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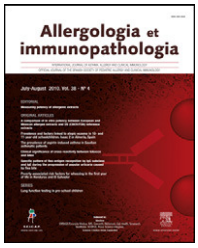

ORIGINAL ARTICLE

# The analysis of Hymenoptera hypersensitive patients in Ankara, Turkey

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Venom immunotherapy;
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**Abstract**
*Background:* Although there are some published data about the prevalence of honeybee and vespid venom allergy from Turkey, there has been no report about Hymenoptera venom immunotherapy practice. Our aim was to determine the characteristics of Hymenoptera venom hypersensitivity and venom immunotherapy practice in Ankara, Turkey.
*Methods:* Demographic and clinical data, intradermal test, and serum specific IgE results of 65 Hymenoptera venom allergic patients who were followed up in our department from February 2005 to August 2009 were analysed.
*Results:* Serum Vespula specific IgE class (p:0.02) and Apis specific IgE class were high (p<0.0001) and Apis intradermal test results were positive (p<0.001) in accordance with the patients' history. However, intradermal test results with Vespula were not consistent with self-reported Hymenoptera type (p:0.15). While Apis specific IgE and intradermal test results were correlated with each other (rho: 0.59, p<0.0001), Vespula specific IgE and intradermal test results were not (rho: 0.2, p:0.17). Intradermal test against Vespula did not discriminate between Apis and Vespula hypersensitive patients. There were no significant differences when the grade of reaction and specific IgE and intradermal test results were compared between Apis and Vespula.
*Conclusions:* Vespula venom hypersensitivity was more common among our patients. However, intradermal tests with Vespula had limited diagnostic sensitivity and were not correlated with serum specific IgE. Based on our results and previous reports, we recommend that negative skin test responses, especially with Vespula, need further investigation.


## Introduction

Hymenoptera venom can lead to systemic allergic reactions in up to 5% of the population in Europe and North America.[1] In Turkey, the lifetime incidence of sting exposure and the incidence of systemic and severe systemic reaction rates

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were estimated to be 94.5%, 7.5%, and 2.2%, respectively.[2] In children aged 6- 13 years, the lifetime cumulative sting prevalence and severe systemic reaction incidence were estimated to be 60.8% and 0.2%, respectively.[3] It has been noted that Hymenoptera stings were most often caused by the honeybee, *Apis Mellifera*, and the vespid, *Vespula Vulgaris*, in Turkey.[4]

To identify species of the offending insect, recommend treatment, and educate patients about avoidance measures, patients should undergo a diagnostic evaluation.[5] A detailed and careful history can usually establish the diagnosis of Hymenoptera sting reaction.[5] However, sensitisation should be confirmed by a skin test reaction to venom or the detection of venom-specific IgE-antibodies to identify the responsible species, because there may be recall bias.[6] The sensitivity and specificity of the utilised diagnostic tests are important for the correct diagnosis. Although there are some published data about the prevalence of honeybee (apis) and vespid (vespula) venom allergy, there has been no report about Hymenoptera venom immunotherapy practice from our country. Our aim was to determine the characteristics of Hymenoptera venom hypersensitivity and venom immunotherapy practice in Ankara, Turkey.

## Materials and methods

Data of 65 Hymenoptera venom allergic patients who were followed up in our department from February 2005 to August 2009 were analysed. Immunotherapy was recommended to the patients with a history of immediate systemic reaction after a sting and demonstrable specific IgE either by skin testing or serum assay. The severity of reactions was graded according to the system proposed by HL Mueller.[7] All patients with Hymenoptera hypersensitivity were recommended self-injectable adrenalin.

To distinguish between honeybee and vespula, patients were asked several questions. Questions were about the patient's activity and location at the time of the sting and if he/she could recognise the insect. The presence of a stinger, which is left primarily by honeybees, is asked. Hymenoptera figures were used for visual identification of the insect, in case of doubt.

Skin tests were performed with Apis and Vespula venom (*Apis Mellifera*, *Vespula*, Stallergenes, France) at least four weeks after the sting reaction.[1,6] Skin prick test (SPT) was carried out with 1 μg/ml of venom concentration, and if negative, then intradermal injections with concentration of tenfold dilutions ranging from 0.001- 1 μg/ml and 0.02 ml of venom were performed on the volar surface of the forearm.[6] The SPT was considered positive if a geometric mean diameter of the resulting wheal was at least 3 mm after 15 min.[8] Intradermal test was read as positive at a mean wheal diameter of at least 5 mm with erythema with concentration of 1 μg/ml or less.[9] Histamine and saline were used as positive and negative controls, respectively. The test was terminated at the positive concentration.

Serum allergen specific IgE levels to Apis and Vespula venom were measured with the immunoCAP system (PhadiaAB, Upsala, Sweeden) according to the manufacturer's instructions. Specific IgE measurement was also performed at least two weeks after the sting reaction. The measuring range of the immunoCAP system is 0.35-100 kU/L. The results were graded as class 0, 1, 2, 3, 4, 5, and 6 with specific IgE levels of <0.35, 0.35-0.7, 0.7-3.5, 3.5-17.5, 17.5-50, 50-100, and >100 kU/L, respectively. Results >0.35 kU/L were considered as positive.

Ultrarush desensitisation was performed to patients who live far from Ankara. Cumulative dose of 101.1 μg/ml in 2.5 hours was administered on the first day in an inpatient setting. The maintenance dose of 100 μg/ml was administered after 15 days and thereafter once a month for a year.[10] Injection interval was six weeks for the second year and eight weeks for the third year until the end of five years.[11] All patients who received immunotherapy had also been prescribed self-injectable adrenalin. Pulse rate, blood pressure and peak expiratory flow rate were recorded during the desensitisation and after each injection during the maintenance phase. Full emergency resuscitation equipment was available. Injections were performed subcutaneously by experienced medical doctors. After each injection, patients were kept under medical observation for at least 30 minutes. Conventional immunotherapy protocol was administered to remaining patients, who live in Ankara, with weekly injections for 16 weeks when the maintenance dose of 100 μg/ml is reached. Maintenance injection intervals were the same as ultrarush protocol.

### Statistical analysis

Descriptive data for categorical and numerical variables were expressed as frequencies and median with range in parenthesis, respectively. Results of specific IgE and intradermal testing were compared between the groups who reported Apis and Vespula sting, respectively. Mann-Whitney U test was used for the statistical testing. P values less than 0.05 was considered as statistically significant. The study protocol was accepted by the local ethical committee.

## Results

A total of 65 patients were included in the analysis. Mean age of the study population was 40 (16-68, SD: 12.4) and 50.8% were females. Mean reaction age of the patients was $32.8 \pm 13.35$ (5-57). According to patients' history, Vespula was responsible for 52.3%, Apis for 27.7%, both Apis and Vespula for 9.2% of the reactions. Responsible Hymenoptera type was not known in 10.8% of the cases. According to Mueller's classification, incidence of Grade 1, 2, 3, 4 reactions were 4.6%, 18.5%, 29.2%, and 47.7%, respectively. Clinical data of patients receiving immunotherapy are shown in Table 1. Forty-six (70%) patients received immunotherapy, 17 (26%) self injectable adrenalin, and two (3%) did not receive any treatment (lost to follow-up). Clinical data of patients who did not receive immunotherapy are shown in Table 2.

Thirty-five patients (53.8%) had accompanying atopic disease, 14 of whom had rhinitis, nine had asthma and rhinitis, seven rhinitis, three aspirin-exacerbated respiratory disease and two drug hypersensitivity.

Twelve patients are on the first, 14 on the second, four on the third, eight on the fourth, and five patients are on the fifth year of immunotherapy. Two patients are lost to

**Table 1** Clinical data of patients receiving immunotherapy*

| | Vespula | Apis |
|---|---|---|
| *Median Age (Range)* | 37.5 (17-56) | 43 (20-59) |
| *Gender* | | |
| Male | 16 | 10 |
| Female | 8 | 5 |
| *Total* | 24 | 15 |
| *Grade of reaction‡* | | |
| 2 | 4 | 1 |
| 3 | 4 | 7 |
| 4 | 16 | 7 |
| *Prick test positivity (1 μg/ml)* | 0 | 1 |
| *Intradermal test positivity* | | |
| 0.001 μg/ml | 1 | 3 |
| μg/ml | 2 | 2 |
| μg/ml | 8 | 5 |
| μg/ml | 3 | 0 |
| *Negative* | 6 | 1 |
| *Not performed* | 4 | 3 |
| *Total* | 24 | 15 |
| *SpIgE class* | | |
| 0 | 1 | 0 |
| 1 | 1 | 1 |
| 2 | 9 | 8 |
| 3 | 8 | 4 |
| 4 | 2 | 2 |
| 5 | 0 | 0 |
| 6 | 2 | 0 |
| *Not performed* | 1 | 0 |
| *Total* | 24 | 15 |
| *Protocol* | | |
| Ultra-rush | 16 | 5 |
| Conventional | 8 | 10 |
| *Total* | 24 | 15 |
| *Adverse reaction†* | | |
| Large local | 3 | 1 |
| Systemic grade 1 | 1 | 1 |
| grade 2 | 0 | 1 |
| grade 3 | 0 | 3 |
| *Total* | 4 | 6 |

Descriptive data for categorical and numerical variables were expressed as frequencies and median with range in parenthesis, respectively.

* Data of seven patients who received immunotherapy with both venoms are not included.

‡ There were no grade 1 and large local reactions in the immunotherapy group.

† No patients had grade 4 systemic reaction after injections.

**Table 2** Clinical data of patients who did not receive immunotherapy*

| | Apis | Vespula | Total |
|---|---|---|---|
| *Responsible bee* | 2 | 13 | 19 |
| *Reaction grade* | | | |
| 1 | None | 2 | 3 |
| 2-3 | 2 | 7 | 12 |
| 4 | None | 4 | 4 |
| *Skin prick test* | None | None | None |
| *Intradermal test* | | | |
| 0.001 μg/ml | - | - | - |
| 0.01 μg/ml | - | - | - |
| 0.1 μg/ml | - | 2 | 2 |
| 1 μg/ml | 1 | 3 | 4 |
| Negative | - | 3 | 3 |
| Not performed | 1 | 5 | 6 |
| *SpIgE class* | | | |
| 0 | 1 | 4 | 5 |
| 1 | - | - | - |
| 2 | - | 5 | 5 |
| 3 | 1 | 3 | 4 |
| 4 | - | 1 | 1 |
| *Immunotherapy recommended* | 2 | 5 | 7 |

* Data of two patients that the bee type is not known and two patients that Apis and Vespula were indicated are not included.

follow up and one patient left treatment due to financial problems. Twelve patients were restung during immunotherapy and among them only two had systemic reactions, one grade 2, and the other one grade 3. These two patients self injected adrenalin.

Serum Vespula specific IgE class (p:0.02) and Apis specific IgE class were high (p<0.0001) and Apis intradermal test results were positive (p<0.001) in accordance with the patients' history. However, intradermal test results with Vespula were not consistent with self-reported Hymenoptera type (p:0.15). Eight out of 25 patients revealed negative skin test result with Vespula, five of whom had positive serum specific IgE, whereas intradermal test with Apis revealed only one negative result out of 15 Apis hypersensitive patients. In patients who underwent immunotherapy, both intradermal tests and serum specific IgE for Apis and Vespula were significantly correlated with immunotherapy bee type (p: 0.04, p<0.0001 and p<0.0001, p<0.0001, respectively). Vespula venom allergic patients tended to have high values of specific IgE against Apis. While Apis specific IgE and intradermal test results were correlated (rho: 0.59, p<0.0001), Vespula specific IgE and intradermal test results were not (rho: 0.2, p:0.17). Intradermal test against Vespula did not discriminate between Apis and Vespula hypersensitive patients. These results suggest that intradermal test for Vespula have limited diagnostic sensitivity. The association between test results and bee type are shown in Table 3.

Nineteen patients did not receive immunotherapy, seven of whom were recommended and 12 of whom were not. Twelve patients were not recommended immunotherapy because tests were negative in six, concomitant severe disease was present in four, reaction was a mild urticaria in one, and no reaction in the second sting in one patient. In those 12 patients, self-reported Hymenoptera was vespula in nine, was not known in one and both apis and vespula in two patients. Among 19 patients who did not receive immunotherapy, 17 received self-injectable adrenalin only,

**Table 3** The association between test results and reported bee type[†]

| | Vespula | Apis | p |
|---|---|---|---|
| Vespula class | n: 33<br>3 (1-3) | n: 18<br>2 (1.25-2.75) | 0.025 |
| Apis class | n: 32<br>1 (0-2) | n:18<br>2 (2-3) | <0.0001 |
| Intradermal Vespula[*] | n: 25<br>1 (0-2) | n: 12<br>0 (0-1) | 0.15 |
| Intradermal Apis[*] | n: 13<br>0 (0-0) | n:15<br>2 (1.25-3) | <0.0001 |

[†] The analysis is performed based on self-reported bee type; median and interquartile ranges are given.
[*] 0: negative test result, 1: 1 μg/ml positive, 2: 0.01 μg/ml positive, 3: 0.1 μg/ml positive.

13 of whom had been stung with Vespula, two patients who were recommended immunotherapy with Apis were lost to follow up. The high number of patients in the only self-injectable adrenalin group who had been stung with Vespula also indicates that the diagnostic tests, especially the skin tests with Vespula may have limited diagnostic sensitivity.

There were no significant differences when grade of reaction and specific IgE and intradermal test results were compared between Apis and Vespula. Serum specific IgE and intradermal test results of Grade 4 reaction were not different from Grade 1-2-3 reactions.

## Discussion

Honeybees usually do not sting without provocation; they mostly sting in defence of their nest and their queen, whereas yellow jackets tend to sting with minimal provocation. They are highly aggressive and will sting for no apparent reason, particularly in the autumn, when larger populations compete for limited food supplies.[12,13] Among our Hymenoptera hypersensitive patients, based on self-reported Hymenoptera type, Vespula was responsible for 52.3% of the reactions, and Apis for 27.7%. Previous studies also reported higher incidence of Vespula hypersensitivity.[14,15]

For SPT, venom concentrations in the range of 1.0 μg/mL are usually performed before intracutaneous tests, but are not used by all allergists.[5,6] Even at 100 μg/ml the sensitivity of SPT is lower than that of intradermal test.[16] We performed SPT with 1 μg/ml venom concentration and only one patient with honeybee allergy was SPT positive. Although our study population is small and we performed SPT with 1 μg/ml venom concentration, we can assume that prick testing rarely seems to be positive.

The most striking finding in our study population was the low sensitivity of intradermal test with Vespula. Because of the irritative effect of commercially available venom preparations, concentrations higher than 1 μg/ml cannot be used. Low level of specific IgE, however, can lead to systemic reactions, and may not be detected with intradermal testing with 1 μg/ml concentration of the Vespula venom preparation.[17] Based on our results and previous reports, we recommend that negative skin test responses especially with Vespula, need further investigation.

We previously reported that a history of seasonal and perennial rhinitis, food allergy, and physician-diagnosed asthma in Turkish beekeepers were associated with systemic sting reactions. The risk of systemic reaction increases approximately threefold when one atopic disease is present and eleven fold when two or more concurrent atopic diseases are present compared to having no atopic disease.[18] In this study, 53.8% of our patients had concurrent atopic disease in accordance with our previous finding in beekeepers. Müller also reported that atopy may increase the risk and severity of systemic reactions in beekeepers and their family members.[19] However, Birnbaum et al. reported that the percentage of atopic subjects defined by clinical symptoms and positive skin tests to common aeroallergens was not higher among patients with a history of anaphylactic reaction to Hymenoptera sting than in controls with no such history. They suggested that atopy is not a risk factor for the occurrence of an anaphylactic reaction to Hymenoptera sting, at least among non-beekeepers.[20] We think that this issue should be further investigated.

We analysed our Hymenoptera venom hypersensitive patients and we found some interesting data. However, there are some limitations of our analysis. First, our study population is small. Second, we performed the analysis based on self-reported Hymenoptera type and a considerable number of patients (10.8%) could not remember the responsible Hymenoptera and data of these patients are not included to the analysis. Also, there may have been recall bias. Nevertheless, we showed that if self-reported Hymenoptera is Apis, then the patient's report is very likely to be true.

In conclusion, our aim was to determine the characteristics of Hymenoptera venom hypersensitivity and venom immunotherapy practice in Ankara, Turkey. We analysed the data of our patients and found that Vespula venom hypersensitivity was more common among our bee stung patients. Intradermal tests with Vespula have limited diagnostic sensitivity and are not correlated with serum specific IgE. In the case of negative skin test results, especially with Vespula, it should be kept in mind that it is not possible to fully exclude a future sting reaction.

## Conflict of interest

The authors have no conflict of interest to declare.

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