

Clinical Features of Children with Venom Allergy and Risk Factors for Severe Systemic Reactions

S. Tolga Yavuz Umit M. Sahiner Betul Buyuktiryaki Ozge U. Soyer
Cansin Sackesen Bulent E. Sekerel Ayfer Tuncer

Pediatric Allergy and Asthma Unit, School of Medicine, Hacettepe University, Ankara, Turkey

Key Words

Bee allergy · Children · Hymenoptera venom anaphylaxis · Insect venom hypersensitivity · Systemic reactions · Venom immunotherapy · Wasp allergy

Abstract

Background: Severe systemic reactions (SRs) to insect venom are rare in childhood and there are few data on this study population. The aim of our study is to analyze the clinical features and to document the risk factors for severe SRs in children with insect venom allergy. **Methods:** Children with SRs after Hymenoptera sting were analyzed. The diagnosis was based on medical history, skin tests and/or specific IgE testing. **Results:** Seventy-six children were included [57 boys (75%), mean age 9.8 ± 3.4 years]. The mean age of children at the time of SR was 8.3 ± 3.4 years. Reactions were accounted for *Vespula* (wasp) venom in 58 (76%) and *Apis mellifera* (bee) venom in 18 (24%) patients. Twenty-six percent of patients had aeroallergen sensitization and 33% had atopic disease, whereas 66% had experienced previous stings. The upper limb was the most frequent area of sting (43%), and the cutaneous system (99%) was the most frequent involved system. SRs occurred in 59% of patients. Multivariate logistic regression analysis revealed eosinophilia ($>5\%$) [odds ratio (OR) 12.6; confidence interval (CI) 1.5–109.7; $p = 0.022$], female sex (OR 6.4; CI 1.5–26.9; $p = 0.011$) and accompanying atopic dis-

ease (OR 3.4; CI 1.2–12.3; $p = 0.016$) as significant risk factors for severe SRs. Ninety-seven percent of patients were admitted to the emergency department; however, epinephrine was used in only 46% of patients. **Conclusions:** There was a high frequency of hypersensitivity to wasp venom among the study population, and severe reactions were related to mild eosinophilia, female sex and concomitant atopic diseases. A better understanding of the risk factors may lead to effective utilization of health care sources in the future.

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Introduction

Systemic allergic reactions caused by stinging insects of the order Hymenoptera including the honey bee and wasp are one of the most frequent IgE-mediated diseases and may have life-threatening consequences. They are reported to be responsible for 1.5–34% of emergency department admissions due to any cause of anaphylaxis in children and adults [1]. Moreover, the latest studies report an incidence of insect sting mortality due to anaphylaxis ranging from 0.03 to 0.48 fatalities per 1,000,000 inhabitants per year that accounts for nearly 20% of fatal anaphylaxis cases [1]. Fatalities are usually reported to occur in adults >45 years of age; however, children are also known to be at risk [2, 3].

Insect sting allergy in children is usually reported to have some unique features. Severity and the prevalence of systemic reactions (SRs) are reported to be less common than in adults [4]. Epidemiological studies worldwide report a prevalence of SRs ranging from 0.34 to 6.5% in schoolchildren [5–7]. In Turkey, the lifetime cumulative sting prevalence and severe SR incidence in children aged 6–13 years was estimated to be 60.8 and 0.2%, respectively [8]. Results of previous studies indicate that Hymenoptera stings were most often caused by the honey bee, *Apis mellifera*, and the wasp, *Vespula vulgaris*, in Turkey [8–11].

Along with the very few published data about the prevalence of Hymenoptera venom allergy in childhood, there are few data regarding the demographic features and risk factors for severe SRs in children with venom allergy. The aim of this study was to determine the characteristics of Hymenoptera venom hypersensitivity in children and to identify risk factors for severe SRs in this population.

Materials and Methods

Patients

Children who were referred to the largest pediatric allergy outpatient department of Turkey with a history of SR after Hymenoptera sting from August 2007 to August 2011 were enrolled and analyzed. All children and their parents were interviewed and a questionnaire including information about insect sting history, medication and demographic data was performed. Picture cards of insects (wasps and bees) were displayed to the children and parents who had noticed the sting in order to determine the implicated insect. For all sting reactions, the presence of detailed systemic symptoms were queried and recorded. The study had approval from the Institutional Review Board of Hacettepe University, and written informed consent forms were obtained from the children and their parents.

Diagnosis of IgE-Mediated Venom Allergy

Children with a history of previous systemic sting reaction were diagnosed as having IgE-mediated venom allergy after confirming the sensitization by a skin test reaction [intradermal and prick tests with *Vespula* (wasp) and *A. mellifera* (bee) venoms at least 2 weeks after the reaction] and/or the detection of specific IgE (sIgE) to the implicated type of insect, as shown by elevated titers of sIgE (>0.35 kU/l) measured by the Pharmacia CAP System method (Uppsala, Sweden) [12]. Patients with inconsistent results or in whom no unequivocal diagnosis could be made were excluded from the analysis.

Study Measurements

Skin Tests

Skin tests were performed with bee and wasp venom (*Apis mellifera*, *Vespula*, Stallergenes, France) at least 2 weeks after the sting reaction [12]. A skin prick test with 1 µg/ml of venom con-

centration was performed; if negative, intradermal injections with a concentration of 10-fold dilutions ranging from 0.001 to 1 µg/ml and 0.02 ml of venom were given into the volar surface of the forearm [12]. The skin prick test was considered positive if a geometric mean diameter of the resulting wheal was at least 3 mm after 15 min compared to the negative control. The intradermal test was read as positive at a mean wheal diameter of at least 5 mm with erythema, with a concentration of ≤1 µg/ml. Histamine and saline were used as positive and negative controls, respectively. The test was terminated at the positive concentration. The severity of the results was graded according to the positive reaction concentration. A positive prick test was graded as class 5, whereas a positive intradermal test at 1 µg/ml was graded as class 1 [9].

All participants were also skin prick tested for aeroallergens common to our region along with histamine and negative controls [13]. The tests were applied on the volar surface of both forearms and recorded after 15 min and considered as positive if the mean wheal diameter was ≥3 mm compared to the negative control. 'Aeroallergen sensitization' was defined as 'having at least one positive skin test response' to one of the aeroallergens tested.

In vitro Tests

The serum allergen sIgE level to bee and wasp venom and total IgE levels were measured with the ImmunoCAP system (PhadiaAB, Uppsala, Sweden), according to the manufacturer's instructions. sIgE measurement was also performed at least 2 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 sIgE 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 positive.

Eosinophil counts were performed at least 2 weeks after the reaction and determined by Coulter Counter (Beckman Coulter, Fullerton, Calif., USA) leukocyte measurements.

Diagnosis of Asthma

In children with a history of symptoms suggestive of asthma, the diagnosis was made in the presence of reversible airway obstruction as defined by at least a 12% improvement in forced expiratory volume in 1 s following bronchodilator administration in children capable of doing spirometry or improvement in asthma symptoms after inhaled steroid or leukotriene modifier agent therapy.

Diagnosis of Allergic Rhinitis

Allergic rhinitis is clinically defined by occurrence of symptoms such as rhinorrhea, nasal obstruction, nasal itching and sneezing for at least a minimum duration of 2 years in children with aeroallergen sensitivity.

The presence of allergic rhinitis and/or asthma was defined as accompanying atopic diseases. Pediatric allergy specialists made diagnoses of accompanying diseases.

Severity of Reactions

The severity of reactions was graded according to the system proposed by Mueller [14, 15] who described 4 different grades. A mild SR was defined as a grade I or II reaction, whereas a severe SR as grade III or IV.

Statistical Analysis

The SPSS software package version 18.0 (SPSS Inc., Chicago, Ill., USA) was used for all calculations. Descriptive data for categorical and numerical variables were expressed as frequencies and means with standard deviations for normally distributed variables or as medians with interquartile ranges in parentheses for the non-normally distributed variables. Group comparisons were established using Mann-Whitney tests for medians (and interquartile ranges), Student's *t* test for means (and standard deviations) and the χ^2 test for categorical variables. The association between demographic, symptomatic and laboratory parameters and severe SR was analyzed using logistic regression modeling. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine independent predictors of severe SR. A cut-off value of 5% representing the highest sum of sensitivity and specificity was determined for eosinophilia by constructing receiver operating characteristic (ROC) curves, and sensitivity and specificity were calculated accordingly. Spearman's non-parametric rank correlation test was used to evaluate the correlation between sting reaction severity, skin test results and sIgE levels of the implicated insect. A *p* level <0.05 was considered significant.

Results

Demographic Data

A total of 76 children were included [57 boys (75%), mean age 9.8 ± 3.4 years]. Reactions were accounted for wasp venom in 58 (76%) and bee venom in 18 (24%) patients. Seasonal distribution of the cases resulted in the following: 13 (17.1%) cases in spring, 45 (59.2%) in summer, 17 (22.4%) in autumn, and 1 (1.3%) in winter (fig. 1). Reactions in winter or spring were significantly more frequent with bees (38.9 vs. 12.1%; *p* = 0.017). Three children with bee venom allergy (BVA) were members of beekeep-

er families. The mean age at the time of last SR was significantly lower in children with BVA (6.6 ± 3.2 vs. 8.9 ± 3.3 years; *p* = 0.009). According to the classification of Mueller [14, 15], severe SRs (grades III and IV) were encountered in 45 (59.2%) cases. Aeroallergen sensitization was present in 20 (26.3%) patients. Twenty-five (32.9%) children had concomitant atopic disease, i.e. 19 (25.0%) with bronchial asthma and 13 (17.1%) with allergic rhinitis. Demographic characteristics of the study population are presented in table 1.

Sting Reaction

Previous sting history with the implicated insect was positive in 65.8% of children. Among the 50 children who remembered previous stings, local large reactions were

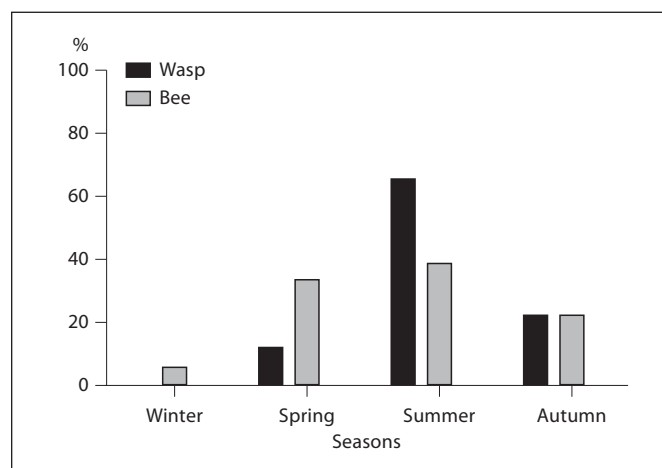


Fig. 1. Seasonal distribution of insect stings.

Table 1. Demographic characteristics of the study population

	All patients (n = 76)	Wasp (n = 58)	Bee (n = 18)
Males	57 (75.0)	42 (72.4)	15 (83.3)
Mean age $\pm$ SD, years	9.8 ± 3.4	10.1 ± 3.3	8.8 ± 3.4
Mean age $\pm$ SD at the time of SR, years	8.3 ± 3.4	8.9 ± 3.3	$6.6 \pm 3.2^*$
Severe SR	45 (59.2)	33 (56.9)	12 (66.7)
Concomitant atopic disease	25 (32.9)	16 (27.6)	9 (50.0)
Asthma	19 (25.0)	13 (22.4)	6 (33.3)
Allergic rhinitis	13 (17.1)	8 (13.8)	5 (27.8)
Aeroallergen sensitization	20 (26.3)	14 (24.1)	6 (33.3)
Family history of atopy	28 (36.8)	22 (37.9)	6 (33.3)
Family history of insect allergy	18 (23.7)	11 (19.0)	7 (38.9)

Figures in parentheses are percentages. * *p* < 0.05.

Table 2. Characteristics of SRs

	All patients	Wasp	Bee
Previous sting	50 (65.8)	35 (60.3)	15 (83.3)
Reaction ¹ during previous sting	37 (74.0)	23 (65.7)	14 (93.3)
Types of previous sting reactions, %			
No reaction	28.0	37.1	6.7
Large local reaction	16.0	20.0	6.7
Mild SR (grades I and II)	46.0	40.0	60.0
Severe SR (grades III and IV)	10.0	2.9	26.7
Previous SR after sting	56.0	42.9	86.7*
Area of sting, %			
Upper limb	43.4	48.3	27.8
Lower limb	22.4	24.1	16.7
Torso	1.3	1.7	–
Head-neck	32.9	25.9	55.6*
Affected organ system, %			
Cutaneous	98.7	100	94.4
Respiratory	74.7	73.7	77.8
Gastrointestinal	41.3	40.4	44.4
Cardiovascular	40.0	40.4	38.9
Severity of SR, %			
Grade I	11.8	13.8	5.6
Grade II	28.9	29.3	27.8
Grade III	50.0	50.0	50.0
Grade IV	9.2	6.9	16.7
Emergency room admission, %	97.3	100.0	88.9
Treatment, %			
Adrenaline	45.8	43.6	52.9
Corticosteroids	79.2	82.1	68.8
Antihistamine	94.4	96.4	87.5

Figures in parentheses are percentages. * $p < 0.05$.

¹ Large local or systemic reaction.

referred to by 16.0% and SRs by 56.0%. Previous SRs after sting were more frequent in children with BVA (86.7 vs. 42.9%; $p = 0.004$). The upper limb was the most frequent area of sting (43.4%) in the study population. Head and neck stings were more frequent in children with BVA (56 vs. 26%; $p = 0.019$). The most frequent symptoms involved the cutaneous system (98.7%), followed, in order of frequency, by respiratory (74.7%), gastrointestinal (41.3%) and cardiovascular (40.0%) symptoms. The most common symptoms in the study population were: angioedema (86.7%), generalized urticaria (84.0%), generalized erythema (77.0%), dyspnea (73.3%), wheezing (33.3%) and nausea (30.7%). Cyanosis was more frequent in children with BVA (38.9 vs. 14.0%; $p = 0.039$). 97.3% of patients admitted to the emergency department; however, adrenaline was used in only 45.8% of patients. Characteristics and comparison of sting reactions and admission symptoms of patients are presented in table 2 and figure 2.

Diagnostic Tests

Specific IgE to wasp and bee venom was positive in 87 and 45% of children, whereas skin tests with wasp and bee venom were positive in 89 and 37% of patients, respectively. Furthermore, sIgE and skin tests were positive for both wasp and bee venom in 35 and 24% of patients, respectively. Skin test positivity to both insects was more frequent in children with BVA (47 vs. 17%; $p = 0.021$). Wasp sIgE and skin tests were positive in 53% of children with BVA. Skin tests were not performed due to the risk of severe anaphylaxis or unwillingness of parents in 6

Table 3. Diagnostic tests of patients

	All patients	Wasp	Bee
Wasp sIgE, kU/l	5.8 (1.9–23.2)	7.0 (2.8–29.0)	0.57 (0.2–9.7)
Bee IgE, kU/l	0.3 (0.04–2.9)	0.26 (0.04–0.6)	21.3 (3.8–90.4)
Positive wasp sIgE, %	86.7	96.6	52.9
Positive bee sIgE, %	45.3	29.3	100.0
Positive sIgE for both insects, %	34.7	29.3	52.9
Total IgE, kU/l	172 (67–443)	138 (65–431)	254 (68–635)
Eosinophils, %	2.8 (1.7–4.4)	3.2 (1.5–4.4)	3.1 (2.2–6.9)
Eosinophil count/mm ³	200 (100–400)	200 (100–300)	300 (175–525)*
Positive wasp skin test, %	88.6	100.0	52.9
Positive bee skin test, %	37.1	17.0	100.0
Positive skin test for both insects, %	24.3	17.0	47.1*

Data are medians with interquartile ranges in parentheses, unless otherwise indicated. Skin tests were performed in 53 of 58 children with wasp allergy and in 17 of 18 children with bee allergy. * $p < 0.05$.

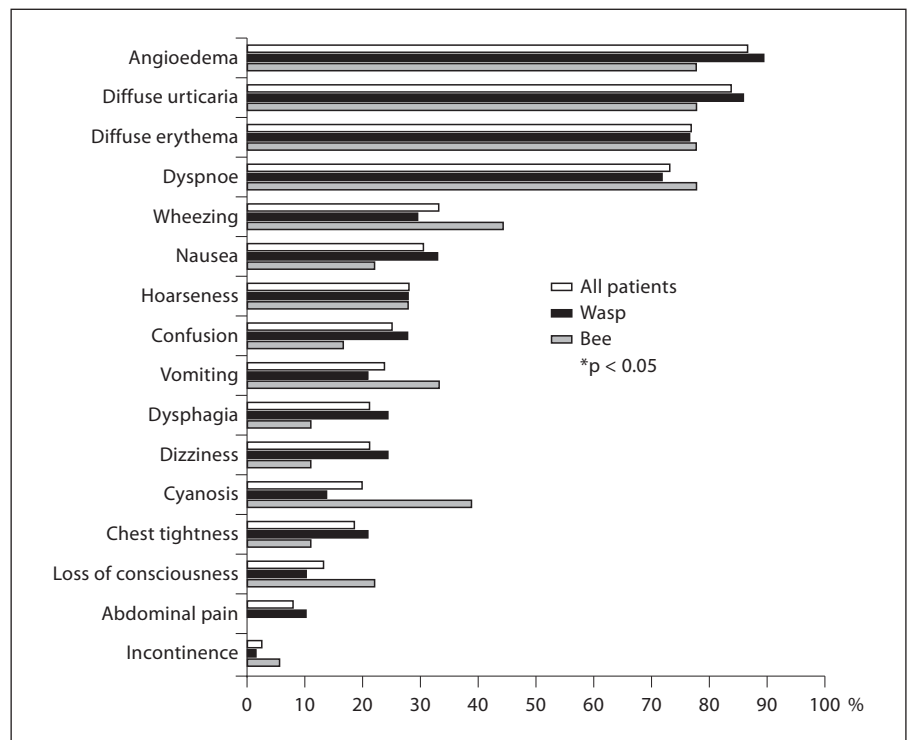


Fig. 2. Admission symptoms of patients.

children (5 wasp and 1 bee). No statistically significant correlations were found between severity of reaction and diagnostic tests, such as sIgE levels and skin test results. However, there were significant correlations between sIgE levels and skin tests for bee ($p = 0.77$, $p < 0.001$) and wasp ($p = 0.54$, $p < 0.001$) venom, respectively. There were no significant differences when sIgE and skin test results were compared between bee and wasp venom. Laboratory test results are presented in tables 3 and 4.

Risk Factors for Severe SR

Comparing the demographic and laboratory characteristics of children, female sex (35.6 vs. 9.7%; $p = 0.01$), concomitant atopic disease (44.4 vs. 16.1%; $p = 0.01$) and head and neck stings (42.2 vs. 19.4%; $p = 0.037$) were significantly more frequent in children with severe SRs. Respiratory symptoms were also more frequent in girls compared to boys (100.0 vs. 66.1%; $p = 0.002$). The medians of eosinophil percentages (3.8 vs. 2.4%; $p = 0.021$) and absolute counts (300 vs. 200; $p = 0.028$) were significantly higher in patients with severe SRs. When analyzed using ROC curves for the best cut-off with the highest combined sensitivity and specificity, a cut-off value of 5% for eosinophil percentage was obtained with an area under the curve of 0.658 ($p = 0.021$) and a sensitivity and speci-

Table 4. Results of sIgE measurements and skin tests for implicated insect

	Wasp	Bee
sIgE class, %		
0	1.7	0
1	3.4	0
2	24.1	17.6
3	37.9	17.6
4	20.7	35.3
5	6.9	11.8
6	5.2	17.6
Prick test positivity (1 µg/ml), %	11.8	29.4
Intradermal test positivity, %		
0.001 µg/ml	3.9	29.4
0.01 µg/ml	52.9	5.9
0.1 µg/ml	23.5	11.8
1 µg/ml	7.8	23.5

ficity of 34 and 97%, respectively. Eosinophilia ($>5\%$) was determined in 34% of the patients with severe SRs and in 3% of those with mild SRs ($p = 0.002$). There was no significant difference between eosinophil counts of children with and without atopy (200, range 108–400, vs. 200, range 100–300; $p = 0.536$), and there was no correlation

Table 5. Logistic regression analysis for severe SR

	Univariate analysis		Multivariate analysis	
	OR	p	OR	p
Age at SR	0.90 (0.79–1.04)	0.151		
Female sex	5.15 (1.35–19.63)	0.016	6.41 (1.53–26.89)	0.011
Implicated insect (bee)	1.51 (0.50–4.59)	0.463		
Aeroallergen sensitization	2.60 (0.83–8.13)	0.101		
Family history of atopy	1.40 (0.53–3.65)	0.492		
Family history of venom allergy	1.10 (0.37–3.27)	0.851		
Concomitant atopic disease	4.16 (1.35–12.80)	0.013	3.37 (1.24–12.25)	0.016
Concomitant asthma	2.35 (0.75–7.39)	0.144		
Previous sting history	1.10 (0.42–2.88)	0.846		
SR during previous sting	0.83 (0.32–2.14)	0.697		
Head and neck stings	3.10 (1.10–8.87)	0.041		
Eosinophilia (>5%)	15.00 (1.86–121.11)	0.011	12.61 (1.45–109.71)	0.022
Total IgE	1.01 (0.99–1.01)	0.200		

Figures in parentheses are CIs.

between atopic status and eosinophil counts either ($r = 0.068$; $p = 0.565$).

Multivariate logistic regression analysis revealed female sex [odds ratio (OR) 6.4; confidence interval (CI) 1.53–26.89; $p = 0.011$], eosinophilia (>5%; OR 12.61; CI 1.45–109.71; $p = 0.022$) and accompanying atopic disease (OR 3.37; CI 1.24–12.25; $p = 0.016$) as significant risk factors for severe SRs (table 5).

Discussion

The results of the present study, conducted in a group of children with Hymenoptera venom allergy, revealed the high frequency of hypersensitivity to wasp venom in the study population. However, SRs with bee venom are encountered in younger ages. The symptoms of SRs to Hymenoptera venom most frequently involved the skin. Severe reactions may be seen in nearly 60% of patients and severe SRs were related with female sex, patients with mild eosinophilia (>5%) and accompanying atopic diseases. Additionally, an underdiagnosis and undertreatment of anaphylaxis were observed in the study group.

Demographic Features

Wasps are frequently found in urban settings. They are known to be more aggressive when getting food and defending themselves. Therefore, more stings are observed especially in the late summer and autumn when the food supply is limited for large populations and peo-

ple eat outdoors. In contrast, honeybees are usually regarded as 'peaceful' and they sting rarely (except near their hives), particularly in cool damp weather [16]. In our study, consistent with the offensive nature of the wasps, there was a high prevalence of wasp-related SRs in parallel with the findings of other studies from Turkey and worldwide [9, 10, 17]. Moreover, the majority of wasp stings occurred in July, August and September, whereas a considerable part of bee stings occurred in cooler seasons such as winter and spring, along with early summer.

As a result of spending more time outdoors leading to an increased risk of exposure to insect stings, a male predominance was present in our study population, as shown in previous childhood studies [5–7, 18, 19]. The upper limb was the most common area of stings in parallel with previous studies [20–22]. Involvement of organ systems, such as the cutaneous system followed by respiratory and gastrointestinal systems, was also consistent with previous childhood data [19, 23]. In the meantime, results of our study revealed some significant differences between demographic features of children allergic to wasp and bee venoms. First of all, the mean age of children at anaphylactic reaction was lower in children with BVA. Moreover, parameters which may be related to severe anaphylaxis such as head and neck stings and cyanosis were also more frequent, and eosinophil counts were higher in children with bee allergy. However, the frequency of severe SR did not differ significantly between groups, although it was higher in children with BVA (66.7 vs. 56.9%).

Bee venom-allergic patients are known to be at a greater risk of an SR for their next sting than children with wasp venom allergy [1]. In accordance with these data, previous reactions were more frequent and severe in children with BVA in our study. This is probably due to the higher amount of venom applied by bee sting [24]. The difference may also be due to higher and earlier exposure in children with BVA in our study group.

Emergency room admission was very high (97.3%) in our study population. However, epinephrine treatment was administered to less than half of the patients (45.8%). Under-represented usage of epinephrine was previously indicated in two large multicenter studies of emergency department visits for insect sting allergies from North America and Central Europe, in which only 12 and 22% of patients with SRs received epinephrine treatment, respectively [22, 23]. Along with our study, misuse of antihistamines and corticosteroids in the initial and acute treatment of anaphylaxis, indicating the need of educational measures for physicians, was present in those studies.

Risk Factors for Severe SRs

Observation of children with insect allergy has led to the perception of milder clinical presentations with a much higher frequency of isolated cutaneous reactions and a lower frequency of severe cardiovascular symptoms, compared to adults [19, 25, 26]. Different classifications for defining severe SRs may lead to varying results; however, the frequency of SRs against wasp (56.9%) and bee venom (66.7%) was relatively high in our study population when compared to previous studies. This finding may be due to the referral of severe cases to our department, and further multi-center studies are needed to confirm these data.

Apart from the established risk factors for SRs in children, we aimed to identify risk factors for severe SRs in children with venom allergy. Results of previous studies which were performed predominantly in the adult population have determined several risk factors, such as history of previous severe sting reactions with significant bronchial obstruction, older age (>40 years), insect type (honeybee, European hornet) pre-existing conditions (cardiovascular diseases, asthma), regular usage of certain drugs (β -blockers, angiotensin-converting enzyme inhibitors), sting site (e.g. head) and conditions with elevated basal serum tryptase concentration [1, 16]. However, risk factors related to more serious SRs in children are not as well demonstrated as in adults.

There are controversial results from adulthood studies investigating the influence of gender on the severity

of SR in patients with insect venom allergy. Some authors have determined male sex as a risk factor for severe SRs [17, 26], whereas a considerable number of studies could not find a difference between genders [11, 21, 27, 28]. Results of a recent study on British beekeepers revealed female sex as a predisposing factor for severe SR [29]. There are relatively few studies in children, and their results did not establish gender as a risk factor for SR [6, 7, 19]. In our study, a higher percentage of girls had severe reactions when compared to boys (84.2 vs. 50.9%; $p = 0.01$), and results of multivariate logistic regression analysis revealed female sex as a significant risk factor for severe SR. To the best of our knowledge, it is a novel finding in children with insect venom allergy. This finding may be important for girls with insect allergy, who are found to be prone to severe SR, to modify dressing and make-up habits as suggested by recent guidelines, although this recommendation may seem controversial due to lack of hard evidence in the entomology literature [30–32].

The prevalence of aeroallergen sensitization in our study population was similar to previous childhood studies [33]. In parallel with several studies on adult populations [11, 21, 28, 34] investigating the relationship between aeroallergen sensitization and severe SRs in patients with insect venom allergy, our results were also far from establishing a significant association. The relationship between atopic diseases such as asthma and allergic rhinitis and severe reactions in venom-allergic patients has also been investigated. Although Solley [20] did not find a significant association, results of various studies [7, 22, 35, 36] have reported significant associations between the presence of atopic diseases – particularly asthma – and severe SR. In parallel with previous studies, severe SRs were more frequent in children with asthma (73.7 vs. 54.4%); however, the difference between the two groups did not reach significance, possibly due to small sample size ($p = 0.131$). Nevertheless, analysis of our data revealed the presence of an atopic disease such as allergic rhinitis or bronchial asthma as a significant risk factor for severe SR in children with insect venom allergy. Respiratory involvement was found to be higher (88 vs. 68%; $p = 0.06$) in children with atopic disease, and contribution of the underlying airway disease to the ongoing reaction may be a plausible explanation for this finding.

Valentine et al. [37] have reported that particularly in children with a history of mild or isolated cutaneous symptoms, more severe SRs with subsequent stings had occurred in only a minor group. Reisman [38] has also reported a lower incidence of subsequent stings resulting

in SR compared to adults. On the other hand, in another childhood study by Setticone and Chafee [39], a history of previous sting reaction was reported as a risk factor for future stings. In our study, a previous sting history was not significantly related with severe SR in the logistic regression analysis. Nevertheless, in parallel with the childhood findings of Solley [20], 57.1% of wasp venom-allergic children and 13.4% of bee venom-allergic children with previous stings had no SR during the previous sting. Therefore, risk of SR during subsequent insect stings in children without insect venom allergy must be kept in mind.

The influence of the sting site on the severity of SR has been reported in a few studies. Mueller [15] has established an association between head, neck or throat stings and severe SR; however, this relationship was not repeated in other studies predominantly including adults [20–22]. In our study, head and neck stings were more frequent (42.2 vs. 19.4%; $p = 0.037$) in children with severe SRs. However, the significant relationship between head and neck stings and severe SR that emerged in univariate analysis was lost in the multivariate analysis. This issue may deserve further investigation.

Several studies which investigated the association of total or sIgE levels and skin test results with severe SR did not find a significant relationship among those parameters [19, 27, 28, 40]. Total IgE levels significantly correlated with sIgE levels in our study (data not shown). However, there was no correlation between diagnostic test results and the severity of sting reactions.

Another novel finding was the mild increase in serum eosinophil levels (both absolute counts and percentages) of the patients which were significantly related with the severity of SR. With the help of ROC curve analysis, we established a cut-off value of 5% eosinophilia to differentiate patients with severe and mild SRs. Eosinophilia ($>5\%$) was present in 34.1% of patients with severe SRs and only in 3.3% of children with mild SRs ($p = 0.002$). However, large confidence intervals in the logistic regression analysis owing to small sample size impair the statistical power of this finding.

A possible explanation for this association may be that eosinophilia is a hallmark of atopic diseases, particularly asthma. The presence of eosinophilia was not associated with asthma, and furthermore, asthma itself did not appear as a risk factor for severe SR at univariate analysis in our study group. However, the relatively small sample size may be a potential reason of this lack of association and needs to be further evaluated in larger groups. The prevalence of asthma (25%) was moderately high in our study

group when compared with previous studies [20, 22, 35]. Moreover, asthma prevalence was higher than the prevalence of allergic rhinitis, conflicting with previous reports [9, 35]. This implies that the study population may be somehow selected for asthma and this would be likely to be associated with eosinophilia and with more SRs to stings, based on existing literature.

The relationship of eosinophilia with aeroallergen sensitization is a well-known issue; however, aeroallergen sensitization did not emerge as a risk factor for severe SR in our study and no correlations were found between atopic status and eosinophil counts. This discrepancy is possibly due to the inadequate sample size of our study group.

Our study group consisted of patients who were referred to the largest tertiary pediatric allergy center in Turkey due to insect venom allergy and initiation of venom immunotherapy. Pediatric allergy specialists performed the interviews and skin tests and diagnosed the accompanying diseases. This is the first childhood study from Turkey, which is a distinguished place for beekeeping and honey production worldwide, and one of the few studies globally documenting the clinical features of patients and risk factors for severe SRs in children with insect venom allergy.

There are also some limitations to our study. First of all, although there are not many large cohort studies worldwide, a relatively small number of children leading to large CIs may impair the accuracy of our results. Secondly, inclusion of well-established parameters that play an important role in the severity of SRs in adults such as baseline tryptase levels may better help to determine risk factors in children [17, 28]. Finally, possible recall biases and predominant referral of severe cases might have led to different results as compared to previous studies regarding the history of previous sting and frequency of severe SRs, respectively, in our study group.

In conclusion, we demonstrated that severe SRs were associated with mild eosinophilia, female sex and concomitant atopic diseases in this highly selected population of children with a history of sting-related SR. Further studies, particularly with a larger group of children, are required, and a better understanding of the natural course of the condition may lead to optimal use of therapeutic interventions in children.

Disclosure Statement

There is no potential conflict of interest.

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