Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
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ORIGINAL ARTICLE

OPEN ACCESS

Safety of 1 mcg/mL as the starting dose in cluster protocol for hymenoptera immunotherapy

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Received 10 June 2024; Accepted 18 November 2024
Available online 1 January 2025

KEYWORDS
cluster protocol;
hymenoptera stings;
hymenoptera venom allergy;
safety;
venom immunotherapy

Abstract
Background: Hymenoptera venom allergy is a potentially severe allergic reaction in the general population. The only preventative approach in these cases is venom immunotherapy (VIT), which follows different protocols. The recommended initial dose is 0.001–0.1 mcg of venom extract. However, few reports have declared the safety of 1 mcg venom as the starting dose.
Methods: The study was conducted on Iranian patients with a history of anaphylaxis to venom. Skin tests confirmed hypersensitivity to honeybee, yellow jacket, and/or paper wasp from subfamily Polistes using *Apis melifera*, *Vespula spp*, and *Polistes spp* venom extracts, respectively. Subsequently, the patients were treated with the cluster protocol.
Results: Twenty-two patients (17 males and 5 females, aged 28.3±11.8 years) were enrolled in the study. Skin prick tests and intradermal tests showed positive results for yellow jacket in 17 (77.3%) and 21 (95.4%) patients, honeybee in 14 (63.6%) and 17 (77.3%) patients, and wasp in 14 (63.6%) and 17 (77.3%) patients, respectively. Upon administering the initial dose of 1 mcg/mL, 40.9% (9 cases) of patients presented mild local reactions, including 7 with yellow jacket allergy, 5 with honeybee allergy, and 3 with wasp allergy. One patient with yellow jacket allergy had a mild systemic reaction. Patients with a positive skin test for wasp had significantly lower rate of reactions after the first dose of venom (p=0.026). Throughout the entire build-up phase, more than 90% (20 of 22) of patients experienced mild local reactions, followed by large local reactions (3 cases, 13.6%), mild systemic reactions (1 case at 1 mcg/mL dose), and moderate-to-severe systemic reactions (3 cases, 13.6%). Large local and moderate-to-severe systemic reactions were detected after injecting 50 mcg (each one case) and 100 mcg (each 2 cases) of venom extracts.

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https://doi.org/10.15586/aei.v53i1.1151

Conclusion: This study recommends 1 mcg/mL of the venom extract as a safe starting dose for VIT. This accelerated protocol could successfully reduce the time and costs of therapy for patients undergoing out-patient cluster VIT.

Introduction

Hymenoptera venom allergy is a potentially severe allergic reaction following a honeybee, vespid, or ant sting. This allergic presentation can range from mild local reactions to life-threatening anaphylaxis.[1] It is estimated that 56-6-94.5% of the general population has been stung at least once in their lifetime, with a higher prevalence among beekeepers. Furthermore, approximately 0.3–7.5% of adults and up to 3.4% of children experience systemic reactions following a hymenoptera sting.[2,3] Currently, the only preventing approach in these cases is venom immunotherapy (VIT), which is reported to be effective in 77–84% of patients for honeybee venom and 91–96% of those receiving vespid venom.[1] However, immunotherapy can also induce serious side effects, even anaphylaxis.[4]

VIT consists of a build-up phase and a maintenance phase to ensure a sustained therapeutic effect. Different VIT protocols are available including conventional, cluster, rush, and ultra-rush protocols.[5] The conventional protocol and its accelerated alternative, the cluster protocol, are administered in outpatient clinics, gradually reaching the maintenance dose over several weeks to months. In contrast, rush and ultra-rush are performed in an in-hospital setting, reaching the maintenance dose within days.[5] Conventional regimens are generally considered to be the best tolerated, with a lower incidence of adverse effects.[1,6] The recommended initiating dose in the build-up phase is between 0.001 and 0.1 mcg of venom extract, reaching the maintenance dose of 100 mcg of venom for both adults and children.[1,7] However, it has been reported that the starting dose of 1 mcg in the rush and ultra-rush protocols was also safe, without being associated with a higher rate of side effects in either adults or children.[8] Nonetheless, further clinical data are warranted to thoroughly evaluate the safety of this approach.

In this study, we scheduled VIT under the cluster protocol for Iranian patients with venom allergy using 1 mcg/mL of venom as the starting dose. We monitored the safety of higher doses of venom during the initial phases of the treatment. The results of this study could reduce the duration and cost of cluster VIT and provide a clearer perspective on this venom regimen for clinical immunologists.

Methods

The study included 22 Iranian patients with a history of anaphylaxis to venom, who were referred to the allergy clinics of Rasool-e-Akram hospital, affiliated with Iran University of Medical Sciences, Tehran, Iran (between April 2011 and 2021). This study was approved by the Ethics Committee of the Iran University of Medical Sciences (IR.IUMS.FMD.REC.1402.065), in accordance with the Declaration of Helsinki. Prior to data collection, written informed consent was obtained from each patient and/or their parents. None of the patients reported a history of taking medications that could influence the results, including betablockers or ACE inhibitors.

Skin prick tests were used to confirm hypersensitivity to honeybee, yellow jacket and/or wasp venom. The tests were performed using 0.001, 0.01, 0.1, and 1 µg/mL venom extract from honeybees (*Apis melifera*), wasps (*Polistes spp.*), and yellow jackets (*Vespula spp.*). Histamine dihydrochloride (1 mg/mL) and albumin 0.03% diluent were used as positive and negative controls, respectively. The choice of extract was based on the patient's history. In cases with double- or triple-sensitized skin tests, immunotherapy was performed using more than one extract. After 15 min, the presence of a wheal ≥ 3 mm in diameter was considered a positive reaction. Subsequently, an intradermal test was performed on the forearm with increasing concentrations from 0.001µg/mL to 1 µg/mL. Positive tests were defined as reactions (wheal ≥ 5 mm in diameter with erythema) occurring after 15 min at a concentration of 1 µg/mL or less.

The patients were scheduled for cluster VIT in out-patient clinics; the protocol began without premedication, with an initial dose of 0.1 mL (1 µg/mL, aqueous, HollisterStier, USA). All injections were administered subcutaneously in the mid-posterolateral upper arm. All patients underwent the cluster protocol with five visits, one week apart. The first course included three injections followed by two injections in the next two courses, and one injection in the last course (Table 1). Vital signs were

Table 1 Modified cluster protocol for bee venom Immunotherapy.

Day	Hour	Injection volume (mL)	Venom Concentration (mcg/mL)	Injection Dose (mcg)
Day 1	0	0.1	1	0.1
	1	0.1	1	0.1
	2	0.1	1	0.1
Day 8	0	0.1	10	1
	1	0.5	10	5
	2	0.1	100	10
Day 15	0	0.2	100	20
	1	0.3	100	30
Day 22	0	0.5	100	50
	1	0.5	100	50
Day 29	0	1	100	100

monitored initially, before, and during each injection. Full emergency resuscitation equipment was readily available at all times. In case of any systemic allergic reactions during the build-up phase, treatment was interrupted until complete recovery, and then restarted with 2-step dose reduction. Venom extract dosage, along with local and systemic reactions, were documented. If a patient developed a large local reaction with distinct erythema and/or swelling (>8 cm in diameter) on both upper arms, the protocol continued without dose reduction.

Statistical analysis

Qualitative variables were reported as absolute numbers and percentages. The mean and standard deviation was used for quantitative variables. The t-test, Chi-square test, or Fisher exact test were utilized for comparisons. All statistical analyses were performed using SPSS version 26.0 (IBM, Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant.

Results

Twenty-two patients (17 males and 5 females, aged 28.3±11.8 years) with a history of anaphylaxis to venom were examined; only one of the patients was a beekeeper. More than 63% of patients (14 of 22) had a previous history of allergy prior to developing anaphylaxis (Table 2). These allergic presentations included allergic rhinitis (8 cases, 36.4%), asthma (4 cases, 18.2%), urticaria (2 cases, 9.1%), atopic dermatitis, dermatographism, and allergic conjunctivitis each in one patient. Skin prick tests were positive for yellow jacket in 17 (77.3%), honeybee in 14 (63.6%), and wasp venom in 14 (63.6%) patients. About 32% (7 out of 22) of patients tested positive for all 3 venoms, 40.9% (9 out of 22) for 2, and 27.3% (6 out of 22) for a single type of venom extract. None of the patients had elevated level of serum tryptase or IgE before VIT. The initial dose of 1 mcg/mL venom was administered for all included patients. At this dose, 40.9% (9 cases) of patients presented mild local reactions, including 7 with yellow jacket, 5 with honeybee, and 3 with wasp allergy. One patient with yellow jacket allergy had also a mild systemic reaction in form of generalized skin rash. Patients with a positive skin test for wasp had significantly lower rate of reactions after receiving the first dose, as determined by the Chi-square test (p=0.026).

Table 2 The basic information of the patients.

Parameters	Patients
Age, y, mean (± SD)	28.3 (±11.8)
Sex, male/female	17/5
Previous history of allergy, n (%)	14 (63%)
Skin prick test, n (%)	
Yellow jacket	17 (77.3%)
Honeybee	14 (63.6%)
Wasp	14 (63.6%)
Basic serum IgE level, IU/mL, mean (± SD)	29.1 (±13.4)

y: year, SD: Standard deviation, n; number, IgE: immunoglobulin E.

During the entire build-up phase, more than 90% (20 of 22) of patients experienced mild local reactions followed by large local (3 cases, 13.6%), mild systemic (one case at ≥1 mcg/mL dose), and moderate-to-severe systemic (3 cases, 13.6%) reactions. Large local and moderate-to-severe systemic reactions were detected after injecting 50mcg (each one case) and 100 mcg (each 2 cases) of venom extracts. Of the 14 patients with honeybee allergy, 13 (92.8%) represented mild local reactions and 3 (21.4%) had moderate-to-severe systemic reactions. Among patients with wasp allergy, mild local reactions were detected in 92.9% (13 cases), large local reactions in 14.3% (2 cases), and moderate-to-severe systemic reactions in 7.1% (one case) of individuals. Mild and large local reactions were observed in 88.2% (15 cases) and 17.6% (3 cases) of patients with yellow jacket allergy, respectively; 5.9% (one case) and 11.8% (2 cases) of them experienced mild and moderate-to-severe systemic reactions as well, respectively.

We further analyzed the effect of sex, age, and past allergy history on allergic reactions following VIT. The presence of a previous history of allergy did not significantly influence the development of allergic reactions during the build-up phase ($p=0.515$) or after the initial dose ($p=0.187$). Similarly, no significant difference was detected in the Chi-square test between the sex and age of patients who developed allergic reactions and those who did not, either after the first dose (1 mcg/mL) of venom or during the entire build-up procedure ($p>0.05$).

Discussion

VIT is the standard therapeutic approach for patients with severe allergy to venom; and different VIT regimens have been introduced over nearly four decades.[1] However, the choice of schedule generally depends on the convenience, urgency of the patient's need for treatment, and the experience and comfort of the immunologist with specific protocols.[7] In this study, we used a 1 mcg/mL venom concentration as the starting dose of cluster protocol in Iranian patients with a history of anaphylaxis to venom. Approximately 41% of the patients developed mild local reactions following the initial dose, with one case of mild systemic reactions. During the build-up phase, more than 90% of patients experienced mild local reactions. Mild-to-moderate systemic adverse effects occurred in only three patients at 50 and 100 mcg venom concentrations. These reactions included one patient who received extracts from honey bee, yellow jacket, and wasp; one patient who received only honey bee extract, and the last who received extracts from honeybee and yellow jacket.

A similar outcome has been reported in a previous study conducted on patients undergoing rush and ultra-rush protocols with an initial dose of 1 mcg/mL venom.[8] In accordance with our results, they reported that none of their patients showed systemic reactions after the initial dose, and the majority of the systemic reactions were caused by bee venom at the 100-mcg venom concentration.[8]

Similarly, a previous study revealed no correlation between the initial venom dose (ranging from 0.0001 mg to 1 mg) and the prevalence or severity of systemic allergic reactions induced by VIT.[9] A recent article from Spain investigated the safety of a clustered VIT schedule using a 2-day, 5-dose induction period, reaching the therapeutic target on the 7th day. On the first day, patients received three injections of alum-based depot products (10 mcg, 20 mcg, 20 mcg). Only mild local and systemic reactions were detected during the immunotherapy, which were resolved with antihistamine.[10]

Interestingly, it has been consistently reported that honeybee venom is the most important risk factor (3.1 to 6.0-fold higher risk) for systemic adverse events after VIT.[6,8] Other risk factors associated with a higher chance of systemic allergic manifestations include female sex, senior age, mastocytosis, elevated serum tryptase and IgE level, and particularly rapid dose escalation during the build-up phase, as observed in rush and ultra-rush protocols.[11,12] To mitigate the disadvantages of this rapid dose escalation, we implemented the cluster protocol, which is a faster alternative to conventional methods while maintaining a safer approach compared to rush and ultra-rush protocols.[13] We detected no significant difference in the sex and age of patients who did and/or did not experience adverse reactions following VIT. Additionally, a previous history of allergic diseases was not significantly associated with VIT reactions. However, it should be noted that these VIT risk factors are generally correlated with systemic reactions.[11,12] Nevertheless, the majority of allergic reactions in our patients were limited to local manifestations, with only few systemic reactions, which impedes an accurate assessment of the risk factors. A limitation of our study was that we did not have access to component-resolved diagnosis and were unable to distinguish the single-allergen patients from double- or triple-sensitized ones if they tested positive for more than one type of venom. Therefore, we set every positive test as a criterion for immunotherapy with that extract. Given the possibility of injecting all three extracts for patients in one session, in case of developing a reaction, we were unable to identify the exact extract causing that reaction. Furthermore, it should be noted that this is a pilot study conducted on a small number of patients, and therefore, the current work lacks sufficient data to thoroughly discuss the risk factors involved in VIT allergic reactions during the build-up phase.

This study was conducted to examine the safety of a higher initial dose of venom immunotherapy compared to the standard dose. By demonstrating the significance of this approach in this pilot study, allergists and patients may be able to plan a more efficiently designed VIT regimen, reducing both time and costs.

Conclusion

In summary, our results indicate that administrating 1 mcg/mL venom concentration as the starting dose in a VIT protocol is safe. Increasing the initial dose up to 10 times may accelerate the build-up phase and shorten the duration of cluster VIT, which is a safe out-patient protocol. In addition to reducing the time required for VIT, this approach also reduces therapy costs for patients by minimizing the number of injections. Further studies involving larger populations and varying VIT protocols are necessary to validate these findings.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Iran University of Medical Sciences (IR.IUMS.FMD.REC.1402.065), according to the Declaration of Helsinki. Prior to data collection, a written informed consent has been obtained from each patient and/or their parents.

Consent for Publication

Informed consent was obtained from the parents of the patients prior to being included in the study.

Availability of Data and Materials

The datasets supporting the conclusions of this article are included within the article.

Author Contributions

MB: interpreted the data, designed the work, and drafted or substantively revised the manuscript. SA, MF, SS, MN: acquired and interpreted the data and revised the manuscript. NS: analyzed and interpreted the data, drafted or substantively revised the manuscript. SB: made substantial contributions to the conception, acquisition of data, and interpretation of data, and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of Interests

The authors declare that they have no conflict of interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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