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A New Approach to Eliminate Hymenoptera Venom Grading Sensitization Test in the North Iran: Cross-Sectional Study

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Abstract

Background: Anaphylaxis is an allergic reaction which occurs with or without the stimulation of the immune system. Hymenoptera stings are common causes of anaphylaxis in the world. Skin tests are the first-line diagnostic measure for Hymenoptera anaphylaxis. The present study aimed to evaluate the safety of a single-step approach in sensitization testing for Hymenoptera venom.

Methods: This cross-sectional study was conducted in 2019 in Golestan province the north of Iran. The sample population consisted of 140,000 individuals covered by 84 rural healthcare centers in the vicinity of Gorgan, Iran. Thirty-three patients agreed to receive the diagnostic test. In this research, in contrast to the 2011 ACAAI guideline, the extracts of venom of three types of Hymenoptera were injected intra-dermally without any dilution at the concentration of 1 µg/ml.

Results: The results of the skin test in the patients bitten by honey bee, yellow jacket, and paper wasp were negative in 15.2%, 15.2%, and 21.2% of the cases, respectively. After the test, no allergic reaction was observed, with the exception of a minor skin reaction, which improved within a short time. These preventive measures were taken during the test for the following four hours when the patient was present at the test site and up to 48 hours afterward via follow-up from the healthcare center to the home of the patient.

Conclusion: The results of our study showed that the non-diluted single injection of the Hymenoptera sting was accompanied by no side effects.

Keywords: Anaphylaxis, Skin Tests, Venoms, Bites and stings, Hymenoptera

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Introduction

Anaphylaxis is a severe, life-threatening hypersensitivity reaction characterized by the rapid progression of airway and throat involvement, laryngeal and respiratory tract edema, bronchospasm, tachypnea, hypotension, and skin and mucosal involvement in most cases. Anaphylaxis could be an allergic reaction that occurs with or without the stimulation of the immune system through unknown mechanisms, and several factors could potentially cause anaphylaxis, such as insect bites (1).

Hymenoptera stings are a common cause of anaphylaxis worldwide (2). Bees belong to the family of Hymenoptera and their stings have long been recognized as a major cause of anaphylaxis in humans (3). The incidence rate of fatal anaphylaxis due to Hymenoptera has been estimated at 0.03-0.48 mortality per million per year (4). In the United Kingdom, one in every 1,333 people experiences anaphylaxis in their lifetime.

Based on skin tests in adults, the prevalence of sensitization to Hymenoptera is reported to be 26%, which reaches 30-40% people with a history of Hymenoptera stings. Moreover, the prevalence of systemic reactions to Hymenoptera stings is estimated to be 1% in children and 3% in

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↑What is "already known" in this topic:
However, consecutive skin testing is time-consuming and difficult for patients and medical staff, while affordable skin testing provides rapid results and a reliable and safe test in vivo. Skin tests attracted great attention in 1990 and 1996, while its safety consequences are to be further clarified.

→What this article adds:
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adults (4). The most common responses to the Hymenoptera stings include transient pain, itching, and swelling, which could lead to severe local/systemic reactions in some cases. According to previous studies, 5% of people developed extensive local swelling at the sting site following a Hymenoptera sting (3, 4).

In Hymenoptera stings, the sting organ and the venom system often remain in the skin and continuously release the venom. However, the paper wasp may usually remove its sting system after the bite (4, 5, 6). The molecular properties of the toxins found vary in the Hymenoptera family (7, 8, 9). In addition to the acute systemic reactions, a delayed response to hymenoptera stings may occur in some cases in the form of serum sickness, encephalitis, peripheral and central neuropathy, glomerulonephritis, myocarditis, and Guillain-Barre syndrome (9, 10).

A common diagnostic measure to predect Hymenoptera anaphylaxis is a skin test for the Hymenoptera venom (11). According to the current guidelines, skin testing should be performed in several stages at an interval of 15-20 minutes between the injections. The method has been developed based on the results of previous studies and has been used for decades. However, consecutive skin testing is time-consuming and difficult for patients and medical staff, while affordable skin testing provides rapid results and a reliable and safe test in vivo. Skin tests attracted great attention between 1990 and 1996, while its safety consequences are to be further clarified (2). The present study aimed to report the results of skin tests as a single step and intradermal injection at the concentration of 1µg/ml.

Methods

Sample collection and processing

This cross-sectional study was conducted in 2019 to provide a detailed epidemiological study in the north of Iran. The sample population included 140,000 individuals covered by 84 rural health centers in the vicinity of Golestan, Iran. The preliminary history of the patients was collected via interviews, and 201 patients with a history of anaphylaxis were identified as cases of bee stings.

Data were collected and recorded on demographic characteristics, history of Hymenoptera stings, bite season, bite time, sting complications, and history of treatment (12). At the next stage, 201 patients with a history of anaphylactic reaction to individual stings were invited to partake in the diagnostic test. In total, 33 provided informed consent participated in the study. The test was carried out at the comprehensive round-the-clock health center in Jalin city, Gorgan city, and Golestan province. In order to prevent possible complications of the test, all facilities including: emergency room, laboratory, presence of the resuscitation team, etc., were provided.

The objectives and procedures of the research were explained to the participants, and they were assured of confidentiality terms regarding their personal information, as well as the right to withdraw. In this study, there was no age limit, and only patients with a history of anaphylaxis following bee stings were examined. The inclusion criteria were people who have been bitten by bees and have severe allergy symptoms (anaphylaxis). The exclusion criteria were patients with non-systemic reactions caused by bee stings, patients whose anaphylaxis is caused by fire ant venom allergy, and the patient's lack of consent to provide information.

We used the latest guidelines of the American College of Asthma, Allergy, and Immunology (A practice parameter update 2011) for the most susceptible insects, which were published in 2011. The intradermal test is performed within the range of 0.001-0.01 mg/ml. With each 10 fold increase in dilution, the skin test results are positive to reach a maximum of 1 µg/ml (13).

According to the study by Strohmeier et al. (14) and in accordance with the feedback of a panel of experts, the intra-cutaneous tests were eliminated from the stages for one year, and various intradermal dilutions were performed in the patients referring to the allergy clinic. After the submission of the documents and the final approval of the Ethics Committee 33 patients were enrolled and tested. In addition, the study protocol was approved by the University with the coordination and provision of a complete resuscitation team in the presence of a resident anesthesiologist and coordination with experienced resuscitation staff in the presence of allergists. In addition, the required forensic physician licenses were obtained for the study. The tests were performed at 8 AM in the presence of a team of 50 experienced staff, including physicians, nurses, laboratory managers, and university officials.

There is a view that states injection is not uncomplicated (14), which has been in place since 2018 with the issuance of a license for the ethics group of Hazrat-Rasoul Allergy Clinic in a completely restricted manner. Therefore, we selected 4-5 subjects each month to remove various dilutions to evaluate the allergies to bee stings. This process continued until it was revealed that testing various dilutions was uncomplicated. At the next stage and in a large population, we performed a door-to-door plan for the diagnosis of anaphylaxis. This method was selected based on conducting extensive tests, control and protection measures, and recording tests using audio-visual aids. Unlike conventional methods, this approach was performed without the extract dilution of three types of Hymenoptera (Honeybee (Apis mellifera), Yellowjacket (Vespula spp) and the Paper wasp (Polistes spp)) along with the intradermal injection of the concentration of 1 µg/ml.

Statistical analysis

Data analysis was performed in SPSS Version 21 using descriptive statistics, such as mean, standard deviation, frequency distribution tables, and Chi-square tests at the significance level of 0.05.

Results

Thirty-three persons participated in this study. The mean age of them was 36.39 ± 16.03 years old. The age range was 7-63 years old. In this population, 16 (48.5%) of the cases were female and the males were involved in 17 (51.5%) cases.

The time of stings and the severity of anaphylaxis were evaluated in the subjects. The frequency distribution of cases based on these variables is shown in Table 1. In the

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studied population, most of the cases occurred in summer. Mostly, the participants were bitten at noon. 54.2% of the patients had a severe anaphylaxis. In most cases, skin symptoms manifested and the patients were treated with corticosteroids.

The results of the skin test in the patients bitten by honey bees, yellow jackets, and paper wasps were negative in 15.2%, 15.2%, and 21.2% of the cases, respectively (shown in Table 2). After the test, no registrable allergic complication was detected, with the exception of a minor skin complication, which improved within a short time. No other complications were observed during the test until the next four hours when the patient attended the test site and even up to 48 hours afterward at the health center and during the follow-up period.

Our method was compared with other skin testing methods in Table 3 and Table 4. There were no significant differences in adverse effects and possible allergic reactions between the methods. There were no significant differences between the four methods in number of adverse complications ($P = 0.603$) and possible complications ($P = 0.482$).

Table 1. The frequency distribution of bitten cases based on the season and the time of bites and their complications

Variable	Variable Level	Frequency (%)
Bite Season	Spring	12 (36.4)
	Summer	15 (45.5)
	Autumn	6 (18.2)
Bite Time	Morning	2 (6.1)
	Noon	21 (63.6)
	Evening	7 (21.2)
	Night	3 (9.1)
Severity of Anaphylaxis	Mild	3 (9.1)
	Moderate	12 (36.4)
	Severe	18 (54.5)
History of Treatment	Antihistamine	27 (81.8)
	Corticosteroids	28 (84.8)
	Serum Therapy	13 (36.4)
	Epinephrine	1 (3)
Clinical Symptoms Following Bee Sting	Pruritus	21 (63.6)
	Urticaria	22 (66.7)
	Flushing	10 (30.3)
	Angioedema	3 (9.1)
	Rhinitis	11 (33.3)
	Hypotension	21 (63.6)
	Skin	26 (78.8)
	Respiratory	24 (72.7)
	Cardiovascular	22 (66.7)
	Neurological	10 (30.3)

Table 2. Skin test positivity by venom based on Hymenoptera types

Bee type	Positivity grade	Frequency (%) of cases
Honey Bee	Negative	5 (15.2)
	+1	20 (60.6)
	+2	7 (21.2)
	+3	1 (3)
Yellow Jacket	Negative	5 (15.2)
	+1	15 (45.5)
	+2	6 (18.2)
	+3	6 (18.2)
	+4	1 (3)
Paper Wasp	Negative	7 (21.2)
	+1	19 (57.6)
	+2	3 (9.1)
	+3	4 (12.1)

Discussion

Single-step venom allergy testing has some significant advantages for patients and the health care system such as fewer injections, reduction in cost to the health care system and reduction in time of tests (13). The present study aimed to eliminate various dilutions of bee susceptibility tests, and the obtained results indicated that contrary to conventional methods, single-dose injection has no side effects on patients. This is consistent with the results obtained by Strohmeier, which indicated that 98.7% of the patients had uncomplicated intradermal test results and only 0.6% showed an allergic reaction during the test (14).

The standardized method modified in 1989 (10) and 1996 (15) is primarily based on control, and dilution was only performed to extract venom. In our research and the study by Strohmeier, a different method was used. Intradermal experiments were performed in 1989 and 1996 with the control and dilution of a maximum of two and three toxins, respectively. In the study by Strohmeier, the toxin was extracted with a maximum of four dilutions, while the toxin

Table 3. Comparison of skin testing methods with the proposed method

Procedure	Method			
	Standard Method[10] 1989	Modified Method[15] 1996	Strohmeier Method[2] 2013	Proposed Method 2020
Prick Test	Controls and one venom concentration	Controls and one venom concentration	Not done	Not done
Intradermal Test	Controls and up to 4 venom concentrations	Controls and 2 venom concentrations	Controls and 4 venom concentrations	Controls and one venom concentration
Administration	Sequential; 20 minutes per concentration	Partly simultaneous; 20 minutes prick test, 20 minutes intradermal tests	Simultaneous	Simultaneous
Volume per Concentration (ml)	0.05	0.03	0.02	0.02
Patients	3236	446	478	33
Total Adverse Reactions N (%)	64 (2.0)	9 (2.0)	6 (1.3)	0
Presumed Allergic Reactions N (%)	45 (1.4)	5 (1.1)	3 (0.6)	0
Total Time (min)	100	40	20	20

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Table 4. *P*-Value of total adverse reaction and presumed allergic reactions between different methods

Procedure	*P*-Value
Total Adverse Reactions (four methods)	0.601
Presumed Allergic Reactions (four methods)	0.482
Total Adverse Reactions (our method and standard method)	0.523
Presumed Allergic Reactions (our method and standard method)	0.651

was diluted with the maximum concentration of each extract in the current research. Contrary to the study by Strohmeier (14) in which honeybee and paper wasp extract solutions were used, we utilized three extracts in the present study as well as the extract of yellow jackets. Furthermore, previous studies have mostly used the standard method, while we only performed one injection. The total number of adverse reactions was only one, and the duration of the study was 20 minutes.

Our findings indicated that there were no significant differences in adverse effects and possible allergic reactions between the methods. The results of a study by Quirt et al, 2016, along with the presented results in here. They showed a single-step venom allergy intradermal testing protocol with a 1 µg/mL concentration of venom extracts without adverse effects (13).

One of the limitations of the study is the small sample size, so it is suggested to conduct studies with a large sample size.

Conclusion

According to the results, the protocol that we used in our study could be incorporated into various diagnostic measures without complications in a cost-efficient, stress-free manner through a one-step test for the diagnosis of bee allergies to 1/1 with three types of bee extracts that could reduce the additional costs of care and would increase the speed of diagnosis. Also, The skin test protocol with different concentrations and simultaneous injection is much cheaper, faster and safer and helps the physician in decision-making in the treatment process of the patient is a reflection of the sensitivity of the person helping. To evaluate the safety of the venom extract, the pharmacokinetics of the drug should be assessed with a study of a large number of patients.

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Ethical consideration

This study was approved by the research ethics committee of Golestan University of Medical Sciences (IR.IUMS.FMD.REC2018.9411568001).

Authors' contributions

All authors have contributed equally to developing the concept, implementation, processing of results, and writing the article. We declare that this material has not been published before and is not under consideration by other publishers.

Conflict of Interests

The authors declare that they have no competing interests.

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