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The role of basophil activation test in venom immunotherapy: comparative evaluation with specific IgE and skin prick tests, innovative approaches

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Venom Immunotherapy; basophil activation test; specific IgE test; skin prick test.

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IMPACT STATEMENT

The basophil activation test (BAT) offers high accuracy in diagnosing insect venom allergy and making immunotherapy decisions, providing significant insights for clinical practices.

Summary

Background. In diagnosing insect venom allergy and making immunotherapy decisions, clinical history, skin tests, and specific serum IgE levels are commonly utilized. This study aims to emphasize the clinical significance of using the basophil activation test in accurately identifying sensitivities in individuals with insect venom allergy and to compare its effectiveness with other testing methods. **Methods.** This study included a total of 43 patients, who experienced at least one systemic allergic reaction following insect stings and were deemed suitable for immunotherapy. Basophil activation test, specific serum IgE levels, and skin prick test results utilized in making immunotherapy treatment decisions were recorded. **Results.** Our study determined that the overall clinical sensitivities of the basophil activation test (BAT), specific serum IgE (spIgE), and skin prick test (SPT) for *Apis mellifera* were 95.5%, 95.7%, and 48.4% respectively, while for *Vespula vulgaris*, they were 83.3%, 100%, and 33.3%. Based on these results, the prediction of systemic reactions to bee stings is ordered as spIgE > BAT > SPT. Additionally, early-stage skin prick tests showed a sensitivity of 67% and specificity of 50% at a cut-off value of 1.5 mm, and 33% sensitivity and 83% specificity at 2.5 mm. **Conclusions.** This study demonstrates that the basophil activation test (BAT) can provide a high positive predictive value in immunotherapy treatment decisions and offer significant insights in clinical practices.

Introduction

Hypersensitivity to Hymenoptera stings, affecting a considerable segment of the population (56-94%), poses a potential life-threatening risk (1). Systemic allergic reactions to such stings have been reported in up to 7.5% of adults and 3.4% of children (2). Venom immunotherapy (VIT) remains the sole effective treat-

ment for patients showing severe reactions following Hymenoptera stings, reducing the risk of a serious systemic reaction to a sting by approximately 90% (3, 4).

For the detection of hypersensitivity to Hymenoptera venom, skin tests and the measurement of specific IgE antibodies in serum are the commonly employed methods, proving effective

in confirming diagnoses in various cases. Nonetheless, there can be instances of test results not aligned with clinical histories. Precise identification of a patient's sensitivity before initiating VIT is critically important for the success of the treatment. In such scenarios, there arises a need for alternative testing approaches, like cellular *in vitro* tests, that can yield more definitive outcomes. With the limitations of traditional diagnostic methods such as specific IgE and skin prick tests in mind, the significance of the Basophil Activation Test in diagnosing venom allergy is increasingly being acknowledged (5).

Presently, the basophil activation test is utilized in determining clinical sensitivity at the commencement of venom immunotherapy, in patients with conflicting or negative skin test or specific IgE results, for allergen selection in patients with dual sensitivities for VIT, and in the monitoring and evaluation of VIT's efficacy (6, 7). To date, there has been a limited number of studies in literature on this subject and none from our country.

The main objective of our study is to showcase the applicability and effectiveness of the Basophil Activation Test in detecting hypersensitivity to Hymenoptera venom. In particular, this study provides a comparative evaluation of this test with skin prick tests and allergen-specific serum IgE measurements, in terms of both clinical sensitivity and positive predictive values. The study highlights the necessity for current approaches in the more accurate and effective detection and management of hypersensitivity conditions resulting from Hymenoptera stings.

In this regard, the role of the basophil activation test in the immunotherapy process for bee allergy is thoroughly compared with traditional approaches, such as specific IgE measurement and Skin Prick tests. The study delves into the advantages and limitations of these three distinct testing methods and evaluates the potential contribution of BAT in diagnosing Hymenoptera venom allergy.

Materials and methods

Study population and design

This study included a total of 43 patients who exhibited systemic allergic reactions following *Apis mellifera* and *Vespula vulgaris* were consequently treated with venom immunotherapy (VIT). Demographic data, clinical characteristics, and the severity of reactions of the patients were recorded (**table I**). Anamnesis information included details about the type of stinging bee and reaction characteristics. The severity of the patients' reactions was graded according to the Muller classification (2).

Skin prick test

Standardized purified venom antigens of *Apis mellifera* and *Vespula vulgaris* (ALK-Abello, Horsholm, Denmark) were used for skin tests. Application was performed at the recommended standard dosage of 100 µg/mL concentration. Patients underwent skin

Table I - Demographic information of patients.

	n	Percentage %
Gender		
Male	34	79.1
Female	9	20.9
Age		
Below 10 years old	6	14.0
Between 10-20 years old	24	55.8
Above 20 years old	13	30.2
Time to Initiate VIT after Bee Sting		
Below 1 month	4	9.3
Between 1-2 months	18	41.9
Between 2-6 months	5	11.6
Between 6 months to 1 year	12	27.9
Above 1 year	4	9.3
Age of Starting VIT		
Below 5 years old	3	7.0
Between 5-10 years old	18	41.9
Between 10-15 years old	7	16.3
Above 15 years old	15	34.9
Incidence of Bee Sting during VIT		
No	21	48.8
Yes	22	51.2
Incidence of Sting by Treated Bee Species		
No	1	4.5
Yes	21	95.5
Reaction Type		
None	14	63.6
Local	6	27.3
Systemic	2	9.1
Duration of VIT		
1 year	5	11.6
2 years	4	9.3
3 years	5	11.6
4 years	8	18.6
5 years	18	41.9
6 years	3	7.0

prick tests, and intradermal test methods were not employed. Positive test results were defined according to the recommendations of the European Academy of Allergy and Clinical Immunology. Intradermal test results were considered positive if the difference from the negative control was greater than 3 mm (2).

Specific IgE antibody determination

The levels of allergen-specific IgE in serum samples were measured using the ImmunoCAP 1000 system manufactured by Phadia (Sweden). For each serum sample, IgE levels against Honeybee (*Apis mellifera*, I1) and Wasp (*Vespula* Spp, I3) were measured using the ImmunoCAP test kit. A specific test (test code: 6759) for the Bee Venom Components IgE panel was applied. The levels of Allergen-Specific IgE (spIgE) were classified according to a predetermined evaluation scale. Values below 0.10 kU/L were considered negative, while values above 0.10 kU/L were considered positive.

Basophil activation test

BATs were conducted using Flow CAST (Bühlmann Laboratories AG). Venous blood was collected in 10 mL EDTA tubes and stored at 4 °C for no longer than 24 hours. For each patient and allergen, polystyrene tubes were prepared with different concentrations of allergens (bee and wasp venom) and diluted in stimulation buffer. The Flow CAST method was employed for *Apis mellifera* (BAG2-I1) and *Vespula* spp (BAG2-I3). The cut-off point for CD63 activation was determined as 11.5 ng/mL or higher concentrations at $\geq 10\%$. Positive controls included monoclonal anti-Fc ϵ RI antibody and N-formyl-methionyl-leucine-phenylalanine (2 mM), and the negative control used only the stimulation buffer. Cells were analyzed by flow cytometry using a FACSCalibur flow cytometer (Becton-Dickinson Biosciences GmbH, Heidelberg, Germany). Basophilic cells were selected from the lymphocyte population using anti-CCR3 and the upregulation of the activation marker CD63 was calculated as the percentage of CD63 cells in the total basophilic cell population. The cut-off point was set at 10% CD63 cells, as recommended by the supplier.

Statistical evaluation

After encoding the data obtained from the research, it was transferred to the computer and analyzed using the SPSS (Statistical Package for Social Sciences) software package (Version 22 for Windows, SPSS Inc, Chicago, IL, USA). Frequency (categorical) data were expressed in numbers and percentages (%). The diagnostic decision-making characteristics (sensitivity, specificity, *etc.*) of SpIgE, BAT, and SPT results in predicting *Apis mellifera* and *Vespula vulgaris* stings were assessed through Receiver Operating Characteristic Curve (ROC) analysis. In the evaluation of Area Under the Curve (AUC) values in ROC analysis, a test was considered statistically significant when $p < 0.05$.

Ethical committee

The ethical approval for this study was obtained from the Clinical Research Ethics Committee of Ondokuz Mayıs University (number: 2021000609-1). Our study was conducted in accordance with the principles of good clinical practice based on the Helsinki Declaration. Ethical approval confirms that research studies are conducted in compliance with ethical standards and human rights, and that the rights of participants are protected.

Results

Of the 43 patients included in the study, 79.1% were male and 20.9% were female. The ages of the patients at the start of venom immunotherapy are presented in **table I**.

Based on anamnesis, 33 (70.2%) cases were attributed to *Apis mellifera* stings and 14 (29.8%) to *Vespula vulgaris*. Among the patients who reacted to *Apis mellifera* stings, 42.4% displayed Grade 3 reactions and 39.4% Grade 4, while for those reacting to *Vespula vulgaris* stings, 21.4% were Grade 3 and 71.4% Grade 4. Skin Prick Test (SPT) was administered to all 43 patients. Immediately after bee stings, in the first presentation, only 19 skin prick tests were positive (14 *Apis mellifera*, 5 *Vespula vulgaris*). Therefore, those who tested negative among the patients who applied within the first 8 weeks were retested. Sensitivity and specificity were evaluated according to these results. Positive reactions to *Apis mellifera* were observed in 31 patients, while the remaining 12 showed positive reactions to *Vespula vulgaris*. Dual sensitivity was observed in 17 patients. The sensitivity of SPT in predicting *Apis mellifera* stings was 48.4% with a positive predictive value (PPV) of 65.2%. For *Vespula vulgaris* stings, the sensitivity was 33.3% with a PPV of 20.0% (**table II**).

SpIgE assessment was conducted in 31 patients. Among 23 patients who showed systemic reactions to *Apis mellifera* stings, 22 had positive SpIgE results, while all 8 patients with systemic reactions to *Vespula vulgaris* stings had positive results. The sensitivity of SpIgE for systemic reactions caused by *Apis mellifera* and *Vespula vulgaris* stings was determined as 95.7% with a PPV of 100.0% for *Apis mellifera*, and 100.0% with a PPV of 88.9% for *Vespula vulgaris* (**table II**). In 17 patients, dual sensitivity was detected in the DPT test, while in 7 patients, dual sensitivity was detected in the spIgE test.

BAT assessment was carried out in 28 patients. Of the 22 patients stung by *Apis mellifera*, 21 were confirmed by BAT results, and 5 of 6 patients stung by *Vespula vulgaris*. The sensitivity of BAT in predicting *Apis mellifera* stings was 95.5%, with a PPV and Likelihood Ratio (LR) of 95.5% and 5.72, respectively. For *Vespula vulgaris* stings, the sensitivity of BAT was 83.3%, with a PPV and LR of 83.3% and 18.51, respectively (**table III**). Dual sensitivity was not detected.

In terms of diagnostic efficacy in identifying systemic reactions to *Apis mellifera* and *Vespula vulgaris* stings, the diagnostic supe-

Table II - Diagnostic values of diagnostic tests in predicting stings from the *Apis mellifera* and *Vespula vulgaris*.

	Diagnostic test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR
<i>Apis mellifera</i>	SPT	48.4	33.3	65.2	80.0	0.73
	SpIgE	95.7	100.0	100.0	11.1	NA
	BAT	95.5	83.3	95.5	16.7	5.72
<i>Vespula vulgaris</i>	SPT	33.3	48.3	20.0	34.8	0.64
	SpIgE	100.0	95.7	88.9	NA	23.26
	BAT	83.3	95.5	83.3	4.5	18.51

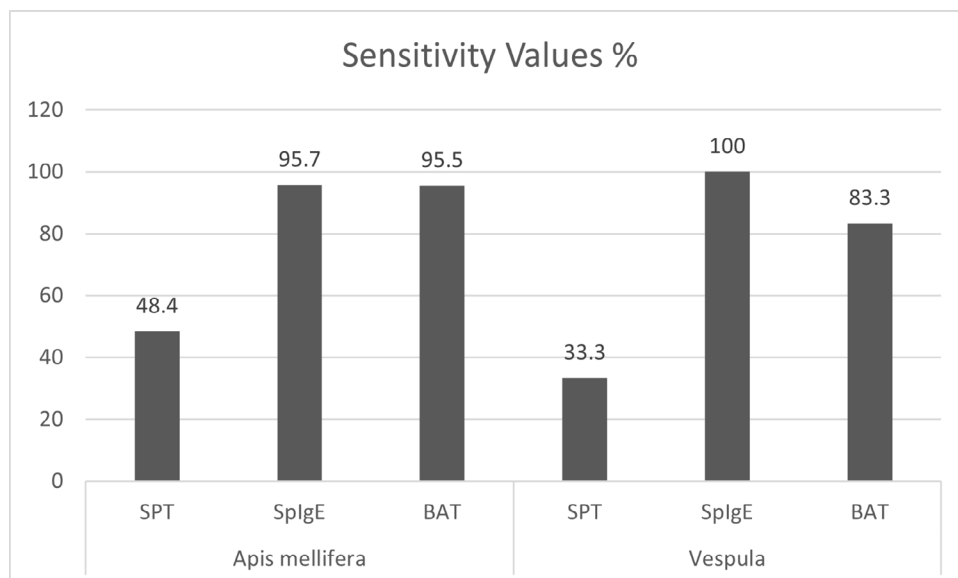
Positive predictive value; negative predictive value; likelihood ratios.

Table III - Comparison of basophil activation test sensitivity results with skin prick test and specific IgE results by bee species.

Variables			Sensitivity results of BAT		P-value*	P-value**
			Positive n (%)	Negative n (%)		
<i>Apis mellifera</i>	SPT	Positive	8 (38.1)	0 (0.0)	NA	NA
		Negative	13 (61.9)	0 (0.0)		
	SpIgE	Positive	16 (94.1)	1 (100.0)	1.00	0.817
		Negative	1 (5.9)	0 (0.0)		
<i>Vespula vulgaris</i>	SPT	Positive	3 (60.0)	0 (0.0)	NA	NA
		Negative	2 (40.0)	0 (0.0)		
	SpIgE	Positive	5 (100.0)	0 (0.0)	NA	NA
		Negative	0 (0.0)	0 (0.0)		

*Fisher's Exact test; **Spearman correlation analysis; NA: no analysis done.

Figure 1 - Sensitivity values for *Apis mellifera* and *Vespula vulgaris*: comparing Basophil Activation Test (BAT), Specific Serum IgE (spIgE), and Skin Prick Test (SPT).



riority ranking was established as Specific IgE (SpIgE) > Basophil Activation Test (BAT) > Skin Prick Test (SPT) (figure 1).

Determining the optimal cut-off value for SPT through ROC analysis (figure 2)

Patients were categorized based on the time intervals following bee stings for conducting the skin prick test: within the first 4

weeks, between 4 and 8 weeks, and beyond 8 weeks. ROC analyses were performed on the measurements in millimeters obtained from the skin prick test results according to this categorization. Diagnostic power of SPT was evaluated considering the patients' time of presentation. For cases presented ≤ 4 weeks, a cut-off = 1.5 mm resulted in a sensitivity of 67% and specificity of 50%. When the cut-off was set at 2.5 mm under the same conditions,

Figure 2 - Diagnostic evaluation of SPT in distinguishing *Apis mellifera* from *Vespula vulgaris* using ROC analysis.

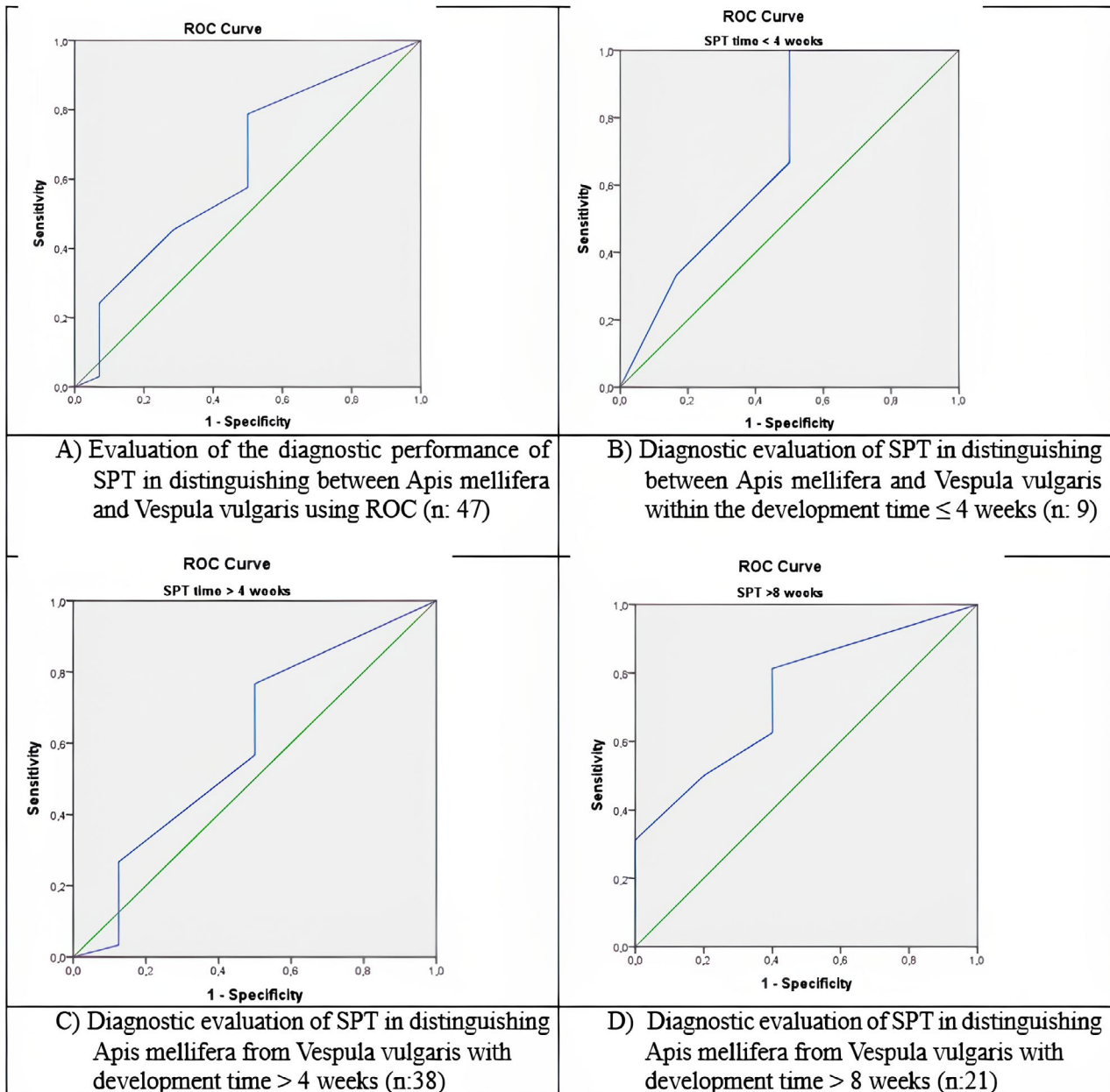


Table IV - Diagnostic values of SPT in distinguishing *Apis mellifera* from *Vespula vulgaris*.

Application time	Cut off value	Sensitivity	Specificity	AUC	SE	P-value	95% Confidence Interval (Minimum - Maximum)	
≤ 4 weeks	1.5	67%	50%	0.69	0.18	0.36	0.33	1.00
	2.5	33%	83%					
> 4 weeks	1.5	57%	50%	0.60	0.12	0.39	0.36	0.84
	2.5	47%	62%					
	3.5	27%	87%					
> 8 weeks	1.5	63%	60%	0.73	0.12	0.11	0.49	0.97
	2.5	50%	80%					
	3.5	31%	100%					

the sensitivity was 33% and specificity was 83% (AUC = 0.69; $p = 0.36$; 95%CI 0.33-1.00). In cases presented > 4 weeks, the statistical evaluation yielded a sensitivity of 57% and specificity of 50% for a cut-off = 1.5 mm (AUC = 0.60; $p = 0.39$; 95%CI 0.36-0.84). The calculated cut-off values and diagnostic values for SPT according to the patients' presentation times are shown in **table IV**.

In our study, irrespective of the patients' time of presentation, ROC analysis was conducted to determine the most appropriate cut-off point for distinguishing between *Apis mellifera* and *Vespula vulgaris* species based on the reaction diameters measured in SPT. In this assessment, the area under the curve (AUC) was calculated as 0.633 (95%CI 0.45-0.81), and a sensitivity of 86% and specificity of 93% were found for a 3.5 mm cut-off value ($p = 0.153$)

Discussion and conclusions

Venom immunotherapy (VIT) is highly effective, with 77% to 84% of patients protected from anaphylaxis after bee venom VIT, and this rate increases to 91% to 96% following wasp venom VIT (2, 8). However, VIT is expensive and time-consuming, requiring a treatment duration of at least 3 to 5 years. According to the guidelines of the European Academy of Allergy and Clinical Immunology for specific immunotherapy for Hymenoptera venom allergy, allergen-specific immunotherapy is recommended for children and adults who have systemic allergic reactions exceeding general skin symptoms, with documented sensitivity to the culprit insect's venom, determined by skin tests and/or specific serum IgE (sIgE) tests and/or basophil activation test (BAT) (9, 10). All three test methods can provide valuable information in the immunotherapy process for bee allergy. While the basophil activation test offers a sensitive approach to determining cellular response, specific IgE tests and Skin Prick tests are more commonly used methods with quicker results. The combination of these tests and consideration of clinical symptoms are import-

ant in determining the treatment plan. The advantages and limitations of each test method should be considered to select the most appropriate diagnostic approaches for individual patients. The bee species identified in a patient's history may not always align with the results from diagnostic tests, or there may be sensitivities to multiple bee species. This can be due to a person's sensitivity to multiple bees or cross-reactivity between bee venom allergens or both (11). Basophil activation test (BAT) helps in diagnosing clinically relevant venoms in cases where routine tests (specific IgE, skin tests) are inconclusive in Hymenoptera venom allergy. A study in our country identified bee venom as the most common cause of anaphylaxis in adults, accounting for 60.8% of cases. However, the basophil activation test (BAT) is not yet widely used as a first-line test in venom allergy diagnosis and is only available in a few centers in our country and worldwide. Nevertheless, the role of BAT in the diagnosis of Hymenoptera venom allergy (HVA) is well known. When basophils are activated, surface markers such as CD63 and CD203C increase. Measurement of these markers by flow cytometry is a reliable method in allergy diagnosis.

In our study, the sensitivity of BAT for *Apis mellifera* was found to be 95.5%, with a PPV of 95.5% and an LR of 5.72. Similarly, for *Vespula vulgaris*, the sensitivity was 83.3%, PPV 83.3%, and LR 18.51. A study in the literature found the sensitivity of BAT to be between 83-92% and specificity between 80-100% (12, 13). In our study, dual sensitivity was detected in 39.5% of patients in the DPT test and in 22% of patients in the sIgE test, while no dual sensitivity was observed in the BAT test. Therefore, BAT plays a crucial role in treatment decisions in cases of dual sensitivity. While additional diagnostic tests are not mandatory when sIgE and skin prick test results are definitive and consistent, in "difficult cases" where sIgE and DPT results are negative or contradictory, the use of BAT is recommended. This is especially true in cases of double positivity to wasp and bee venoms (14). In a previous study, 19 out of 26 patients (73%) who

had systemic allergic reactions, with negative skin prick tests and undetectable specific IgE, had positive BAT results for a single venom (6 for bee venom, 7 for wasp venom), and six were positive for both venoms (15). BAT has been found applicable in patients with very low sIgE levels where inhibition tests are not possible. It offers additional advantages over specific IgE tests, as basophils are not activated by clinically insignificant IgE antibodies. 75% of 47 sIgE negative patients had a positive reaction in the basophil activation test (15).

In our study, 39.5% of participants had positive Skin Prick Tests (SPT) for both bee species. For patients with double sensitivity, basophil activation test results were considered before starting immunotherapy treatments. Moreover, 4 patients had both skin test and sIgE results negative. Nevertheless, based on clinical history and basophil activation results, venom immunotherapy against *Apis mellifera* was initiated.

A study in the literature indicated that the general clinical sensitivities of the basophil activation test, specific serum IgE, and skin test were 90%, 76%, and 64%, respectively. The same study found the PPVs for these three tests for bee venom were 79%, 73%, and 78%, for wasp venom 86%, 59%, and 43%, and for both venom types 84%, 77%, and 22%, respectively (16).

In our study, the clinical sensitivities for *Apis mellifera* were determined as 95.5%, 95.7%, and 48.4%; for *Vespula vulgaris* as 83.3%, 100%, and 33.3%, respectively. SpIgE demonstrated high sensitivity and PPV ranging between 95.7% and 100%. BAT similarly showed high sensitivity and PPV values. However, SPT had some limitations with lower sensitivity and PPV. These results should be considered significant factors in the selection of tests for the diagnosis of venom allergy and should guide future research in this field. Following a systemic reaction to venom, a skin prick test may be conducted depending on the patient's clinical condition and stability. Skin prick testing can provide rapid, cost-effective, and clinically valuable results. Typically, reaction diameters below 3 mm are considered negative in the literature. However, it is generally advisable to wait for a certain period after a systemic reaction before performing a skin prick test. This waiting period usually ranges from 4 to 6 weeks but may occasionally yield negative results for up to 6 months.

Beekeeping is prevalent in our region, with many patients being beekeepers or their children. Patients prefer to commence treatment as soon as they seek medical attention. However, in our study, skin prick tests were negative in 55% of patients upon initial presentation. Hence, our aim was to establish a new cutoff value for early-stage skin prick tests. In cases with a history of systemic reaction, a cutoff of 1.5 mm was accepted for both bee species in instances lasting 4 weeks or less, with a sensitivity of 67% and specificity of 50%. Conversely, a cutoff of 2.5 mm resulted in a sensitivity of 33% and specificity of 83% (AUC = 0.69; $p = 0.36$; 95%CI = 0.33-1.00). The absence of these sensitivities in healthy individuals underscores a limitation of our study.

In contemporary medical practice, component-resolved diagnosis (CRD) methodologies represent a significant advancement in bee venom immunotherapy, employing molecular diagnostic techniques. CRD facilitates the identification of specific IgE sensitivities to bee venom components, enabling treatment processes to be more targeted and personalized. Compared to traditional skin prick tests or total IgE tests, CRD more effectively distinguishes cross-reactivity situations and simplifies the management of patients with sensitivities to multiple venoms. This method allows for the development of specialized immunotherapy formulations for individuals sensitive to specific venom components. The implementation of CRD aids in predicting the response to immunotherapy and reduces the need for potentially dangerous allergen skin tests, thereby enhancing safety for patients at high risk of severe allergic reactions. However, the widespread adoption of CRD is hampered by challenges such as high costs and limited accessibility. These challenges are particularly pronounced in healthcare systems with limited resources, inhibiting the broad utilization of CRD. Therefore, further research into the cost-effectiveness and accessibility of CRD is necessary. In our country, the access to these tests is still not at the desired level, which constitutes one of the limitations of our study. This shortfall highlights the importance of strategic planning and resource allocation for future advancements (18,19). In our study, we evaluated the relationship between basophil activation test, specific serum IgE, and skin prick test in the diagnosis of Hymenoptera venom allergy. We emphasize that each of these tests has its own significant advantages and limitations. We concluded that sIgE could be superior to BAT and SPT in terms of sensitivity and specificity in some cases. However, our study demonstrated that BAT could play a significant role, especially in situations of diagnostic uncertainty and in decisions regarding immunotherapy. We proposed that SPT is critical in determining early-stage reactions and in immunotherapy decisions, yet its cutoff values need reevaluation. Consequently, we believe the combined use of these three testing methods is important for a more comprehensive and accurate diagnosis of Hymenoptera venom allergy. This study can be considered a critical step in advancing diagnostic and treatment methodologies.

Fundings

None.

Contributions

ŞİKK, AY: data curation, writing – original draft, writing – review & editing. ŞİKK, EB, EA: conceptualization, data curation, writing – original draft. ŞİKK, DÖ, ÖT, RS, AY: conceptualization, writing – original draft, writing – review & editing. ŞİKK, RS, AY: conceptualization.

Conflict of interests

The authors declare that they have no conflict of interests.

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