Archives of Razi Institute, Vol. 77, No. 1 (2022) 359-365

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DOI: 10.22092/ARI.2021.356679.1892

Original Article

Investigation on the Effect of Age and Gender on Hypersensitivity Reactions due to Allergens Injection in Iraqi Population

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Received 1 November 2021; Accepted 22 November 2021
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Abstract

Hyperactivity of the immune system due to the insertion of allergens into the living body has been known as an allergic reaction. Some substances, such as pollen grains, insects' venom, house dust mite, foods, and medicines, can induce allergic responses. Therefore, this study was designed to shed light on the role of gender and age in allergic reactions resulting from some organic and chemical allergens. A total of 200 individuals participated in this study, including 70 males and 130 females. A skin test was performed by subcutaneously injecting allergens, namely amoxicillin, cefotaxine, gentamicin, *Vespula* spp., and *Apis mellifera.* All the chemicals were purchased from Sigma-Aldrich unless otherwise stated. The spot of injection was sterilized by ethyl alcohol (70%) and well dried; subsequently, 0.05 mL of each allergen (antigen) was injected via a 1-mL medical syringe. The results showed that 140 cases were allergic. Anti-cefotaxine occupied the highest percentage among the studied drug allergens. The highest percentage of males (37.5%) that were allergic was at the age range of 28-35 years, whereas the highest percentage of females (18.5%) that were allergic was at the age range of 17-27 years. Sensitivity to amoxicillin accounted for 12.5% of males at the age range of 28-37 years and 3.7% of females at the age range of 17-27 years. Gentamicin triggered the highest percentage of sensitivity in 12.5% and 7.4% of males and females aged 48-57 years and 17-27 years, respectively. The results showed that honey bees had the highest percentage of total sensitivity at 40%. The highest sensitivity rate stood at 37.5% in males at the age range of 28-37 years and 18.5% in females at the age range of 17-27 years. Wasps recorded a total sensitivity rate of 17.1%, with the highest percentage at 37.5% in males who were aged 17-27 years and 3.7% in females at the age ranges of 17-27 and 48-57 years. The results of the statistical analysis indicated that there were significant ($P \leq 0.05$) differences for all allergens that were studied regarding gender and age.

Keywords: Allergy, *Apis mellifera, Vespula* spp.

1. Introduction

Allergies comprise a group of complex disorders and are characterized by abnormal immune responses due to foreign antigens called allergens. Most of us are exposed to allergens daily, such as food, pollen, animal dander, and medications. Even minuscule components of any of these allergens are capable to trigger allergies. The prerequisite to understanding the allergic responses would be investigating the induction of the adaptive immune response through T-helper cells type 2 and immunoglobulin E (IgE) (1). Allergic rhinitis and asthma are the most common allergic diseases in children. Age, environment, and hereditary genes all are considered the main factors that participated in the development of allergies (2).

Allergy to any stimulus has a sudden onset, usually lasts less than 24 h, and involves one or more physiological function(s). More than 30% of the population in modern countries suffers from various types of allergies, leading to social and economic consequences (3). Insect stings and injections that are considered to work against allergens cause anaphylaxis, resulting in the constriction of blood vessels, airway obstruction, or sometimes both (4). Sensitivity resulting from insect stings constitutes approximately 42% of all cases of allergic reactions (5).

One of the allergy cases is the order of Hymenoptera presented by the families of bees (Apoidea), wasps (Vespoidea), and ants (Formicidae), whose venom containing the cross-reactive carbohydrate determinant (CCD) interacts with antibodies. Immunoglobulin E (IgE) directed to the determinant (i.e., CCD) is believed to be the reason for the discovery of multiple sensitivities (5). Insect bites cause itching and swelling, accompanied by pain and symptoms, which can vary from patient to patient. Some patients have local reactions, whereas others have systemic and even fatal reactions (5).

Apis mellifera bees are bred to produce honey, royal jelly, wax, and venom and enhance the pollination of various types of cultivated plants. When they sting a person or an enemy, they inject a toxic substance, leading to losing their ability to sting and even to live. This species is unlike wasps and ants that can sting more than once without dying. There is great interest in identifying allergens caused by the venom of insects belonging to the Vespoidea (wasps), Apoidea (bees), and Formicidae (ants) families. In 1897, Langer discovered that bee venom called apitoxin consisted of the main active and haemolytic components, namely peptides, melittin, apamin, phospholipase A2, hyaluronidase, noradrenalin, histamine, and dopamine.

Phospholipase A2, which is also found in snake and spider venom and acts as neurotoxins and muscle toxins, and hydronidase are the allergenic proteins of venom. Mast cell degranulating peptide works to degrade the peptide granules, releasing histamine and causing inflammation in this process (6, 7). The venom of *Vespula* spp. contains some small peptides, such as mastoparn, which is a major component of venom and Vespula Quinin. Vespula Quinin are vasoactive glycopeptides containing nine long amino acids. These small peptides have such effects as releasing histamine in body cells, lowering blood pressure, and inducing pain. Therefore, it is similar to bee and wasp venom for comprising of phospholipase A2, melittin, and hyaluronidase. Wasp venom is distinguished from bee venom by the presence of antigen 5 and the two *Vespula vulgaris* and *Vespula germanica* types that belong to *Vespula* spp., which is the most sensitivity-inducing of all types (8-10). The quantity of protein in the venom per bite varies between hymenopteran species, ranging from 1.7 to 3.1 Mg in *Vespula* spp., being approximately 17 Mg in *Polistes* spp., and reaching 59 Mg in honeybees (10).

Reactions from immunization drugs account for around 6-10% of adverse drug reactions. Allergen-related antibiotics, especially beta-lactams, are the most common drugs. Beta-lactams are estimated to be responsible for more than 40% of allergic reactions. In 1990, the results of a study showed that people sensitive to amoxicillin do not necessarily have an allergy to benzylpenicillin. This finding changed the perception that patients with hypersensitivity reactions to a particular type of penicillin should avoid penicillin altogether. In the mentioned study, only 290 people were allergic to penicillin and 57.9% were allergic to amoxicillin (11). Adverse drug reactions (ADRs) affect 10-20% of hospital patients and more than 7% of the general population. Immunological drug reactions, such as allergic reactions, are estimated to account for about one-third of ADRs, and antibiotic allergy is found to be the most common drug allergy (12). Amoxicillin has common adverse reactions; however, no known factors exist that can help predict amoxicillin allergy in children. In addition, the methods used to diagnose amoxicillin allergy are not standardized and their role in diagnosis is unclear (13).

This study aims to detect insect and drug allergies by examining skin and eosinophil percentage and highlighting the effect of age and gender on hypersensitivity reactions resulting from injected allergens in the population of Mosul city, Iraq.

2. Materials and Methods

The study sample (n=200) included 70 males and 130 females from Mosul city who were tested for allergies, among whom, 140 (32 males and 108 females aged 17-77 years old) cases showed a positive allergic response.

2.1. Allergy Check Tests

2.1.1. Skin Test

A skin test was performed by subcutaneously injecting allergens, including amoxicillin, cefotaxine, gentamicin, *Vespula* spp., and *Apis mellifera*. All the chemicals were purchased from Sigma-Aldrich (USA) unless otherwise stated. The spot of injection was sterilized by ethyl alcohol (70%) and well dried; subsequently, 0.05 mL of each allergen (antigen) was injected via a 1-mL medical syringe. The skin prick test method was performed using the available injected allergens and the same amount of allergen (0.05 ml) was placed on the skin using a syringe; afterward, the antigen was introduced (14). Sensitivity detection was accomplished by measuring the red circle around the injection area (15).

2.1.2. Differential White Blood Cell Eosinophil Count

A 2-mL blood sample was taken from each participant, placed in a test tube containing Ethylenediaminetetraacetic acid; followingly, the blood slide was dyed with Leishman's stain, and eosinophils were counted per 100 white blood cells (16).

2.2. Statistical Analysis

The ANOVA test (X^2) was used for between-group comparisons at the significant level of 0.05 ($P \leq 0.05$) (17).

3. Results and Discussion

It is worth noting that the hallmark of allergy is tissue infiltration with increased numbers of eosinophils (18). Eosinophils are granular white blood cells with a diameter of usually 10-16 µm. Their nuclei are bilobed, and the proportion of cytoplasm is about 30%. They imbibe eosinophilic elements and originate from bone marrow. Bone marrow was first described by the scholar Paul Ehrlich in 1879. He stated that the number of bone marrows increased in patients with asthma, allergies, and helminth infections (19). Eosinophil biology may be indirectly affected by IgE (20). There are data to support the view that eosinophils reduce the allergic process and play an important role in the disorder repair that leads to weakening the impact of some allergic diseases (18). Table 1 and figure 1 show the percentage of people with allergies according to the number of eosinophils. The results indicated that the percentages of people with more than, equal to, and less than 6 eosinophils were 57%, 25%, and 18%, respectively.

Table 1. Percentage of people with allergies by number of eosinophils

Number of eosinophils	>6	6	>3
Number of people	80	35	25
Percentage of people	57.14%	25%	17.86%

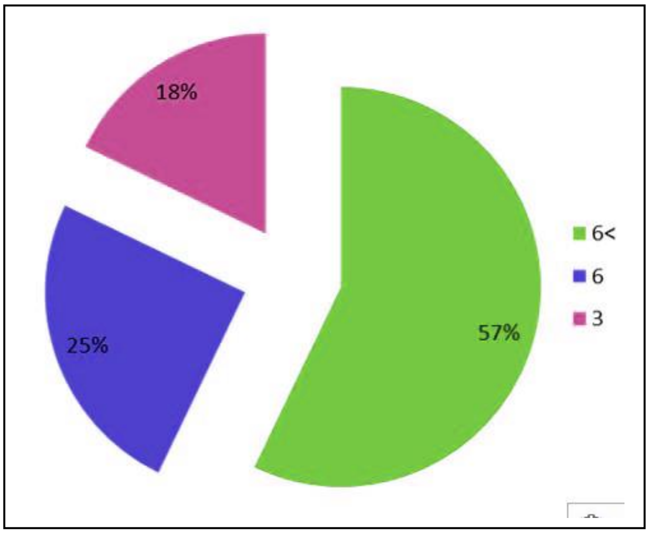

Figure 1. Percentage of people with allergies by eosinophil count

The diameter of the red circle around the area where the injection was administered was 5 mm or more, indicating the person's positive response. A larger diameter circle indicates greater sensitivity (15). The results showed that the percentage of people with a red circle diameter of 5 mm stood at 7.14%. Moreover, the percentage of subjects showing a diameter of approximately 10 mm and 20 mm was obtained at 17.9%. It was also revealed that 57.14% of the cases had a diameter of 15 mm, as shown in table 2 and figure 2.

Table 2. Percentage of people with allergies according to the red circle

Diameter of red circle	5mm	10mm	15mm	20mm
Number of people	10	25	80	25
Percentage	7.14%	17.9%	57.14%	17.9%

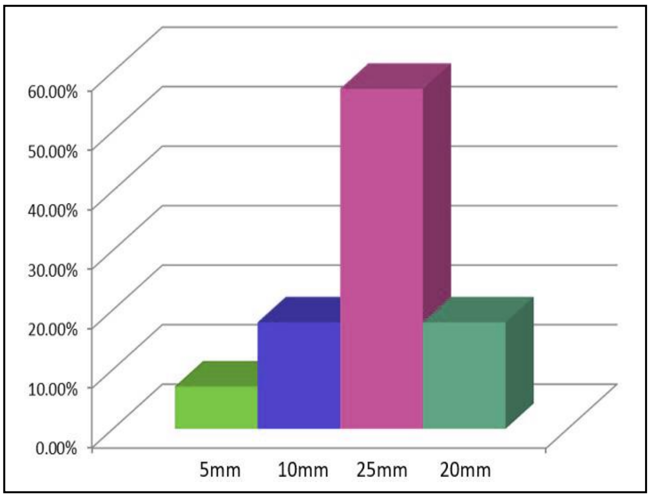

Figure 2. Percentage of red circle diameters in allergic individuals

Adverse drug reactions affect 10-20% of hospital patients and more than 7% of the general population. Immunological drug reactions, such as allergic reactions, are estimated to account for about one-third of ADRs, and antibiotic allergy is the most common drug allergy (12). The results of the current study showed that the highest percentage of sensitivity to amoxicillin was 12.5% and 3.7% for males and females aged 28-37 and 17-27 years, respectively. The total percentage was estimated at 5.7%, as shown in table 3, which was not in agreement with the results of a study carried out by Montañez, Ariza (11), in which the percentage of people allergic to amoxicillin was 90% in Spain. In a study conducted by Faitelson, Boaz (13), which included a skin prick test on 133 children, the results were 2% immediate sensitivity and 5% belated sensitivity to amoxicillin.

Cefotaxine is considered the first choice in penicillin-sensitive patients and those requiring antibiotic treatment during surgery. The current study recorded the highest sensitivity to cefotaxine at 37.5% in males aged 28-37 years, followed by 18.5% in females aged 17-27 years. The total percentage stood at 37.1% as shown in table 3, which was higher than the percentage recorded in America, accounting for 1.7% in those who were not allergic to penicillin and 3-5% in those who were allergic to penicillin (21). Another study was conducted in Iran on 51 patients (22 males and 29 females) with the age range of 1-65 years. In the mentioned study, 36, 15, 8, and 5 subjects were allergic to drugs, penicillin, cefotaxine, and amoxicillin-penicillin, respectively (12).

The highest rate of sensitivity to gentamicin in the current study was 12.5% in males aged 48-57 years and 11.11% in females aged 28-37 years. The total percentage was estimated at 17.1%, as shown in table 3, which was lower than that recorded in a recent study conducted in Germany, in which the sensitivity rate was 68% (i.e. 14 out of 25 patients). Among these cases, one person had a joint operation, during which, the doctor injected the solution gentamicin 80 HEXAL SF to prevent infection. The patient contracted allergic dermatitis due to the application of intra-articular gentamicin (22). In the UK and Italy, the allergy rate reached 36.9% in a total of 16,329 patients aged 18-64 (23). During statistical analysis, it was noticed that there were significant differences for all sensors in table 3 regarding gender and age at the probability level of $P \leq 0.05$.

Stings of the Hymenoptera were found to cause systemic allergic reactions in 1-5% of the population in Europe and North America (24). The results of a recent study have shown that sensitivity resulting from hymen wing stings accounts for 0.3-7.5% of the general population who are affected by systemic anaphylaxis. Honey bee venom causes allergy in 84% of patients with hepatitis in Europe (9). However, the results of the current study showed that *Apis mellifera* recorded the highest rate of allergy in males at 25% and in females at 18.5% for the age range of 17-27. The total percentage was 40% as shown in table 4, which was similar to that recorded in a study performed by Fehr, Micaletto (9). In the mentioned research, the sensitivity rate to *Apis mellifera* was calculated at 31.3%, which was equivalent to 150 patients. However, the results of the current study were higher than those found in a study carried out by Antonicelli, Bilò (25). In which, out of a total of 157 patients with *Apis mellifera* allergy, only 25 subjects showed sensitivity to *Apis mellifera*.

Based on the finding of the current study, *Vespula* spp. recorded the highest sensitivity rate at 37.5% in males aged 17-27 and 3.7% in females aged 17-27 and 48-57. The total percentage was estimated at 17.1%, as shown in table 4, which was lower than that reported in the study conducted by Fehr, Micaletto (9), in which, the rate of sensitivity to *Vespula* spp. was 68.8% (i.e. 330 patients). In another study conducted on 157 patients with Hymenoptera sensitivity, 97 individuals showed sensitivity to *Vespula* spp. and 35 patients were allergic to *Vespa crabro* (25). In a study conducted on 54 people suffering from Hymenoptera sting allergy, it was revealed that only 7 (13%) patients suffered from sensitivity to *Vespula* spp. (24), which was in line with the results of the current study.

It was observed from the results of the current study that the sensitivity rate against honey bees was higher than the percentage of wasp allergy, which was inconsistent with the results of a study performed by Cross, Choudhury (8) reporting that the patients allergic to wasp venom rarely had an allergy to honey bees. In looking through vis-à-vis cases of allergic reactions to the order Hymenoptera in the study carried out by Cross, Choudhury (8), it was reported that a 45-year-old man was previously in good health and developed an acute myocardial infarction after being repeatedly stung by a hornet. The patient had a history of atopy, leading to the possibility that Hymenoptera stings were severe in individuals with atopy. Statistical analysis using the Chi-square test showed significant differences for all allergens regarding gender and age at the probability level $P \leq 0.05$ (Table 4).

Table 3. Relationship between gender, age, and sensitivity to amoxicillin type antibiotics (cefotaxine and gentamicin) by performing a skin test

Age / Injected medicine		17-27		28-37		38-47		48-57		58-67		68-77		Percentage of total injected
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♀+♂
Amoxicillin	+	0 0%	4 3.7%	4 12.5%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	8 5.7%
	-	16 50%	20 18.5%	8 25%	44 40.7%	0 0%	20 18.5%	4 12.5%	8 7.4%	0 0%	4 3.7%	0 0%	8 7.4%	
Cefotaxine	+	0 0%	20 18.5%	12 37.5%	12 11.11%	0 0%	0 0%	4 12.5%	4 3.7%	0 0%	0 0%	0 0%	0 0%	52 37.1%
	-	16 50%	4 3.7%	0 0%	32 29.6%	0 0%	20 18.6%	0 0%	4 3.7%	0 0%	4 3.7%	0 0%	8 7.4%	
Gentamicin	+	0 0%	8 7.4%	0 0%	12 11.11%	0 0%	0 0%	4 12.5%	0 0%	0 0%	0 0%	0 0%	0 0%	24
	-	16 50%	16 14.8%	12 37.5%	32 29.6%	0 0%	20 18.6%	0 0%	8 7.4%	0 0%	4 3.7%	0 0%	8 7.4%	17.1%

Significant level of ≤ 0.05 using the Chi-square

Table 4. Relationship between gender, age, and sensitivity to some types of insects (*Apis mellifera* and *Vespula* spp.) by performing a skin test

Age / Injected medicine		17-27		28-37		38-47		48-57		58-67		68-77		Percentage of total injected
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♀+♂
Apis mellifera	+	0 0%	8 25%	20 18.5%	12 37.5%	16 14.8%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	56 40%
	-	16 50%	8 25%	4 3.7%	0 0%	28 25.9%	0 0%	20 18.6%	4 12.5%	8 7.4%	0 0%	4 3.7%	0 0%	
Vespula spp.	+	0 0%	12 37.5%	4 3.7%	4 12.5%	0 0%	0 0%	0 0%	0 0%	4 3.7%	0 0%	0 0%	0 0%	24 17.1%
	-	16 50%	4 12.5%	20 18.5%	8 25%	44 40.7%	0 0%	20 18.6%	4 12.5%	4 3.7%	0 0%	4 3.7%	0 0%	

Significant level of ≤ 0.05 using the Chi-square

Authors' Contribution

Study concept and design: I. N. L.
Acquisition of data: I. N. L.
Analysis and interpretation of data: I. N. L.
Drafting of the manuscript: I. N. L.
Critical revision of the manuscript for important intellectual content: I. N. L.
Statistical analysis: I. N. L.
Administrative, technical, and material support: I. N. L.

Ethics

All studies were performed in compliance with the rules of humane treatment of University of Mosul, Mosul, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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