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Prick and intradermal skin tests in patients with severe hymenoptera sting allergy using commercial versus in-house allergen extracts

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Abstract

Background: Fire ant, honey bee, and wasp allergen extracts are useful in the diagnosis and treatment of severe Hymenoptera allergic patients.

Objective: To evaluate the result of skin prick test (SPT) and intradermal test (ID) compared between local and commercial insect allergen extracts in patients with severe Hymenoptera sting allergy.

Methods: SPT and ID using local and commercial insect allergen extracts were performed. Specific IgE (sIgE) to honey bee, wasp, and fire ant; component-resolved diagnosis (CRD); (rApi m1, rApi m2, rApi m3, rApi m5, rApi m10, rVes v5, rPol d5, and rVes v1); and, cross-reactive carbohydrate determinant (CCD) were performed.

Results: Twenty-seven patients were included. Twenty-five had anaphylaxis, and 2 had severe systemic skin reaction. Positive skin test (SPT and/or ID) result from local and commercial allergen extracts was 74% *vs.* 67% for fire ant, 48% *vs.* 59% for honey bee, and 52% *vs.* 74% for yellowjacket. Local and commercial allergen extracts showed substantial agreement for fire ant (k = 0.647, p = 0.001) and honey bee (k = 0.632, p = 0.001), and moderate agreement for wasp (k = 0.547, p = 0.001). When compared with sIgE subtracted with CCD and/or CRD, skin test results of local fire ant allergen extract showed higher sensitivity (87% *vs.* 67%), specificity (42% *vs.* 33%), and accuracy (67% *vs.* 52%) than commercial extract. Commercial honey bee and wasp showed higher sensitivity (62% *vs.* 50%, 85% *vs.* 65%) and accuracy (63% *vs.* 52%, 78% *vs.* 70%), respectively.

Conclusion: SPT and ID with local or commercial insect venoms could help in confirming and/or identifying the causative insects.

Key words: Allergen extracts, Anaphylaxis, Component-resolved diagnosis, Insect allergy, Specific IgE to venom

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Abbreviations/acronyms

SPT	skin prick test
ID	intradermal test
sIgE	specific IgE
CRD	component-resolved diagnosis
CCD	cross-reactive carbohydrate determinant

Introduction

Severe allergic reaction from Hymenoptera insect sting is not uncommon. A study from Thailand found that 7.7% of anaphylaxis cases in the emergency room were caused by insect sting allergy.[1] A retrospective study conducted at Siriraj Hospital (Bangkok, Thailand) reported that 11% of anaphylaxis cases were suspected to be insect-related.[2] Determination of the causative insect is important for preventing future stings, and for treatment with immunotherapy. Sting history and clinical manifestations are useful in the diagnosis of Hymenoptera sting allergy, but identification of the causative insect is often quite difficult. To confirm the causative insect in severe allergic cases, although sting challenge tests considered being the gold standard in the diagnosis. However, it is not recommended as a routine diagnostic method, thus skin tests (skin prick test [SPT] and intradermal test [ID]) and/or the determination of specific immunoglobulin E (sIgE) to honey bee, wasp, hornet, and fire ant venoms should be performed.[3,4]

SPT and ID with insect venoms and/or quantification of sIgE to insect venoms and component-resolved diagnosis (CRD) are useful methods for identifying the causative insect.[5] Skin test is easier, less expensive, and more sensitive[6] than sIgE (RAST), but it cannot be performed shortly after severe allergic reaction, in patients currently taking antihistamines, or in patients with extensive skin lesions. Commercial allergen extracts are periodically unavailable. However, honey bee, common wasp, white-faced hornet, yellow hornet, and yellow-jacket venom extracts and fire ant whole body extract were commercially available at the time this study was conducted. These extracts are used not only for the diagnosis via skin testing, but also for specific treatment with immunotherapy. The aforementioned periodic lack of availability of commercially available allergen extracts can prevent diagnosis by skin test, and can interrupt the continuity of immunotherapy treatment. Improvement in the availability of venom extracts in our region is urgently needed.

To improve the availability of these allergen extracts, we developed extracts from the common stinging insect species in Thailand,[7-9] including honey bee venom extract from *Apis dorsata*, wasp venom extract from *Vespa affinis*, and fire ant whole body extract from *Solenopsis geminata*. The purpose of developing these extracts is to ensure a reliable source for extracts in case commercial extracts are unavailable, and to reduce the cost of these extracts so that patients in Thailand and other countries in the region will have access to diagnosis of and treatment for insect allergy. However, the developed extracts must be tested both *in vitro* and *in vivo* to evaluate their efficacy and safety. After *in vitro* testing for safety and efficacy, *in vivo* evaluation must be performed to compare our locally-produced extracts with commercially available insect allergen extracts.

The purpose of this study was to evaluate the result of SPT and ID compared between local and commercial insect allergen extracts in patients with severe hymenoptera sting allergy, and to compare the results of SPT and ID of both local and commercial allergen extracts to the result of CRD.

Materials and Methods

The research protocol was approved by the Institutional Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (COA no. 450/2559 [EC3]). This study was registered with ClinicalTrials.gov (NCT03645291). Written informed consent from parents or guardians and assent from children older than 7 years of age were obtained.

Preparation of local insect allergen extracts

Wasps (*Vespa affinis*) were collected from Nakhon Ratchasima province, which is located in lower Northeastern Thailand. Honey bees (*Apis dorsata*) and fire ants (*Solenopsis geminata*) were collected from Chiang Mai province, which is located in central Northern Thailand. Adult insects were verified by an entomologist. The freshly collected mature insects were kept frozen until allergen extract processing.

For fire ant whole body extraction, fire ants were ground by mortar and pestle, homogenized by sonication in PBS with 0.8% phenol, and then centrifuged. The supernatant was collected, filled with glycerol 1:2 v/v (50% final concentration), and filtered with a 0.2 micron membrane filter. The fire ant whole body extract was maintained at 2-8°C until use.

For wasp and bee venom extraction, the venom sac was removed from each insect using forceps and scissors, after which it was washed with phosphate buffered saline (PBS) pH 7.4 and then placed into a small volume of buffer with 0.8% phenol. The venom was collected from each sac, pooled, and centrifuged at 12,000 × g for 20 minutes at 4°C. The supernatant was collected and the protein concentration was measured. The venom solution was diluted to 200 µg/ml with PBS containing 0.8% phenol, filled with glycerol 1:2 v/v (50% final concentration), and filtered using a membrane filter (pore size 0.2 µm). Wasp and bee venom extracts were maintained at 2-8°C until use.

Individual batch of the extracts was determined protein quantity and quality by Bradford assay and SDS-PAGE, respectively. Protein content of the extracts in each batch was standardized according to the commercial extract. Sterility testing was determined according to the medical device standards. In addition, skin testing to all local insect allergen extracts were performed in ten healthy control subjects and the results were all negative.

Studied population

This study in patients with severe allergic and anaphylactic reactions to stinging insects was performed at the Division of Allergy and Clinical Immunology, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during the June 2018 to May 2019 study period. Severe allergic reaction was defined as a generalized reaction that could be life-threatening, such as generalized urticaria with angioedema. Anaphylaxis was defined as an acute severe reaction involving more than 1 anatomical system, including cutaneous, respiratory, circulatory, and/or gastrointestinal components.[10]

Table 1. Source and concentration of local and commercial insect allergen extracts.

Allergenic Extract	Local		Commercial	
	Source	Concentration	Source	Concentration
Whole body fire ant	*Solenopsis geminata*	1:10 w/v	*Solenopsis invicta*	1:100 w/v**
Honey bee venom	*Apis dorsata*	100 µg/ml	*Apis mellifera*	120 µg venom protein/1.2 ml*
Yellow jacket venom	*Vespa affinis*	100 µg/ml	*Vespula vulgaris*	120 µg venom protein/1.2 ml*

* Jubilant HollisterStier LLC
** ALK-Abello, Inc.

Study methods

Patient sting history was collected and recorded. SPT with local and commercial insect allergen extracts was performed in all patients. The commercial insect allergen extracts used in this study were fire ant (*Solenopsis invicta*) whole body extract (ALK-Abello, Port Washington, NY, USA), honey bee (*Apis mellifera*), wasp (*Polistes fuscatus*), white-faced hornet (*Dolichovespula maculate*), yellow hornet (*Dolichovespula arenaria*) and yellowjacket (*Vespula vulgaris*) venom extracts (Jubilant all 5 from HollisterStier LLC, Spokane, WA, USA). Comparison of the source and concentration of local and commercial insect allergen extracts is shown in **Table 1**. Skin prick tests were performed by applying one drop of 1 µg/mL venom extract to the forearm, and then pricking the skin through the surface of the drop with a sterile lancet. Skin response was assessed after approximately 15-20 minutes. In patients who had negative SPT to 1 µg/mL concentration of insect allergen extract, ID for that insect allergen extract was performed starting at a concentration of 0.001 µg/mL. A 1 mL tuberculin syringe with a short 27-gauge needle was used to deliver a volume of 0.05 mL for intradermal testing. The needle was introduced into the superficial layers of the skin, bevel down, until the bevel was completely buried. A 0.05 mL aliquot of the venom dilution was then slowly injected, which resulted in the development of a small bleb. If a negative skin reaction was obtained within 20 minutes, ID testing was continued using 10-fold increases in allergen extract concentration until a reaction consisting of a 5-10 mm wheal and 11-20 mm erythema was obtained, or until a concentration of 1 µg/mL was tested – whichever occurred first. A patient should be considered sensitive to the test venom when a skin response of 5-10 mm wheal and 11-20 mm erythema or greater occurs at a concentration of 1 µg/mL or less.[11]

Specific IgE levels of fire ant (i70), honey bee (i1), yellowjacket (i3), and paper wasp (i77); component-resolved sIgE: rApi m1 (i208), rApi m2 (i214), rApi m3 (i215), rApi m5 (i216), rApi m10 (i217), rVes v5 (i209), rPol d5 (i210), and rVes v1 (i211); and, cross-reactive carbohydrate determinant (MUXF3 CCD, i214) were measured by ImmunoCap (UniCAP 100; Phadia AB, Uppsala, Sweden). Positive results of sIgE, CRD, and CCD were interpreted when the levels were ≥ 0.35 kUA/L.

Fire ant was concluded to be the causative insect if fire ant sIgE -CCD was > 0.35 kUA/L. Honey bee was concluded to be the causative insect if honey bee sIgE subtracted with CCD (sIgE -CCD) was > 0.35 kUA/L, and/or if any of the honey bee CRDs (rApi m1, m2, m3, m5, or m10) was positive. Wasp was concluded to be the causative insect if wasp sIgE-CCD was > 0.35 kUA/L, and/or if any of the wasp CRDs (rVes v5, rPol d5, or rVes v1) was positive.

Mastocytosis was clinically evaluated according to WHO classification.[12] Baseline serum tryptase was assayed by ImmunoCap to rule out mastocytosis. Serum tryptase level exceeding 20 mcg/L is one of the WHO minor criteria for diagnosing systemic mastocytosis.[12]

A flow diagram of the study protocol is shown in **Figure 1**.

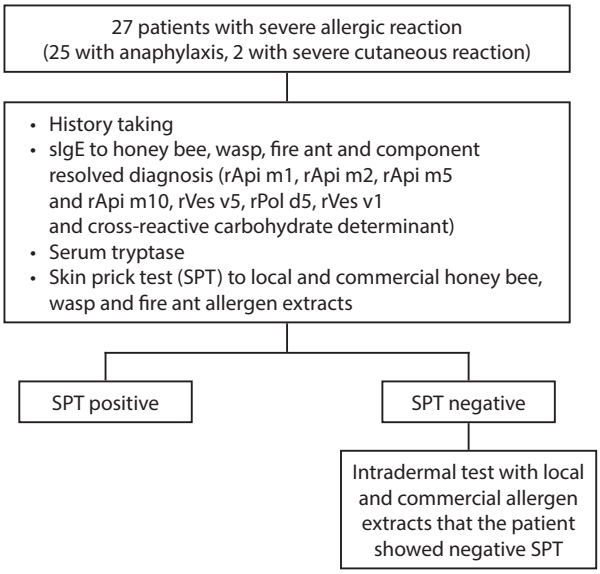

Figure 1. Flow of the participants in the study.

Statistical analysis

Patient demographic and clinical data are presented as number and percentage (%), mean ± standard deviation (SD), or median and range (minimum, maximum), as appropriate. The agreement of positive and negative results between local and commercial allergen extracts was evaluated using Cohen's kappa statistic. The kappa value was categorized as no agreement (< 0.00), none to slight (0.01-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), and almost perfect agreement (0.81-1.00).[13] Sensitivity, specificity, and accuracy were used to evaluate the validity of the results of SPT to local and commercial allergen extracts, using CRD or sIgE-CCD as a reference standard test. Association between history of causative insects and the results of sIgE -CCD or CRD was analyzed by Fisher's exact test. A *p*-value less than 0.05 was considered statistically significant. All statistical analyses were performed using PASW Statistics (SPSS) 18.0 (SPSS, Inc., Chicago, IL, USA).

Results

Twenty-seven patients with anaphylaxis or severe systemic skin reaction to Hymenoptera insects (fire ant, honey bee, wasp, and hornet) were included in this study. The mean age of patients was 13.8 years (range: 5-25). Anaphylaxis was diagnosed in 25 patients. The most common organ involvement in anaphylactic patients was skin (24/25 patients, 96%). Respiratory symptom was the second most common (19/25 patients, 76%) followed by circulatory symptoms (6/25 patients, 24%) and gastrointestinal symptoms (4/25 patients, 16%). Two patients with severe systemic skin reaction suffered from generalized urticaria with severe angioedema. More than 60% of patients developed symptoms within 15 minutes after being stung, and 93% developed symptoms within one hour after being stung. The demographic and clinical characteristics of study patients are presented in **Table 2**. The causative insects by history were fire ant in 12/27 patients (44.4%), honey bee in 9/27 patients (33.3%), and wasp or hornet in 9/27 patients (33.3%) (**Table 3**). The duration from the last sting or bite was 5 years among all patients, and 3 years among 80% of patients. The most common location where the sting occurred was home, followed by school and garden. The most common part of the body to be stung was foot (37.4%), followed by hand or finger (29.6%). Seventy-eight percent of patients were admitted to the hospital at least once due to insect sting or bite.

Among all patients, SPT to local fire ant whole body allergen extract showed 63% positivity, and the commercial extract showed 55% positivity. SPT to local honey bee venom allergen extract showed 22% positivity, and the commercial version showed 33% positivity. SPT to local yellowjacket venom allergen extract showed 33% positivity, whereas commercial common wasp, white-faced hornet, yellow hornet, and yellowjacket showed positive 33%, 44%, 26%, and 44%, respectively.

Table 2. Demographic data of studied population. (n = 27)

Clinical characteristic	n (%)
Mean age (years) ± SD, range	13.8 ± 5.0, 5-25
Sex: Male	16 (59.2%)
Symptoms of insect allergy	
Anaphylaxis	25 (92.6%)
Severe systemic skin reaction	2 (7.4%)
Other allergic diseases*	
Asthma	5 (18.5%)
Allergic rhinitis	13 (48.1%)
Allergic rhino-conjunctivitis	4 (14.8%)
Atopic dermatitis	1 (3.7%)
Drug allergy	3 (11.1%)
Food allergy	5 (18.5%)
Family history of allergy in first degree cousins	10 (37%)
Causative insects by history*	
Fire ants	12 (44.4%)
Bee	9 (33.3%)
Wasp and hornet	9 (33.3%)
No. of stinging (Times)	
1–2	22 (81.5%)
3–4	2 (7.4%)
4–5	2 (7.4%)
> 5	1 (3.7%)
Last stinging before enrollment (Year)	
0–1	8 (29.6%)
> 1–3	14 (51.9%)
> 3–5	5 (18.5%)
Time from stinging to symptom (minutes)	
0–15	18 (66.7%)
> 15–30	3 (11.1%)
> 30–60	4 (14.8%)
> 60	2 (7.4%)
Treatment with epinephrine injection	26 (96.3%)
Prophylaxis with epinephrine auto-injected	25 (92.6%)
Treatment with antihistamine	24 (88.9%)
Treatment with systemic corticosteroid	12 (44.4%)

*Some patients have more than one diagnosis and causative insect by history

Table 3. Comparison of skin test results using commercial and local insect allergen extracts.

No	Age (year)	Atopy family history	Causative insect by history	Bee SPT + ID Commercial	Bee SPT + ID Local	Bee CRD	Wasp SPT+ID Commercial	Wasp SPT + ID Local	Wasp CRD	FA SPT+ID Commercial	FA SPT + ID Local	FA IgE -CCD
1	15	+	F, W	+	+	+	+	+	+	+	+	+
2	11	-	B	+	+	+	+	-	-	-	-	-
3	16	-	F	-	-	+	+	+	+	+	+	+
4	7	-	W (oral)	+	+	+	+	+	+	+	+	+
5	7	+	B	+	-	+	+	+	+	+	-	-
6	17	-	B	+	+	+	+	+	+	+	+	+
7	14	-	F	-	+	+	-	-	+	+	+	-
8	8	-	B	-	-	-	-	-	-	-	+	+
9	11	-	F	-	-	-	-	-	-	-	+	+
10	18	+	W, F	+	+	+	+	+	+	+	+	+
11	19		F	+	+	+	+	+	-	+	+	+
12	9	Unidentified	Not known	-	-	-	-	-	-	-	-	+
13	16	-	B, W	+	+	+	+	+	+	+	+	-
14	13	-	W	+	-	+	+	-	+	+	+	-
15	10	+	W	+	+	+	+	+	+	+	+	+
16	24	+	W	+	+	+	+	+	+	+	+	-
17	11	+	B	+	-	+	+	-	-	-	-	-
18	8	+	F	-	-	+	-	-	+	-	-	+
19	25	-	F	-	-	+	-	-	-	-	-	-
20	15	+	B	+	-	+	+	+	+	+	+	-
21	14	-	B	+	+	+	+	-	+	+	+	-
22	15	+	B	+	+	+	+	+	-	+	+	+
23	15	+	F	-	-	+	+	-	+	-	+	+
24	12		F	-	-	+	+	-	+	+	+	-
25	8	+	B	+	+	+	-	-	-	-	-	-
26	11	-	F	-	-	+	+	+	+	+	+	+
27	23	+	F	-	-	+	+	+	+	+	+	+

Abbreviation: B, honey bee; W, wasp; F, fire ant; CCD, Cross-reactive carbohydrate determinant; CRD, Component resolved diagnosis; ID, Intradermal test; SPT, Skin prick test

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Table 4. Agreement analysis between commercial and local allergen extracts. (n = 27)

4a. Skin prick test result.

Commercial	Local		*p*-value	Kappa
	Negative	Positive		
Fire ant			0.542	0.004
Negative	8 (30%)	4 (15%)		
Positive	2 (7%)	13 (48%)		
Bee			0.364	0.049
Negative	16 (59%)	2 (7%)		
Positive	5 (19%)	4 (15%)		
Common wasp			0.500	0.009
Negative	15 (56%)	3 (11%)		
Positive	3 (11%)	6 (22%)		
White- faced hornet			0.615	0.001
Negative	14 (52%)	1 (4%)		
Positive	4 (15%)	8 (29%)		
Yellow hornet			0.471	0.013
Negative	16 (59%)	4 (15%)		
Positive	2 (7%)	5 (19%)		
Yellow jacket			0.308	0.100
Negative	12 (45%)	3 (11%)		
Positive	6 (22%)	6 (22%)		

4b. Skin prick test combined with intradermal test result.

Commercial*	Local		*p*-value	Kappa
	Negative	Positive		
Fire ant			0.001	0.647
Negative	6 (22%)	3 (11%)		
Positive	1 (4%)	17 (63%)		
Bee			0.001	0.632
Negative	10 (37%)	1 (4%)		
Positive	4 (15%)	12 (44%)		
Wasp			0.001	0.547
Negative	7 (26%)	0 (0%)		
Positive	6 (22%)	14 (52%)		

*Commercial wasp skin prick test combined with intradermal test results from Table 4b were the combination of the results of wasp, white- faced hornet, yellow hornet and yellow jacket

The SPT result of local fire ant whole body allergen extract showed moderate agreement with commercial fire ant whole body allergen extract (k = 0.542, p = 0.004). The SPT result of local honey bee venom allergen extract showed fair agreement with commercial honey bee venom allergen extract (k = 0.364, p = 0.049). The SPT result of local yellowjacket venom allergen extract showed moderate agreement with commercial wasp venom allergen extract (k = 0.500, p = 0.001) and yellow hornet venom allergen extract (k = 0.471, p = 0.013), and showed substantial agreement with white-faced hornet (k = 0.615, p = 0.001). Agreement between local yellowjacket venom allergen extract and commercial yellowjacket venom was fair (k = 0.308, p = 0.100), as shown in **Table 4a**.

When the results of SPT and ID were combined (**Table 3** and **Table 4b**), the positivity of local allergen extract increased to 74% for fire ant, to 48% for honey bee, and to 52% for yellowjacket. The positivity of commercial allergen extract also increased to 67% for fire ant, to 59% for honey bee, and to 74% for the wasp group. Positive SPT or ID of any commercial extract of common wasp, white-faced hornet, yellow hornet, or yellowjacket was reported as a positive skin test (SPT + ID) result. Agreement analysis between local and commercial extracts showed significant agreement for fire ant SPT and ID (k = 0.647, p = 0.001) and honey bee SPT and ID (k = 0.632, p = 0.001), and moderate agreement for wasp SPT and ID (k = 0.547, p = 0.001).

When SPT and ID were compared with sIgE subtracted with CCD (sIgE-CCD) and/or CRD (as gold standard), we found that ID increased the sensitivity and accuracy of SPT to fire ant, honey bee, and wasp, but not specificity. The validity of SPT and ID of local and commercial fire ant, honey bee, and wasp allergen extract when compared with sIgE-CCD of fire ant and sIgE-CCD or CRD of honey bee and wasp as a gold standard is shown in **Tables 5a, 5b,** and **5c**, respectively. Positive SPT or ID of any commercial extract of common wasp, white-faced hornet, yellow hornet, or yellowjacket was reported as a positive skin test (SPT + ID) result. Combined SPT and ID result of local fire ant whole body extract showed higher sensitivity (87% *vs.* 67%), specificity (42% *vs.* 33%), and accuracy (67% *vs.* 52%) than commercial fire ant whole body extract. History alone showed 64% sensitivity, 70% specificity, and 67% accuracy when compared with fire ant sIgE-CCD as a gold standard. Combined SPT and ID result of commercial honey bee venom extract showed higher sensitivity (62% *vs.* 50%) and accuracy (63% *vs.* 52%) than local honey bee venom extract with equal specificity (100%) when compared with sIgE-CCD and/or CRD of honey bee as a gold standard. The result of bee and wasp skin tests compared with only CRD as gold standard was similar to when compared with both sIgE-CCD and/or CRD as gold standard. History alone showed low sensitivity and accuracy, but high specificity.

Table 5. Validity of skin prick test and intradermal test of local and commercial insect allergen extract when compared with specific immunoglobulin E and/or component-resolved diagnosis as gold standard.

5a. Fire ant whole body allergen extract

	sIgE-CCD (Gold standard)		
	Sensitivity	Specificity	Accuracy
SPT (C)	8/15 (53%)	5/12 (42%)	13/27 (48%)
SPT + ID (C)	10/15 (67%)	4/12 (33%)	14/27 (52%)
SPT (L)	11/15 (73%)	6/12 (50%)	17/27 (63%)
SPT + ID (L)	13/15 (87%)	5/12 (42%)	18/27 (67%)
History alone	9/14 (64%)	7/10 (70%)	16/24 (67%)

5b. Bee venom allergen extracts.

Gold standard	Sensitivity		Specificity		Accuracy	
	CRD	IgE-CCD or CRD	CRD	IgE-CCD or CRD	CRD	IgE-CCD or CRD
SPT (C)	9/24 (38%)	9/26 (35%)	3/3 (100%)	1/1 (100%)	12/27 (44%)	10/27 (37%)
SPT + ID (C)	16/24 (67%)	16/26 (62%)	3/3 (100%)	1/1 (100%)	19/27 (70%)	17/27 (63%)
SPT (L)	6/24 (25%)	6/26 (23%)	3/3 (100%)	1/1 (100%)	9/27 (33%)	7/27 (26%)
SPT + ID (L)	13/24 (54%)	13/26 (50%)	3/3 (100%)	1/1 (100%)	16/27 (59%)	14/27 (52%)
History alone	8/21 (38%)	9/21 (39%)	2/3 (67%)	1/1 (100%)	10/24 (42%)	10/24 (42%)

5c. Wasp venom allergen extracts.

Gold standard	Sensitivity		Specificity		Accuracy	
	CRD	IgE-CCD or CRD	CRD	IgE-CCD or CRD	CRD	IgE-CCD or CRD
SPT (C)*	9/18 (50%)	9/20 (45%)	9/9 (100%)	7/7 (100%)	18/27 (67%)	16/27 (59%)
SPT + ID (C)*	16/18 (89%)	17/20 (85%)	5/9 (56%)	4/7 (57%)	21/27 (78%)	21/27 (78%)
SPT (L)	9/18 (50%)	9/20 (45%)	9/9 (100%)	7/7 (100%)	18/27 (67%)	16/27 (59%)
SPT + ID (L)	12/18 (67%)	13/20 (65%)	7/9 (78%)	6/7 (86%)	19/27 (70%)	19/27 (70%)
History alone	5/17 (29%)	7/19 (37%)	6/8 (75%)	6/6 (100%)	11/25 (44%)	13/25 (52%)

*Commercial wasp skin prick test combined with intradermal test results from Table 5c were the combination of the results of wasp, white- faced hornet, yellow hornet and yellow jacket

Abbreviations: C, commercial insect allergen extract; CRD, component-resolved diagnosis; ID, intradermal test; L, local insect allergen extract; SPT, skin prick test, sIgE-CCD, specific immunoglobulin E subtracted with cross-reactive

The combined SPT and ID result of commercial wasp venom extract showed higher sensitivity (85% *vs.* 65%) and accuracy (78% *vs.* 70%) than local honey bee venom extract, but lower specificity (57% *vs.* 86%) when compared with sIgE-CCD or CRD of wasp as a gold standard. When compared with only CRD, the results were similar. History alone showed low sensitivity, but high specificity.

No systemic reaction from SPT or ID was observed when using either local or commercial insect allergen extracts. The only side effect was local swelling, pain, and/or itching, which was mild and improved with antihistamine. Serum tryptase level was less than 6 μg/L in all studied patients.

Discussion

This study showed that SPT and ID with local and commercial insect allergen extracts in patients with severe insect allergic reaction were comparable in sensitivity and accuracy, and that they showed significant agreement. In patients with negative SPT, the additional ID gave the skin test higher sensitivity and accuracy in patients with fire ant, honey bee, or wasp allergy.

SPT positivity was found in more than 50% of patients with severe fire ant allergic reaction, but in less than 40% of patients with severe honey bee and wasp allergic reactions. Positivity of SPT with local and commercial yellowjacket venom allergen extract was 33% and 44%, respectively.

The higher rate of the positive SPT results to fire ant than when using only the clinical history might due to the fact that there is some *in vitro* cross-reactivity between bee, wasp, and fire ant. Patients with bee, and wasp allergy could also have a positive SPT results to fire ant. However, when using sIgE-CCD as a gold standard to diagnose fire ant allergy, the sensitivity increase, but lower in specificity when using SPT and ID, as compared to those with history alone. In contrast, for patients with bee, or wasp allergy, double positive sensitization is not uncommon. Therefore, diagnosis of those insect allergy cannot be done by using only the clinical history.

Differences in the species of insects used in the preparation of extracts might be the cause of differences in skin test results. Skin test with local allergen extracts should be preferred over commercial versions since the insects used in commercial extracts may not be the same species as the insects in other areas. Ideally, local insect allergen extracts should be prepared from the common local insects that are the common cause of insect allergy in that area. In our study, *Solenopsis geminata* was used instead of *Solenopsis invicta* for preparing fire ant whole body allergen extract; *Apis dorsata* was used instead of *Apis mellifera* for preparing bee venom allergen extract; and, *Vespa affinis* was used instead of *Vespula vulgaris* for preparing yellowjacket venom allergen extract. These species of insects are the common causative insects for insect sting allergy in Thailand.[7-9] The venom allergens of different honey bee species are highly similar and highly cross-reactive.[14] Cross-reactivity between *Polistinae* and *Vespinae* venoms (especially yellowjacket venom) is frequently observed.[15,16] Different ant venoms share some common protein components, but each group has a number of unique allergen components.[17]

Athough there were only 27 participants in this study, all of them had severe allergic reaction and 25 cases had anaphylaxis. By limiting the study population to only those with severe insect allergic reaction, we reduced the effect that variation in severity would have had on the test result. The high proportion of negative SPT to local and commercial honey bee and wasp venom allergen extracts (78%, 66%, 67%, and 56-74%, respectively) may have been due to the younger age of patients in this study (mean age: 13.8 years. range: 5-25). Children had lower skin test reactivity to culprit venom, and lower ID sensitivity than adults.[18] There were no differences between venom sIgE levels in relation to anaphylaxis grade, regardless of age. The mean level of bee venom sIgE was highest in the youngest children, with an observed subsequent decrease in sIgE up to age 30 years but there was no correlation between wasp venom sIgE and age.[18]

This study was performed within 5 years after the last sting, and 81% of our patients had their last sting within 3 years. This could affect the rate of positivity of sIgE and skin test, but it should not affect the comparison result since we compared skin test and sIgE in the same patients. Specific IgE was found to decrease during 1 to 4 years after Hymenoptera venom anaphylaxis, and it may fall below the level of detection with very long latency periods.[5] The rate of loss of sensitization to Hymenoptera venom in skin tests was 12% per year, with 33% of skin tests becoming negative after 2.5 years.[19] Negative skin test results and failure to detect venom sIgE in patients with a convincing history of Hymenoptera venom anaphylaxis may merely reflect a long latency period between sting event and diagnostic testing,[18,19] especially in children since they have milder reactions and better recovery due to having a strong cardiovascular system.[18] However, sensitization can remain detectable for many years in a number of patients.

To evaluate the sensitivity, specificity, and accuracy of skin test results from local and commercial insect allergen extract, we used CRD as a gold standard. Sting challenge with live insect is a gold standard in diagnosis of insect allergy, but it is too dangerous in anaphylactic insect sting allergic patients and should not be used in untreated patients.[20] CRD was used as a gold standard similar to a previous study that showed that rApi m 1, rApi m 10, rVes v 1, and rVes v 5 facilitated identification of the culprit venom with good agreement with skin testing.[21] Identification of the causative insect improved with additional CRDs. However, severity of sting reaction was not associated with results obtained by skin testing, venom-specific IgE levels, or molecular diagnosis.[5,21] Venom skin test responses are negative in many patients with a history of systemic allergic reactions to insect stings, and may be associated with positive serologic test responses to venom-specific IgE antibodies. Venom skin test responses should be repeated when negative along with a serologic IgE antivenom test, and better diagnostic skin test reagents are urgently needed.[22]

The concentrations of local and commercial allergen venom extracts of honey bee and wasp were the same at 100 mcg/ml each. The concentration of fire ant used in the local extract was 10 times higher than the concentration in the commercial extract (1:10 *vs.* 1:100 w/v) in order to get the similar positive result in pilot study. Skin test with both concentrations showed no significant side effects. This might be due to differences in the fire ant species used in extract preparation. The higher concentration of local fire ant allergen extract might explain the higher number positive skin tests and higher sensitivity of local fire ant allergen extract over the commercial version.

In this study, sIgE, CRD, and CCD were considered positive when levels were ≥ 0.35 kUA/L, which is consistent with the internationally accepted cut-off level of 0.35 kU/l for detecting sIgE. The analytical sensitivity of modern assays is 0.10 kU/l[23] due to the finding that venom sIgE within the range of 0.10 to 0.35 kU/l may be clinically relevant in patients with low total IgE, and this must be evaluated in the context of the patient's history. However, the level 0.10 kU/l showed lower specificity, and more false-positive results. The limitation of our study is related to CRD or sIgE-CCD were used as a reference standard test, instead of the sIgE alone because of the potential cross-reactivity between insects across the CCD, and commercially unavailable of the CRD to fire ant.

Future study should be conducted to compare of types of causative insects in nearby regions, cross-reactivity among species of insects, the major allergens in each insect allergen extract, and their role in causing insect allergy.

Conclusions

SPT and ID with insect venoms could help in confirming and/or identifying the causative insects with the sensitivity of < 75%, except for fire ant 87%, and specificity < 70%. Compare between local and commercial extracts, local fire ant whole body extract showed higher sensitivity, whereas for honey bee, and yellowjacket, commecial extracts showed higher sensitivity.

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Conflict of interest declaration

The authors declare no conflicts of interest.

Clinical trial registration

ClinicalTrials.gov NCT03645291

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