

# The correlation between anti phospholipase A<sub>2</sub> specific IgE and clinical symptoms after a bee sting in beekeepers

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Adv Dermatol Allergol 2016; XXXIII (3): 206–210

DOI: 10.5114/ada.2016.60613

## Abstract

**Introduction:** Beekeepers are a group of people with high exposure to honeybee stings and with a very high risk of allergy to bee venom. Therefore, they are a proper population to study the correlations between clinical symptoms and results of diagnostic tests.

**Aim:** The primary aim of our study was to assess the correlations between total IgE, venom- and phospholipase A<sub>2</sub>-specific IgE and clinical symptoms after a bee sting in beekeepers. The secondary aim was to compare the results of diagnostic tests in beekeepers and in individuals with standard exposure to bees.

**Material and methods:** Fifty-four individuals were divided into two groups: beekeepers and control group. The levels of total IgE (tIgE), venom-specific IgE (venom sIgE), and phospholipase A<sub>2</sub>-specific IgE (phospholipase A<sub>2</sub> sIgE) were analyzed.

**Results:** Our study showed no statistically significant correlation between the clinical symptoms after a sting and tIgE in the entire analyzed group. There was also no correlation between venom sIgE level and clinical symptoms either in beekeepers or in the group with standard exposure to bees. We observed a statistically significant correlation between phospholipase A<sub>2</sub> sIgE level and clinical signs after a sting in the group of beekeepers, whereas no such correlation was detected in the control group. Significantly higher venom-specific IgE levels in the beekeepers, as compared to control individuals were shown.

**Conclusions:** In beekeepers, the severity of clinical symptoms after a bee sting correlated better with phospholipase A<sub>2</sub> sIgE than with venom sIgE levels.

**Key words:** honeybee venom, phospholipase A<sub>2</sub>, diagnostic tests, sting.

## Introduction

Allergy to *Hymenoptera* venom, including honeybee venom, is an important problem in allergological practice [1]. Stinging by insects of *Hymenoptera* order represents one of the main causes of anaphylaxis [2]. In Central Europe, most of post-stinging anaphylactic reactions are induced by honeybees (*Apis mellifera*) and wasps (*Vespula vulgaris*, *Vespula germanica*), less frequently by other *Hymenoptera*, such as hornets (*Vespa crabro*) and bumblebees (*Bombus* spp.) [3].

Stinging by a honeybee usually causes local erythema and pain, but in a person sensitized to the venom

large local reactions (LLR) and systemic reactions (SYS), the anaphylactic shock may occur. Severe systemic symptoms, particularly concerning the respiratory system, may induce a fear of a subsequent sting and the fear may significantly impair the quality of life. In all patients with a history of systemic reactions after a sting, diagnostic tests such as skin tests and venom specific IgE determination are recommended. However, results of these tests do not correlate with severity of clinical symptoms, which has already been documented in the literature [3–6]. For these reasons, it is important to find a more

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**Received:** 21.07.2014, **accepted:** 5.05.2015.

sensitive and specific diagnostic tool that yields the results corresponding to the clinical signs after a sting.

Bee venom contains many substances, such as peptides, proteins, and biogenic amines [7–9]. So far, 12 allergens from the bee venom have been identified [10], with phospholipase A<sub>2</sub> (Api m1) being the major one. Available data suggest that phospholipase A<sub>2</sub> specific IgE determination using recombinant Api m1 is a more specific diagnostic test than the bee venom specific IgE [11].

### Aim

The aim of our study was to assess the correlation between total IgE, venom- and phospholipase A<sub>2</sub>-specific IgE and clinical symptoms after a bee sting in beekeepers. Another objective of this study was to compare the results of diagnostic tests in the beekeepers and in the individuals with standard exposure to bees. Beekeepers are a group of people with high exposure to honeybee stings and with a very high risk of allergy to the bee venom. Therefore, they are a convenient population to study the correlation between the clinical symptoms and the results of diagnostic tests.

### Material and methods

The study involved 54 individuals recruited at the meetings of beekeeping associations. All volunteers were divided into two groups: study group (beekeepers, *n* = 30) and control group (individuals with infrequent contact with bees, *n* = 24). Detailed characteristics of the study and control group are presented in Table 1. The study was conducted with the approval of the Bioethics Committee of Poznan University of Medical Sciences, Poland (Resolution No. 324/11).

#### Surveys

The data on clinical symptoms after a bee sting (patient history) were obtained from study questionnaires. The severity of clinical symptoms after a bee sting was classified as a normal reaction (NR), a LLR or a SYS.

#### Laboratory tests

In all individuals, serum levels of total IgE (tIgE), venom specific IgE (venom sIgE) and phospholipase A<sub>2</sub> specific IgE (phospholipase A<sub>2</sub> sIgE) were determined. The diagnostic tests were performed after a minimum of 6 weeks following the last bee sting. All the determinations were conducted using ImmunoCap system, UniCap 100, Phadia.

#### Statistical analysis

Statistica 10.0 software was used. The level of significance, adopted for all the analyses, was *p* < 0.05. Normal distribution of the analyzed data was checked using

Shapiro-Wilk test. The results obtained in the beekeepers and control group were compared by means of Mann-Whitney *U* test. Kruskal-Wallis ANOVA by ranks test was employed to assess the correlation between clinical signs after a sting and results of *in vitro* tests. A correlation between the classes of venom- and phospholipase A<sub>2</sub>-specific IgE was analyzed using Pearson's  $\chi^2$  test. The same test was also used to analyze the correlation between the classes of venom- and phospholipase A<sub>2</sub>-specific IgE and clinical signs after a sting.

**Table 1.** Characteristics of the study group (beekeepers, *n* = 30) and control group (individuals with infrequent contact with bees, *n* = 24)

Characteristic	Study group		Control group		
	N	%	N	%	
Gender	Male (M)	25	83.3	8	33.3
	Female (F)	5	16.7	16	66.7
Age [years]	1–10	–	–	3	12.5
	11–20	–	–	2	8.3
	21–30	2	6.7	4	16.7
	31–40	7	23.3	3	12.5
	41–50	2	6.7	3	12.5
	51–60	7	23.3	3	12.5
	61–70	7	23.3	3	12.5
Atopic diseases	71–80	3	10.0	2	8.3
	> 80	2	6.7	1	4.2
Cardiovascular diseases	Yes	3	10.0	10	41.7
	No	27	90.0	14	58.3
Therapy with $\beta$ -blockers	Yes	13	43.3	6	25.0
	No	17	56.7	18	75.0
Therapy with ACEI	Yes	5	16.7	2	8.3
	No	25	83.3	22	91.7
Clinical symptoms after a sting	Yes	7	23.3	2	8.3
	No	23	76.7	22	91.7
	NR	23	76.6	15	62.5
Number of stings received daily in case of beekeepers	LLR	5	16.7	6	25.0
	SYS	2	6.7	3	12.5
	< 1	15	50	–	–
	1–5	7	23.3	–	–
of beekeepers	6–10	4	13.3	–	–
	> 10	4	13.3	–	–

NR – normal reaction, LLR – large local reaction, SYS – systemic reaction.

## Results

In all the individuals, tIgE, venom sIgE (level and class) and phospholipase A<sub>2</sub> sIgE (level and class) were determined. The raw data on all concentrations are available on request.

### Levels of tIgE, venom sIgE and phospholipase A<sub>2</sub> sIgE vs. clinical symptoms after a sting

Significant differences were observed in venom specific IgE and phospholipase A<sub>2</sub> specific IgE levels between the group of beekeepers and the control group. Therefore, the analysis of correlation between the results of *in vitro* tests and clinical symptoms after a sting (based on survey data) was performed separately in these two groups. However, an assessment of the correlation between clinical symptoms and tIgE level was performed for all the individuals together.

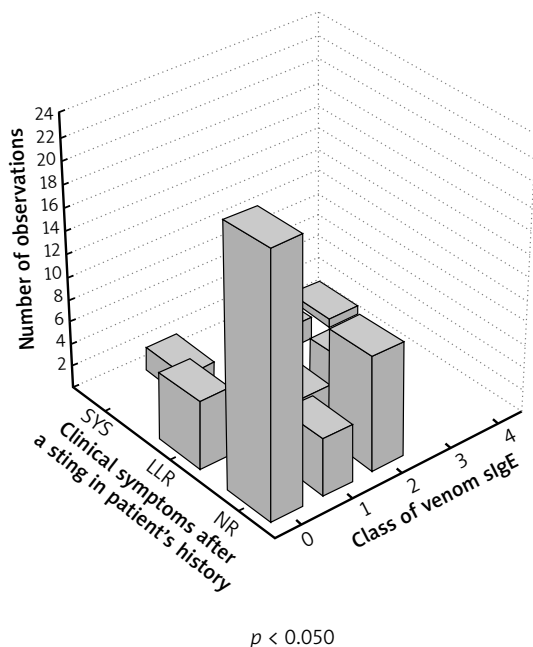
Our study showed no significant correlation between the clinical symptoms after a sting and tIgE ( $p = 0.582$ ) in both analyzed groups. There was also no correlation between venom sIgE level and clinical symptoms either in beekeepers ( $p = 0.124$ ) or in the group with standard exposure to bees ( $p = 0.099$ ). We observed a significant correlation between phospholipase A<sub>2</sub> sIgE level and clinical signs after a sting in the group of beekeepers ( $p = 0.040$ ), whereas no such correlation was found in the control group ( $p = 0.105$ ).

### Classes of venom sIgE and phospholipase A<sub>2</sub> sIgE vs. clinical symptoms after a sting

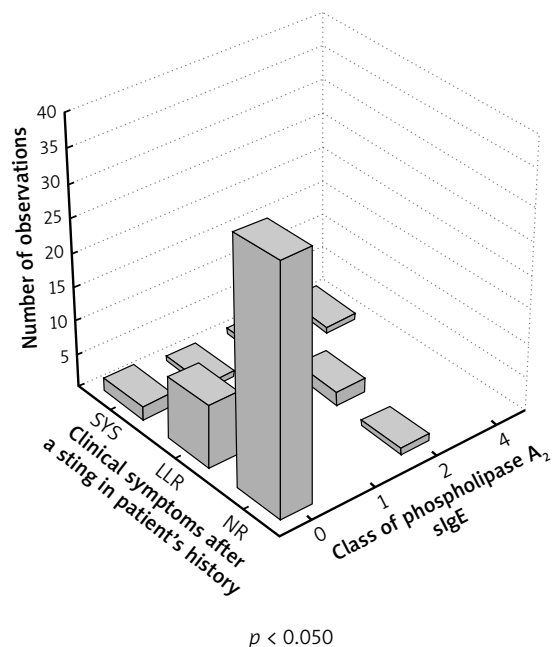
This analysis was performed in all the participants, because no significant differences were previously obtained between the analyzed groups (beekeepers and controls), concerning the classes of venom sIgE or phospholipase A<sub>2</sub> sIgE. Our study showed a significant correlation between clinical symptoms after a sting and both venom sIgE ( $p < 0.050$ ) and phospholipase A<sub>2</sub> sIgE ( $p < 0.050$ ) class. Thus, the severity of clinical signs after a bee sting was greater in the individuals with higher class of venom sIgE and phospholipase A<sub>2</sub> sIgE (Figures 1 and 2). Additionally, the correlation between the class of venom sIgE and phospholipase A<sub>2</sub> sIgE was examined and a strong correlation between the analyzed variables was obtained ( $p < 0.050$ ) – Figure 3.

### tIgE, venom sIgE (level and class) and phospholipase A<sub>2</sub> sIgE (level and class) vs. contact with bees

The results of *in vitro* tests (tIgE, venom sIgE (level and class) and phospholipase A<sub>2</sub> sIgE (level and class)) were compared in the analyzed groups of beekeepers and control individuals. We found no significant differences in tIgE levels ( $p = 0.383$ ), in venom sIgE classes ( $p = 0.292$ ) and phospholipase A<sub>2</sub> sIgE classes ( $p = 1.000$ ) between the groups. However, our study demonstrated significant



**Figure 1.** Clinical symptoms after a sting (NR – normal reaction, LLR – large local reaction, SYS – systemic reaction) vs. class of venom sIgE analyzed using Pearson’s  $\chi^2$  test ( $n = 54$ )



**Figure 2.** Clinical symptoms after a sting (NR – normal reaction, LLR – large local reaction, SYS – systemic reaction) vs. class of phospholipase A<sub>2</sub> sIgE analyzed using Pearson’s  $\chi^2$  test ( $n = 54$ )

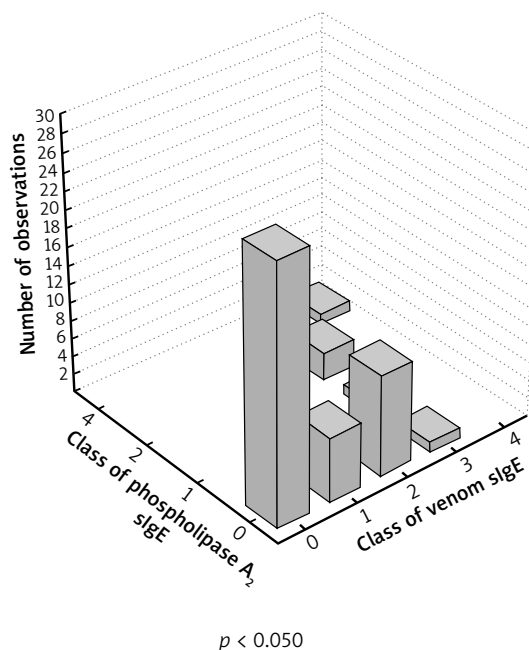
differences in the levels of venom sIgE ( $p = 0.038$ ), with significantly higher values observed in the beekeepers than in the volunteers with standard exposure to bees. In the case of phospholipase A<sub>2</sub> sIgE levels, a limiting level of significance ( $p = 0.055$ ) was obtained. This result indicates a trend for higher levels of phospholipase A<sub>2</sub> sIgE in the beekeepers than in the control individuals.

## Discussion

Determination of total IgE is a basic test performed in the patients with suspected allergy. However, this is a very non-specific test, which does not clearly indicate the allergic cause of the disease [12, 13]. Routinely used *Hymenoptera* venom allergy tests include determination of venom specific IgE and skin tests [3, 4, 14, 15]. Moreover, in the case of suspicion of honeybee (*Apis mellifera*) venom allergy, a determination of venom constituents specific IgE (e.g. phospholipase A<sub>2</sub> specific IgE) may be performed [16]. Earlier studies revealed that in 97% of people with the whole honeybee venom specific IgE, phospholipase A<sub>2</sub> (Api m1) specific IgE was also present [7]. However, these tests are not used in routine diagnosis. Meanwhile, determination of phospholipase A<sub>2</sub> specific IgE reduces the incidence of false positive results. An introduction of recombinant forms of allergens, such as recombinant phospholipase A<sub>2</sub>, also increased specificity of allergy diagnostic tests. Previous clinical studies showed that 15% of subjects with no allergic reaction in the past had positive test results indicating an allergy to bee venom, while none of them exhibited a positive response to recombinant venom allergens, including phospholipase A<sub>2</sub> [11].

The aim of this study was to assess the correlation of clinical symptoms after a bee sting with the results of diagnostic tests in the beekeepers. The correlation between clinical symptoms after a sting and tIgE remains ambiguous. The available literature data indicate that severe systemic signs after a sting are more common in the patients with low levels of tIgE. It was reported that a loss of consciousness occurred in the patients with low (< 50 kU/l) or moderate (50–250 kU/l) levels of tIgE. Mild symptoms related to a sting were observed in those with high tIgE levels (> 250 kU/l) [17]. Other studies showed no influence of tIgE level on the reactions after a sting. However, severe systemic reactions after a sting were observed more frequently in the elderly, in whom tIgE level is physiologically reduced [6]. Our study showed no correlation between the tIgE level and clinical manifestations of a bee sting in either the beekeepers or the control group.

A lack of correlation between the venom sIgE level and the severity of clinical symptoms after a sting has been repeatedly reported in the literature. However, the studies with a provoked sting in patients with an elevated exposure to stings and without a history of severe



$p < 0.050$

**Figure 3.** Class of venom sIgE vs. class of phospholipase A<sub>2</sub> sIgE analyzed using Pearson's  $\chi^2$  test ( $n = 54$ )

anaphylactic reaction showed that an elevated level of venom-specific IgE was a risk factor for a systemic anaphylactic reaction (SAR) [18]. There are no literature data on the correlations between clinical symptoms after a sting and phospholipase A<sub>2</sub> sIgE levels in beekeepers. Therefore, this study examined clinical manifestations of a bee sting and the level of phospholipase A<sub>2</sub> sIgE. Its outcomes revealed no significant correlations between the venom sIgE level and clinical symptoms after a sting, either in the beekeepers or in the control group. However, a correlation between clinical symptoms after a bee sting and the level of phospholipase A<sub>2</sub> sIgE was observed in the beekeepers. Contrary to that, there was no correlation between the clinical symptoms and phospholipase A<sub>2</sub> sIgE level in the individuals with standard exposure to bees. Thus, it can be assumed that in the beekeepers the severity of clinical symptoms after a bee sting correlated better with phospholipase A<sub>2</sub> sIgE level than with venom sIgE level.

Apart from the statistical analyzes, the correlations between clinical symptoms occurring after a sting and the classes of venom sIgE and phospholipase A<sub>2</sub> sIgE were investigated.

We found significant correlations between clinical signs after a sting and the classes of venom sIgE and phospholipase A<sub>2</sub> sIgE in both groups. Thus, the severity of clinical symptoms after a bee sting was greater in the participants with higher classes of both venom sIgE and phospholipase A<sub>2</sub> sIgE. The correlation between the

classes of venom sIgE and phospholipase A<sub>2</sub> sIgE was also examined and it was found to be significant. Therefore, it can be concluded that a patient with a particular class of venom sIgE has also a similar or the same class of phospholipase A<sub>2</sub> sIgE.

Beekeepers are a group of people particularly exposed to honeybee venom allergy [19, 20]. According to current data, 30–60% of beekeepers have positive results of diagnostic tests to bee venom (skin tests and venom sIgE) [21]. However, there are no reports in the available literature on the differences in tIgE levels between the beekeepers and the general population. There are also no data on phospholipase A<sub>2</sub> specific IgE levels in this group.

Therefore, in this study, we determined the levels of tIgE, venom sIgE, phospholipase A<sub>2</sub> sIgE and the classes of venom sIgE and phospholipase A<sub>2</sub> sIgE in two groups – the beekeepers and individuals with standard exposure to bees. Statistical analyses showed no significant differences in tIgE levels and classes of venom sIgE and phospholipase A<sub>2</sub> sIgE between the two analyzed groups. On the contrary, we found that venom specific IgE levels were significantly higher in the beekeepers than in the control group. In the case of phospholipase A<sub>2</sub> sIgE levels, a limiting level of significance obtained indicated a trend toward higher levels of these antibodies in the beekeepers than in the individuals with standard exposure to bees. These results demonstrate that a contact with bees is an important factor influencing the levels of both venom sIgE and phospholipase A<sub>2</sub> sIgE.

## Conclusions

Summing up the findings of this work, it can be concluded that in the beekeepers the severity of clinical symptoms after a bee sting correlated better with phospholipase A<sub>2</sub> sIgE levels than with venom sIgE levels. Moreover, venom sIgE and phospholipase A<sub>2</sub> sIgE levels were higher in the beekeepers than in the control group. This means that the beekeepers differ from the general population, not only in their exposure to a sting, but also in the response of their immunological system to bee venom.

## Acknowledgments

The project was supported by the National Science Centre (2012/05/B/NZ7/O2535).

## Conflict of interest

The authors declare no conflict of interest.

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