johnson and careless weed extracts showed 91.2%, 45.6% and 54.4% positive SPTs, respectively, while for the imported ones 73.5%, 45.6% and 54.4% SPTs were positive, respectively. No adverse reaction was found in all procedures. The concentration of 10,000 BAU/mL of Bermuda grass, 1:20 w/v or 10,000 PNU/mL of Johnson grass and 1:40 w/v or 10,000 PNU/mL of careless weed yielded the most positive SPT results. There was no significant difference in mean wheal diameter (MWD) yielded from using local and imported extracts. Significant correlation was found between MWDs of imported pollen extracts and serum sIgE levels ( $p < 0.01$ ).

**Conclusion:** No significant difference between SPT results of local and imported pollen allergen extracts was found. Significant correlation was found between MWDs of imported pollen extract SPT and serum sIgE levels.

**Keywords:** Local allergen extract, skin prick test, specific IgE, grass pollen, weed pollen

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## Introduction

The prevalence of respiratory allergy in Thailand has been increasing and the Thai population is classified as a moderately high prevalence group by the International Study of Asthma and Allergy in Childhood (ISAAC).<sup>1</sup> The prevalence of asthma is 15% in Thai children<sup>2</sup> and 10% in adults.<sup>3</sup> The prevalence of allergic rhinitis is 32.6% in children aged 6-7 years, 43.4% in children aged 13-14 years<sup>4</sup> and 26% in adults.<sup>5</sup>

The frequency of sensitization in Thai patients, aged 10-59 years, to dust mites [*Dermatophagoides pteronyssinus* (*Dp*) and

*Dermatophagoides farina* (*Df*)] is 76-79%, American cockroach 60%, cat 29%, dog 28%, mold 26%, Johnson grass 21%, Bermuda grass 17% and weed 16%.<sup>6</sup> Another study looking at allergic sensitization in 736 Thai adult patients in 2002-2004 revealed that house dust mite was the most common causative allergen (64.7%) followed by Bermuda grass (52.3%), cockroach (49.8%), para grass (49.4%), sedge (45.9%), careless weed (45.4%), dog (44.2%), cat (39.3%) and *Cladosporium* (38%).<sup>7</sup> Pollens are the most common outdoor allergens that cause sensitization.<sup>7,8</sup>

Pollen allergy is very common in many parts of the world, but is believed to be less common in East Asia, Latin America and tropical areas.<sup>8</sup> The specific pollens responsible for sensitization are different geographically because their nature and number vary with location, temperature and climate.<sup>9</sup> Recent studies have established an association between hospital admissions for asthma and airborne grass pollen allergen levels<sup>10</sup> as well as a causal relationship between grass pollen challenge and asthma exacerbation.<sup>11</sup>

The skin prick test (SPT) is approved as a valid tool for evaluating allergic sensitization in atopic diseases. SPT is simple, accurate, safe and inexpensive compared to specific IgE (sIgE) measurement.<sup>12</sup> The incidence of adverse reaction from SPT is no more than 0.04 %<sup>13</sup> and the reaction is usually mild. Severe side effects from SPT are very rare.<sup>14</sup> No fatal case has been reported from the utilization of aeroallergen SPT.<sup>12-15</sup> A previous study in 5,879 Thai patients revealed no adverse systemic reactions from 82,306 SPTs.<sup>16</sup>

In Thailand, imported allergen extracts are expensive and not always available so our group has spent some time studying and developing the process of making pollen allergen extracts in our GLP laboratory with the hope that, if the developed allergen extracts are comparable to the imported ones, we can have cheaper and readily available extracts and will be able to produce extracts from pollens which are only found in the Asian region.

Therefore, the aim of this study was to verify whether pollen allergen extracts prepared by our method yield the same efficacy and safety as extracts imported from the West. The SPT and serum sIgE were used to compare the mentioned parameters of the extracts.

## Methods

### Preparation of our pollen allergen extracts<sup>17</sup>

Pollen from Bermuda grass (*Cynodon dactylon*), Johnson grass (*Sorghum halepense*) and careless weed (*Amaranthus hybridus*) grown naturally near Bangkok were collected and identified by a qualified botanist. In order to be used as a source material for making allergen extract, each type of pollen has to be at least 99% pure. The extraction process developed by researchers at Mahidol University has been patented by the Thai authority. The in-house extracts were prepared according to the international immunotherapy preparation guidelines rules and regulation advice on the formulation of allergenic extracts with international standards.<sup>18</sup> This is a step to further confirming research before entering the clinic. Our pollen allergen extracts used in this study were subsequently produced by Greater Pharma Company Limited, a GMP certified manufacturing company in Bangkok, Thailand.

The imported pollen allergen extracts (Bermuda grass, Johnson grass and careless weed) were purchased from ALK Laboratories, Port Washington, New York, USA. The concentration of our Bermuda grass is the same as the imported one (10,000 BAU/mL). The concentration of the imported Johnson grass and careless weed pollen allergen extracts are 1:20 w/v and 1:40 w/v, respectively, while the concentration of our Johnson grass and careless weed pollen allergen extracts is 10,000 PNU/mL which is very close to the concentration of

imported extract.

### Subjects

This double-blinded, self-controlled study was approved by the Institutional Ethics Committee of Siriraj Hospital, Mahidol University. The study was performed in volunteers with respiratory allergy aged 18–60 years who had a positive SPT to at least one kind of pollen (Bermuda grass, Johnson grass or careless weed). Subjects with acute asthma exacerbation, severe skin diseases, chronic disease (such as autoimmune disease, immune deficiency and cancer) or who were pregnant were excluded. The use of antihistamines, systemic corticosteroids ( $\geq 20$  mg/day) and topical corticosteroids was discontinued for at least 7 days before testing. Written informed consent was obtained from all volunteers. The study was registered with ClinicalTrials.gov (NCT02764827).

### Skin prick test

A blood lancet (Vitrex® steel, Vitrex Medical A/S, Herlev, Denmark) was used for SPT in this study. SPT was performed on the upper back of subjects by an experienced technician in a room fully equipped with resuscitation equipment. Subjects were pricked with different concentrations of imported and local Bermuda grass, Johnson grass and careless weed pollen allergen extracts (1:1, 1:3.3, 1:10 and 1:33.3 dilution). Histamine dihydrochloride (10 mg base/mL) and sterile glycerinated saline were used as positive and negative controls, respectively. Wheals and flares induced were recorded 10 min after histamine testing and 15 min after allergen extract testing. Mean wheal diameters (MWDs, the longest diameter plus the perpendicular diameter and then divided by 2) were calculated. A wheal diameter at least 3 mm larger than the negative control was considered positive. All of the subjects were observed for at least 30 min after SPT for adverse reactions. If the MWD was more than 10 mm, the subject received low sedating antihistamine immediately and the observation time was increased to 2 h or until MWD decreased. Serum sIgE levels of Bermuda grass, Johnson grass and careless weed were measured using ImmunoCAP® (UniCAP 250, Instrument Pharmacia Diagnostic AB, Uppsala, Sweden). The sIgE result was interpreted as positive when the level was  $\geq 0.35$  kUA/L.

### Statistical analysis

The data were analyzed using SPSS software version 18 (SPSS Inc., Chicago, IL, USA). Characteristics data are presented as the median (range) for continuous data or number and percentage for categorical data. Agreement between SPT results of local and imported allergen extracts was evaluated using kappa and intraclass correlation.<sup>19</sup> Correlation between the sIgE and MWD of allergen extracts was evaluated using Spearman's rho correlation. A p-value of  $< 0.05$  was considered statistically significant.

## Results

Sixty-eight cases with a positive SPT to at least one kind of imported pollen extract were included in the study. The mean age was 23 (18–60) years. Females were predominant (64.7%).

Allergic rhinitis was found in 91.2% of the cases as shown in **Table 1**.

**Table 1. Characteristic of study population (N = 68)**

Characters	n (%)
Mean age (minimum-maximum) years	23 (18-60)
Sex	
Male	24 (35.3)
Female	44 (64.7)
Diagnosis	
Asthma	18 (26.5)
Asthma alone	1 (1.5)
Asthma + AR*	16 (23.5)
Asthma +AR+ Urticaria	1 (1.47)
Allergic rhinitis	62 (91.2)
AR alone	36 (52.9)
Asthma + AR	16 (23.5)
AR + Atopic dermatitis + Urticaria	2 (2.9)
AR + Urticaria	8 (11.8)
Atopic dermatitis	3 (4.4)
Urticaria	11 (16.2)
Food allergy	15 ( 22.1)
Drug allergy	12 ( 17.6)
Smoking	1 (1.5)
Alcoholic drinking	3 (4.4)

\*AR = Allergic rhinitis

The SPT results to different concentrations of local and imported pollen allergen extracts are shown in **Table 2**. The higher the concentration of pollen allergen extract, the

more positive SPT results were observed. The concentration of both our extracts and imported extracts that yielded the significantly highest positive numbers of SPT results was undiluted concentrations, 10,000 BAU/mL of Bermuda grass, 10,000 PNU/mL of our Johnson grass and careless weed and 1:20 w/v of imported Johnson grass and 1:40 w/v of careless weed pollen allergen extracts. No adverse reaction was found in all subjects.

At the highest SPT positive concentration (undiluted concentrations), our Bermuda grass pollen allergen extract showed more cases with a positive SPT than the imported one, 91.2% vs. 73.5%. In contrast, the number of cases with a positive SPT for careless weed pollen allergen extract was higher for the imported allergen extract than our extract, 45.6% vs. 36.8%. The positive SPT results for Johnson grass were equal between local and imported allergen extracts, 54.4%.

However, the MWDs of local and imported pollen allergen extracts were not statistically significantly different. Slight agreement was found between MWDs from SPTs of local and imported Bermuda grass ( $\kappa = 0.153$ ) while fair agreement was found between MWDs from SPTs between local and imported Johnson grass and careless weed ( $\kappa = 0.407$  and  $0.337$ , respectively) as shown in **Table 3**. The correlation coefficients between local and imported Bermuda grass, Johnson grass and careless weed pollen allergen extracts were very low (0.042, 0.059 and 0.048, respectively) while the ICCs showed poor to fair agreement (0.173, 0.001 and 0.309, respectively).

Concerning serum sIgE, the mean  $\pm$  SD sIgE levels for Bermuda grass, Johnson grass and careless weed were  $3.26 \pm 6.19$  (maximum level 35.10),  $3.06 \pm 7.15$  (max 42.50) and

**Table 2. Positive skin prick test to our extracts and imported extracts of Bermuda grass, Johnson grass and careless weed pollen**

Diluted concentration*	Positive skin prick test n (%)					
	Bermuda		Johnson		Careless weed	
	Our	Imported	Our	Imported	Our	Imported
Undiluted	62 (91.2)	50 (73.5)	37 (54.4)	37 (54.4)	25 (36.8)	31 (45.6)
Diluted 1:3.3	55 (80.9)	39 (57.4)	22 (32.4)	26 (38.2)	14 (20.6)	15 (22.1)
Diluted 1:10	45 (66.2)	30 (41.1)	14 (20.6)	18 (26.5)	9 (13.2)	8 (11.8)
Diluted 1:33	30 (44.1)	24 (35.3)	9 (13.2)	16 (23.5)	8 (11.8)	4 (5.9)

\*Undiluted concentrations; Imported and our Bermuda grass: 10,000 BAU/mL; Imported Johnson grass 1:20 w/v; our Johnson grass 10,000 PNU/mL; Imported Careless weed 1:40 w/v, our Careless weed 10,000 PNU/mL

**Table 3. Correlation between skin prick test results of our extracts and imported extracts of Bermuda grass, Johnson grass and careless weed pollen at undiluted concentrations**

	Positive skin prick test number (%)		Mean wheal diameter mm. (maximum)		kappa	Correlation coefficient	ICC
	Our	Imported	Our	Imported			
Bermuda	62 (91.2)	50 (73.5)	4 (18)	3 (17)	0.153	0.042	0.173
Johnson	37 (54.4)	37 (54.4)	3 (19)	3 (15)	0.407	0.059	0.001
Careless weed	25 (36.8)	31 (45.6)	3 (16)	3 (23)	0.337	0.048	0.309

ICC, intraclass correlation

**Table 4. Comparison of positive results of serum specific IgE and skin prick test with our extracts and imported extracts of Bermuda grass, Johnson grass and careless weed pollen at undiluted concentrations**

		SPT + sIgE +	SPT + sIgE -	SPT - sIgE +	SPT - sIgE -	Concordance (%)	P
Bermuda:	Our	37	25	5	1	55.9	0.395
	Imported	36	14	6	12	70.6	<b>0.004</b>
Johnson:	Our	21	16	10	21	61.8	0.430
	Imported	27	10	4	27	79.4	<b>&lt;0.001</b>
Careless weed:	Our	13	12	16	27	58.8	0.234
	Imported	20	11	9	28	70.6	<b>0.001</b>

SPT, Skin prick test; sIgE, serum specific IgE

1.08 ± 2.33 (max 12.10) kUA/L, respectively. When comparing sIgE levels to the MWD of local and imported pollen allergen extracts, the correlation coefficient for Bermuda grass was negative in our extract (-0.083) and good in the imported extract (0.568). The correlation coefficients for Johnson grass and careless weed were fair in local extracts (0.423) and good in imported extracts (0.660).

When comparing the positive and negative results of SPT with sIgE (Table 4), we found that the overall concordance was more than 50% and the highest concordance (79.4%) was found between positive results of sIgE and SPT with imported Johnson grass. A significant correlation,  $p < 0.01$ , was found between positive sIgE and positive SPT results of all imported pollen extracts.

## Discussion

The concentrations of local and imported pollen allergen extracts that yielded the highest positive number of SPT results were the undiluted extracts: 10,000 BAU/mL of Bermuda grass, 1:20 w/v or 10,000 PNU of Johnson grass and 1:40 w/v or 10,000 PNU of careless weed.

The number of cases with a positive SPT to our Bermuda grass pollen allergen extract was higher than to the imported one although the species of Bermuda grass are the same. The difference may be due to the dissimilarity of antigenicity of plant species grown in different geographical areas, the preparation methods or the longer storage time of the imported extract before reaching the customer.

In contrast, the number of cases with a positive SPT to careless weed pollen allergen extract was higher in the imported allergen extract than in our extract. This may be due to the different species of careless weed used for extract production. The careless weed used in the imported allergen extract is *Amaranthus palmeri* while the one used in our extract is *Amaranthus hybridus* since it is a common careless weed in Thailand.<sup>20,21</sup> The species used for preparation of imported allergen extracts and sIgE measurement of Bermuda grass, Johnson grass and careless weed were the same (*Cynodon dactylon*, *Sorghum halepense* and *Amaranthus palmeri*, respectively). This may be a reason why the concordance between the sIgE and SPT results of our careless weed extract was lower than that of the imported one. The case between the two *Amaranthus* species makes it possible to postulate that the two species have different allergenic proteins. This postulation

needs further investigation.

Serum sIgE is not a gold standard in the diagnosis of allergen sensitization since it does not provide a strong correlation with clinical manifestations.<sup>22</sup> Skin tests remain the most sensitive and specific test available compared to physician clinical assessment.<sup>22</sup> Nasal challenge is the gold standard used to compare in vivo and in vitro methods. However, nasal challenge is difficult to perform and there is no consensus yet regarding the criteria for a positive test. Therefore, in this study serum sIgE was used as a reference to compare SPT results from different extract preparations.

In Western countries, pollens are present in the air only in certain seasons. In contrast, in a tropical climate, such as the one in Thailand, which consists of three seasons, i.e. summer (from March to June), rainy season (from July to October) and winter (from November to February), pollens are present in the air all year round with the greatest frequency observed during the winter months.<sup>23</sup> The most recent aeroallergen survey in five areas of Bangkok (2012–2013) showed that the peak of the pollen season is in September and the most common types of pollen identifiable were grass (< 40 µm), Typhaceae, sedge, grass (> 40 µm) and Amaranthaceae.<sup>21</sup>

In Thai patients, Bermuda grass is the most common pollen allergen sensitization (54.9%).<sup>7,24</sup> This is the same as the study of 628 patients in Mexico in which the most common sensitizing pollen allergen was Bermuda grass (26%).<sup>25</sup> In contrast, studies in South Korea demonstrated that Timothy grass is more common than Bermuda grass<sup>26</sup> and in Taiwan, sensitization to Johnson grass is more prevalent than Bermuda grass.<sup>27</sup>

Of note is that Timothy grass is not found in Thailand while para grass and *Typha* which are common in our country are not found in the West. Therefore, we cannot find imported allergen extracts for para grass and *Typha* to use in our allergy practice. This may also be true for other kinds of pollen which grow specifically in Asia. From this study, we feel that our developed extraction process is acceptable and will be beneficial for our patients with respiratory allergy because we will be able to produce all kinds of pollen allergen extracts which are the significant cause of their symptoms.

Some drawbacks in this study are 1) the absence of the nasal provocation test which may be a more accurate reference than sIgE and 2) the allergenic composition of the pollen is not included. The allergenic composition of some pollens is being studied in our center and will be subsequently reported.

## Conclusion

The SPT results for our pollen allergen extracts and imported pollen allergen extracts showed no significant difference, with low agreement and correlation. Significant correlation was found between the MWD of imported pollens extracts and serum sIgE levels.

## Acknowledgements

The authors would like to thank Mr. Suthipol Udompunturak for statistical analysis and Wisuwat Songnuan, Kitipong Soontrapa, Chaiphichit Phayangkhe and Koravit Somkid for pollen allergen extract preparation. This study was supported by the Research Development Fund and a Chalermprakait grant from the Faculty of Medicine, Siriraj Hospital, Mahidol University, Thailand and by an NSTDA Chair Professor grant (P-1450624) funded by the Crown Property Bureau.

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