

Molecular profile of specific IgE to allergenic components in allergic adults using allergen nano-bead array

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Abstract

Background

An increasing interest in the field of molecular diagnosis of allergy has been developed in recent years and it goes to be as the routine in vitro protocol in allergy diagnosis. friendly allergen nano-bead array (FABER) is a new multiplex assay for the evaluation of specific IgE against 244 allergens including whole extracts and allergenic molecules. The research intended to assess the pattern of IgE sensitization to allergenic components of allergens in allergic adults using FABER 244.

Methods

Sixty patients with allergic diseases entered this cross-sectional study. Specific IgE to 122 whole allergens extracts and 122 allergenic components were assessed using an allergen nano-bead array (FABER) for all patients. This test includes inhalant and food allergens.

Results

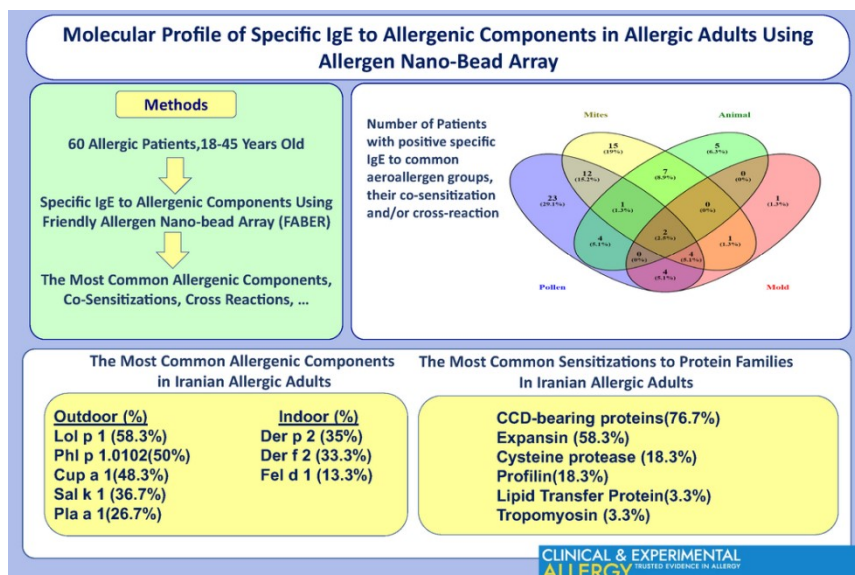
Thirty-seven patients were male (61.7%). The mean (SD) age of patients was 30.73(±6.87) years. As the allergen nano-bead array results showed, *Lolium perenne* (63.3%), *Phleum pratense* (60%) and *Platanus acerifolia* (51.7%) were considered as the

most common IgE sensitizations to the aeroallergen extracts. Moreover, Lol p 1, Phl p 1.0102 and Cup a 1 were found as the most frequent allergenic components in our allergic patients. Among protein families, CCD-bearing proteins, expansin, cysteine protease and profilin families illustrated the highest allergic sensitization.

Conclusions

The results of the present study demonstrated that despite the higher prevalence of sensitization to *Salsola kali* (47.2%) using extract-based assays in the previous phase of this research, allergenic components of grasses (Lol p 1, Phl p 1.0102), Cup a 1 as well as Sal k1 as the major components of *Cupressus arizonica* and *Salsola kali* showed the higher sensitization, respectively, in adults' allergic patients using FABER test.

Graphical Abstract



Keywords

allergenic components | molecular diagnosis | specific IgE

Key messages

- Molecular profile of IgE sensitization to allergenic components was determined in Iranian allergic adults for the first one using allergen nano-bead array.
- Allergenic components of outdoor aeroallergens including Lol p 1, Phl p 1.0102, Cup a 1 and Sal k 1 were considered as the most common allergens in Iranian allergic adults.
- IgE sensitization to allergenic components of indoor allergens such as Der p 2, Der f 2 and Fel d 1 were the most common in Iranian allergic adults.

1 INTRODUCTION

Allergic sensitization is mainly regarded as a risk factor for developing immediate hypersensitivities, especially respiratory hypersensitivity.¹⁻³ Epidemiologic studies to determine the IgE sensitization to allergens have been mostly performed using allergen extract-based conventional tests in vivo or in vitro in the different communities. Moreover, the simultaneous presence of genuine allergenic molecules, cross-reactive pan-allergens, and cross-reactive minor components, as well as non-allergenic proteins in allergen extracts could lead to difficulty in exact diagnostics of the relevant allergen.^{4,5} Comprehensive information on allergenic molecules is not obtained using extract-based methods.⁶

The measurement of specific IgE against the natural or recombinant allergenic components are called molecular diagnosis of allergy (MA) or component-resolved diagnosis (CRD).⁷ Allergenic components of various sources such as foods, pollens, mites, animals and moulds belong to the different protein families.^{8,9} For example, the most frequent protein families of pollens are expansin C and N, profilin, EF-hand, Ole 1-like and pectate lyase.⁸ The interest of researchers and physicians in field of allergy diagnosis using molecular methods has considerably increased during the past twenty years¹⁰ and it goes to be a routine in vitro protocol in allergy diagnosis.¹¹ The literature review shows the importance of this in vitro approach to differentiate the cross-reactivity from primary sensitization especially in poly-sensitized patients, the specific profile of allergic sensitization for each subject, identification of high-risk components, and the precise selection of proper allergens for specific immunotherapy.^{7,12,13} The epidemiologic studies regarding the assessment of IgE sensitization profile to allergenic molecules have been influenced by the molecular diagnosis progression.¹⁴ Moreover, a higher predictive value has been reported for molecular-based techniques rather than extract-based methods.^{15,16}

The development and progress of the in vitro methods (especially multiplex assay) with a low volume of sample and allergen for allergy diagnosis has resulted from DNA technology, biochemistry advances, purification and production of recombinant allergens.^{17,18} A new multiplex diagnostic technique based on nanotechnology has been developed to quantify specific IgE to more than 240 whole allergen extracts and molecules known as friendly allergen nano-bead array (FABER).¹⁰

This paper attempts to evaluate the profile of IgE sensitization against allergenic components as well as the protein families of aero and food allergens in Iranian adult allergic patients using FABER as a new multiplex technique by the molecular diagnosis. To the best of our knowledge, no epidemiological research about the sensitization to allergenic components using the allergen nano-bead array technique has been conducted in Iran so far.

2 METHODS

2.1 Patients

Sixty patients whose allergy were confirmed by the specific questionnaire and extract-based method entered this cross-sectional study. In the earlier phases of this study, a specific questionnaire was fulfilled for volunteers referred to the Blood Transfusion Organization and students and staff of Tehran University of Medical Sciences aged 18 to 45 years. According to the responses of the participants to the queries, patients with asthma, allergic rhinitis, allergic conjunctivitis and atopic symptoms entered for the specific IgE assay [Immunoblot, RIDA qline Allergy (R-Biopharm,

Germany)].^{1,19} Sixty patients with clinical allergic symptoms and allergy diagnosis by the specific questionnaire and the positive specific IgE were randomly selected for the measurement of specific IgE using the FABER test. This study was jointly conducted by two centres including Immunology, Asthma, and Allergy Research Institute (IAARI), Tehran University of Medical Sciences, Iran, and Centri Associati di Allergologia Molecolare (CAAM) in Rome, Italy from 2013 to December 2016. Demographic data and clinical symptoms of patients with asthma, allergic rhinitis, allergic conjunctivitis and atopic dermatitis were recorded in a particular questionnaire.¹⁹

Ethics: The protocol of this research was approved by the Ethics Committee of IAARI (No: 412/P/280). Informed written consent was taken from all subjects.

2.2 Friendly allergen nano-bead array (FABER)

As previously described,^{1,19} a three-mL blood sample had been taken from allergic patients after obtaining informed consent and their serum was stored in -70°C until assay time. The molecular assessment of IgE sensitization was done by a multiplex technique called FABER 244 (ADL S.r.l., Latina, Italy). FABER is the first laboratory multiplex assay for the measurement of specific IgE to 122 whole extracts (including food, outdoor and indoor allergens,...) and 122 allergenic molecules, simultaneously.¹⁰ All serum samples were tested in the molecular allergology laboratory of CAAM in Rome, Italy (Supported by Research Fellowship award 2014 from EAACI). Specific IgE to 122 whole allergens extracts and 122 allergenic components was measured. Allergenic components and extracts were attached to chemically activated nanoparticles. Analysis and interpretation of the FABER test were performed using CAAM Digital Reporting System (CDRS). The cut-off of IgE concentration in this method is 0.30 FIU. The value of less than 0,01 FIU/ml and $>0,01/<0,30$ FIU/ml are considered as negative and doubtful respectively. The name of aeroallergens and their components are listed in Tables 1 and 2.

TABLE 1 The frequency and the percentage of IgE sensitization to the extracts and components of outdoor and indoor aeroallergens in subjects with allergic diseases

Outdoor Allergens	Components	N (%)	Indoor Allergens	Components	N (%)
<i>Ambrosia artemisiifolia</i> (Ragweed)	Amb a Amb a 1	9 (15) -	<i>Alternaria alternata</i>	Alta1 Alt a 6.0101	6 (10) -
<i>Artemisia vulgaris</i> (Mugwort)	Art v Art v 1	15 (25) 5 (8.3)	<i>Aspergillus fumigatus</i>	Asp f	5 (8.3)
<i>Betula verrucosa</i> (Birch)	Bet v Bet v 1.0101 Bet v	15 (25) 2 (3.3) 9 (15)	<i>Aspergillus niger</i>	Asp n	5 (8.3)

Outdoor Allergens	Components	N (%)	Indoor Allergens	Components	N (%)
	2.0101				
<i>Corylus avellana</i> (Hazelnut)	Cor a [Pollen] Cor a 1.0103	12 (20) 2 (3.3)	<i>Aspergillus restrictus</i>	Asp r 1	2 (3.3)
<i>Cryptomeria japonica</i> (Japanese Cedar)	Cry j	5 (41.7)	<i>Blattella germanica</i> (German Cockroach)	Bla g Bla g 1 Bla g 2 Bla g 4 Bla g 5	6 (10) 1 (1.7) 2 (3.3) 1 (1.7) -
<i>Cupressus arizonica</i> (Arizonica Cypress)	Cup a 1	29 (48.3)	<i>Canis familiaris</i> (Dog)	Can f Can f 1 Can f 2 Can f 3 Can f 5	2 (3.3) - 1 (1.7) 1 (1.7) -
<i>Lolium perenne</i> (Rye grass)	Lol p [Pollen] Lol p 1	38 (63.3) 35 (58.3)	<i>Candida albicans</i>	Cand a	2 (3.3)
<i>Mercurialis annua</i> (Annual Mercury)	Mer a 1	11 (18.3)	<i>Cladosporium herbarum</i>	Cla h	2 (3.3)
<i>Olea europaea</i> (Olive Tree)	Ole e [Pollen] Ole e 1	17 (28.3) 11	<i>Dermatophagoides farinae</i>	Der f 1 Der f 2	2 (3.3) 20 (33.3)

Outdoor Allergens	Components	N (%)	Indoor Allergens	Components	N (%)
	Ole e 2	18.3 7 (11.7)			
<i>Parietaria judaica</i> (Parietaria)	Par j Par j 2	12 (20) -	<i>Dermatophagoides pteronyssinus</i>	Der p 1 Der p 10 Der p 2 Der p 23.0101 Der p 7 Der p 9	2 (3.3) 1 (1.7) 21 (35) 5 (8.3) 3 (5) 2 (3.3)
	Phl p		<i>Euroglyphus maynei</i>	Eur m 2	
<i>Phleum pratense</i> (Timothy Grass)	Phl p 1.0102 Phl p 2.0101 Phl p 5.0101 Phl p 6.0101 Phl p 7.0101	36 (60) 30 (50) 4 (6.7) 4 (6.7) 1 (1.7) 1 (1.7)	<i>Felis domesticus</i> (Cat)	Fel d Fel d 1 Fel d 2	14 (23.3) 8 (13.3) 1 (1.7)
<i>Platanus acerifolia</i> (Plane Tree)	Pla a Pla a 1	31 (51.7) 16 (26.7)	<i>Penicillium chrysogenum</i>	Pen ch	4 (6.7)
<i>Quercus alba</i> (Oak)	Que a [Pollen]	12 (20)	<i>Periplaneta americana</i>	Per a Per a 7	- 2 (3.3)

Outdoor Allergens	Components	N (%)	Indoor Allergens	Components	N (%)
<i>Salsola kali</i> (Russian thistle)	Sal k 1	22 (36.7)			

TABLE 2 The frequency and the percentage of IgE sensitization to the extracts and components of food allergens in patients with allergic diseases

Food Allergens	Components	N (%)	Food Allergens	Components	N (%)
<i>Actinidia chinensis</i>	Act c	6 (10)	<i>Glycine max</i>	Gly m	7 (11.7)
	Act c 11	0		Gly m 1	0
	Act c Chitinase IV	3 (5)		Gly m Agglutinin	0
<i>Actinidia deliciosa</i>	Act d	9 (15)		Gly m TI	3 (5)
	Act d 1	1 (1.7)	<i>Homo sapiens</i>	Hom s LF	13 (21.7)
	Act d 10	0	<i>Hordeum vulgare</i>	Hor v [Seed]	13 (21.7)
	Act d 2	0	<i>Juglans regia</i>	Jug r [Seed]	12 (20)
	Act d 5	1 (1.7)		Jug r 2	0
<i>Aedes communis</i>	Aed c	8 (13.3)		Jug r 3	0
<i>Allium cepa</i>	All c	11 (18.3)	<i>Lactuca sativa</i>	Lac s	12 (20)
<i>Allium porrum</i>	All p	15 (25)	<i>Lens culinaris</i>	Len c	13 (21.7)
<i>Allium sativum</i>	All s	15	<i>Linum usitatissimum</i>	Lin us	12 (20)

Food Allergens	Components	N (%)	Food Allergens	Components	N (%)
		(25)			
<i>Ananas comosus</i>	Ana c 2	8 (13.3)	<i>Litopenaeus vannamei</i>	Lit v 1	
<i>Anacardium occidentale</i>	Ana o	2 (3.3)	<i>Lupinus albus</i>	Lup a [Seed]	
	Ana 0 3	0	<i>Malus domestica</i>	Mal d [Fruit]	14 (23.3)
<i>Apium graveolens</i>	Api g	13 (21.7)		Mal d 1.0108	2 (3.3)
	Api g 1.0101	1 (1.7)	<i>Oryza sativa</i>	Ory s [Seed]	
<i>Arachis hypogaea</i>	Ara h	9 (15)	<i>Ovis aries</i>	Ovi a [Meat]	1 (1.7)
	Ara h 1	1 (1.7)		Ovi a [Milk]	1 (1.7)
	Ara h 2	0		Ovi a 6	1 (1.7)
	Ara h 3	0	<i>Pandalus borealis</i>	Pan b	1 (1.7)
	Ara h 6	0	<i>Persea americana</i>	Pers a	13 (21.7)
	Ara h 8	0	<i>Pinus pinea</i>	Pin p [Seed]	
	Ara h Agglutinin	0	<i>Pistacia vera</i>	Pis v [Seed]	8 (13.3)
<i>Bos domesticus</i>	Bos d (Meat)	0	<i>Prunus armeniaca</i>	Pru ar [Fruit]	16 (26.78)

Food Allergens	Components	N (%)	Food Allergens	Components	N (%)
	Bos d (Milk)	0	<i>Prunus dulcis</i>	Pru du [Seed]	15 (25)
	Bos d 4	0	<i>Prunus persica</i>	Pru p [Peel]	20 (33.3)
	Bos d 5	0		Pru p [Pulp]	17 (28.3)
	Bos d 6	0		Pru p 3	2 (3.3)
	Bos d 8	0		Pru p 7	0
	Bos d CA	0	<i>Punica granatum</i>	Pun g	1 (1.7)
	Bos d Gelatin	0			
	Bos d LF	0			
<i>Bubalus bubalis</i>	Bub b [Milk]	0		Pun g 1	0
<i>Camelus dromedarius</i>	Bub b [Milk]	0		Pun g 14	0
<i>Castanea sativa</i>	Cas s	11 (18.3)		Pun g 5	0
<i>Ceratonia siliqua</i>	Cer si	15 (25)		Pun g 7	0
<i>Chenopodium quinoa</i>	Che qu	1 (1.7)	<i>Sesamum indicum</i>	Ses i	5 (8.3)
<i>Cicer arietinum</i>	Cic a	7 (11.7)	<i>Sinapis alba</i>	Sin a [Seed]	6 (8.3)
<i>Citrus reticulata</i>	Cit r [Fruit]	13	<i>Solea solea</i>	Sol so	0

Food Allergens	Components	N (%)	Food Allergens	Components	N (%)
		(21.7)			
<i>Corylus avellana</i>	Cor a [Seed]	12 (20)	<i>Solanaceae, Solanum lycopersicum</i>	Sola l [Fruit]	11 (18.3)
	Cor a 1.0103	2 (3.3)		Sola l [Seed]	10 (16.7)
	Cor a 14	1 (1.7)		Sola l 6	0
	Cor a 8	0	<i>Solanaceae, Solanum melongena</i>	Sola m	15 (25)
	Cor a 9	0	<i>Solanaceae, Solanum tuberosum</i>	Sola t	11 (18.3)
<i>Coturnix coturnix</i>	Cot c [Egg white]	0		Sola t 1	11 (18.3)
	Cot c [Egg Yolk]	0	<i>Spinacia oleracea</i>	Spi o	15 (25)
<i>Daucus carota</i>	Dau c	20 (33.3)	<i>Thunnus albacares</i>	Thu a [Meat]	0
<i>Equus asinus</i>	Equ as [Milk]	1 (1.7)	<i>Triticum aestivum</i>	Tri a [Seed]	12 (20)
<i>Equus caballus</i>	Equ c [Milk]	1 (1.7)		Tri a 7k-LTP	0
	Equ c 3	0		Tri a 18	1 (1.7)
	Equ c Myoglobin	0		Tri a 28	0
<i>Fagopyrum esculentum</i>	Fag e	14 (23.3)		Tri a Gliadin	1 (1.7)

Food Allergens	Components	N (%)	Food Allergens	Components	N (%)
<i>Gadus morhua</i>	Gad m [Meat]	0	<i>Triticum polonicum</i>	Tri tp	13 (21.7)
<i>Gallus domesticus</i>	Gal d [Egg white]	0	<i>Vitis vinifera</i>	Vit v [Fruit]	11 (18.3)
	Gal d [Egg yolk]	0	<i>Zea mays</i>	Zea m [Seed]	8 (13.3)
	Gal d [Meat]	0	<i>Zea mays</i>	Zea m 14	0
	Gal d 1	0			
	Gal d 2	0			
	Gal d 3	0			
	Gal d 4	0			
	Gal d 5	0			

Abbreviation: N, number.

2.3 Statistical analysis

The data were analysed by SPSS version 20 (IBM). The frequency and percentage of the categorical variables were determined. Mean (SD) or median (P25, P75) was calculated for quantitative variables. Association between categorical variables was determined by the chi-square test. GraphPad Prism 8 (GraphPad.

Software) and Venny 2.1²⁰ were used for to the graph drawing.

3 RESULTS

The mean (SD) age of the participants was 30.73 (6.87) years among them, and 37 patients were male (61.7%). The frequency of allergic diseases was as follows: asthma (34.5%), allergic rhinitis (90%), allergic conjunctivitis (37.5%) and atopic dermatitis (17.2%).

As the allergen nano-bead array results showed the most common sensitizations to the aeroallergen extracts included *Lolium perenne* (63.3%), *Phleum pratense* (60%) and *Platanus acerifolia* (51.7%) respectively. Moreover, IgE sensitization to allergenic molecules showed Lol p 1(58.3%),

Phl p 1.0102 (50%) and Cup a 1(48.3%) as the most frequent. Der p 2 (35%), Der f 2 (33.3%) and Fel d 1 (13.3%) were considered as the highest sensitizations to indoor aeroallergens components (Table 1).

The results of IgE reactivity to food allergens using FABER showed *Daucus carota* (Carrot) and *Prunus persica* (Peach) as the most common sensitizing foods (33.3%) in these patients. Interestingly, IgE sensitization to an allergenic component of potato (*Solanum tuberosum*) called Sola t1 (a CCD-bearing protein) was observed in 18.3% of patients (Table 2).

Figure 1 illustrates the prevalence of sensitization to the main outdoor and indoor aeroallergens components according to sex. The prevalence of allergic sensitization to outdoor components was higher in males while the higher frequency of sensitization to indoor allergenic components was observed in females. These differences in frequency of outdoor and indoor allergens between males and females were significant. Moreover, the findings obtained from the prevalence of IgE sensitization to different protein families in allergic patients are presented in Figure 2. It is apparent from this figure that CCD-bearing proteins, expansins, cysteine proteases and profilins were the most frequent protein families sensitizing our patients.

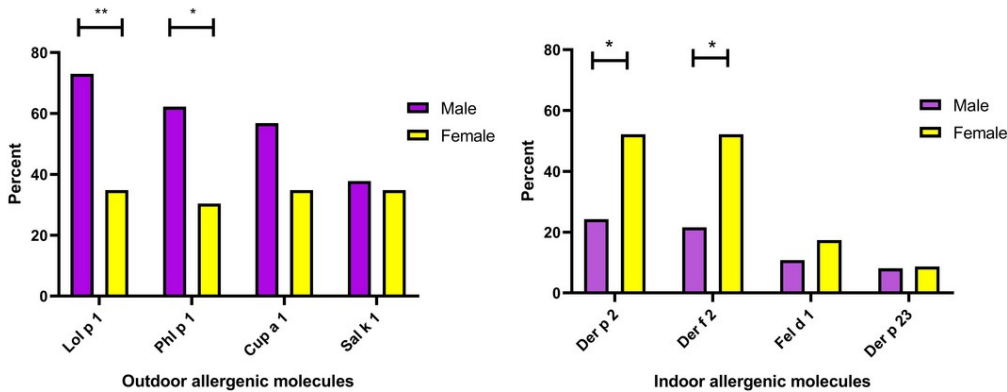


FIGURE 1Percentage of IgE sensitization to allergenic molecules of outdoor and indoor aeroallergens according to the sex. * $p < .05$, ** $p < .01$

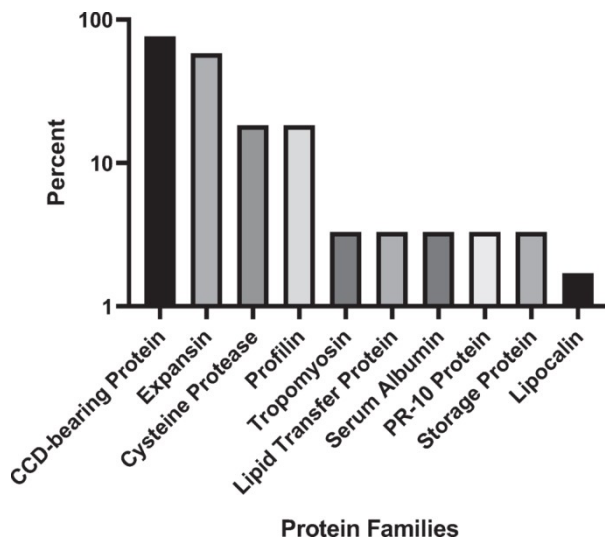


FIGURE 2Prevalence of IgE sensitization to different protein families in allergic patients

As shown in Figure 3, co-sensitization or cross-reactivity to Lol p 1 and Phl p 1 was observed in 50% of subjects. Grass allergenic components demonstrated the highest cross-reactivity among pollen allergens.

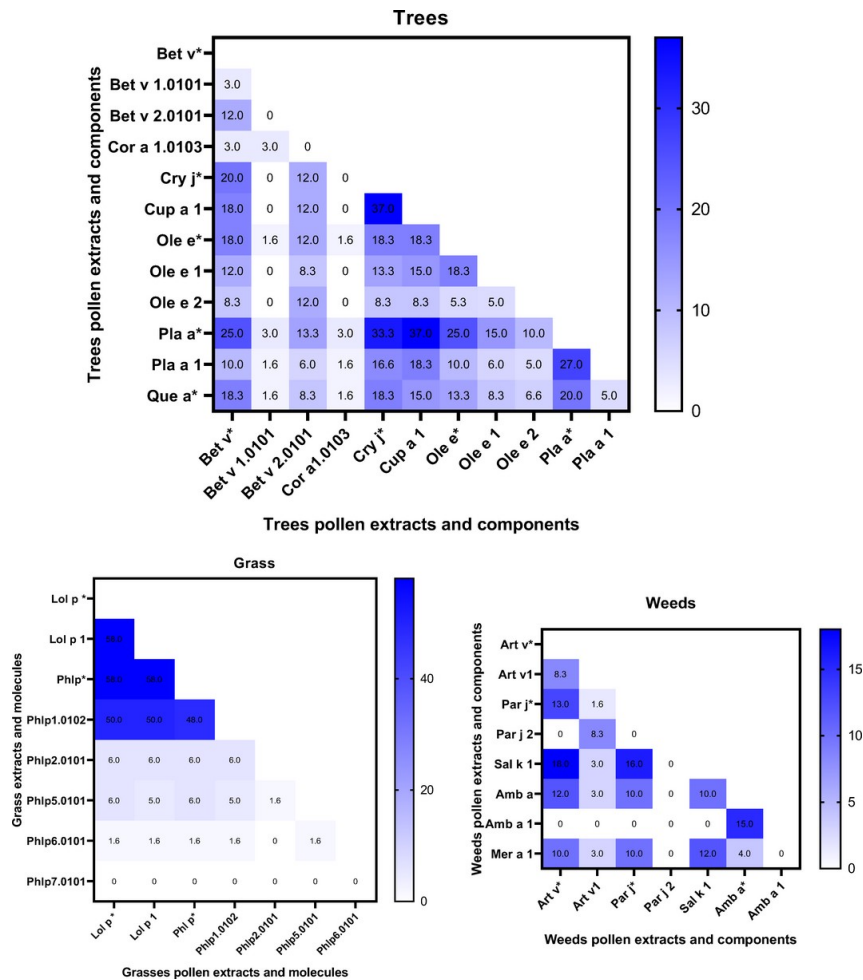


FIGURE 3 Heat map of co-sensitization or cross-reactivity between aeroallergens extracts or molecules.
* Pollen whole extract

The frequency of IgE sensitization to the different kinds of aeroallergens including pollens, mites, moulds and animals is shown in Figure 4A. Among aeroallergens, exclusive IgE sensitization to pollens and mites was seen in 23 and 15 patients respectively. Twenty-four patients had allergic sensitization to all pollens including grasses, trees and weeds (Figure 4B). Moreover, Figure 4C highlights IgE reactivity to CCD and profilin in pollen-sensitized individuals.

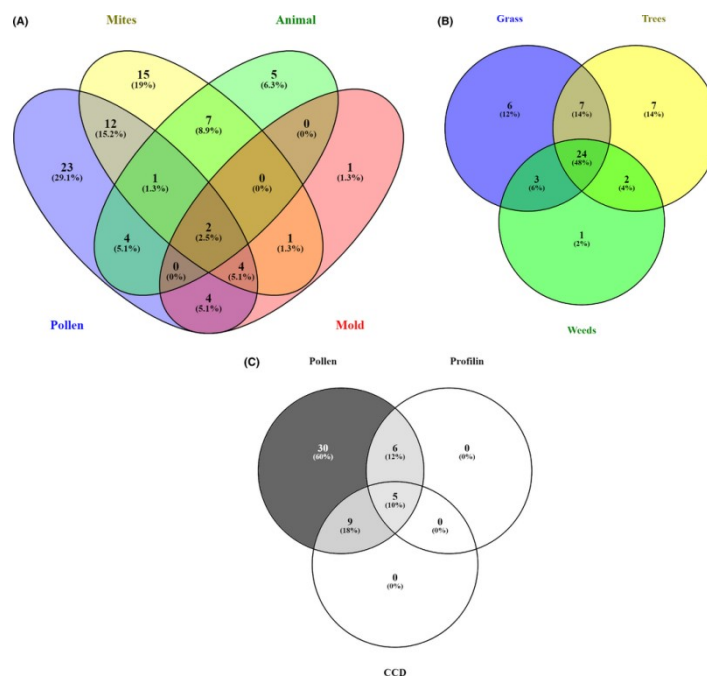


FIGURE 4 Venn diagram of (A) the number of patients with positive specific IgE to pollens, mites, moulds and animals, (B) the number of patients with IgE sensitization to grasses, weeds and trees pollen (C) the number of subjects with IgE sensitization to pollens, profilin (including Bet v 2, Mer a 1, Hev b 8 and Ole e 2) and cross-reactive carbohydrates determinants (Ana c 2, Hom S LF and Amr HRP)

Figure 5 presents IgE reactivity to cross-reactive carbohydrate determinants (Ana c 2, Hom S LF and Amr HRP) in subjects with positive specific IgE to Lol p 1, Cup a 1 and Ole e 1.

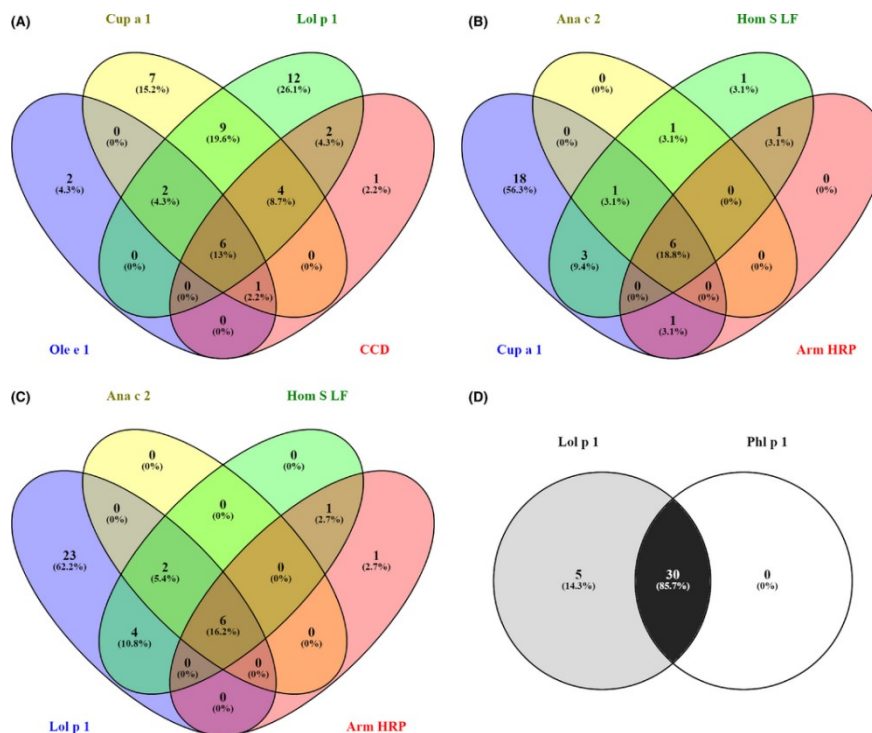


FIGURE 5 Venn diagram of (A) the frequency of patients with IgE sensitization to Cup a 1, Lol p 1, Ole e 1 and cross-reactive carbohydrates determinant (Ana c 2, Hom S LF and Amr HRP) (B) the frequency of subjects with IgE sensitization to Cup a 1 and cross-reactive carbohydrates determinant (Ana c 2, Hom S LF and Amr HRP), (C) the number of individuals with positive IgE reactivity to Lol p 1 and cross-reactive carbohydrates determinant (Ana c 2, Hom S LF and Amr HRP) (D) the number of patients with cross-reactivity between expansins including Lol p 1 and Phl p 1

The quantitative results of specific IgE concentration demonstrated a non-normal distribution of specific IgE level. So, the median (P25, P75) was considered for the results of specific IgE. As the results of allergen-specific IgE in positive patients to related allergens showed the median (P25, P75) of specific IgE to Pla a1: 11.02(1.96, 63.28), Ole e1: 8.74(2.74, 19.93), Fel d1: 5.08(1.42, 16.70), cup a1: 4.97(2.19, 12.08), Lol p 1: 3.82(1.20, 21.99), Sal k1: 2.33(1.41, 4.38) FIU.

4 DISCUSSION

In the current study, the IgE molecular profile showed Lol p 1, Phl p 1.0102 and Cup a 1 as the most common allergenic components. Moreover, we found that outdoor allergenic especially pollen components developed higher sensitization compared with indoor and food allergenic components in Iranian adult allergic patients. Furthermore, the CCD-bearing proteins (such as Cup a 1, Lol p 1), expansin (Lol p 1, Phl p 1.0102), cysteine protease and profilin demonstrated the highest IgE sensitization among protein families.

One of the most significant reasons for the pollen-induced allergic reactions all over the world is members of the Pooideae subfamily.^{21,22} Most allergenic molecules used for the molecular diagnostics of grass pollen sensitization stem from *Phleum pratense*.²³ In our study, the results of IgE sensitization to two members of this subfamily including *Lolium perenne* and *Phleum pratense* demonstrated their high frequency among our patients. This subfamily shows wide IgE cross-reactivity together.²⁴ Lol p1²⁵ and Phl p 1 as the major allergens of *Lolium perenne* and *Phleum pratense* are a member of the group 1 grass allergens. They are glycoproteins (CCD-bearing protein) that appear in high homology with the β -expansin proteins.²¹ Positive-specific IgE to Phl p 1 acts as an indicator of exact sensitization” to Poaceae grasses family. Phl p 2, p 3, p 5 and p 6 are recognized as genuine markers for the Pooideae subfamily.⁵ As our findings revealed, CCD-bearing proteins and expansin showed the highest prevalence of sensitization among protein families. Contrary to our findings and European studies^{14,26} regarding the high prevalence of IgE reactivity to Phl p 1, Phl p 4 was considered as a major allergenic molecule of *Phleum pratense* and diagnostic indicator of grass sensitization in southern China.^{27,28} Twelve out of the 35 our Lol p 1-sensitized patients were positive CCD. As the heat map (Figure 3) and Venn diagram (Figure 5) showed, high cross-reactivity was observed between Lol p 1 and Phl p 1.

The results of the present study indicated Cup a1 as the most common allergenic component of tree pollens with a 48.3% frequency. Cup a1 is a pectate lyase protein (45 kDa) and CCD-bearing protein which is regarded as a genuine allergen in the Cupressaceae family. This molecule is extracted from *Cupressus Arizonica* and is considered a major allergen in *Cupressus arizonica*-allergic patients.²⁹⁻³¹ In other studies, IgE sensitization to Cup a 1 was 42.7% and <5% in Italy and Austria respectively.^{6,15} Cupressaceae allergenic components (such as Cup a1, Cry j1, Cup s 1...) display high homology and IgE cross-reactivity.^{5,32} For instance, 37% cross-reactivity was seen between Cup a 1 and Cry j (*Cryptomeria japonica*) in our study. Besides, 38% of cup 1 a -sensitized patients demonstrated IgE reactivity to CCD indicators. In a study by Marchuet et, allergic sensitization to Cup a 1 was observed in all Cry j 1-sensitized subjects.³²

In addition to *cupressus arizonica*, another allergen extract originating from tree pollens named plane tree (*Platanus acerifolia*) exhibited high IgE sensitization (51.7%) in our patients, but the frequency of sensitization to Pla a1 as its major allergenic component of *Platanus acerifolia*³³ was only 26.7%. It could be attributed to other major allergens of *P.acerifolia* (such as Pla a 2)³⁴ or cross-reactive components.³⁵ Pla a1 acts as an invertase inhibitor and shows high similarity to Pla or 1 in *P. orientalis*.⁵ This result is in agreement with the Stemeseder study in Austria that reported a similar sensitization rate to Pla a1 (24.9%).¹⁵ Furthermore, a 15.5% prevalence of IgE sensitization

to Pla a 2 as another major molecule of *P. acerifolia* was reported by Panzner et al in the Czech Republic.¹⁴

The findings of this study demonstrated that although the sensitization to the whole extract of *Salsola kali* was higher using the immunoblotting assay in the previous phase of this project,¹ Sal k1 as one of the major allergenic components of *Salsola kali* was considered as the fourth common allergenic molecule. It is mentioned that Assarzaghan et al reported Sal K3 as another major allergenic component in Iranian patients with *Salsola kali* allergy³⁶ while this component is not included in the FABER test.

House dust mites (HDM) are considered as one of the most important indoor aeroallergens developing allergic diseases³⁷ which with about 39 and 38 allergenic molecules has been being recognized for *Dermatophagoides farina* and *Dermatophagoides pteronyssinus* respectively (www.allergome.org). According to a meta-analysis by Shokouhi et al, a 38% prevalence of HDM sensitization has been reported in Iran.³⁸ Among house dust mite allergens in the FABER profile, Der p 2 and Der f 2 which belong to the NPC2 protein family³⁹ were the most prevalent. The study of Batard et al depicts similar findings regarding the dominance of IgE sensitization to mites group 2 rather than mites group 1.³⁷ The diagnostic sensitivity of HDM sensitization improves as a result of simultaneous evaluation of specific IgE to groups 1 and 2 of mites allergens (as major allergens for HDM).⁴⁰ In the present study, the IgE profile to Der p 7, 9, 10 and 23 also was assessed but allergic sensitization to them was low (1.7%–8.3%).

IgE sensitization to the whole extract of cat has been recognized in 23.3% of our patients, but IgE sensitization to Fel d1 as a major and primary component of the cat was 42.9% in cat-sensitized patients and 13.3% in all subjects. According to the literature review, the sensitization to this component has been shown in about 90% of patients with cat allergy and is from diagnostic predictor in immunotherapy with cat extract in patients. In addition to Fel d 1, this source has other allergenic components such as Fel d 2 and Fel d 4.⁴¹ Although only one of our patients showed a positive specific IgE to Fel d 2, the findings of another study demonstrated that Fel d 2 as a serum albumin protein has shown cross-reactivity with other serum albumin allergens such as Can f3 (dog), Ecu c 3(horse), Bos d 6(cow).⁴¹

As the FABER results show, *Daucus carota* (Carrot) and *Prunus persica* (Peach) were recognized as the most common sensitizing foods (33.3%) in our patients however it seems that IgE sensitization to them has been developed as a result of the food-pollen syndrome. According to the Popescu study, individuals with respiratory allergic reactions show cross-reactivity between pollen and food allergens. Allergic sensitization to Apiaceae foods including carrot or celery could be because of cross-reactivity to profilin, Bet v 1-homologous proteins.⁴² Additionally, IgE reactivity to Sola t1 was identified in 18.3% of patients. Sola t 1 is a CCD-bearing protein and is considered as a CCD marker in some molecular-based techniques (www.allergome.org).

It is apparent that a relatively small sample size of patients could be considered as one limitation in this study.

5 CONCLUSION

This is the first study in which the molecular diagnosis of allergy using an allergen nano-bead array has been used to determine the most common allergenic components in Iranian allergic adults. Regarding the higher prevalence of IgE sensitization to the whole extract of *Salsola kali* in the previous phase of this study,¹ allergenic molecules of grasses including Lol p 1, Phl p 1.0102, and

cup a 1 and Sal k1 as the major molecules of *Cupressus arizonica* and *Salsola kali* showed higher sensitization in adults' allergic patients, respectively, by allergen nano-bead array test. Therefore, this research extends our knowledge of IgE reactivity to protein families including the CCD-bearing proteins, expansin, cysteine protease and profilin.

6 ETHICS STATEMENT

The protocol of this research was approved by the Ethics Committee of IAARI (No: 412/P/280; 15 Dec 2012). Informed written consent was obtained from all subjects.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Raheleh shokouhi Shoormasti contributed to the conception, design, acquisition, performing tests, analysis, interpretation of data and drafting the manuscript. Chiara Rafaiani contributed to the acquisition, performing tests and analysis. Mohammad Reza Fazlollahi contributed to the conception, design, acquisition, and interpretation of data. Anoshirvan Kazemnejad contributed to the design, statistical analysis and interpretation of results. Maryam Mahloojirad, Majid Moslemi and Javid Morad Abbasi contributed to the acquisition and interpretation of data. Mostafa Moin and Adriano Mari contributed to the conception, design, acquisition, analysis and interpretation of results. Zahra Pourpak contributed to the conception, design, acquisition, analysis and interpretation of results, drafting and revising the manuscript. All authors revised the manuscript critically for important intellectual content. They approved the final version to be published and agreed to be accountable for all aspects of the work.

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