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Clinical and immunological characteristics of bee venom hypersensitivity among beekeepers in Thailand

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Abstract

Background: Bee venom (BV) hypersensitivity can be severe and potentially life-threatening. Beekeepers heavily exposed to bee stings and are thus at a high-risk group. The data on bee sting reactions among beekeepers in Thailand is limited.

Objectives: To determine the prevalence, clinical and immunological characteristics, and the knowledge of BV hypersensitivity in Thai beekeepers.

Methods: A self-reported questionnaire survey about BV reactions in beekeepers were conducted. Further blood test for immunological parameters: serum BV-specific IgE (BV sIgE), phospholipase A2-specific IgE (Api m1 sIgE), and BV-specific IgG4 (BV sIgG4) were compared between non-allergic beekeepers, patients with a history of bee sting anaphylaxis and the non-allergic control group.

Results: A total of 202 out of 447 questionnaires (response rate 45%) were returned. The median age was 46.7 years. Systemic reactions were documented in 6.4%. Younger than 45 years was found to be a factor associated with systemic reactions (OR, 4.35; 95% CI, 1.16-16.31). The BV sIgE and Api m1 sIgE were significantly higher in the anaphylaxis group (p = 0.001). The median of BV sIgG4 was significantly higher in non-allergic beekeepers (p = 0.001). For the knowledge of BV hypersensitivity, 56.4% recognized that BV hypersensitivity could be fatal but only 6% knew about epinephrine auto-injector device.

Conclusions: The prevalence of systemic reactions after stings among Thai beekeepers was not high, which might be due to the tolerance induced by natural exposure via sIgG4. The level of knowledge of BV hypersensitivity among beekeepers was insufficient, more education must be provided.

Key words: bee venom, venom allergy, bee stings, beekeepers, Thailand

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Introduction

Bee stings are a common outdoor nuisance. In most cases, bee stings are just annoying, and home treatment is all that is necessary to ease the pain. However, people who are allergic to bee stings or have been stung numerous times may have a serious life-threatening reaction that requires emergency treatment.[1] The prevalence of bee sting hypersensitivity varies between geographic locations and ethnic groups. The rate of systemic allergic reactions to bee stings in the general population ranges from 0.3% to 7.5%, and annual mortality due to bee stings ranges from 0.03 to 0.48 per million inhabitants.[2,3]

The risk of a severe reaction increases with the degree of exposure. Beekeepers and their family members are heavily exposed to honeybee stings, and are at a higher risk. Therefore, Beekeepers are an interesting population for the study of epidemiology and immunopathogenesis of bee venom allergy. Data from literature suggest that 17% to 43% of beekeepers are allergic to bee venom.[4,5] Previous studies have found the frequencies of systemic reactions among beekeepers to range from 4% to 38%,[2-9] which were higher than the general population.

Apis mellifera, the European honeybee, was first imported into Thailand in the 1940s for agricultural pollination, producing honey and beeswax.[10] Nowadays, it is the most popular species of bee for apiculture in Thailand and worldwide. Northern regions are the largest apicultural area in Thailand. The number of beekeepers is gradually increasing,[10] and bee venom allergy has become an increasingly important health concern. However, the data on bee sting reactions among beekeepers in Thailand is limited.

The main objectives of the study were to determine the prevalence, clinical characteristics of bee venom hypersensitivity, and risk factors for systemic reactions among beekeepers in Northern Thailand. Moreover, the differences of immunological parameters between the beekeepers, patients with bee venom-induced anaphylaxis, and non-allergic control groups were compared.

Materials and Methods

A cross-sectional study was conducted between February 1, 2020, to December 30, 2020. The beekeepers' names and contact lists were obtained from the Agricultural Technology Promotion Center (Economic Insects section), Chiang Mai Province, Thailand. Four hundred and forty-seven beekeepers living in Northern Thailand could be reached. Two hundred and two (45%) beekeepers who responded and completed the questionnaire were enrolled in the study. The questionnaire includes demographic information, concomitant diseases, duration of beekeeping, number of active working days in a week, the average number of bee stings in a year, clinical symptoms, and family history of bee venom allergy. The descriptions and pictures of local reactions, large local reactions, and systemic reactions had been shown to the participants to determines the severity and type of reactions. The most severe reactions after bee stings were recorded. The beekeepers were additionally asked about their level of knowledge of bee venom hypersensitivity and management.

The definitions of reactions[1] were as follows: 'Local Reactions (LR)' were reactions limited to the area of the sting site. These reactions may consist of pain, swelling, redness, and itching; 'Large Local Reactions (LLR)' were characterized by swelling and redness that extended from the sting site with a diameter exceeding 10 cm and may last for more than 24 hours; 'Systemic Reactions (SR)' were symptoms and signs in one or multiple organ systems distant from the site of the stings. Participants who fulfilled one of the three clinical diagnostic criteria of the 2006 National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network (NIAID-FAAN) symposium were diagnosed with anaphylaxis.[11] The systemic reactions due to venom were classified according to the Ring-Messmer classification.[12] The participants were inquired about the course of symptom severity. The severity is defined as 'decrease' if the latter sting reactions were reducing in severity, e.g., LLR or SR occurred in the previous sting reactions turn to minor LR. On the other hand, 'increase' in severity is defined as the reactions from minor LR turn to LLR or SR later.

For the immunological evaluation, the three groups of volunteers were enrolled. These included 20 beekeepers without SR after bee stings who were invited from the questionnaire survey group, 20 individuals with a history of anaphylaxis after bee stings, and 20 healthy control who had a history of mild local reactions after bee stings. Although age and gender were matched between control and anaphylaxis groups, the baseline characteristics-match between the beekeeper group and the others could not be done due to the predominant male gender and elder age. A sample of at least 27 participants in each group was estimated to provide 90% power for declaring differences in immunological markers.[15] A testing laboratory supply and budget limitation resulted in the trial having fewer participants than anticipated for the immunogenicity analyses. Blood samples were collected. The serum levels of honeybee venom-specific IgE (*A. mellifera* venom; i1), phospholipase A2-specific IgE (rApi m 1; i208), tryptase, and honeybee venom-specific IgG4 were measured using ImmunoCAP (Thermo Fisher Scientific) according to the manufacturer's instructions.

The analyses were performed using SPSS version 26.0 (SPSS, Chicago, IL, USA). The demographic data were reported using the median, range, and interquartile ranges (IQR) for quantitative variables without normal distribution. Mean and standard deviation (SD) for variables with normal distribution. Numbers and percentages for categorical variables. The normal distribution of the analyzed data was tested using the Shapiro-Wilk test. Mann-Whitney U tests were used to compare numerical data without normal distribution. Kruskal-Wallis ANOVA by ranks was used to compare quantitative variables without normal distribution. The risk factor of systemic reactions was tested using binary logistic regression analysis. P-value < 0.05 was considered as statistically significant.

The study was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University Hospital, Chiang Mai University (070/2020). Written informed consent were obtained from all participants. This study was registered at the Thai Clinical Trial Registry (No TCTR20210531007).

Results

A total of 202 out of 447 completed questionnaires (response rate, 45.2%) were returned. The demographic data, bee sting reactions, and family history of bee venom allergy are shown in **Table 1**. The median age of participants was 46.70 (IQR 36.05-58.75) years and 68.3% were male. The concomitant allergic diseases were present in 18 (9%) and non-allergic diseases in 50 (24.8%). While 66.3% had no underlying conditions. Time of beekeeping varied in duration. Ninety (44.6%) beekeepers have worked for more than 10 years. The median number of active working days in a week was two (range 1–4) days. The average number of stings in a year and the clinical symptoms after being stung are summarized in **Table 1**. Most beekeepers have been stung more than 100 times and the course of symptoms severity seemed to be decreasing. The onset of the reactions (95%) was within four hours.

Table 1. Baseline Characteristics, Level of Knowledge and Management of Reactions (n = 202)

Characteristics	N (%)
Age (years) (median; IQR)	46.70 (36.05–58.75)
Sex	
Male	138 (68.3)
Female	64 (31.7)
Concomitant allergic diseases	**18 (9)**
Asthma	8 (4.0)
Allergic rhinitis	7 (3.5)
Food allergy	2 (1.0)
Atopic dermatitis	1 (0.5)
Concomitant non - allergic diseases	**50 (24.8)**
Hypertension	26 (12.9)
Diabetes mellitus	5 (2.5)
Dyslipidemia	3 (1.5)
Dyspepsia	5 (2.5)
Others	11 (5.4)
No concomitant diseases	**134 (66.3)**
Beekeeping duration (years)	
1–5	52 (25.7)
5–10	60 (29.7)
> 10	90 (44.6)
No. of active working days in a week (days; ranges)	2 (1 - 4)
No. of stings in a year	
< 10 times	8 (4.0)
11 to 100 times	51 (25.2)
> 100 times	143 (70.8)
Course of symptoms after being stung, over the years	
Decrease in severity	174 (86.1)
No change	27 (13.4)
Increase in severity	1 (0.5)
Onset of reaction after bee stings	
< 4 hours	192 (95.0)
4 to 24 hours	4 (2.0)
> 24 hours	6 (3.0)
The type of reaction where the bee stings	
Local reaction	**189 (93.6)**
Local reaction	166 (82.2)
Large local reaction	23 (11.4)
Systemic reactions	**13 (6.4)**
Severity[a]	
Grade I: generalized urticaria	8 (4.0)
Grade II: anaphylaxis	5 (2.5)
Grade III/IV: shock/cardiac arrest	0 (0)
Family history of bee venom allergy	32 (15.8)
Level of knowledge about bee sting reactions	
Might bee venom allergy be life-threatening? Yes	114 (56.4)
Have you heard of epinephrine auto-injector/ pre-filled syringe? Yes	12 (5.9)
Management after bee stings	
Hospital visit	12 (5.9)
Epinephrine injection	3 (1.5)
Doing nothing	143 (70.8)
Apply a medicine to sting site	27 (13.4)
Antihistamine and paracetamol tablets	21 (10.4)
Cold compression to sting site	1 (0.5)
Apply Thai herbs	7 (3.5)

[a]According to Ring and Messmer 1977

SR, LLR, and LR were reported in 13 (6.4%), 23 (11.4%), and 166 (82.2%) of beekeepers, respectively. Among beekeepers with SR, eight beekeepers had a Grade 1 reaction and five beekeepers had a Grade 2 reaction according to the Ring-Messmer classification. The severe life-threatening allergic condition (Grades 3 and 4) had not been reported. Moreover, we found that 32 (15.8%) of beekeepers' family members had allergic reactions to bee stings. Twenty-two had SR and five of them were diagnosed with anaphylaxis to bee stings.

Beekeepers' Level of Knowledge and Their Management of Bee Venom Reactions

One hundred and fourteen (56.4%) beekeepers recognized that bee venom hypersensitivity could be fatal and only 6% knew about epinephrine auto-injector or epinephrine prefilled syringe devices. Surprisingly, hospital visits after bee stings were reported in only 12 (5.9%) beekeepers. Among those, three of them received epinephrine treatment intramuscularly. 70.8% of the participants did not seek any treatment, 13.4% applied to medicine or balm to sting site, 10.4% took antihistamine or paracetamol tablets, 3.5% applied for Thai traditional medicine and 0.5% applied ice pack. The beekeepers' knowledge and management of venom reactions are summarized in **Table 1**.

Table 2. Factors Associated with Systemic Reactions Among Beekeepers (n = 202)[a]

Risk factors	Systemic reactions (n = 13)	Local reaction (n = 189)	*P*-value	OR (95% CI)
Age (years): < 45	10 (76.9)	82 (43.4)	0.03	4.35 (1.16–16.31)
Sex: male	8 (61.5)	130 (68.8)	0.59	0.73 (0.23–2.31)
Concomitant diseases of atopy				
Asthma	0	8 (4.2)	0.99	0
Allergic rhinitis	1 (7.7)	6 (3.2)	0.41	2.54 (0.28–22.85)
Food allergy	0	2 (1.1)	0.99	0
Atopic dermatitis	0	1 (0.5)	0.99	0
Family member with bee venom allergy	2 (15.4)	30 (15.9)	0.96	0.96 (0.20–4.57)
Stings in a year ≤ 100 times	3 (23.1)	56 (29.6)	0.62	0.71 (0.19–2.69)
Total time beekeeping ≤ 10 years	8 (61.5)	104 (55.0)	0.65	1.31 (0.41–4.14)

[a]Factors associated with systemic reactions generated using a logistic regression model.
Abbreviations: CI, confidence interval; OR, odds ratio.

Table 3. Baseline Characteristics among Beekeeper, Anaphylaxis and Control group
Values are presented as median (range), or number (%).

Characteristic	Beekeeper Group (n = 20)	Anaphylaxis Group (n = 20)	Control Group (n = 20)
Age (years) (median; IQR)	62.2 (55.2–71.7)	34.9 (27.0–46.0)	34.0 (26.5–44.2)
Sex			
Male	15 (75)	8 (40)	8 (40)
Female	5 (25)	12 (60)	12 (60)
Concomitant allergic diseases	0	8 (40)	5 (25)
No. of stings per lifetime			
1	0	4 (20)	1 (5)
1 to 10 times	1 (5)	14 (70)	18 (90)
11 to 20 times	0	0	1 (5)
21 times or more	19 (95)	2 (10)	0
Course of symptoms after being stung, over the years			
Decrease in severity	19 (95)	0	3 (15)
No change	1 (5)	8 (40)	17 (85)
Increase in severity	0	12 (60)	0
Family history of bee venom allergy	6 (30)	0	1 (5)
The site of the bee sting			
Head and neck	4 (20)	9 (45)	3 (15)
Hand and upper limb	16 (80)	7 (35)	15 (75)
Trunk	0	2 (10)	0
Lower limb	0	2 (10)	2 (10)

Table 4. Comparison of Immunological Tests

Immunological tests	BK (n = 20)	A (n = 20)	C (n = 20)	*P*-value[b]			*P*-value[b]
				BK vs A	BK vs C	A vs C	
Bee venom sIgE (kUA/l)[a]	0.57 (0.25–2.43)	3.90 (2.94–15.45)	0.16 (0.05–0.76)	< 0.001	0.079	< 0.001	< 0.001
Api m1 sIgE (kUA/l)[a]	0.25 (0.08–0.57)	1.72 (0.38–7.15)	0.02 (0.01–0.18)	0.003	0.038	< 0.001	< 0.001
Serum Tryptase (µg/l)[a]	3.90 (2.74–6.21)	3.69 (3.06–4.93)	3.12 (2.75–4.04)	0.735	0.114	0.120	0.186
Bee venom sIgG4 (mgA/l)[a]	25.41 (9.23–31.65)	0.17 (0.04–1.01)	0.01 (0.01–0.04)	< 0.001	< 0.001	0.026	< 0.001
Bee venom sIgE/sIgG4[a] (kUA/L)/(mgA/l)	0.03 (0.01–0.21)	29.58 (12.60–105.63)	6.28 (2.00–12.25)	< 0.001	< 0.001	0.065	< 0.001

[a]Median (range)
[b]Kruskal-Wallis test
Values are presented as median (range), The significance level is *$p < 0.05$.
Abbreviations: sIgE, specific immunoglobulin E; BV, bee venom; BK, beekeepers; A, anaphylaxis; C, control; kUA/l, kilo allergy unit per liter; µg/l, microgram per liter; mgA/l, milligram allergy per liter

Factors Associated with SR Following Bee Stings Among Beekeepers

The factors associated with reported SR following bee stings among beekeepers are demonstrated in **Table 2**. The only statistically significant factor that increased the risk for the SR was found to be the age < 45 years. SR among beekeepers aged < 45 years were four times higher compared to those older ones (OR, 4.35; 95% CI, 1.16-16.31, $p = 0.03$). Whereas, sex, concomitant disease of atopy, the family member with bee venom allergy, number of stings in a year and beekeeping duration were not a risk factor for SR.

Comparison of Immunological Tests

There were three groups of participants including, beekeepers who did not have a systemic reaction after bee-sting, patients with a history of bee venom-induced anaphylaxis, and non-bee venom allergic control group. The characteristics of participants involving in the immunological test were shown in **Table 3**. The median age in the beekeepers' group was higher than other groups and 75% were male. We found a high prevalence of concomitant atopic disease in the anaphylaxis group. Beekeepers had the highest number of bee stings. The severity of symptoms was decreased by time course in beekeeper populations. The two most common sites of bee sting were the head/ neck and hand area.

The immunological tests showed statistically significant differences in venom-sIgE levels and Api m1 sIgE ($p < 0.001$). The median venom-sIgE and Api m1 sIgE levels were highest in anaphylaxis group. Analyses showed no statistically significant differences in the levels of baseline serum tryptase ($p = 0.186$) among the three groups. The bee venom-sIgG4 in beekeepers was significantly higher than anaphylaxis and control group ($p < 0.001$). The median of bee venom-sIgE/sIgG4 ratio also showed a statistically significant difference among the three groups ($p < 0.001$). The median ratio in anaphylaxis, beekeepers, and control groups were 29.58, 0.03, and 6.28, respectively. The immunological data were shown in **Table 4**.

Discussion

This cross-sectional study has demonstrated that the prevalence of bee sting SR among Thai beekeepers was 6.4%. The LR were found to be very frequent of 93.6%. Younger than 45 years was found to be a factor associated with systemic reactions (OR, 4.35; 95% CI, 1.16-16.31, $p = 0.03$). The knowledge of bee venom hypersensitivity among beekeepers seemed to be insufficient. Fifty-six percent recognized that bee venom allergy could be fatal, but only 6% knew about epinephrine auto-injector devices. The immunological tolerance among beekeepers via the presence of bee venom sIgG4 was showed in this study.

Our study was the first data in Thailand that focused on bee sting hypersensitivity in populations at high risk. The severity of reactions after bee stings varies from mild LR to SR including anaphylaxis, which can be life-threatening.[1] 6.4% of our beekeepers reported SR. It is within the ranges of previous studies, in which the occurrence of SR in beekeepers varied from 4% to 38%.[2-9] The variation in SR rates have been attributed to climate, geographical location, race, data collection technique, the definition of an allergic reaction, and degree of exposure.[1-3,5,7] The low SR rate found by the German study[9] was due to the high mean age of participants (63.7 years). They have been showing the statistically significant inverse relationship between SR and age. It was supported by the Turkish study,[13] SR were found to be common among young beekeepers. These are in line with our finding that SR was 4.35 times higher in beekeepers younger than 45 years. The explanation of the correlation might be due to the older beekeepers have been exposed to bee venom allergens longer than the younger ones, which causes natural desensitization. The older beekeepers might have higher sIgG4 levels that provide protection. Therefore, the severity of symptoms after bee stings decreases with age.[5,14,15]

Most of the beekeepers in our study reported > 100 stings in a year and the clinical severity seemed to be decreasing over time. In agreement with Bousquet et al.,[4] they reported

that the degree of sensitization of beekeepers against bee stings was strongly related to the annual number of stings, and the beekeepers who were stung often appeared to be protected against stings. In addition, we also found that the shorter duration of beekeeping was related to a higher prevalence of SR, but the result did not reach statistically significant. Most of the beekeepers in our study reported sting reactions to occur most frequently following the first year of beekeeping.

Numerous studies reported a history of atopy as a risk factor for SR.[13,16,17] A clinical history of atopic diseases, such as asthma, allergic rhinitis, eczema, and food allergy were reported between 20% to 52% of beekeepers.[17] Annila et al.[18] reported the presence of upper respiratory tract symptoms while working at hives significantly increases the risk of SR. Celikel et al.[13] also reported the risk of SR increases approximately three-fold when one atopic disease is present and eleven-fold when two or more concurrent atopic diseases are present concerning no atopic disease. The present study, however, did not support this finding. This might be due to a low rate of concomitant atopic diseases among our participants (8%).

Regarding the level of knowledge, our study revealed that the awareness of venom hypersensitivity is low among Thai beekeepers. We found that 56% of beekeepers aware of the possible lethal effects of bee venom and only 5.9% of beekeepers heard about the epinephrine auto-injector device. In contrast to the study in Turkey,[19,20] 80% of the beekeepers recognized that bee venom could be lethal, and 30% were aware of the epinephrine auto-injector.

For the treatment of sting reactions, most of the beekeepers (70.8%) explained that their reactions were not severe and self-limited without any treatment. Various treatments with Thai traditional herbs and balm, consisting of lime, garlic, shallot, were commonly used for local reactions. Self-treatment modalities and mild severity might partially explain the low number of hospital visits. Only 12 (5.9%) went to the hospital after bee stings. There were five beekeepers (2.5%) who reported anaphylaxis after bee stings. None of them were referral to an allergy specialist for assessment and treatment with venom immunotherapy. The awareness of venom allergy in this high-risk population was low, and they were poorly informed about the treatment options. Furthermore, 15% of the beekeepers' family members reported that they were allergic to bee stings. Some of them had anaphylactic reactions. Beekeepers, their family members, and community were at an increased risk of sting anaphylaxis and therefore need especially careful education concerning the avoidance of re-exposure, anaphylaxis action plan, epinephrine auto-injector, and allergen immunotherapy with bee venom.[1,5,20]

In the present study, a significantly higher level of bee venom-sIgE and Api m1 sIgE were found in the anaphylaxis, compared to other groups. These results indicate that the levels of bee venom-sIgE and sIgE for Api m1 may be positively correlated with the severity of clinical symptoms.[21] The present study revealed that most patients with a history of *Apis mellifera* hypersensitivity responded to bee venom sIgE. Thus, sIgE might be useful for diagnosis bee venom allergy and for identifying sensitization when venom immunotherapy is being considered.[6] Although, the Component Resolved Diagnostic (CRD) test, Api m1 sIgE, was used to distinguish multiple- or double-sensitization from cross-reaction in insect sting allergy.[22,23] The additional benefit of Api m1 sIgE from bee venom sIgE in the determination of true honeybee venom allergy remains unclear.[22,24,25] We also found a significantly higher level of venom-sIgG4 in the beekeepers, compared to the control and anaphylaxis groups. The results confirmed that the number of bee stings in heavily exposed population influenced venom-sIgG4 levels.[5,6,26] It stimulates natural tolerance to bee venom. Hence, the beekeepers who had frequent stings and a longer duration of beekeeping might have a high concentration of protective sIgG4 antibodies and low clinical allergic symptoms, same as the patients who underwent treatment with venom immunotherapy.[5,14] Moreover, previous studies showed that high level of baseline serum tryptase is one of the risk factors for severe allergic reaction to bee stings.[26,27] Nevertheless, we did not find significant differences in baseline serum tryptase among these three groups.

This study was the first survey of hypersensitivity reactions among beekeepers, conducted in the largest apicultural area in Thailand. The major limitation of the present study is the low response rate. Even though many beekeepers continued beekeeping despite a history of venom allergy, a proportion of individuals likely withdrew from their work after severe hypersensitivity reactions. In contrast, patients who have experienced SR may have been more likely to complete the questionnaire. Only a small number underwent a further immunological investigation. The study population in immunological part represents a convenience sample from the survey in beekeepers, and we cannot rule out that an unintended selection occurred. The results of these may not indicate to the study population. Another limitation is a recall bias due to the study design of the questionnaire survey.

Conclusions

The prevalence of systemic reactions to bee venom hypersensitivity among beekeepers in Northern Thailand was not high. This might be due to the protective effect of the high frequency of bee stings and immunological tolerance via sIgG4. Young beekeepers were associated with SR. The level of knowledge of bee venom hypersensitivity was low, therefore, more education in the beekeepers' community is required.

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