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ORIGINAL ARTICLE

Immune and clinical response to honeybee venom in beekeepers

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Abstract

Objective. The aim of the study was to assess immune response to honeybee venom in relation to the degree of exposure, time after a sting and clinical symptoms.

Materials and method. Fifty-four volunteers were divided into 2 groups: beekeepers and a control group. The serum levels of total IgE (tIgE), bee venom-specific IgE (venom sIgE), phospholipase A_2-specific IgE (phospholipase A_2 sIgE), tryptase and venom-specific IgG4 (venom sIgG4) were determined. In beekeepers, diagnostic tests were performed within 3 hours following a sting and were repeated after a minimum of 6 weeks from the last sting. In individuals from the control group, the tests were performed only once, without a sting.

Results. The tests showed significant differences in venom sIgE (beekeepers' median = 0.34 kUA/l, control group median = 0.29 kUA/l), baseline serum tryptase (beekeepers' median = 4.25 µg/l, control group median = 2.74 µg/l) and sIgG4 (beekeepers' median = 21.2 mgA/l, control group median = 0.14 mgA/l), confirming higher levels of the tested substances in the beekeepers than in the control group. A significant positive correlation was observed between phospholipase A_2 sIgE concentration and severity of clinical symptoms after a sting in the group of beekeepers. It was also demonstrated that the clinical symptoms after a sting became less severe with increasing age of the beekeepers.

Conclusions. The differences in the immune response to a bee sting between the beekeepers and individuals not exposed to bees were probably due to the high exposure of the beekeepers to honeybee venom allergens. This may suggest a different approach to the bee venom allergy diagnostic tests in this occupational group.

Key words

allergens, diagnostics, honeybee sting, beekeeping

INTRODUCTION

Allergy to *Hymenoptera* venom, including honey bee (*Apis mellifera*), is one of the main causes of anaphylaxis, both in adults and children. Beekeepers are individuals particularly exposed to stings and honeybee venom allergy [1, 2, 3]. Data from the literature suggest that 17–43% of beekeepers are allergic to bee venom [4]. According to the literature data, the percentage of systemic reactions after a sting in this group ranges from 4.4% [4] – 43% [5], whereas the incidence of large local reactions reaches 38% [5, 6].

Clinical studies reveal certain predispositions that affect the severity of the allergic reaction to *Hymenoptera* venom [7, 8, 9, 10, 11]. The main risk factors are: age, cardiovascular diseases, administered drugs, particularly beta-blockers and angiotensin-converting enzyme (ACE) inhibitors, mastocytosis, elevated baseline serum tryptase (BST), previous episodes of severe anaphylactic reaction (SAR) after a sting, and a short period of time after the last sting. Additional risk factors which have been identified to-date in the beekeepers include: allergic symptoms in the upper respiratory tract during work in an apiary, asthma, length of time spent in an apiary, the number of stings received per year and seasonality (more severe reactions at the beginning than at the end of the beekeeping season) [5, 6, 12, 13, 14, 15, 16].

The results of currently used skin tests and determination of venom-specific IgE in the diagnosis of allergy to *Hymenoptera* venom do not correlate with severity of clinical symptoms [7, 8, 17, 18]. To estimate the risk of severe allergic reactions after a subsequent sting, the determination of serum tryptase is additionally applied [7, 8, 9, 19, 20, 21, 22, 23]. The measurement of serum tryptase level is recommended within 15 minutes to 3 hours after the beginning of an anaphylactic reaction [24]. It was proved that serum tryptase level correlated with the severity of clinical symptoms. Another test, used in the diagnosis of allergy to bee venom, involves determination of venom-specific IgG4 level that reflects the exposure to insect stings. However, no correlation of sIgG4 level with the severity of clinical symptoms after a sting has been demonstrated [25]. There are no studies on the potential relationship between clinical symptoms and phospholipase A_2 – specific IgE level (phospholipase A_2 – Api m 1 is the major allergen of bee venom).

Beekeepers, being highly exposed to bee stings, provide a very interesting group to study correlations between clinical symptoms after a sting and the diagnostic tests. However, the recent literature contains only few reports on clinical studies focused on the reactions to honeybee venom in beekeepers. Therefore, the aim of this study was to assess the immune response to the honeybee venom in relation to the degree of exposure, time after a sting and clinical symptoms.

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MATERIALS AND METHOD

The study was conducted between June – October 2011 and involved 54 individuals. The participants were volunteers recruited at meetings of beekeeping associations. The volunteers were divided into 2 groups: 1st group – beekeepers (N = 30) and 2nd group – control group (N = 24) consisting of individuals with standard exposure to bees). The study was conducted with the approval of the Bioethics Committee of the University of Medical Sciences in Poznan, Poland (Resolution No. 324/11).

Blood samples were collected twice in the group of beekeepers (immediately after a bee sting and 6 weeks after the last sting) and once in the control group (without a sting).

Data on clinical symptoms after a bee sting were obtained from a questionnaires filled-in by the volunteers involved in the study. The questionnaires were developed for the purpose of this study and consisted of a set of multiple choice questions. All the individuals were asked about the symptoms occurring after a sting in the past. Moreover, when the beekeepers were diagnosed directly after a sting, they filled-in a questionnaire regarding also the recent sting (Tab. 1). The beekeepers were additionally asked about their work in the apiary: number of colonies in the apiary, number of years working in the apiary, number of stings received daily, number of days in the apiary per week and the use of protective clothing (Tab. 2).

Table 1. Clinical symptoms after a bee sting in patients (N = 54) divided into 2 groups: 1st group – beekeepers (N = 30) and 2nd group – control group (N = 24). NR-normal reaction, LLR-large local reaction, SYS-systemic reaction

Clinical symptoms		Participants N=54		1st group N=30		2nd group N=24	
		N	%	N	%	N	%
After a sting in the past	NR	38	70.4	23	76.6	15	62.5
	LLR	11	20.4	5	16.7	6	25.0
	SYS	5	9.2	2	6.7	3	12.5
After a recent sting in beekeepers	NR	–	–	25	83.3	–	–
	LLR	–	–	3	10.0	–	–
	SYS	–	–	2	6.7	–	–

***In vitro* tests.** The serum levels of total IgE (tIgE), bee venom-specific IgE (venom sIgE), phospholipase A_2-specific IgE (phospholipase A_2 sIgE), tryptase and venom-specific (sIgG4) were determined. In the beekeepers, the diagnostic tests were performed within 3 hours after a sting (during the beekeeping season) and were repeated after a minimum of 6 weeks after the last sting (after a minimum of 6 weeks from the end of the beekeeping season). In the control individuals, the tests were performed only once and without a sting.

All the determinations were conducted using ImmunoCap system, UniCap 100, Phadia, Sweden.

Statistical analysis. Statistical analyses were performed using Statistica 10.0 software. In all analyses the level of $p < 0.05$ was assumed as statistically significant. Normal distribution of the analyzed data was tested using the Shapiro-Wilk test. The sequence pairs Wilcoxon test was used to compare the results obtained within 3 hours after a sting and at least 6 weeks after a sting. The results in the participants experiencing increased contact with bees and those in the control group were compared by U Mann-Whitney test. Kruskal-Wallis ANOVA by ranks was used in the beekeeper group for the analysis of sIgG4 *vs.* the following variables: number of years working in the apiary, number of stings received daily, number of working days in the apiary per week, and the use of protective clothing. The same test was also used to assess the correlation between clinical symptoms after a sting and the results of *in vitro* tests.

Table 2. Characteristics of the beekeepers; N = 30

Characteristic		N	%
No. of colonies in the apiary	1 – 0	6	20.0
	21 – 40	18	60.0
	more than 40	6	20.0
No. of years working in the apiary [years]	1 – 10	9	30.0
	11 – 20	10	33.3
	more than 20	11	36.7
No. of stings received daily	less than 1	17	56.7
	1 – 5	5	16.7
	6 – 10	4	13.3
	more than 10	4	13.3
No. of days in the apiary per week	1 – 2	16	53.3
	3 – 4	10	33.3
	5 – 6	4	13.3
Use of protective clothing	some elements	13	43.3
	complete outfit	17	56.7

RESULTS

The presented study focused on the determination of parameters associated with the immune response to the honeybee venom (tIgE, venom sIgE and phospholipase A_2 sIgE, serum tryptase, sIgG4) in a group of 54 individuals experiencing variable exposure to bee stings.

Period of time after a bee sting *vs.* results of diagnostic tests. To check the effects of the period of time after a sting on the *in vitro* tests results (tIgE, venom sIgE and phospholipase A_2 sIgE, serum tryptase and sIgG4), the diagnostic tests were performed twice in 30 stung beekeepers. Analyses showed no significant differences in the levels of tIgE (p=0.324) (median not stung = 22.80 kU/l; median stung = 28.40 kU/l), venom sIgE (p=0.386) (median not stung = 0.34 kUA/l; median stung = 0.33 kUA/l), phospholipase A_2 sIgE (p=0.099) (median not stung = 0.09 kUA/l; median stung = 0.11 kUA/l), serum tryptase (p=0.929) (median not stung = 4.25 µg/l; median stung = 4.45 µg/l) and sIgG4 (p=0.288) (median not stung = 21.20 mgA/l; median stung = 24.50 mgA/l), determined within 3 hours after a bee sting, and after a minimum of 6 weeks after the sting (according to current recommendations).

Degree of exposure to bee stings *vs.* results of diagnostic tests. The results obtained in the beekeepers after at least 6 weeks were compared with those from the control group. Analysis showed no statistically significant differences in tIgE between the 2 groups (p=0.383). The tests showed significant

differences in venom sIgE (p=0.038) (beekeepers median = 0.34 kUA/l, control group median = 0.29 kUA/l), baseline serum tryptase (p=0.013) (beekeepers median = 4.25 µg/l, control group median = 2.74 µg/l) and sIgG4 (p<0.050) (beekeepers median = 21.20 mgA/l, control group median = 0.14 mgA/l), confirming significantly higher levels of the investigated parameters in the beekeepers, compared to the control group. In the case of phospholipase A_2 sIgE, the level of significance p=0.055 was obtained (beekeepers median = 0.09 kUA/l, control group median = 0.08 kUA/l), which confirmed lack of significant differences in phospholipase A_2 sIgE level in the study groups.

Clinical symptoms after a bee sting *vs.* results of diagnostic tests. Due to significant differences in venom-specific IgE, phospholipase A_2-specific IgE, baseline serum tryptase (BST) and specific IgG4 levels in the beekeepers and control groups, the statistical analyses between *in vitro* tests and clinical symptoms after a sting were performed separately for these 2 groups. Only the correlation between clinical symptoms and tIgE was assessed for all the individuals (beekeepers and controls). For these analyses, the data on clinical symptoms after a bee sting in the past (data from a patient's history) were used. Furthermore, the correlation between serum tryptase (sT) level, determined within 3 hours after a sting, and clinical symptoms after a recent sting in the beekeepers was assessed.

There was no statistically significant correlation between tIgE, venom sIgE and clinical symptoms after a sting in any of the analyzed groups. A statistically significant correlation was observed between phospholipase A_2 sIgE and clinical symptoms after a sting in the group of beekeepers (p=0.040), but there was no correlation in the control group. These results indicate that the level of phospholipase A_2 sIgE in beekeepers is positively correlated with severity of clinical symptoms after a sting.

There was also no significant correlation between serum tryptase (sT) level and clinical symptoms directly after a sting, and no significant correlation between baseline serum tryptase (BST) and clinical symptoms in the group of beekeepers. Also, no significant correlation was observed between BST and clinical symptoms after a sting in the control group.

No correlation between sIgG4 and clinical symptoms after a bee sting was observed in all the individuals.

Venom-specific IgG4 level. The correlations between venom-specific IgG4 and the following factors were examined: number of years working in the apiary, number of stings received daily, number of working days in the apiary per week and the use of protective clothing. The analyses were performed in the group of beekeepers. Strong positive correlations between increased sIgG4 levels and: number of years working in the apiary (p=0.041) (Fig. 1), number of stings received daily (p=0.008) (Fig. 2) and number of working days in the apiary per week (p=0.039) (Fig. 3) were confirmed. Moreover, a negative correlation was shown between sIgG4 levels and the use of protective clothing (p=0.028) (Fig. 4).

Clinical symptoms after a bee sting *vs.* age. The analyses were performed separately for the group of beekeepers and the control group. A significant correlation was observed between age and clinical symptoms after a bee sting (p=0.029) in the beekeepers. This correlation showed that the clinical symptoms after a sting became less severe with increasing age (Fig. 5).

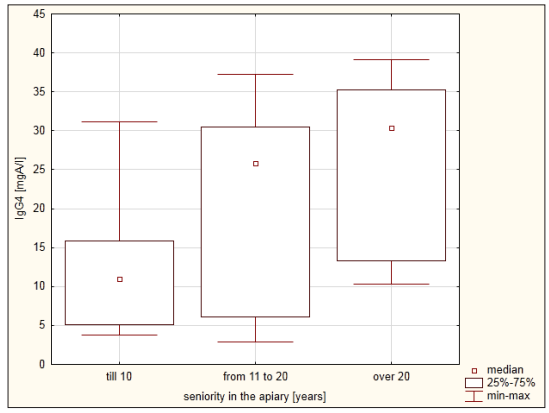

Figure 1. IgG4 *vs.* number of years working in the apiary

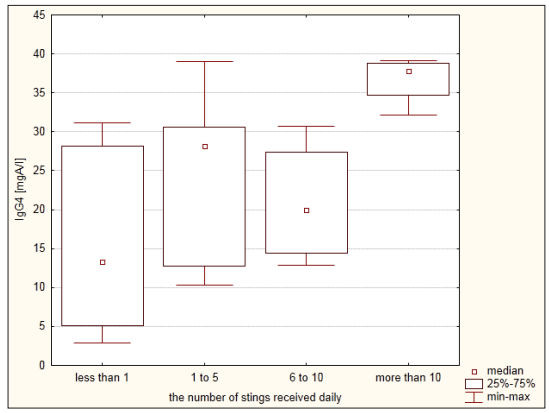

Figure 2. IgG4 *vs.* number of stings received daily while working in the apiary

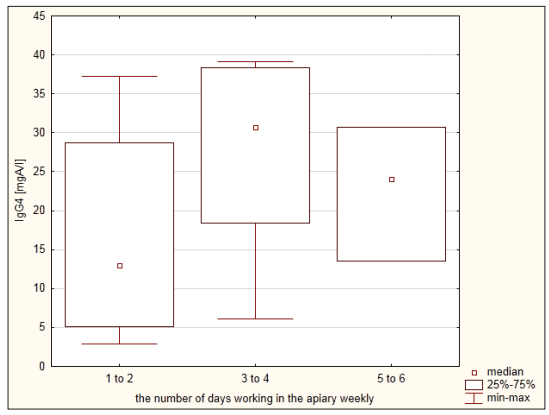

Figure 3. IgG4 *vs.* number of days working in the apiary per week

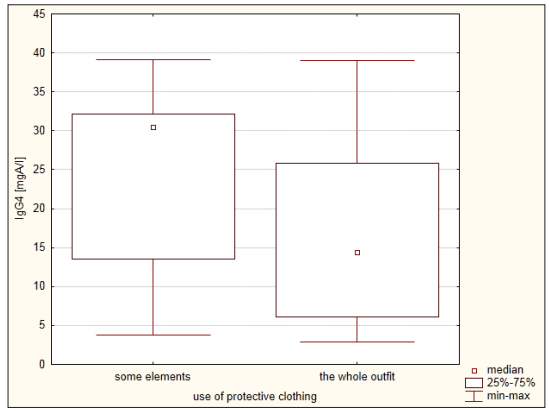

Figure 4. IgG4 *vs.* use of protective clothing

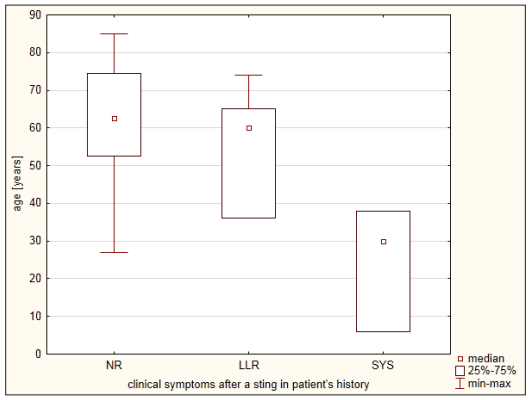

Figure 5. Clinical symptoms (NR – normal reaction; LLR – large local reaction; SYS – systemic reaction) after a sting in patient's history *vs.* age (group of people with increased contact with bees)

There was no significant correlation between clinical symptoms after a sting and age (p=0.102) in the control group. However, Figure 6 shows a tendency to increased severity of sting related clinical symptoms with advancing age.

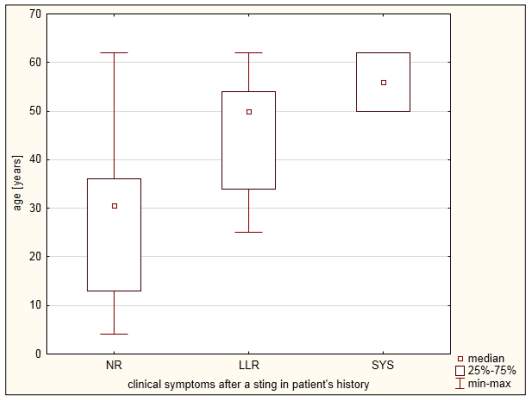

Figure 6. Clinical symptoms (NR – normal reaction; LLR – large local reaction; SYS – systemic reaction) after a sting in patient's history *vs.* age (group of people without increased contact with bees)

DISCUSSION

According to recent recommendations, the diagnosis of allergy to the bee venom should be performed 4–6 weeks after an allergic reaction, because the level of venom-specific IgE (venom sIgE) may be very low or undetectable in the first days after a sting and increase gradually in the following days and weeks [8, 25, 26]. In order to confirm anaphylaxis, the level of serum tryptase (sT) should be determined no later than 3 hours after a sting. Currently, the scientific literature lacks data on the correlation between the levels of total IgE, venom-specific IgE, phospholipase A_2-specific IgE and venom-specific IgG4, and the period of time after a sting.

In the presented study of 30 stung beekeepers, the honeybee venom allergy diagnostic tests were performed twice: immediately after a sting (during the beekeeping season) and after 6 weeks following the last sting. It was found that the levels of tIgE, venom sIgE, phospholipase A_2 sIgE, serum tryptase and sIgG4 directly after a bee sting, and minimum 6 weeks after the sting did not differ in the analyzed individuals. However, it should be emphasized that the stung individuals were beekeepers, and these results should therefore be related only to this group.

The second aim of the study was to compare the results of honeybee venom allergy diagnostic tests in the beekeepers and the people exposed to standard contact with bees. The beekeepers, due to their work, represent a group particularly exposed to stinging and bee venom allergy [4, 25]. Based on previous studies, it is known that the positive results of diagnostic tests, including skin tests and venom-specific IgE determination, are found in 30–60% of people in this group [27]. There are no reports in the available literature documenting differences between beekeepers and the general population in tIgE, venom sIgE, phospholipase A_2 sIgE and baseline serum tryptase levels. It is known that specific IgG4 level is particularly high in the beekeepers group, as it is increasing owing due to the frequent stings.

In this study, a significantly higher level was found of bee venom-specific IgE, baseline serum tryptase and specific IgG4 in the beekeepers, compared to the control group. In contrast, no significant differences were found in total IgE and phospholipase A_2-specific IgE level between these 2 groups.

The correlation between the clinical symptoms after a sting and atopy markers, including tIgE level, is ambiguous and still disputable. It has been repeatedly emphasized that there is a lack of correlation between venom sIgE level and the severity of clinical symptoms after a sting. In the available literature, there are no data on the correlation between the clinical symptoms and phospholipase A_2 sIgE level. Clinical studies performed to-date on phospholipase A_2 sIgE have been related to the use of recombinant phospholipase A_2 (Api m 1) to distinguish double sensitization from cross-reaction in venom allergy [28, 29, 30]. However, there is insufficient information about phospholipase A_2 sIgE determination in bee venom allergy diagnostics. Serum tryptase determination immediately after a sting is a widely recognized test confirming anaphylaxis. Moreover, it was proved that an elevated level of baseline serum tryptase (BST) was a risk factor for severe allergic reaction after a subsequent sting [31]. However, a correlation between specific IgG4 level and the clinical symptoms after a sting has not been demonstrated [25].

In the current study, the levels of total IgE and venom-specific IgE did not correlate with the severity of clinical symptoms after a bee sting in the individuals from either group. The correlation between clinical symptoms and phospholipase A_2-specific IgE level was obtained only in the beekeepers. This suggests a higher diagnostic value of phospholipase A_2-specific IgE in comparison to the venom-specific IgE.

There was no correlation between the severity of clinical symptoms after a bee sting and sT level determined within 3 hours after a sting. The discrepancy between the results obtained in current study and the data from the literature, which prove the correlation between sT and the severity of clinical symptoms, may be due to the fact that the stung individuals in this study were professional beekeepers who spent a lot of time in the apiary. It must be emphasized that the data from the literature refer to the general population, and sT levels in the beekeepers directly after a bee sting has not yet been studied. In the presented study, no correlation was detected between the severity of the clinical symptoms after a bee sting and BST, neither in the beekeepers or in people having standard contact with bees.

Additional factors affecting the level of venom sIgG4 in the group of beekeepers were assessed in this study. It was shown that the sIgG4 level increased in the beekeepers with the increasing length of time spent in the apiary, higher

number of stings received daily while working in the apiary, greater number of working days in the apiary per week, and that it was higher in the beekeepers who did not use protective clothing while working in the apiary. These results confirmed that the factors directly related to the number of received bee stings influenced specific IgG4 levels, which is in agreement with the literature data [1, 7, 8, 14, 32].

The correlation between clinical manifestation of a sting and age was additionally examined. It was observed that clinical symptoms after a bee sting were less severe with the increasing age of the beekeepers. These results challenge those reported in the literature, according to which the risk of severe allergic reaction after a sting increases with age. This correlation may be due to the fact that older beekeepers have higher sIgG4 levels that provide better protection. The older beekeepers have been exposed to bee venom allergens longer than the younger ones, which causes natural desensitization. Therefore, the severity of symptoms after a sting may decrease with the beekeeper's age. However, the literature reports involve only the general population, not beekeepers in particular. In fact, in the control group of people who had standard contact with bees, a tendency to more severe clinical symptoms after a bee sting was observed with advancing age. These observations are consistent with the literature data on the general population.

Summing up, it should be emphasized that the immune response to honeybee venom significantly differs between the beekeepers and the people with standard exposure to bees. Therefore, the results obtained in this study may not be broadly applicable to the general population, but only to the group of beekeepers. The beekeepers had significantly higher levels of venom-specific IgE and BST. Moreover, the higher the number of stings in the beekeepers, the higher the concentration of protective sIgG4 antibodies. This triggers a natural tolerance to honeybee venom in the beekeepers and may suggest the need for a different approach to bee venom allergy diagnostic tests in this occupational group.

Acknowledgments

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