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Immunochemical Characterization of *Amaranthus retroflexus* Pollen Extract: Extensive Cross-reactive Allergenic Components among the Four Species of Amaranthaceae/Chenopodiaceae

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ABSTRACT

The importance of *Amaranthus retroflexus* pollen in causing respiratory allergy has been well ascertained in many countries including Iran with a high positive rate (69%) among Iranian allergic patients. The aim of the present study is to identify the allergenic properties of *A. retroflexus* pollen. Sixteen patients with allergy to *A. retroflexus* pollen were selected for the study. The antigenic and allergenic profiles of the *A. retroflexus* pollen extract as well as pollen extracts from other species of the Amaranthaceae/Chenopodiaceae family, including *Chenopodium album*, *Kochia scoparia*, and *Salsola kali*, were evaluated by ELISA, immunoblotting, and immunoblot inhibition assays.

The resolved protein fractions on SDS-PAGE ranged from 10–85 kDa. Several allergenic components (MW 85, 45, 39, 18, 15, and 10 kDa) of the *A. retroflexus* pollen extract were recognized by using patients' sera by specific antibody of IgE class using ELISA and immunoblot assays.

The IgE reactivity of the *A. retroflexus* pollen extract was partially inhibited by all three pollen extracts tested. The inhibition by the *S. kali* pollen extract was more than those by other pollen extracts. Moreover, the wheal diameters by the *A. retroflexus* pollen extract were highly correlated with those by *C. album*, *K. scoparia* and *S. kali* pollen extracts.

In conclusion, three proteins with apparent MWs of 39, 45, and 66 kDa are suggested as the common allergenic components among the four pollens from the Amaranthaceae/Chenopodiaceae family. It appears that there are some common (similar) epitopes among the four common allergenic pollens.

Key words: Allergens; Amaranthaceae/Chenopodiaceae; *Amaranthus retroflexus*; Cross-reactivity; IgE; Immunoblotting; Pollen; SDS-PAGE

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INTRODUCTION

Amaranthus retroflexus (Redroot Pigweed) is a species of flowering plant from the Amaranthaceae/Chenopodiaceae family which is found throughout the world. In Iran, it is an abundant annual weedy plant in the moorlands and in farms.¹ The main flowering season of this plant is from August to October. Allergy to pollens from Amaranthaceae/Chenopodiaceae has been recognized as a severe problem in desert and semi-desert areas of countries such as Saudi Arabia, Iran, and Kuwait.²⁻⁴ The importance of the *A. retroflexus* pollen in causing respiratory allergy has also been well ascertained in Iran with a high positive rate (69%) among Iranian allergic patients.³

The characterization of single allergen components which are specifically reactive to the immunoglobulin E (IgE) of pollen-allergic patients necessitates clinical diagnosis, the design of patient-adapted immunotherapy, and the clarification of sensitization mechanisms to various allergens.^{5,6}

Protein analysis of the Amaranthaceae/Chenopodiaceae pollens, including *A. retroflexus* pollen, revealed several components ranging from 8 to 97 kDa.⁷⁻¹¹ Furthermore, using Indian patients' sera, Singh et al. reported seven allergic components with 14-70 kDa molecules from *Amaranthus spinosus* pollen.¹¹

Despite its clinical implications in the elicitation of allergic disorders, the antigenic and allergenic properties of *A. retroflexus* pollen have not yet been systematically determined. Therefore, the present study aimed to identify the antigenic and allergenic profile of *A. retroflexus* pollen and to evaluate the IgE cross-reactivity between *A. retroflexus* pollen and other pollens from the Amaranthaceae/Chenopodiaceae family.

PATIENTS AND METHODS

Pollens and Extract Preparation

As a naturally growing annual weed, *Amaranthus retroflexus* grows on vacant and wastelands. Samples of the polliniferous material (Fig.1. A.) were collected from the field between July–September from the wastelands of Mashhad. Identification of the species was confirmed by the Ferdowsi University of Mashhad Herbarium (FUMH, Mashhad, Iran). After pollen separation, the protein extract was prepared from the gathered pollen along with the *A. retroflexus* pollen

purchased from Allergon AB (Välinge, Sweden). Both extracts were then electrophoresed on a 12.5% polyacrylamide gel. The results (not shown) indicated that the SDS-PAGE pattern of the two tested pollen were almost similar; however, due to the high purity of the pollen from Allergon AB (Fig.1. B.), it was preferred to use in this study.

Pollens from three other species of the Amaranthaceae/Chenopodiaceae family including *C. album*, *K. scoparia* and *S. kali* were also purchased from Allergon AB (Välinge, Sweden). Each pollen extract was then prepared as described earlier⁷. The protein content of each extract was then determined using the Bradford's method¹² (Fig.1).

Patients

Sixteen adult respiratory allergic patients were enrolled in this study from the Outpatient Allergy Clinic of Mashhad University of Medical Sciences. The patients were asked to complete a detailed questionnaire. They were considered as having a history of allergy if they reported at least one ophtalmic, nasal, or respiratory symptoms to common allergens such as house dust, domestic animals, foods, or pollens.

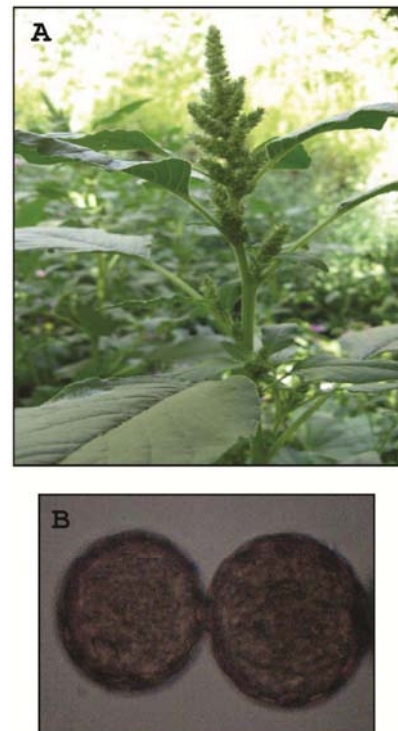


Figure 1. (A) *Amaranthus retroflexus* weed collected from the wastelands of Mashhad. (B) *Amaranthus retroflexus* pollen

The patients were also evaluated by a clinical examination and a skin prick test (SPT) with common aeroallergens. Seven healthy subjects who presented negative SPTs and no specific IgE to the *A. retroflexus* pollen extract (Numbers 17-23) were assigned as negative controls. With an informed written consent from each patient on record, the Human Ethics Committee of the institute approved the study protocol.

Skin Prick Test

Skin prick test (SPT) was performed as previously described.⁷ Four pollen extracts which were common allergens of the area³ were selected including *A. Retroflexus*, *C. album*, *K. scoparia* and *S. kali* (All from Hollister-Stier Laboratories LLC, Spokane, WA, US). Histamine diphosphate (10 mg/ml) as a positive control was also used to make sure of no anti-histamines have been taken which can interfere with the testing results. As a negative control, 50% glycerin solution was applied to make sure the patient was not dermatographic and falsely being identified as sensitive to *A. retroflexus*. A wheal at least 3 mm larger in diameter than the negative control, surrounded by an erythema, was considered as a positive SPT. The patients have been asked to discontinue antihistamine therapy at least for three days prior to SPT.

Enzyme-linked Immunosorbent Assays (ELISAs)

Total serum IgE levels were measured by means of a commercially available ELISA kit according to the manufacturer's instructions (Radim, Italy).

To measure the levels of specific IgE to *A. retroflexus* pollen in patients' sera, an indirect ELISA was developed as described earlier.^{7, 13} Briefly, 2 µg of *A. retroflexus* pollen extract in 100 µl carbonate buffer (15 mM Na₂CO₃ and 35 mM NaHCO₃, pH 9.6) was incubated at 4 °C overnight per well of a 96-well microtiter plate (Nunc MaxiSorp, Denmark). Each well was then blocked for 1 h at 37°C with 150 µl of 2% BSA in PBS following by incubation for 3 h with serum (1:5 diluted with PBS) at room temperature with shaking. Each well was then incubated for 2 h at room temperature with 1:1000 dilution of biotinylated goat anti human IgE antibody (KPL, MD, US) in 1% BSA. Each incubation step was followed by five washes with PBS-T (PBS containing 0.05% Tween 20). Wells were added by 100 µl of a 1:30000 dilution of horseradish peroxidase-conjugated streptavidin (Bio-Rad, US). Following five washes, 100 µl of chromogenic substrate

was added to each well and the plate was incubated for 15 min in the dark. The plate was read at 450 nm with an ELISA reader. Optical density (OD₄₅₀) four times greater than the median values of the negative controls were considered to be positive.

SDS-PAGE and IgE Immunoblotting Assays

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of all pollen extracts was performed according to Laemmli¹⁴ using 12.5% or 15% acrylamide separation gels under reducing conditions. Separated protein bands from the electrophoresis of the four pollen extracts were electro-transferred to Polyvinylidene difluoride (PVDF) membranes (Immobilon P, Millipore Corp., Bedford, MA, US), as previously described.¹⁵ In brief, after the trans-blotting, the membranes were stained, cut into strips and blocked with 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS, 0.15 M, pH 7.4) and left overnight on a shaker, at 4°C. Each strip was then incubated overnight with each of patients' sera in PBS at 4°C on a shaker. Strips were washed with PBS four times each time for 5 minutes and then incubated for 3 h at room temperature with 1:1000 dilution of biotinylated goat anti human IgE antibody (KPL, MD, US) in 1% BSA.

Strips were then washed under the same conditions followed by the incubation for 1 h at room temperature with a 1:10000 dilution of HRP-conjugated streptavidin (Bio-Rad, US). After several washes in PBS, strips were incubated with Supersignal West Pico Chemiluminescent Substrate Kit (Pierce, US) for 5 min, and proteins were then visualized by chemiluminescence using G-Box gel documentation system (Syngene, Cambridge, UK).

Immunoblotting Inhibition Assays

Inhibition experiments were performed using four pollen extracts from Amaranthaceae/Chenopodiaceae, including *A. retroflexus*, *C. album*, *K. scoparia*, and *S. kali*, as well as BSA. Different amounts of each pollen extract (Fig.5) or BSA were pre-incubated with a pooled serum (from patients 8, 9, and 11) for three hours at 37°C. The pre-adsorbed sera were then used for immunoblotting assays.

Statistical Analysis

Statistical analyses were performed using SPSS, v.15 software. The strength of association between the wheal diameters was analyzed using the Spearman rank order

correlation test, and the correlation coefficient was calculated. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Patients

Sixteen patients, eight women and eight men (mean age, 26.94 ± 2.27 years; age range 19-43 years), suffering from respiratory allergy, as well as seven control subject without respiratory allergy, were included in the study. Case histories with respect to respiratory allergy are summarized in Table 1. Rhinitis

and rhinoconjunctivitis were the most prominent clinical manifestations among these patients (Table 1).

Skin Prick Test

Mean diameters of positive wheal sizes were: *A. retroflexus*: 12.38 ± 1.02 mm; *C. album*: 10.81 ± 0.95 mm; *K. scoparia*: 10.67 ± 1.36 mm; *S. kali*: 16.31 ± 1.07 mm. Positive correlation coefficients were attained between the *A. retroflexus* and *C. album* ($r=0.67$, $p < 0.01$), *A. retroflexus* and *K. scoparia* ($r=0.84$, $p < 0.01$), and *A. retroflexus* and *S. kali* ($r=0.54$, $p < 0.05$) pollen extracts for wheal diameter on the SPT (Pearson's rank test, Fig.2).

Table 1. Clinical characteristics, total and specific IgE values and skin reactivity of patients and controls

Patient No.	Age (years)/Sex	Symptoms *	Total IgE (IU/ml)	Specific IgE**	Diameters (mm) of the papules obtained by prick test†					
					Histamine	Glycerin	<i>A. retroflexus</i>	<i>C. album</i>	<i>K. scoparia</i>	<i>S. kali</i>
1	43/M	R	830	0.968	10	0	14	16	na‡	18
2	40/M	R	720	0.422	12	0	18	12	14	18
3	23/F	R	710	1.345	9	0	10	10	8	11
4	41/F	RC	249.8	1.269	12	0	14	12	10	14
5	31/F	R	202.4	0.733	12	0	7	7	8	20
6	41/F	RC/CD	439.5	0.437	12	0	15	10	11	28
7	20/F	R	570	1.313	7	0	9	9	na	12
8	21/M	RC	716	1.06	10	0	14	11	na	15
9	19/M	RC	1120	1.05	13	0	16	16	na	18
10	21/M	R	102	0.78	10	0	8	7	na	13
11	19/M	RC	896	1.1	9	0	8	1	7	14
12	22/M	RC	580.7	0.49	10	0	6	10	8	11
13	19/F	RC	706.1	1.1	15	1	18	15	20	18
14	22/F	RC	580	0.278	8	0	18	12	na	20
15	22/M	RC	275	1.06	14	0	12	14	10	16
16	27/F	R	366.5	1.069	10	0	11	11	na	15
Controls										
17	21/M	-	69.8	0.18	10	0	0	0	0	0
18	22/M	-	630	0.315	9	0	0	0	0	0
19	20/M	-	44.1	0.351	11	0	0	0	0	0
20	21/M	CD	172	na	8	0	0	0	0	0
21	40/M	-	10	0.18	9	0	0	0	0	0
22	22/F	CU	426.9	na	8	0	0	0	0	0
7	35/M	-	63.3	0.455	6	0	0	0	0	0

* AR, Allergic rhinitis; RC, Rhiniconjectivits; CD, Contact Dermatitis; CU, Chronic Urticaria.

** Levels of specific IgE to *A. retroflexus* Pollen Extract by ELISA (optical density at 450nm).

† The mean wheal diameters are displayed in mm. Histamine diphosphate (10 mg/ml)-positive control; Glycerin-negative control.

‡ na, not available

Characterization of *Amaranthus retroflexus* Pollen

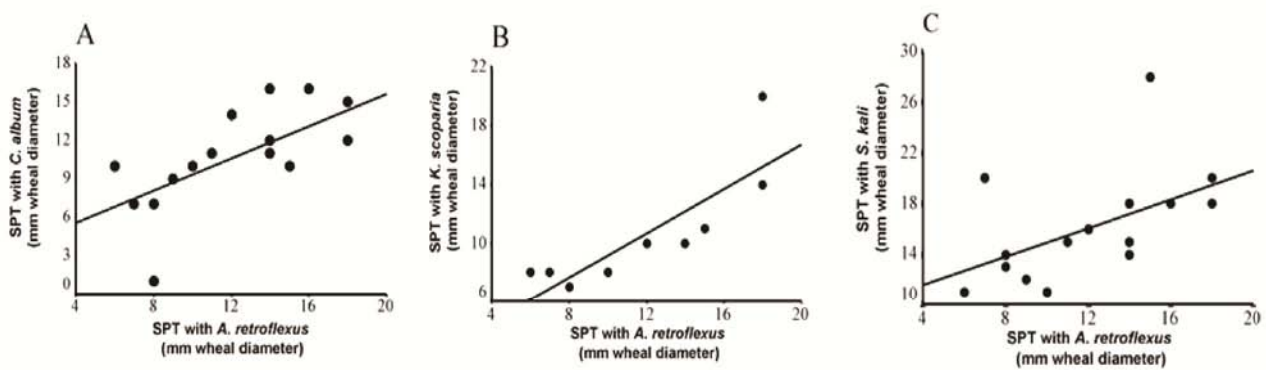


Figure 2. Linear Correlation between the diameters of SPT wheals by *A. retroflexus* pollen extract and those by other pollen extracts; Positive correlation between the *A. retroflexus* and *C. album* pollen extracts (A) ($r = 0.67, p < 0.01$), between the *A. retroflexus* and *K. scoparia* pollen extracts ($r = 0.84, p < 0.01$) (B), and between the *A. retroflexus* and *S. kali* pollen extracts (C) ($r = 0.54, p < 0.05$).

Specific IgE levels

The specific IgE values to *A. retroflexus* pollen extract were determined in sixteen individual patients' sera (Table 1); all sixteen patients had detectable specific IgE levels to the total extract of *A. retroflexus* pollen.

Protein Profiles of Pollens

SDS-PAGE revealed at least seven bands from the *A. retroflexus* pollen extract with estimated molecular weights (MWs) of 85, 66, 50, 45, 39, 25, 18, 15, and 10 kDa (Fig.3), five of which (85, 66, 45, 39, and 15 kDa bands) were also detected by the electrophoresis of three other pollen extracts (Fig.3).

IgE-binding Profile of Pollen Extracts

IgE-reactivity of the separated protein bands from the electrophoresis of the four pollen extracts was

determined via immunoblotting assays. Specific IgE binding fractions probed with sera from all sixteen allergic patients are shown in Figure 4. The results showed several IgE reactive bands ranging from 10 to 85 kDa. Table 2 shows the apparent MW of each protein fraction and the prevalence of each one among all sixteen sera. As shown in Table 2, the most frequent IgE reactive bands among the patients' sera were approximately 45 and 39 kDa bands from the *A. retroflexus* pollen extract (81.25% and 62.5%, respectively), 66 and 18 kDa bands from the *C. album* pollen extract (50.0% and 43.7%, respectively), 66, 45 and 39 kDa bands from the *K. scoparia* pollen extract (66.6%, 41.6%, and 41.6%, respectively), 41.6%, and 39 and 45 kDa bands from the *S. kali* pollen extract (62.5% and 56.2%, respectively). However, when the pooled sera of normal volunteers were used, no IgE binding fractions were observed (Fig.4).

Table 2. Frequency of the IgE-binding of each protein component of pollens from Amaranthaceae/Chenopodiaceae family following immunoblotting assays*

Allergenic components (kDa)	<i>A. retroflexus</i>	<i>C. album</i>	<i>K. scoparia</i>	<i>S. kali</i>
15	3/16 (18.7 %)	6/16 (37.5 %)	0/12 (0.0 %)	2/16 (12.5 %)
18	3/16 (18.7 %)	7/16 (43.7%)	1/12 (8.3 %)	2/16 (12.5 %)
39	10/16 (62.5 %)	4/16 (25.0 %)	5/12 (41.6 %)	10/16 (62.5 %)
45	12/16 (81.25%)	3/16 (18.7 %)	5/12 (41.6 %)	9/16 (56.2 %)
66	4/16 (25.0 %)	8/16 (50.0 %)	8/12 (66.6 %)	6/16 (37.5 %)
85	6/16 (37.5 %)	6/16 (37.5 %)	1/12 (8.3 %)	6/16 (37.5 %)

*The most immunoreactive proteins are shown in bold.

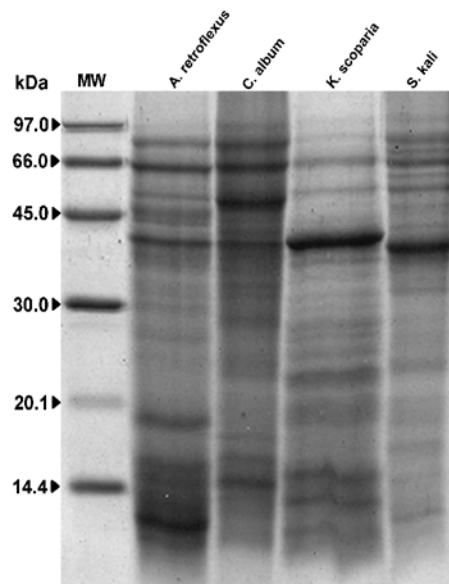


Figure 3. SDS-PAGE analysis of crude extracts of four pollens from Amaranthaceae/Chenopodiaceae family (10 µg each) on a 12.5% polyacrylamide gel with Coomassie Brilliant Blue staining. Lane MW, low MW (Amersham, Buckinghamshire, UK)

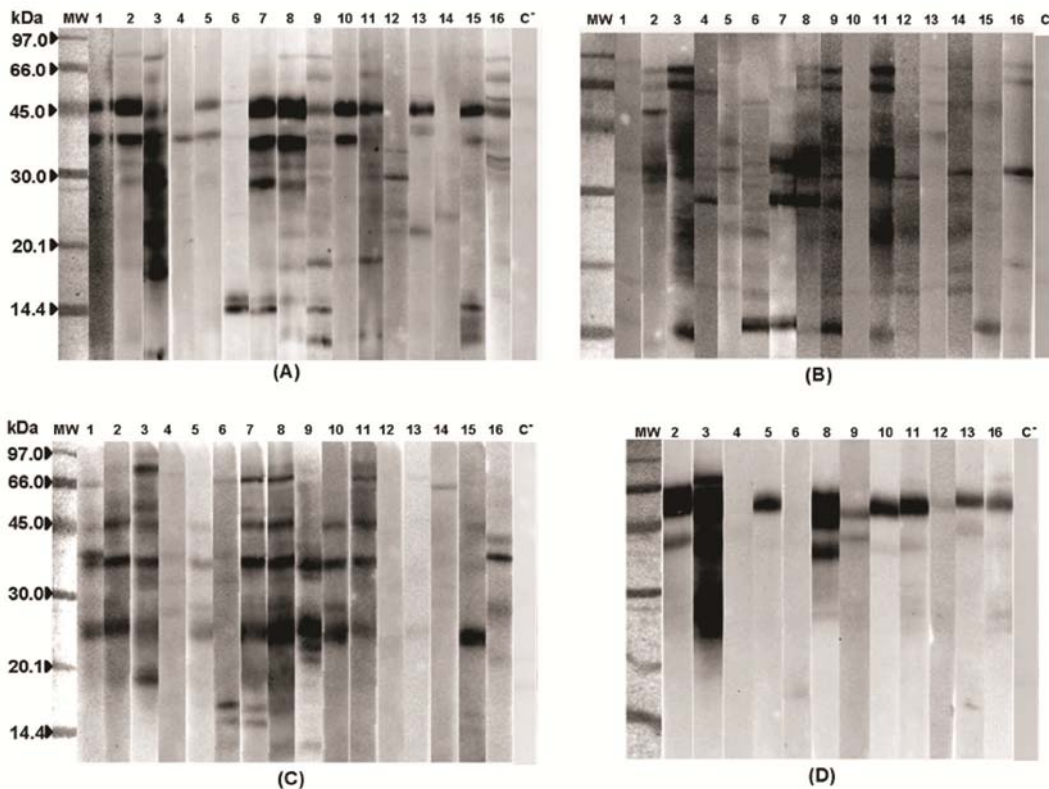


Figure 4. Immunoblotting of four cross-reactive pollen extracts from the family of Amaranthaceae/Chenopodiaceae. Each strip was first blotted with 10 µg of pollen extract from *A. retroflexus* (A), *C. album* (B), *S. kali* (C), or *K. scoparia* (D). All strips were then incubated with the sera from 16 allergic patients (1–16; numbers as in Table 1) and detected for IgE reactive protein bands. MW, low molecular weight (Amersham, Buckinghamshire, UK). C', Negative control.

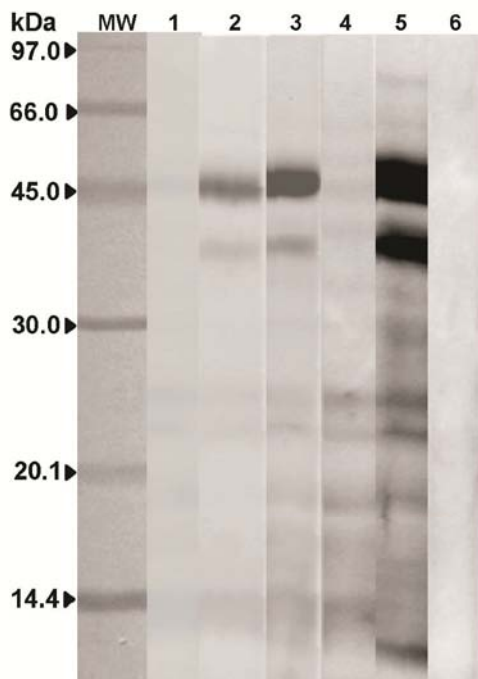


Figure 5. Immunoblotting inhibition assays. Lane MW, low MW (Amersham, Buckinghamshire, UK); Lane 1, *A. retroflexus* protein strip incubated with pooled serum containing 70 µg of *A. retroflexus* pollen extract as inhibitor; Lane 2, *A. retroflexus* protein strip incubated with pooled serum containing 70 µg of *C. album* pollen extract as inhibitor; Lane 3, *A. retroflexus* protein strip incubated with pooled serum containing 70 µg of *K. scoparia* pollen extract as inhibitor; Lane 4, *A. retroflexus* protein strip incubated with pooled serum containing 160 µg of *S. kali* pollen extract as inhibitor; Lane 5, *A. retroflexus* protein strip probed with pooled serum with 1%BSA as inhibitor; Lane 6, *A. retroflexus* protein strip probed with 1%BSA, without inhibitor.

Immunoblot Inhibition Assays

In order to evaluate the IgE cross-reactivity between *A. retroflexus* and three other pollen extracts, an immunoblot inhibition was carried out with the *A. retroflexus* pollen extract as the solid phase. As shown in Figure 5, IgE reactivity to most of the allergenic proteins of *A. retroflexus* pollen was inhibited when the *S. kali* pollen extract was used as inhibitor. However, when *C. album* pollen extract was employed as an inhibitor, the 15 and 18 kDa bands were almost completely inhibited whereas the 10, 39, 45 and 85 kDa bands were partially inhibited. When the *K. scoparia* pollen extract was used as an inhibitor, the protein band of approximately 10 kDa was completely inhibited, while the other bands were barely inhibited (Fig.5).

DISCUSSION

In the present study, the immunochemical characterization of the *A. retroflexus* pollen extract, a member of the Amaranthaceae/Chenopodiaceae family was performed to identify the IgE-binding proteins responsible for type I allergic disorders. In addition, IgE cross-reactivity between the pollen extract from *A. retroflexus* and those from three related species, including *C. album*, *K. scoparia*, and *S. kali*, were evaluated.

SDS-PAGE revealed several bands from the *A. retroflexus* pollen extract with estimated MWs of 85, 66, 50, 45, 40, 25, 18, 15 and 10 kDa (Fig.3). Among those bands, six IgE binding protein fractions with apparent MWs of 85, 66, 45, 40, 18, 15, and 10 kDa were detected from the blot (Fig.4. A). Moreover, the results of SDS-PAGE showed similar patterns of migration by protein components of the four tested pollens, mainly those of 39-97 kDa. Concurrently, immunoblotting assays with the four tested pollens showed almost similar patterns of migration by the allergenic proteins, especially those of 39-85 kDa (Fig. 4 and Table 2). These results collectively suggest that in these pollen extracts, protein components with higher MWs play a greater role in cross-reactivity compared to those with lower MWs.

Two bands with apparent MWs of 39 and 45 kDa were found as the most frequent IgE reactive proteins both in the pollen extracts of *A. retroflexus* (62.5% and 85%, respectively) and *S. kali* (62.5% and 56.2%, respectively) pollen extracts. However, this was not the case for *C. album* or *K. scoparia* (Table 2), in which a protein band of approximately 66 kDa was apparently the most common IgE reactive protein. Similar results had been previously obtained from *S. kali* pollen.⁷

Two other studies had also indicated a major allergen of *S. kali* pollen, designated Sal k 1, with a MW of 40-43 kDa.^{8,9} Moreover, Würtzen et al. showed that among fourteen IgE reactive proteins of *A. retroflexus* pollen, protein band of approximately 49 kDa was the most frequent.¹⁰

In addition, a new allergen from *S. kali* pollen, with cobalamin-independent methionine synthase (MetE) characteristics, was recently designated as Sal k 3 by the WHO/IUIS Allergen Nomenclature Subcommittee (<http://www.allergen.org/Allergen.aspx>).¹⁶

The molecular weight of the whole molecule is 85 kDa; it has however, been proposed to break it into a 39

kDa and a 45 kDa fragments. Therefore, IgE reactive proteins with estimated MWs of 39 and 45 kDa by the current study may correspond to two fragments of that newly designated allergen. In addition, a faint band with an apparent MW of 85 kDa was observed in the blot of all pollen extracts, but not from the *K. scoparia* pollen extract (Fig.4. A-D).

Another IgE binding protein band with an estimated MW of 15 kDa was also detected by immunoblotting of all pollen extracts (ranging from 12.5 to 37.5%, Figure 5), but not that of *K. scoparia* pollen (Fig.4. A-D). However, when strips were probed with the *A. retroflexus* pollen extract, this protein band seemed to be equally inhibited by all three pollen extracts (Fig.5). It was then suggested that this protein band may be a common minor allergen among the four tested pollens and that the difference in the results obtained from immunoblotting and inhibition by the *K. scoparia* pollen extract may be due to the small number of tested sera. Also, two allergens belonging to the profilin family with apparent MWs of 14 to 15 kDa were designated Sal k 4 from *S. kali* pollen, according to WHO/IUIS Allergen Nomenclature Subcommittee,¹⁶ and Che a 2 from the *C. album* pollen,¹⁷ respectively. These two allergens may be homologous with the 15-kDa IgE reactive band which was uncovered by the present study.

Finally, the 18-kDa band was inhibited mostly by the *C. album* pollen extract which suggests this protein is a minor allergen in *A. retroflexus* and *C. album* pollen extracts. Concomitant with this result, two allergens related to the Ole e 1-like protein family have been discovered, namely Che a 1 from *C. album* pollen¹⁸ and Sal k 5 from *S. kali* pollen,¹⁶ with MWs of 17 and 18.2 kDa, respectively.

Singh et al. reported seven IgE reactive proteins ranging from 30-70 kDa from pollen extract of *A. spinosus*, another species of the Amaranthaceae family, including proteins with apparent MWs of 14, 30, 40, 50, 60, 66, and 70 kDa.¹¹ The allergenic profile of *A. spinosus* pollen appears to be almost similar to that of *A. retroflexus*; although, Singh et al. did not mention the frequency of each allergenic protein to patients' sera. Furthermore, the protein band of approximately 85 kDa was not reported as allergenic by Singh et al. while in the present study, this band reacted with IgE from 38% of patients' sera.

The results of immunoblotting inhibition revealed that the IgE binding reactivity of the *A. retroflexus* pollen extract was more or less inhibited by all three

pollen extracts from the Amaranthaceae /Chenopodiaceae family. As shown in Figure 5, IgE reactivity to most of the allergenic proteins of *A. retroflexus* pollen was inhibited when the *S. kali* pollen extract was used as an inhibitor. This indicates a significant IgE cross-reactivity between the two pollens and is also in line with the results of the SPTs (see below).

SPTs with *A. retroflexus*, *C. album*, *K. scoparia*, and *S. kali* pollen extracts were carried out. The results indicated highly significant correlations between the wheal diameters from the *A. retroflexus* pollen extract and those from other pollen extracts (*A. retroflexus* and *C. album* ($r = 0.67$, $p < 0.01$), *A. retroflexus* and *K. scoparia* ($r = 0.84$, $p < 0.01$), and *A. retroflexus* and *S. kali* ($r = 0.54$, $p < 0.05$), (Fig.2). These results suggested an extensive IgE cross-reactivity among the four tested pollens and were concurrent with the results of immunoblotting and inhibition experiments performed by the present study, as well as by other previous studies.^{10, 19}

In conclusion, this study showed that in spite of antigenic and allergenic differences among pollens from four species related to the Amaranthaceae /Chenopodiaceae family, there are significant IgE cross-reactivities among allergenic proteins of the pollens from the four species, especially those proteins with higher MWs. Moreover, three proteins with apparent MWs of 45, 39 and 66 kDa are suggested as the common allergenic components among pollens from these species.

Considering the high prevalence of allergy to pollens from the Amaranthaceae/Chenopodiaceae family in Iran and neighboring countries, identification and production of the recombinant forms of common allergens of this family may lead to the exploration of new guidelines for diagnostic, therapeutic, and preventive purposes. Efforts are now underway to clone cDNAs encoding allergenic cross-reactive proteins from *A. retroflexus* pollen as well as other related pollens.

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