CAT AND DOG DANDER ALLERGENS CAUSING TYPE I HYPERSENSITIVITY IN ASTHMATIC AND OTHER ALLERGIC PATIENTS

Abeer L. Mohammed, Fawzia A. Abdulla, Hadi Al-Fyadh

ABSTRACT

Allergens extracts from cats and dogs dander were prepared by extraction, followed by purification and fractionation by gel filtration. One major peak was obtained from cat dander with a molecular weight 35KDa, where as two major peaks were obtained from dog dander with a molecular weight 21 and 18 KDa. Total and specific IgE ELISA test was performed on 137 patient's sera samples. The rate of the total IgE positive ELISA results was (72.99%). From the total IgE positive, it was found that the rate of the specific IgE positive ELISA results was 49% in case of patients examined with cat dander and 29% in patients examined with dog dander. There was significant difference ($P<0.05$) among different age groups regarding the number of patients who had specific IgE positive results, where the highest rates were observed in male and female of 10-30 years of age. In addition, a significant difference ($P<0.05$) was observed between cat and dog allergy and also among the people who were living in urban and rural area. There is a cross-reaction between the cat and dog danders, extracts and the IgE binding capacity of cat and dog danders, protein extract was high since lower concentration of these extracts was needed to inhibit up to 50% of IgE specific binding sites to the allergosorbent.

INTRODUCTION

Allergy to cats can range from inflammation of the nose and eye to asthma attacks. The symptoms of cat allergy may include congested runny nose, sneezing, conjunctivitis, contact dermatitis, hives, dyspnea and wheezing[1]. Dog allergy has been reported to cause acute symptoms of allergic conjunctivitis and hay fever[2]. The development of respiratory allergies infers the presence of the allergens in an airborne form[3]. Skin scraping (dander) allergens are sticky and they adhere to the surfaces. Dander on smooth surfaces such as a wall can be easily wiped off[4]. But dander in soft materials such as carpets, mattresses, upholstered furniture and clothing can persist in that fabric for long periods of time[5]. Generally the pet dander can remain in a home for up to six months after the pet has been removed[6]. Six millions Americans were allergic to cat dander and approximately one third of them have cats in their home[7]. The aqueous allergen extract used in testing for IgE antibodies in allergic patients may contain a number of allergens in addition to non allergenic components so the purification and characterization of these allergenic components became very important for development of specific reagents for diagnosis and treatment as immunotherapy[8]. *In vitro* different methods have been devised for quantization of allergens in crude extract[9] such as ELISA and ELISA inhibition which are a non radioactive methods based on the same principle to detect IgE depending on the assay design of inhibition methods where it is possible to measure all allergens in a crude extract or single allergen[10]. *The aims of the study were to prepare and characterize cat and dog dander allergens and to compare these allergens with standard cat and dog dander ones. To measure total and specific IgE in patients sera. To know if there is cross reaction between the cat and dog dander allergens.*

MATERIALS AND METHODS

Antigens Preparation:

Cat dander allergen (CDA) and dog dander allergen (DDA) were prepared essentially as described by Duffort et al[11]. The cat and dog dander were collected by skin scraping of 5 cats and 6 dogs. These materials were defatted in diethyl ether and dried at room temperature. Each of the collected materials was mixed with phosphate buffer saline PBS, pH 7.2 at 5/100 w/v. Both of mixtures were clarified by centrifugation (10000 rpm) for 30 minutes at 4°C. The supernatant solution was sterilized by millipore filter (0.45 μm) and stored at 4°C.

Abeer L. Mohammed*, Fawzia Abdulla*, and Fyadh, Hadi Al-Fyadh**
*Department of Microbiology, College of Veterinary Medicine, University of Basrah, Iraq.
**Center of Asthma and Allergic Diseases, Basrah.

The purification and fraction of protein extract on G-75 sephadex:

The gel chromatography was used for the isolation and purification of protein extract into different molecular size using G-75 sephadex according to the method of Leslic and Frank[12].

Determination of protein content:

The protein content of each protein extract was estimated according to whitaker and Granum method[13]. 3 ml of each extract were pipetted in a quartz cuvattes. The absorbance value was measured spectrophotometrically at 235 and 280 nm. The protein content in mg/ml

Was calculated by the following equations:

Protein content mg/ml = A_{235}-A_{280} /2.51.

A_{235} = Absorbition value at 235nm

A_{280} = Absorbition value at 280nm

Determination of the sterility of the protein extract:

The sterility of protein extract was determined according to method of Macckie and Maccartney[14], by inoculation of the extract into duplicate plates of nutrient and blood agar, then these plates were incubated aerobically and anaerobically at 37°C.

Inoculated plates were observed daily for 7 days after inoculation to determine the culture sterility.

PATIENTS AND METHODS

Blood samples were collected from 137 patients, *(91 from urban area and 46 from rural area)* attending the Center of Asthma and Allergic Diseases in Basrah. The patients were of both sexes *(58 males and 79 females)*. Their ages ranged from 10 to 60 years. They were complaining of rhinitis or asthma or conjunctival allergy diseases or urticaria. The control blood samples were collected from forty individuals seen in Basrah General Hospital and 10 veterinarians who were all free of allergic diseases.

Enzyme-Linked Immunosobent Assay (ELISA)

Total IgE ELISA technique:

Total IgE was quantitavely determined according to the method of Biomaghreb kit. Briefly, kit assay buffer (100 µl) was added to each well of microtiter plates which was coated previously with mouse monoclonal anti-human IgE followed by the addition of 20 µl of kit control to the first and second well of the first vertical row. Then to other six wells of first vertical and to the four well of second vertical row standard IgE at the concentration (2,5, 50, 200 and 500µl) were added and the patient sera (20µl) were added to the rest of wells. Plates were then covered with plastic film, homogenized by shaking at 300 rpm and incubated at 37°C for 90 minutes followed by washing with PBS-Tween 20(0.05%). After washing (100µl) of goat anti-human IgE alkaline phosphates conjugates was added to each well. The plates were then covered with plastic film and incubated at 37°C for 90 minutes. After that the plates were washed and freshly prepared para-nitro-phenyle-phosphate solution (100µl) was added to each well. Then the plates were incubated at room temperature for 30 minutes in the dark and 100µl of the stopping solution (2N NaoH) was added to each well. The absorbency of each well was read at 450 nm using microplate reader (Dynatch, microplate reader, and models MR 600, USA).

Specific IgE ELISA technique:

Specific IgE was determined according to the method of Biomaghreb kit (Tunisia). Briefly the reference disc D allergen (*Dermat pteron*) was added to well of micrometer plate started with 3rd well of first vertical row to 8th well of second vertical row followed by the addition of reference serum calibrator (A-H) in which IgE concentration was (52.50, 17.50, 3.50, 0.70, and 35µ1) to the reference D disc. Filter paper discs were prepared, sterilized by autoclaving at 121C° for 15 minutes and saturated with locally prepared cat and dog allergen extracts. The protein content of these was determined according to the protein content of the standard cat and dog allergen discs (Biomaghreb, Tunisia). The locally prepared antigen and standard cat and dog allergen discs were added to the bottom of the rest of wells. The protein content of each cat and dog disc allergen was 0.03 mg/ml and 0.02 mg/ml respectively. Serum samples (50µl) were added to all locally & standard cat and dog allergen discs. Other steps of ELISA technique were performed as in the total IgE ELISA.

ELISA inhibition

For competition experiments microtiter 96 well plates were coated with allergen extract discs at 0.03 (cat) and 0.02 (dog) for I hour at 37C°[15]. Then 25 µl of the serum pool and 25 µl of cat or dog allergen extract at 3 protein concentration 0.003, 0.03, 0.3 mg/ml (cat) and 0.002, 0.02, 0.2 mg/ml (dog) were added to the wells and incubated for 2 hours at room temper. Other steps of ELISA inhibition were performed as in the total IgE ELISA.

Statistical methods:

For the determination of statistical significant the qi-square test was used.

RESULTS

Purification and fraction of protein extract:

On fractionating cat dander one major peak was observed with molecular weight (35 KDa). Dog dander extract was fractionated in two major peaks with molecular weight (18, 21 KDa). (Fig 1,2,3).

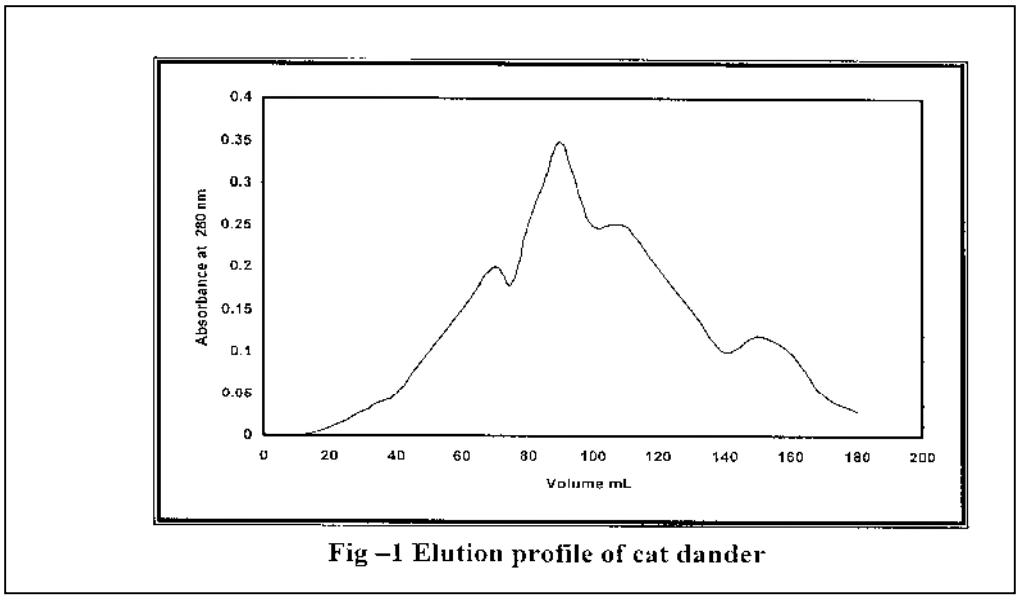

Fig 1. ***Elution profile of cat dander.***

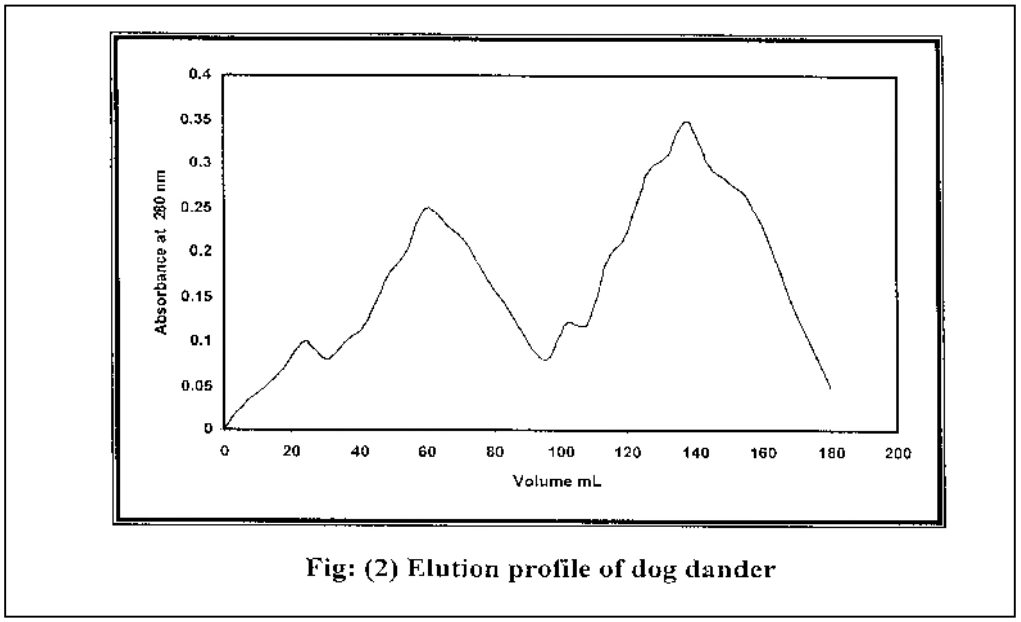

Fig 2. ***Elution profile of dog dander.***

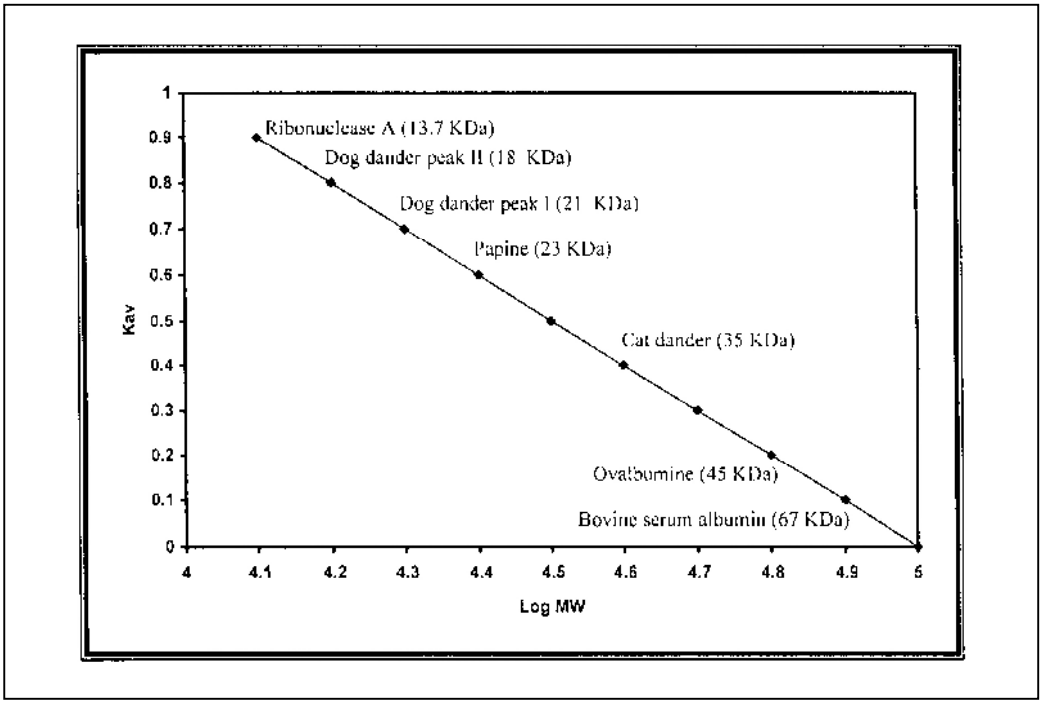

Fig 3. ***The calibration curve of protein extract using some standard proteins for estimation of protein molecular weight.***

ELISA results

Total IgE ELISA:

High total value was observed in 100 patients (72.99%) and those patients tested by specific IgE ELISA using standard and locally prepared cat and dog dander allergens (Table 1,2).

Specific IgE ELISA:

The High rate of positive ELISA results were observed in patients tested with cat dander allergen (49%) in comparism with those tested with dog dander allergen (29%). Also the high rate of positive results was observed in males examined with both cat dander allergen (56%) and dog dander allergen (48%) in comparison with females, (Table-3).

According to age and sex of patients, the highest rate of positive specific IgE ELISA result, in patients tested with cat dander allergens, were observed in first and second age groups in both males and females and the rate decreased gradually with age. Same results were observed in patients tested with standard cat and dog dander allergens, (Table-1,2). There were no significant differences in OD value between males and females and among difference age groups (Table-4,5).

The rate of positive specific IgE ELISA results were observed in patients examined with cat and dog dander allergens were higher in people living in urban area (76.9%) in comparison with rural area (27.0%), (Table-6).

ELISA inhibition:

Cat and dog dander allergen could inhibit to a high extend, the binding of specific IgE to the extracts of cat and dog dander allergen. Cat dander allergen inhibits the binding of IgE to the extract of dog dander allergen at the protein concentration (0.003, 0.03, 0.3 mg/ml) and the percentage of IgE binding inhibition was 38%, 61%, 77% respectively. On the other hand, dog dander allergen inhibits the binding of IgE to the extract of cat dander allergen at the protein concentration (0.002, 0.02, 0.2 mg/ml) and the percentage of IgE binding inhibition was (41%, 58%, 75%) respectively. Furthermore, the IgE binding capacity of cat and dog allergen was found to be high, since lower concentration of these proteins extract were needed to inhibit up to 50% of the IgE specific binding sites to allergosorbent phases (Table-7).

Table 1. ***Comparison of total and specific IgE results in patients examined with locally prepared allergen of cat dander and standard cat dander***

Age group years	ELISA								Total
	Males				***Females***				
	Exam. No	***Total IgE No.***	***Specific IgE C.D No.(%)***	***Specific IgE S.C.D No.(%)***	***Exam. No***	***Total IgE No.***	***Specific IgE C.D No.(%)***	***Specific IgE S.C.D No.(%)***	
10-20	10	8	7 (87.5)	7 (87.5)	20	15	10 (66.6)	10 (66.6)	30
21–30	18	13	9 (69.2)	9 (69.2)	20	16	8 (50.0)	8 (50.0)	38
31-40	20	12	5 (41.6)	5 (41.6)	15	13	4 (30.7)	4 (30.7)	35
41-50	5	5	2 (40.0)	2 (40.0)	16	10	3 (30.0)	3 (30.0)	21
51-60	5	3	0 (0.0)	0 (0.0)	8	5	1 (20.0)	1 (20.0)	13
	58	41	23	23	79	59	26	26	137

Patients number: 137
S.C.D.: Standard cat dander
C.D.: Cat dander

Table 2. ***Comparison of total and specific IgE results in patients examined with locally prepared allergen of dog dander and standard dog dander***

Age group years	ELISA								Total
	Males				***Females***				
	Exam. No	***Total IgE No.***	***Specific IgE D.D No. (%)***	***Specific IgE S.D.D No. (%)***	***Exam. No***	***Total IgE No. (%)***	***Specific IgE D.D No. (%)***	***Specific IgE S.D.D No. (%)***	
10-20	10	8	7 (87.5)	7 (87.5)	20	15	4 (26.6)	4 (26.6)	30
21–30	18	13	6 (46.1)	6 (46.1)	20	16	3 (18.7)	3 (18.7)	38
31-40	20	12	4 (33.3)	4 (33.3)	15	13	1 (7.6)	1 (7.6)	35
41-50	5	5	2 (40.0)	2 (40.0)	16	10	1 (10.0)	1 (10.0)	21
51-60	5	3	1 (33.3)	1 (33.3)	8	5	0 (0.0)	0 (0.0)	13
	58	41	20	20	79	59	9	9	137

Patients number: 137
S.D.D.: Standard dog dander
D.D.: Dog dander

Table 3. ***Rate of positive ELISA results in patient examined with cat and dog allergens.***

Protein extract	Positive response No. %		Total
	Males	*Females*	
Cat dander	23/41 (56.0)%	26/59 (44.0)%	49/100 (49.0)%
Dog dander	20/41 (48.7)%	9/59 (15.2)%	29/100 (29.0)%
Cross reaction between them	18/41(43.9)%	8/59 (13.5)%	26/100 (26)%

Patients number = 100

Table 4. ***The ELISA results in patients with positive responses to locally prepared allergen and standard cat allergen.***

Age group years	Optical Density			
	Males		Females	
	Mean ± SD		*Mean ± SD*	
	C.D.	S.C.D.	C.D.	S.C.D.
10-20	24.23±24.00	16.54 ± 8.46	12.46 ± 12.21	10.35 ± 9.20
21–30	28.91±16.08	24.76 ± 20.70	23.91 ± 19.17	19.42 ± 17.27
31-40	16.40±8.69	13.66 ± 7.41	18.60 ± 9.72	15.64 ± 8.61
41-50	101.77±100.85	86.61 ± 80.66	16.46 ± 4.61	14.21 ± 5.79
51-60	Nil	Nil	Nil	Nil

Patients number: 49
SD: Standard division
C.D.: Cat dander
S.C.D.: Standard Cat dander

Table 5. ***The ELISA results in patients with positive responses to locally prepared allergen and standard dog dander allergens.***

Age group years	Optical Density			
	Males		Females	
	Mean ± SD		*Mean ± SD*	
	D.D.	S.D.D.	C.D.	S.D.D.
10-20	14.83 ± 8.00	12.80 ± 6.54	12.373 ± 9.76	10.02 ± 7.15
21–30	9.83 ± 6.83	7.89 ± 5.62	15.87 ± 11.22	14.19 ± 13.05
31-40	14.78 ± 9.48	13.68 ± 8.74	Nil	Nil
41-50	8.73 ± 7.09	5.38 ± 5.00	Nil	Nil
51-60	Nil	Nil	Nil	Nil

Patients number: 29.
SD: Standard division.
D.D.: Dog dander.
S.D.D.: Standard dog dander.

Table 6. ***The rate of positive ELISA results in urban and rural area.***

	The distribution		Total
	In urban area No. (%)	In rural area No. (%)	
Cat allergy	39 (79.5)	10 (20.4)	49
Dog allergy	21 (72.4)	8 (27.5)	29
Total	60 (76.9)	18 (27.0)	78

Table 7. ***Cross-reactivity of specific IgE binding between cat and dog dander allergen using ELISA inhibition.***

allergosorbent	Inhibitors		
	Protein conc. 1 ***Inhibition (%)***	***Protein conc. 2*** ***Inhibition (%)***	***Protein conc. 3*** ***Inhibition (%)***
Cat dander	0.003 38	0.03 61	0.03 77
Dog dander	0.002 41	0.02 58	0.2 75

Conc Concentration.

DISCUSSION

Purification and fractionation of protein extracts

Gel filtration analysis of cat and dog dander protein extract demonstrated one major peak of cat dander and two major peaks of dog dander. The molecular weight of the eluted protein was in line with the finding of Dreborg et al[16] and Marsh and Norman[17], who reported that allegen usually has molecular weight of 5000-70000 Daltons. These proteins were predominant both in the intensity and the frequency of their recognition by human allergic sera. Therefore, this component can be regarded as a major allergic component of cat and dog dander extract. This finding was in line with Dreborg et al[16] who reported that the major allergens are acidic globular molecules that are stable to enzymatic digestion or chemical denaturation. Often a major allergen is a very abundant protein in the source material and is most readily extracted from the source.

ELISA results

The rate of positive specific IgE ELISA results in patients examined with cat and dog dander allergen is low in comparison to the finding of Bannelos et al[18], who reported 59% and 33% respectively. The explanation of this discrepancy is based on difference in animals species, geographic area, climates and genetic factors[19]. The rate of cat dander allergic patients (49%) is higher than the dog dander allergic patient's (29%) and this discrepancy explained by Murray, et al[1], who reported that exposure to cats was significantly more frequent than the exposure to dogs resulting in greater frequency of sensitization to cat. There is a significant difference regarding the rate of positive specific IgE ELISA results among females and males who were examined with both cat and dander allergen. The possible explanation for these differences may be referred to the hormonal differences between males and females which affect the natural immunity[20]. On the other hand, there is a significant difference among age groups of patients examined with cat and dog dander allergens and these results are in agreement with that reported by Hattevige et al[21], who found that allergen reactivity acquired progressively during childhood peaking 15 and 25 years and declining gradually.

ELISA inhibition

Cat and dog dander allergen cross-reacted in IgE binding sites to completely inhibiting the binding of specific IgE to each other. It seems clear that both proteins bear the same allergenic epitopes. These findings are in line with those reported by Gonzales et al[22], that cross-reaction may extend to the presence of carbohydrate structure shared by several components of the same protein extract. Also these results are in agreement with many other studies as the study of Valenta et al [23], and Ree Van et al[24], who reported that at least part of the cross-reactivity is due to the panallergen, profilin which is present in almost all eukaryotic organisms including human and the study of Hoffman[25] and Markussen et al[26], who reported that mammalian allergens of different species have been shown to cross-react, as allergen extracts from cat epithelium cross react with dog dander extract. Likewise, horse hair and dander allergen cross-react with dog, cat, cow and guinea pig hair extract.

In conclusion, the allergic patients are more sensitive to cat dander allergen than dog dander allergen and there are cross-reactions between cat and dog dander protein extract where there is no difference between ELISA results of locally prepared cat and dog dander allergens and standard cat and dog dander allergens.

REFERENCES

1. Murray AB, Forguson AC. Morrison BJ. The frequency and severity of cat allergy VS. dog allergy in a topic children. J. Allergy Clin. Immunol. 1993; 72: 145-149.
2. Ford AW, Kemeny DM. The allergens of dog identification and partial purification of a major dander allergen. Clin. Exp. Allergy. 1992; 22: 793-703.
3. Mantyjarvi R, Rautiainen J, Virtanen T. Review Lipocalins as allergens. Biochimica et Biophyscia. Acta. 2000; 1482: 308-317.
4. Erwin EA, Wood folk JA, Cusris N, et al. Animal danders. Immunol. Allergy. Clin. North. Am. 2003; 23: 496-481.
5. Custovic A, Green R, Fletcher A, et al. Aerodynamic properties of the major dog allergen, can f l; distribution in homes concentration and particle size of allergen in the air. Am.J. Resp. Crit. Care. Med. 1997; 155: 94-98.
6. Custovic A, Taggart SCO, Woodcock A. House dust mite and cat allergen in different indoor environments. Clin. Exp. Allergy. 1994; 24: 1164 -1168.
7. Gelber L, Seltzer LH, Bouzoukis JK. Sensitization and exposure to indoor allergens as risk factor for asthma among patients presenting to hospital. Am. Rev. Resp. Dis. 1993; 147: 573- 578.
8. Ortalani G, Ispnoa M, Bastorello EA, et al. Comparison of results of skin prick test (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. J. Allergy Clin. Immunol, 1989; 83: 683-690.
9. Weeke B, Lowenstein H. Allergens identification radioimmunoelectrophoresis. Scond. J. Immunol. 1973; 10: 149-153.
10. Kholer G, Milstein C. Continuous culture of cells secreting antibody of predefined specificity. Nature. 1975; 256: 495-497.
11. Duffort O, Carreira J, Lombardero M. Characterization of the main IgE binding components of cat dander. Int. Arch. Allergy. Appl. Immunol. 1987; 84: 339.
12. Leslic H, Frank CH. Isolation and structure of immunoglobulin in Practical Immunology, 3rd ed., Black Well Scientific Publication, Oxford, London. 1976: 1156.
13. Whitakev RJ, Granum EP. An absolute method for protein determination based on difference in absorbance of 235 and 280 nm. Analytical biochemistry. Log1980: 156-159.
14. Macckie TJ, Mccartney JE. Practical medical microbiology. 13th ed. Churchill living stone LTD. Edindurrgh, London, Uk, 1989.
15. Virtanen T, Louhelainen K, Mantyjavim R. Enzyme Linked Immunsorbent Assay (ELISA) inhibition method to estimate the level of airborne bovine epidermal antigen in cowshed. Int. Arch. Allergy Appl. Immunol 1086; 81: 253-257.
16. Dreborg S, Einarsson R, Longbottom JL. The chemistry and standardization of allergen in Weir, D.M. (ed. 1. Hand book of experimental. Immunology) 4th ed. Black well Scientific publication, Oxford, 1986; 10. 1 - 10.28.
17. Marsh D, Norman PS. Antigen that cause atopic diseases in Somter, M. Talmage; D. W.; Frank M. W.; Austen, K.F. and Claman, H. N. (eds.). Immunological diseases. 4ed. Little- Brown and company, Toranto. 1988; 981-1008.
18. Bannelos AA, Montano Veluzques B, Band Ayala Balboa JC. Skin tests, serum specific IgE and total IgE in the diagnosis of patients with perennial allergic rhinitis. Res. Allerg. Mex. 2003; 50: 147-153.
19. Breitendr H, Sheiver O. Environmental pollution and pollen allergy. A possible link. Allergo. 1990; 13: 934.
20. Benjamini, E, Coico R, Sunshine G. Immunology. A short course. 4th (ed.). 2000; 279-300.
21. Hattevige G, Kjeliman B, Bjorksten B. Skin tests in clinical practice and epidemiology. Clin. Exp. Allergy 1992; 2: 881-882.
22. Gon Zales R, Polo F, Zapatero L. et al. Purification and characterization of major inhalant allergens from soybean hulls. Clin. Exp. Allergy 1992;. 22: 748-755.
23. Valenta R, Yssel H, Goldstein RJ, et al. The future of immunotherapy. Therapeutic vaccines. Allergy 1988; 53: 995.
24. Ree Van, Volenko V, Loouwen WA, et al. Profilin is a cross reactive allergen in pollen vegetable foods. Int. Arch. Allergy. Immunol. 1992; 98: 97-104.
25. Hoffman DR. Dog and cat allergens, urinary protein or dander protein? Ann. Allergy. 1980; 45: 205-206.
26. Makussen B, Lowenstein H, Week B. Allergen extracts of horse hair and handruff. Quantitative immunoelectrophoretic characterization of the antigen. Int. Arch. Allergy. Immunol. 1976; 51: 25-37.