10.22059/ijvm.2020.260482.1004906

Iranian Journal of Veterinary Medicine
Volume 14- Issue 04

Original Article

Online ISSN: 2252-0554

Study of Human IgG and IgE Antibodies Against Bee (*Apis mellifera*) Venom

Sedigheh Nabian[1*], Mohammad Taheri[2], Mina Babaei[3], Parastoo Yousefi[2], Abbas Gerami Sadeghian[4], Zahra Asadollahi[4], Ramin Mazaheri Nezhad Fard[5]

[1] *Section of Honey Bee, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[2] *Rastegar Reference Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[3] *Graduated from the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[4] *Section of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[5] *Division of Food Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

Abstract

BACKGROUND: Bee venom contains various biomolecules, such as enzymes, peptides, and amines. The immune system produces IgG antibodies against bee venom proteins. However, IgE antibodies may also be developed in allergic individuals.

OBJECTIVES: In this study, immune responses, including IgG and IgE reactions to bee venom were assessed in various individuals, using the immunoblotting technique.

METHODS: Serum samples were collected from 20 people of three major groups, namely beekeepers, allergic individuals, and normal people. Venom samples of honey bees and wild bees were collected from the suburbs of Tehran, Iran. Furthermore, commercial honey bee venom samples extracted from *Apis mellifera* and samples of wild bees extracted from *Polistes* and *Vespula* were purchased from France. Immunoblotting was carried out using the sera of subjects and anti-human IgG and IgE coupled to horseradish peroxidase.

RESULTS: The results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed similar protein bands in Iranian and European honey bee venoms, including α-glucosidase (170 kDa), Api m (100 kDa), acid phosphatase (49 kDa), hyaluronidase (43 kDa), phospholipase A2 (17 kDa), and melittin (2 kDa). In wild bees, two bands were found with the molecular weights of 35 and 25 kDa belonging to antigen 5 and phospholipase A1, respectively. These were not observed in honey bee venoms. Immunoblot analysis revealed that all the mentioned proteins were immunogenic and allergenic in different individuals. Hyaluronidase, as well as phospholipases A1 and A2, were the major allergens in most individuals, while IgE reaction to melittin was only reported in one person.

CONCLUSIONS: In conclusion, studies on antibodies against bee venoms can be useful in immunotherapy. Different people indicated distinct allergenic patterns. Therefore, further similar assays are recommended before, during, and after immunotherapy.

KEYWORDS: *Apis mellifera*, Bee venom, IgG, IgE, *Polistes*, *Vespula*

Correspondence

Sedigheh Nabian, Section of Honey bee, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Tel: +98 (021) 61117072, Fax: +98 (021) 66933222, Email: nabian@ut.ac.ir

Received: 2020-05-02

Accepted: 2020-08-24

Copyright © 2020. This is an open-access article distributed under the terms of the Creative Commons Attribution- 4.0 International License which permits Share, copy and redistribution of the material in any medium or format or adapt, remix, transform, and build upon the material for any purpose, even commercially.

How to Cite This Article

Nabian, S ., Taheri, M., Babaei, M., Yousefi, P., Sadeghian, A., Babaei, M., & *et al.*, A. (2020). Study of Human IgG and IgE Antibodies Against Bee (*Apis mellifera*) Venom. *Iranian Journal of Veterinary Medicine*, 14(4), 412-420

Introduction

Bee venom is a collection of different compounds, such as enzymes, peptides, and physiologic amines. Some of the physiologic amines include hyaluronidase, phospholipase A, phosphatase acid, α-glycosidase, protease inhibitors, melittin, apamin, mast cell degranulating peptides, adolapin, cardiopep, procamine, tertiapin, secapine, histamine, dopamine, and noradrenaline (Ali, 2012; Mammadova and Topchiyeva, 2017).

Epidemiological studies have shown that 57%–90% of people may be stung at least once in their lives by various types of bees, such as honey bees, vespids (mainly *Vespula* spp.), and bumblebees (Mahmoud Abdu 2012). Bee venom injection under the skin usually causes symptoms, including itching, pain, redness, and swelling that diminish after a few hours (Sahiner, 2016; Asai, 2016).

The immune system normally reacts to bee venom proteins by producing IgG antibodies. In allergic individuals, IgE antibodies may also be generated (Komi, 2017; Ollert and Blank, 2015). Both immediate and delayed hypersensitivity reactions are induced in predisposed individuals. Immediate reactions can be categorized into local and systemic responses. Sometimes, local reactions are triggered by IgE-dependent reactions (Zhang *et al.,* 2018). Systemic reactions cause severe symptoms ranging from cutaneous to respiratory, cardiovascular, and gastrointestinal manifestations.

Sensitization of the mast cells by specific IgE can lead to degranulation accompanied by the release of histamine and other pharmacologically active substances. Mild allergic responses due to insect stings can be healed using antihistamines. However, the principal treatment in severe conditions is venom immunotherapy. This treatment restores the normal immune system by inducing regulatory T-cells, which decrease the production of inflammatory cytokines and allergen-specific IgE, in addition to blocking IgG4 antibodies (Reber *et al.*, 2017). It seems that the profiling of bee venom immunogens and allergens by allergic and non-allergic stung people sera can help select appropriate proteins for immunotherapy. This study aimed to assess immune responses, including IgG and IgE to bee venom in various individuals using the immunoblot technique.

Materials and Methods

Sera Collection

Blood samples were collected from 20 individuals of three different groups, including seven beekeepers, seven individuals allergic to bee venom based on their history, and six healthy people, who were not stung by bees as control. Blood samples were centrifuged at 1500 g at room temperature for 5 min to isolate sera.

Bee Venom Preparation

Honey bees (*Apis mellifera*) and wild bees (*Vespula* spp.) were collected from apiaries around Tehran, Iran, and were stored at -20°C until usage. The bee poison sacs were removed manually using a stereomicroscope and were floated in phosphate buffer solution (PBS, pH: 7.2). These were homogenized by pipetting and sonicating. Following centrifugation at 12000 g for 10 min, supernatants were collected and frozen at -20ºC. Commercially available venom of *A. mellifera*, *Polistes*, and *Vespula* were purchased from France (Danesh Pajoh Company, France). Melittin (Cat No. 2272M-1MG) was purchased from Sigma-Aldrich, USA.

SDS-PAGE

To characterize the protein patterns of honeybee venom, electrophoresis was performed using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDSPAGE) and 5% stacking gels under nonreducing conditions. All electrophoresis steps were completed utilizing a mini gel electrophoresis system (BioRad, USA) based on the original proto-

cols by Lamelli (1970). The quantity of the protein samples was assessed based on the Warburg assay. Next, 10 µL (20 µg) of proteins was diluted in SDS-PAGE sample buffer and electrophoresed on gels for 90 min at 80 V. Gels were divided into two parts and one part was stained by Coomassie Brilliant Blue to visualize protein bands. The other part was used for western blotting.

Immunoblotting

Immunoblotting was carried out according to the original protocols by Wang *et al.* with some modifications. Bee venom proteins were separated in 12% SDS-PAGE gels and were transferred to nitrocellulose membranes using a wet system (Bio-Rad, USA) in transfer buffer. The membrane was blocked for 45 min with PBS containing 2.5% of Tween 20 followed by washing steps with PBS containing 0.05% of Tween 20 (3×5 min). Membranes were incubated in sera derived from various subjects (1/20 in PBS containing 0.05% Tween 20) for 1 h at room temperature. Afterwards, membranes were washed and separately incubated with anti-human IgG and IgE coupled to horseradish peroxidase (1/1000 in PBS) for 30 min at room temperature. After washing, the color was generated using diaminobenzidine H_2O_2.

Results

SDS-PAGE

Protein bands with the molecular weights of 2.8–180 kDa were obtained by SDS-PAGE for the venom proteins of honey bees and wild bees (*Polistes* and *Vespula*) and commercial melittin (Figure 1). All venoms showed common bands with the molecular weights of 38–45, 49, and 170 KDa belonging to hyaluronidase, acid phosphatase, and α-glucosidase, respectively. Bands with the molecular weights of 17–20 kDa for phospholipase A2 and 55 KDa for icarapin were detected in the venom specimens of honey bees. On the other hand, bands with molecular weights of 25 and 35 KDa were related to phospholipase A1 and Antigen 5 found in wild bees venom from Iran and France.

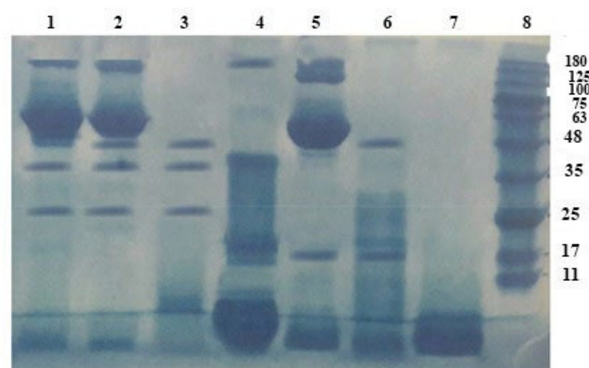

Figure 1. Results of SDS-PAGE for bee venom; 1: *Polistes* (France), 2: *Vespula* (France), 3: wild bee from Iran, 4: *Apis mellifera* from Iran, 5: *Apis mellifera* from France, 6: *Apis mellifera* from Iran (1/5 diluted), 7: commercial melittin, and 8: molecular ladder

Immunoblotting for Venom Specific IgG

Sera samples of 20 individuals from three various groups, including beekeepers who were stung several times, allergic people, and individuals without a sting history as the control group were used for immunoblotting. Results of the analysis of sera from beekeepers and allergic individuals demonstrated immunogenic bands for bee venoms with a range of 2.8–170 KDa. Sera from beekeepers and allergic individuals reacted with two immunogenic bands with the molecular weights of 17–20 and 49 kDa, respectively, belonging to Iranian and European honey bee venoms. Sera reacted with immunogenic bands with molecular weights of 23, 35, and 170 kDa related to wild bee venoms. Moreover, sera reacted with the immunogenic bands of 38–45, and 100 KDa for all venoms. No reactions were reported with the sera from the control group (Figure 2).

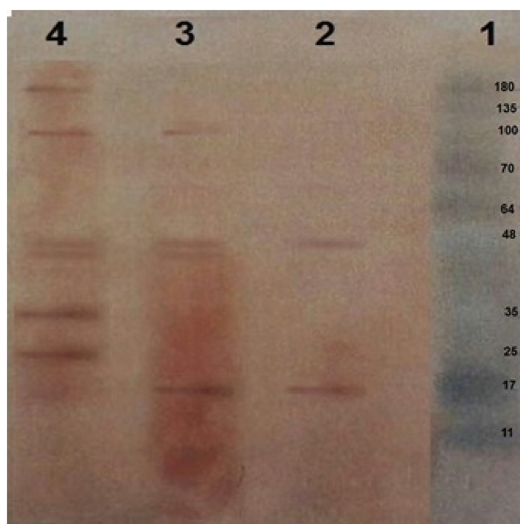

Figure 2. Results of the immunoblot analysis of IgG antibodies against bee venoms using beekeeper sera; 1: molecular ladder, 2: honey bee venom from Iran, 3: honey bee venom from France, and 4: wild bee venom (Polistes spp.)

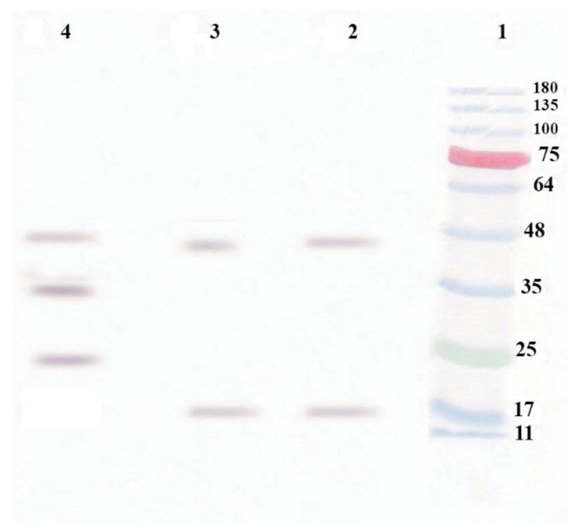

Figure 3. Results for the immunoblot analysis of IgE antibodies against bee venoms using beekeeper sera; 1: Vespula venom, 2: Polistes venom, 3: Apis mellifera venom from Iran, and 4: molecular ladder

Immunoblotting of the Venom Specific IgE

Results of immunoblotting various bee venoms using the sera of beekeepers and allergic individuals and anti-human IgE conjugates are shown in Figure 3. In general, allergic bands with a range of 2.7–49 kDa were observed in bee venoms. Sera from beekeepers and allergic individuals reacted with allergens with the molecular weights of 38–45 kDa belonging to honey bees and wild bees. Furthermore, serum specimens reacted with two other bands of 17–20 and 40–49 kDa related to European and Iranian honey bees, respectively. It is noteworthy that two bands with molecular weights of 23 and 35 kDa were found exclusively in wild bees. No reactions were reported to the serum samples of the control group.

Results of immunoblotting assays are summarized in Tables 1 and 2. As indicated in figures, no IgG or IgE reactions were detected to bee venoms in people with no history of bee stings. However, beekeepers and allergic individuals showed variable positive results for venom specific IgG and IgE against hyaluronidase, phospholipases A1 and A2, acid phosphatase, Apm5, α-glucosidase, Antigen 5, and melittin. Among the bee venom components, hyaluronidase was the most immunogenic and allergen, while melittin was one with the lowest immunogenic capacity.

Table 1. Immunoblotting results using anti-human IgG coupled with horseradish peroxidase

	glocosidase α	Apim5	Phosphotase acid	Hyaluronidase	Ag5	Phospholypase A1	Phospholypase A2	Melittin
Beekeeper 1				+				
Beekeeper 2			+	+		+		
Beekeeper 3	+			+	+	+	+	
Beekeeper 4	+		+	+		+	+	
Beekeeper 5				+			+	

	glocosidase α	Apim5	Phosphotase acid	Hyaluronidase	Ag5	Phospholypase A1	Phospholypase A2	Melittin
Beekeeper 6	+	+	+	+		+	+	
Beekeeper 7	+	+				+	+	
Allergic person 1				+			+	+
Allergic person 2				+				
Allergic person 3				+				
Allergic person 4				+				
Allergic person 5								
Allergic person 6				+				
Allergic person 7				+				
Contro 1								
Control 2								
Control 3								
Control 4								
Control 5								
Control 6								

Table 2. Immunoblotting results using anti-human IgE coupled with horseradish peroxidase

	glocosidase α	Apim5	Phosphotase acid	Hyaluronidase	Ag5	Phospholypase A1	Phospholypase A2	Melittin
Beekeeper 1				+				
Beekeeper 2			+	+		+		
Beekeeper 3	+			+	+	+	+	
Beekeeper 4	+		+	+		+	+	
Beekeeper 5				+			+	
Beekeeper 6	+	+	+	+		+	+	
Beekeeper 7				+				
Allergic person 1				+			+	+
Allergic person 2				+				
Allergic person 3				+				
Allergic person 4				+				
Allergic person 5								
Allergic person 6								

	glocosidase α	Apim5	Phosphotase acid	Hyaluronidase	Ag5	Phospholypase A1	Phospholypase A2	Melittin
Allergic person 7				+				
Control 1								
Control 2								
Control 3								
Control 4								
Control 5								
Control 6								

Discussion

In the current study, human antibodies IgG and IgE were comprehensively investigated against bee venom antigens in three groups of allergic individuals, beekeepers with bee sting history, and controls without bee sting history. Antibodies against honey bee sting usually cause mild symptoms, such as itching, skin redness, and swelling. However, it can induce severe anaphylaxis in allergic individuals. Barnard (1976) reported 400 deaths due to allergies caused by Hymenoptera bites, with the majority of honey bee stings occurring during ten years (Hoffman *et al.*, 1997).

Venom-specific immunotherapy decreased the risk of subsequent sting anaphylaxis (Gihyun and Hyunsu, 2016; Schiener *et al.*, 2017). Detection of relevant allergens helped to prepare immunotherapeutic products for the clinical treatment of allergic people (Blank *et al.* 2017; Matysiak *et al.*, 2016). Jeep *et al.* (1996) carried out immunoblot assays using the sera of allergic people before and after immunotherapy. They concluded that immunoblot results could reflect immune responses to epitopes, which induced IgG and IgE in individuals.

Several studies have shown that bee venom proteins entail a complex mixture of epitopes, some of which being allergens in sensitive individuals. In the present investigation, SDS-PAGE results revealed similar protein bands in Iranian and European honey bee venoms, including α-glucosidase (170 kDa), Api m (100 kDa), acid phosphatase (49 kDa), hyaluronidase (43 kDa), phospholipase A2 (17 kDa), and melittin (2 kDa). In wild bees, two bands with the molecular weights of 35 and 25 kDa belonged to Antigen 5 and phospholipase A1, respectively, which were not found in honey bee venoms. The latter findings are similar to the other studies (Spillner *et al.*, 2014; Muller, 2003).

Immunoblot analysis demonstrated that all the highlighted proteins were variably immunogenic and allergenic in different groups of people. In the current study, beekeepers had significantly higher venom-specific IgE, compared to the control group. According to the previous studies, 30%–60% of beekeepers show positive results for venom-specific IgE (Muller *et al.*, 2005).

In the present study, hyaluronidase and phospholipases A1 and A2 were the major allergens in most individuals, while IgE reaction to melittin was observed exclusively in one serum sample. The previous studies indicated that phospholipases A1 and A2 and hyaluronidase are the most remarkable allergens in the venom of honey bees and wild bees. Phospholipases A1 and A2 are glycoproteins that play the role of cytotoxin (Owen *et al.*, 1990). Hyaluronidase is among the important allergens of bee venom that degrades hyaluronic acid and shows 50% sequence identity with vespid venom hyaluronidase (Markovic-Housely *et al.*, 2000).

The crude honey bee venom dry weight is 50% of melittin and 12% of phospholipase A2. According to Muler *et al.* (2002), only 28% of the allergic people showed IgE reaction to melittin. The data of the current study on serum reaction with melittin were similar to the previous studies. Acid phosphatase, dipeptidyl peptidase IV, and icarapin are the other components of venoms with lower quantities than melittin and phospholipase A2. However, the former substances were shown to have IgE reactivity in more than 50% of the sera of allergic patients (Frick *et al.*, 2016; Kohler *et al.*, 2014).

Information about clinically relevant allergens can help access venom ingredients, which are more useful for immunotherapy. Therefore, western blotting can be useful in the diagnosis of allergens and preparation of standard venom extracts for immunotherapy. Our results along with the results of other studies show that substances with low molecular weight, especially bioactive peptides from crude venoms, are less allergen. Consequently, the removal of these substances via purification steps may be appropriate for immunotherapy in relevant patients without specific IgE reactivity to low-molecular-weight peptides. Variations were found in reactivity to bee venom components between the allergic subjects. As a result, immunoblotting results can be informative before, during, and after immunotherapy.

Acknowledgments

The authors gratefully acknowledge the financial support provided by the Research Council of the Faculty of Veterinary Medicine, University of Tehran, Iran.

Conflict of Interest

The authors declared that there is no conflict of interest.

References

Ali, M.A. (2012). Studies on bee venom and its medical uses. *Int J Adv Res Tech*. 1: 69-83.

Asai, Y., Uhara, H., Miyazaki, A., Saiki, M., Okuyama, R. (2016). Late onset of acute urticaria after bee stings. *Case Rep Dermatol* 8:341-343. [DOI:10.1159/000449033] [PMID] [PMCID]

Barnard, J.H. (1973). Studies of 400 Hymenoptera deaths in the United States. *J Allergy Clin Immunol*. 52: 529. [DOI:10.1016/0091-6749(73)90044-4]

Blank,S., Etzold, S., Darsow, U., Schiener, M., Eberlein, B., Russkamp, D., Wolf, S., Grassel, A., Biedermann, T., Ollert, M., Schmidt- Weber, C. (2017). Component-resolved evaluation of the content of major allergens in therapeutic extracts for specific immunotherapy of honey bee venom allergy. *Hum Vaccine Immunother*. 13(10). [DOI:10.1080/21645515.2017.1323603] [PMID] [PMCID]

Frick, M., Fischer, J., Helbling, A., Ruef, F., Wieczorek, D., Ollert, M., Pfutzner, W., Muller, S., Huss-Marp, J. (2016). Predominant Api m 10 sensitization as risk factor for treatment failure in honey bee venom Immunotherapy. *J Allergy Clin Immunol*. 138: 1663-71. [DOI:10.1016/j.jaci.2016.04.024] [PMID]

Gihyun. L., Hyunsu B. (2016). Bee venom phospholipase A2: yesterday's enemy becomes today's friend. *Toxins*. 8: 48. [DOI:10.3390/toxins8020048] [PMID] [PMCID]

Hoffman, D.R. (1997). Allergy to bee venom: in vitro diagnosis and identification and isolation of allergens. *Cutis*, 19: 763-767. PMID: 872619

Jeep, S., Paul, M., Muller, U., Kunkel, G. (1996). Honeybee venom allergy: immunoblot studies in allergic patients after immunotherapy and before sting challenge. *Allergy*. 51: 540-6. [DOI:10.1111/j.1398-9995.1996.tb00110.x] [PMID]

Kohler J, Blank S, Muller S, Bantleon F, Frick M, Huss- Marp J, Lidholm J, Spillner E, Jakob T. (2014). Component resolution reveals additional major allergens in patients with honey bee venom allergy. *J Allergy Clin Immunol*, 133: 1383-9. [DOI:10.1016/j.jaci.2013.10.060] [PMID]

Komi, D.E.A & Shafaghat, F. & Zwiener, R.D. (2017). Immunology of Bee Venom. Clinic *Rev Allerg Immunol*.

Lameli, UK. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 227: 680-685. [DOI:10.1038/227680a0] [PMID]

Mahmoud Abdu Al-Samie Mohamed A. (2012). Studies on bee venom and its medical uses. *Inter J Adv Res Tech*, 1(2).

Mammadova FZ, Topchiyeva ShA. (2017). Isolation and identification of biologically active components from the honey bee venom *Apis mellifera* L. caucasica. *Moj Toxicol*. 3(7). [DOI:10.15406/mojt.2017.03.00078]

Markovic-Housley Z, Miglierini G, Soldatova L, Rizkallah PJ, Muller U, Schirmer T. (2000). Crystal structure of hyaluronidase, a major allergen of bee venom. *Structure Fold*, 15: 1025-1035. [DOI:10.1016/S0969-2126(00)00511-6]

Matysiak, J., Matysiak, J., Breborowicz, A., Kycler, Z., Dereziriski, P., Kokot, Z. (2016). Immune and clinical response to honey bee venom in beekeepers. *Ann Agric Environ Med*, 23: 120 -124. [DOI:10.5604/12321966.1198506] [PMID]

Muller, U. (2002). Recombinant venom allergens. Allergy. 57: 570-576. [DOI:10.1034/j.1398-9995.2002.02157.x] [PMID]

Muller, U.R. (2003). Hymenoptera venom allergy, recent developments and perspectives in diagnosis and immunotherapy. *Revue Francaise d, Allergologie et Immunologie Clinique*, 44: 282-285.

Ollert, M., Blank S. (2015). Anaphylaxis to in-sect venom allergens: Role of molecular diagnosis. *Cure Allergy Asthma Rep*, 15: 527. [DOI:10.1007/s11882-015-0527-z] [PMID] [PMCID]

Owen, M.D., Pfaff, L.A., Reisman R.E., Wypych, J. (1990). Phospholipase A2 in venom extracts from honey bees (*Apis mellifera* L.). of different ages. *Toxicon*, 28: 813-820. [DOI:10.1016/S0041-0101(09)80004-4]

Reber, L.L., Hernandez, J.D., Galli, S.J. (2017). The pathophysiology of anaphylaxis, Mechanisms of allergic diseases. *J Allergy Clin Immunol*, 140: 335-48. [DOI:10.1016/j.jaci.2017.06.003] [PMID] [PMCID]

Sahiner, U. M., & Durham, S. R. (2019). Hymenoptera venom allergy: how does venom immunotherapy prevent anaphylaxis from bee and wasp stings?. *Frontiers Immunol*, 10, 1959. [DOI:10.3389/fimmu.2019.01959] [PMID] [PMCID]

Schiener, M., Graessel, A. Ollert, M., Schmidt-Weber CB, Blank, S. (2017). Allergen specific immunotherapy of hymenoptera venom allergy-also a matter of diagnosis. *Hum Vaccine Immunother,* 13: 2467-2481. [DOI:10.1080/21645515.2017.1334745] [PMID] [PMCID]

Spillner, E., Blank, S., Jakob, T. (2014). Hymenoptera allergens: from venom to venom. *Frontiers Immunol*. 5: 1-7. [DOI:10.3389/fimmu.2014.00077] [PMID] [PMCID]

Wang, H., Nuttalla, P.A. (1994). Comparison of the proteins in salivary glands, saliva and hemolymph of Rhipicephalus appendiculatus female ticks during feeding. *Parasitol*, 109: 517-523. [DOI:10.1017/S003118200008077X] [PMID]

Zhang, B., Li, Q., Shi, C., Zhang, X. (2018). Drug-induced pseudoallergy: A review of the causes and mechanisms. *Pharmacology*, 101:104-110. [DOI:10.1159/000479878] [PMID]

10.22059/ijvm.2020.260482.1004906

Iranian Journal of Veterinary Medicine
Abstracts in Persian Language
Online ISSN 2252-0554

مجله طب دامی ایران، ۱۳۹۹، دوره ۱۴، شماره ۴، ۴۱۲-۴۲۰

مطالعهٔ آنتی‌بادی‌های IgG و IgE علیه زهر زنبورعسل (*آ پیس ملیفرا*)

صدیقه نبیان[1]، محمد طاهری[2]، مینا بابایی[3]، پرستو یوسفی[2]، عباس گرامی صادقیان[4]، زهرا اسدالهی[4]، رامین مظاهری نژاد فرد[5]

[1] بخش زنبور عسل، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

[2] آزمایشگاه مرکزی رستگار، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

[3] دکترای عمومی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

[4] بخش انگل شناسی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

[5] گروه میکروب شناسی غذایی، گروه پاتوبیولوژی، دانشکده بهداشت، دانشگاه علوم پزشکی تهران، تهران، ایران

(دریافت مقاله: ۱۳ اردیبهشت ماه ۱۳۹۹، پذیرش نهایی: ۰۳ شهریور ماه ۱۳۹۹)

زمینه مطالعه: زهر زنبورعسل دارای بیومولکول‌های مختلف از جمله آنزیم‌ها، پپتیدها و آمین‌ها است. سیستم ایمنی علیه پروتئین‌های زهر زنبورعسل IgG تولید می‌نماید همچنین در افراد آلرژیک ممکن است IgE نیز تولید شود.

هدف: در این مطالعه، پاسخ ایمنی (واکنش‌های IgE وIgG) علیه زنبورعسل با استفاده از روش ایمونوبلاتینگ در افراد مختلف سنجش می‌شود.

روش‌کار: نمونه‌های سرمی، از ۲۰ فرد مختلف در سه گروه زنبورداران، افراد آلرژیک و افراد سالم جمع‌آوری گردید. زهرهای زنبورعسل معمولی و وحشی اطراف شهر تهران جمع‌آوری شد. همچنین زهرهای تجاری زنبورعسل (مستخرج از *آ پیس ملیفرا*) و زنبورهای وحشی (مستخرج از *پولیستس و وسپولا*) از کشور فرانسه خریداری گردید. ایمونوبلات با استفاده از آنتی‌بادی‌های ضدایمونوگلوبولین G انسان و ضدایمونوگلوبولین E انسان کونژوگه شده با HRP انجام شد.

نتایج: نتایج SDS-PAGE حضور باندهای مشترکی را در زهرهای مختلف زنبورعسل ایرانی و اروپایی شامل آلفاگلوکوزیداز (۱۷۰ کیلودالتون)، Api m (۱۰۰ کیلودالتون)، اسید فسفاتاز (۴۹ کیلودالتون۹)، هیالورونیداز (۴۳ کیلودالتون)، فسفولیپاز A2 (۱۷ کیلو دالتون) و ملیتین (۲ کیلودالتون) را نشان داد. در عصاره زهر زنبورهای وحشی دو باند با وزن‌های مولکولی ۳۵ و ۲۵ کیلودالتون مربوط به آنتی‌ژن ۵ و فسفولیپاز A1 نیز مشاهده شد که در زهر زنبورعسل معمولی وجود نداشت. آنالیز ایمونوبلات نشان داد که تمام پروتئین‌های مذکور به نوعی در افراد مختلف ایمونوژن و آلرژن بوده‌اند. اما هیالورونیداز و فسفولیپاز A2 در بیشتر افراد، آلرژن بودند. در حالی که واکنش IgG به ملیتین تنها در یک فرد مشاهده شد.

نتیجه‌گیری نهایی: مطالعه بر روی آنتی‌بادی‌های ضد زهر زنبورعسل می‌تواند در ایمونوتراپی مفید واقع شود. از آن جایی که افراد مختلف، الگوهای آلرژنیکی مختلفی را نشان می‌دهند، لذا به نظر می‌رسد که بررسی‌های مشابه بعدی قبل، بعد و در طی دوره ایمونوتراپی می‌تواند مفید واقع شود.

واژه‌های کلیدی: *آ پیس ملیفرا*، زهر زنبور، *پولیستس*، *وسپولا*

نویسندهٔ مسئول: صدیقه نبیان، بخش زنبور عسل، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران. ایمیل: nabian@ut.ac.ir