

Pattern of sensitization to honeybee venom in beekeepers: A 5-year prospective study

Dimitrios Kalogeromitros, Ph.D., Michael Makris, M.D., Stamatis Gregoriou, M.D.,
Dimitrios Papaioannou, Ph.D., Alexandros Katoulis, Ph.D., and Nicholas G. Stavrianeas, Ph.D. (Greece)

ABSTRACT

Beekeepers are at increased risk for honeybee (Hb) venom allergy and they represent a unique population for Hymenoptera venom studies. The aim of this was to prospectively examine the pattern of Hb venom sensitization over a 5-year period in new beekeepers and define possible predisposing factors. Thirty-five beekeepers were tested every 6 months for 5 years with in vivo and in vitro methods to detect the possible development of sensitization to Hb and common wasp (Cw) venom. Inclusion criteria included the lack of previous beekeeping activity and absence of sensitization or reported reaction to Hymenoptera stings. Subjects with both in vivo and in vitro tests that were definitely positive or with one definitely positive and the other doubtful were considered sensitized. Ten of 35 new beekeepers (28.6%) and 3 of 36 controls (8.3%) developed sensitivity to Hb venom during the 5-year period. The risk ratio in incidence studies was calculated at 3.43 (SE of log risk ratio = 0.61; 95% CI of risk ratio = 1.03–11.42). All sensitized beekeepers were detected within the first 18 months of occupational exposure; 8 of 10 (80%) beekeepers were detected during the initial 12 months and the 2 remaining beekeepers were detected between 12 and 18 months. One of 35 (2.9%) beekeepers and 1 of 36 controls (2.8%) were sensitized to Cw venom. The number of stings per year and atopy had no effect on sensitization rate. Although predisposing factors to sensitization or anaphylaxis could not be identified, beekeepers developed sensitization to bee venom in <18 months.

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Beekeeping and honey production have been flourishing activities in Greece since ancient times as recorded in Homer's epics. Nowadays, a significant part of the rural population raises beehives either as a full-time occupation or as a leisure-time pursuit.

The prevalence of systemic reactions to Hymenoptera stings ranges from 0.8 to 3.3% in the general population,^{1,2} but a Greek study³ estimated that in Greece the sensitization prevalence is almost 27%. Beekeepers are frequently exposed to honeybee (Hb) stings and therefore may be at an increased risk of developing IgE-mediated allergic reactions to Hb venom. Moreover, beekeepers may have higher risk of common wasp (Cw) stings than the general population, because wasps are present eating honey in many hives. The incidence of systemic reactions in beekeepers after an Hb sting is high. Numerous studies^{4–6} have shown that between 14 and 35% of beekeepers have a history of at least one episode of systemic allergic reaction after Hb stings and Bousquet *et al.* reported anaphylactic reactions in 42% of subjects.⁷ Awareness of possible

life-threatening consequences causes anxiety among beekeepers and their relatives.

Nevertheless, beekeepers are a special group of individuals who differ from the general population in clinical and diagnostic parameters of Hb sting allergy. Studies indicate that generalized allergic reactions including urticaria/angioedema and systemic anaphylaxis are most common among the beekeepers receiving less stings; none of those reporting >200 stings/year experienced a systemic allergic reaction whereas 45% of those receiving only up to 25 stings did report a reaction.⁸

An IgE-mediated allergic reaction is a two-step process consisting of a sensitization and reaction phase. Although anaphylaxis is the most severe and, therefore, well-studied, clinical pattern of Hymenoptera sting allergy, the focus of this study is on the natural history of sensitization to Hb venom in new beekeepers.

We conducted this study to explain the pattern of sensitization to Hymenoptera venom and the possible influence of several factors (atopy, number of Hb stings/year, and duration of beekeeping) over a 5-year period, in a selected population of nonsensitized individuals who began beekeeping as their main occupational activity for the first time in their lives.

SUBJECTS AND METHODS

Patients

The study population was collected from individuals that enrolled in the Greek Beekeeper Association be-

From the Department of Allergy, University of Athens, Attikon Hospital, Athens, Greece

Address correspondence and reprint requests to Dimitrios Kalogeromitros, M.D., Ph.D., Allergological Unit, University of Athens, Attikon Hospital, 1 Rimini str, 12462 Xaidari, Greece

E-mail address: mak-mik@hol.gr

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tween 1997 and 1999, to receive a government grant to set up their hives, and were referred to the Allergy Department of the Andreas Sygros Hospital for medical advice about Hb venom allergy. All new beekeepers were informed for the possible dangers of Hb venom allergy and the existing diagnostic procedures for *in vivo* and *in vitro* detection of sensitization. The Scientific Committee of the Andreas Sygros Hospital approved study protocol. After informed written consent was obtained the aforementioned beekeepers were checked for possible sensitization to Hb and Cw venom with *in vivo* and *in vitro* diagnostic procedures as described later. Inclusion criteria were lack of previous beekeeping activity and absence of sensitization and clinical reaction to Hymenoptera stings. Forty-eight individuals interested in the Greek Beekeeping Association grant for new professional beekeepers were referred for medical advice. Twelve of 48 beekeepers were excluded because of already existing sensitization: 7 were sensitized to Hb venom, 4 were sensitized to Cw venom, and 1 was sensitized to both Hb and Cw venom; 2 of these 12 beekeepers had presented clinical reaction (urticaria/angioedema) after an Hb sting in the past. Only one individual who fulfilled criteria chose not to participate in our study—thus minimizing selection biases—and 35 consecutively enrolled beekeepers agreed to undergo allergological evaluation every 6 months for 5 years to determine possible development of sensitization. In addition, at each visit, investigators recorded the number of Hb stings in each subject as well as the number of anaphylactic reactions and large local reactions (LLRs). LLRs were defined as swelling at the sting site with a diameter of at least 10 cm and lasting at least 24 hours. Each participant used personal diary cards for recording field stings and was guided to check the exact number of stings immediately after beekeeping activity. Finally, for methodological reasons, beekeepers were categorized into three groups according to the estimated number of Hb stings per year: group A, <10 stings/year; group B, 11–50 stings/year; group C, >50 stings/year. This specific type of categorization was predefined before starting the study and was based on observational data in numerous beekeepers receiving Hb venom immunotherapy reflecting the range of exposure and differences in personal beekeeping skill. These three groups were compared with a control group which consisted of beekeepers' family members and field workers in agricultural activities who had moderate exposure to possible Hymenoptera stings. Sixty-three subjects were checked as possible controls with the already described diagnostic procedures before enrollment. Twenty-seven out of the 63 subjects were excluded for different reasons: 12 individuals were sensitized to Hb (5 subjects) or Cw venom (7 subjects) and 3 individuals (2 subjects sensitized to Hb

and 1 subject sensitized to Cw) had already presented systemic reaction after Hymenoptera sting in the past; additionally, 12 subjects were reluctant to participate mainly because of the long duration of the study. Finally, 36 subjects fulfilling the inclusion criteria composed the control study group.

Atopy

Presence or absence of atopy was estimated by a detailed clinical history of atopic manifestations (allergic rhinitis, bronchial asthma, and atopic dermatitis) or at least one positive skin-prick test (wheal diameter 7 mm) out of seven common inhalant allergens (*Olea eur*, mixed grasses, *Parietaria* sp., *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cladosporium herbarum*, and *Alternaria alternata*; Stallergenes, Antony, France).

Skin Tests

Intradermal skin tests were performed with Hb and Cw venom extracts as described previously⁹ (Stallergenes) and skin-prick tests were not performed because we felt they were not necessary for safety reasons. A negative (solvent) and a positive control (histamine hydrochloride, 10 mg/mL) were tested concurrently with the venom. The end point concentration (EPC) was determined as the lowest concentration resulting in a wheal of at least 5 mm in diameter with erythema. An EPC of $\leq 10^{-4}$ g/L was considered definitely positive, an EPC of 10^{-3} g/L was considered doubtful, and an EPC of 10^{-2} g/L was considered negative (because of nonspecific skin irritation).

Specific IgE

Specific IgE to Hb and Cw venom was estimated by commercially available radioallergosorbent test (RAST; Pharmacia, Uppsala, Sweden). Results were given in RAST classes from 0 to IV (class 0, <0.35 kU/L; class I, 0.35–0.7 kU/L; class II, 0.7–3.5 kU/L; class III, 3.5–17.5 kU/L; class IV, >17.5 kU/L). Classes II–IV were considered as definitely positive, class I as doubtful and class 0 as negative.

Sensitization Criteria

Intradermal skin test has been used as the main criterion of sensitization because historically it is considered biologically relevant and the most sensitive test for venom-specific IgE antibody. A 2003 rostrum paper from the Insect Committee of the American Academy of Allergy, Asthma and Immunology¹⁰ has refocused the discussion on the relative diagnostic performance of the skin test versus serological assays for venom-specific IgE antibody and reminds readers that no confirmatory test (*in vivo* or *in vitro*) has perfect diagnostic sensitivity and specificity. Having this data in mind,

Table 1 Demographic and other characteristics of study population and control group

	Beekeepers	Controls
Sex (male/female)	30/5	28/8
Age (mean $\pm$ 1 SD)	34.97 $\pm$ 10.48	33.53 $\pm$ 11.33
Atopy (yes/no)	7/28	5/31
Log10 of serum total IgE (mean $\pm$ 1 SD)	1.83 $\pm$ 0.36	1.86 $\pm$ 0.39
Sensitization (yes/no)	10/25	3/33
LLRs (yes/no)	5/35 (14.3%)	6/36 (16.6%)
Systemic reactions (yes/no)	0/35	0/36

Sensitization rate: beekeepers = 28.6% and controls = 8.3%. Risk ratio = 3.43 (SE of log risk ratio = 0.61); 95% CI of risk ratio = 1.03–11.42.

we decided to use a combination of intradermal (ID) testing and venom-specific IgE to define sensitization criteria as follows: sensitized, subjects with both skin tests and RAST definitely positive or with one definitely positive and the other doubtful; nonsensitized, either both examinations negative or one negative and the other doubtful; doubtful, one examination definitely positive and the other negative or both tests with doubtful results. Doubtful subjects were considered nonsensitized for statistical analysis purposes.

Statistical Analysis

The risk ratio (relative risk) in incidence studies and its 95% CI (confidence intervals) were computed. Mantel-Haenszel chi-square procedure¹¹ was applied to examine the possible association between sensitization to Hb venom and degree of exposure to venom after stratification for the atopic status. Different models of logistic regression analysis were used to explore the possible associations of sensitization to Hb venom (as dependent variable) and sex (male/female), age (in years), and log(10) of serum total IgE as independent variables.

RESULTS

The demographics and other characteristics of the study population are presented in Table 1. The two groups had no statistical differences in epidemiological parameters. Ten of 35 beekeepers (28.6%) and 3 out of 26 controls (8.3%) were sensitized to Hb venom during the 5-year period. The risk ratio in incidence studies was calculated at 3.43 (SE of log risk ratio = 0.61; 95% CI of risk ratio = 1.03–11.42). Eight of the 10 sensitized beekeepers (80%) were sensitized during the initial 12 months of occupational exposure and the 2 remaining beekeepers were sensitized sometime between 12 and 18 months after starting occupational exposure. None of the sensitized subjects in both groups reported systemic reaction after being stung. However, 5 of the 35 beekeepers (14.3%)—2 sensitized and 3 nonsensitized—experienced LLR after Hb sting while in the

control group the prevalence was 6 of 36 subjects (16.6%)—all nonsensitized.

Four of 7 (57.1%) atopic beekeepers and 6 of 28 nontopic beekeepers (21.4%) were sensitized. Atopic status was investigated as a possible risk factor for sensitization with the obvious limitation of the small sample of atopic individuals. Odds ratio was calculated at 4.89 with 95% CI (0.85–28.1), reaching no statistical significance.

According to the number of stings per year described previously, 6 of 12 subjects (50%) in group A, 2 of 14 subjects (14.3%) in group B, and 2 of 9 subjects (22.2%) in group C developed sensitization. Statistical evaluation with Mantel-Haenszel chi-square after stratification for atopy (2.2881[df]; $p = 0.130$; Table 2) indicated that the number of stings per year was neither a risk nor a protective factor for sensitization development in the beekeeper population under study.

Using the Mantel-Haenszel procedure (and taking into account atopic background) no association was established between the degree of exposure to Hb venom, as expressed in the number of stings per year and sensitization rate. Consequently, the number of stings per year is neither a risk nor a protective factor for sensitization in the beekeeper population under study. As for Cw sensitization, only 1 of 35 (2.9%) beekeepers was sensitized (at the eighth time point) and in the control group also 1 of 36 subjects (2.8%) was positive (at the sixth control time point).

Various logistic regression models were used to investigate possible association of sensitization development with a number of independent variables including sex, age, total serum IgE (after logarithmic transformation) atopy, and number of stings per year (in three categories, <10, 11–50, >50). Every time only three variables were included in each model, because of the relative small sample size. In all possible combinations, none of the foregoing independent variables was found to be significantly associated with sensitization to Hb venom.

Table 2 Association between sensitization to Hb venom and degree of exposure to venom after stratification for the atopic status

No. of stings per yr	Presence of Atopy			
	Nonatopic		Atopic	
	Negative	Positive	Negative	Positive
0–10	6	4	0	2
10–50	10	0	2	2
>50	6	2	1	0

Mantel-Haenszel chi-square = 2.288 (1 df); $p = 0.130$.

DISCUSSION

Beekeepers present an increased risk for Hb sting allergy as a result of increased exposure.^{8,12,13} However, until now, only case control retrospective study has been reported.^{13,14} This is the first time that the pattern of sensitization development over a 5-year period was investigated in a follow-up prospective study, in a population starting occupational exposure to Hymenoptera venom, without previous sensitization and in comparison with a control group of people with outdoor occupational activities and occasional exposure to Hymenoptera venom.

Sensitization developed in 28.6% of the beekeepers compared with 8.3% of the control group. The relative risk (risk ratio in incidence studies) of developing sensitization to Hymenoptera venom within 4 years in beekeepers starting their occupation was 3.43 (CI = 1.03–11.42). Interestingly, all sensitization developed within the initial 18 months of occupation. Although no study focusing on sensitization exists, our findings are in accordance with a study by Eich-Wanger *et al.*,¹² where the majority of allergic beekeepers reacted shortly after starting their occupation of beekeeping, at a time when stings occurred more frequently than in their previous life.

According to previous surveys, the prevalence of atopy among Hb venom allergic subjects has been 20–44%.^{7,15} In our study population, atopic status did not increase the likelihood of sensitization. Other published studies report atopic background in similar frequency among allergic beekeepers, allergic family members, and allergic controls.^{11,12,13,16} Miyachi *et al.*¹⁷ observed a significantly higher frequency of atopic background in allergic beekeepers than in nonallergic beekeepers. They suggested that during their work, either through inhalation or multiple stings, atopic beekeepers are sensitized more easily than nonatopic beekeepers. This finding is not in contrast to our results because our study refers to sensitization rate and not to clinical reactions; besides, the small sample size is another confusing factor for definite conclusions.

Contrary to previous reports, suggesting that multiple stings per year act as a protective factor for the

presentation of allergic reactions,^{7,18} no association was established between the degree of exposure to Hb venom, as expressed in the number of stings per year, and sensitization rate. On the other hand, we have to point out that 6 of the 12 beekeepers (50%) that reported <10 stings/year were sensitized. One might presume that in a more numerous population sample there could be a statistically significant association.

Surprisingly, none of the sensitized subjects experienced systemic allergic reaction. The small number of our sample (10/35) and the limited duration of reaction recording, almost 3–4 years after sensitization onset, as well as the frequent field stings that might have induced tolerance could represent possible explanations for the lack of systemic reactions in our population.

Furthermore, according to our findings, *in vivo* and *in vitro* sensitization cannot be used as a predictive factor of anaphylaxis. This aspect, although seems misleading must be kept in mind as it represents data in a unique population with frequent and numerous Hb field stings. Sex, age, and total serum IgE also are not associated with the development of sensitization in beekeepers who are starting occupational exposure.

Regarding Cw sensitization rate, the incidence was almost identical in both groups although the only 5-year duration of study and the small sample size may be important misleading factors for epidemiological conclusions. However, in beekeepers' studies the incidence of anaphylactic Cw sting reactions was the same as among the general population.^{1,2} Sensitized beekeepers were counseled to always wear protective clothing while beekeeping, carry an emergency kit (autoinjectable adrenaline, antihistamines, corticosteroids, and inhaled β_2 -agonist), inform family members before attending the bees, and continue follow-up at an allergy unit.

CONCLUSION

In our study, new-onset beekeepers developed sensitization to Hb venom within the first 18 months of beekeeping. Furthermore, the number of stings and the presence of atopy were not risk factors for the devel-

opment of new sensitization. We suggest that beekeepers should regularly be monitored for the development of sensitization and always apply all protective measures while at work. Additionally, because they represent a unique population that may provide important information about the natural history of sensitization and Hymenoptera allergy, future studies are warranted.

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