ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
October 2016; 15(5): 363-371.

Identification and Characterization of Main Allergic Proteins in Cooked Wolf Herring Fish

Mohsen Mohammadi[1,2], Reza Falak[2,3], Kobra Mokhtarian[2], Mohammad Reza Khoramizadeh[1], Esmaeil Sadroddiny[1], and Gholam Ali Kardar[1,4]

[1] Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2] Immunology Research center, Iran University of Medical Sciences, Tehran, Iran
[3] Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[4] Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Received: 2 February 2016; Received in revised form: 31 March 2016; Accepted: 6 April 2016

ABSTRACT

Our aim in this study was to identify and characterize allergic proteins in cooked wolf herring fish. We heated the crude extract alternatively at 50, 60, 70, 80, 90, and 100°C for one hour and results were compared by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Also, proteins were immunoblotted with fish-sensitive patients' sera. The major allergenic proteins were identified via mass spectrometry. These allergenic proteins were then purified by anion exchange chromatography and the IgE-immunoreactivity of the fractions was compared with the crude extracts via disk enzyme-linked immunosorbent assay (ELISA).

SDS-PAGE of the crude extract showed more than 15 distinct protein bands. Five of these proteins, with apparent molecular weights of 12, 18, 24, 38, and 51 kDa, were only observed in the 100°C heated extract. Immunoblotting of the heated extract revealed that the 12 and 51 kDa proteins were IgE-immunoreactive with 88 percent of fish-sensitive patients' sera while the 24 and 38 kDa proteins reacted with 33.3 and 55.5 percent of fish-sensitive patients' sera, respectively. Mass spectrometry of the 12, 38, and 51 kDa proteins revealed that all three were parvalbumin oligomers.

Disk ELISA results showed that 20 of 25 and 14 of 25 fish-allergic patients' sera were IgE-reactive with purified oligomeric parvalbumin-coated and crude extract-coated disks, respectively. Parvalbumin and its oligomers are the main allergenic molecules in cooked fish.

Therefore, an enriched or purified fraction containing this protein could be a useful source of allergen for applications in ELISA-based immunoassays and could discriminate fish-allergic patients who can tolerate cooked fish from those who cannot.

Keywords: Hypersensitivity; Parvalbumin; Thermoresistant allergen; Thermolabile allergen; Wolf herring fish

Corresponding Authors: Reza Falak, PhD;
Department of Immunology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. Tel: (+98 21) 8670 3299, Email: falak.r@iums.ac.ir, rezafalak@gmail.com

Gholam Ali Kardar, PhD;
Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran. Tel: (+98 21) 6691 9587, Email: gakardar@tums.ac.ir

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Seafood, including fish and shellfish, is the most common cause of food allergies, especially in coastal areas where seafood is highly consumed,[1] and is responsible for 0.4% and 2% respectively, of food allergies in the US. However, seafood allergy is more prevalent in eastern countries such as Japan and Singapore.[2]

Immunoblotting of total extracts and application of some confirmatory tests have characterized more than ten different IgE-binding proteins in fish. These proteins range from 11 to 151 kDa and can be categorized as thermolabile or thermoresistant.[3,4] Both types are found in raw fish, but the more stable thermoresistant proteins can trigger allergy symptoms in most fish-sensitive patients. Fish is served both raw and cooked in far eastern countries, including Japan. In fact, consumption of raw seafood, such as sashimi, is common in these countries; however, in some other countries, such as Iran, semi-cooked fish is also eaten. [5]

Current in vivo and in vitro diagnostic methods employ a raw extract that contains both thermoresistant and thermolabile proteins. Raw extract cannot distinguish patients sensitive only to thermolabile allergens who may safely eat cooked fish, from patients who are sensitive to thermoresistant allergens and cannot eat either raw or cooked fish.[6]

Parvalbumin, a 12 kDa protein, is the main heat-resistant protein and also the major allergen in most fish species.[7-13] However, in contrast to previous reports, some recent studies showed that thermolabile allergens such as creatine kinase (42 kDa) and enolase (51 kDa) are major raw fish extract allergens, while parvalbumin was considered to be a minor allergen in these studies. In addition, some fish-allergic patients may tolerate cooked fish because they are sensitive only to thermolabile allergens, which are absent from the cooked fish.[3,12,14-16]

Because sensitivity to both raw and cooked fish is routinely observed, the allergens should be categorized. Allergy diagnostic tests are based on allergen extracts that contain a mixture of proteins, which can reduce the specificity of the tests.[15] Therefore, purification or enrichment of major allergens could aid the diagnosis of allergic disease. In this regard, for differential diagnosis and treatment of fish-allergic patients who can tolerate cooked fish, we see a need to characterize and identify heat-resistant allergens. In the present study, we focused on characterization of heat-resistant allergens from wolf herring fish (Chirocentridae family) which is highly consumed in the Middle East.[17]

MATERIALS AND METHODS

Fish-Allergic Patients' Sera

Sera from 13 male and 12 female children, ranging from 4 month to 4 years, with the mean age±SD of 12.64±10.07 months, and the mean specific IgE±SD of 6.36±3.594 IU/ml were collected at Day General Hospital, Tehran, Iran (demographic data is summarized in Table 1). All participants showed clinical history of fish allergy and positive Enzyme Allergo Sorbent Test (EAST) with a fish mix containing cod, salmon, and tuna. Sera from 7 healthy individuals, ranging from 1 to 3 years of age, with no symptoms suggestive of allergy to seafood and no IgE-reactivity with wolf herring fish crude extract were applied as negative controls. Written informed consent was obtained from the patients' parents. The study protocol was approved by the ethical committee of Tehran University of Medical Sciences (No.140778).

Fish Extract Preparation

Raw extract was prepared from fresh wolf herring muscle. In brief, 500 mg of tissue was homogenized with a three-fold volume of 20 mM Tris-HCl pH 8.5 containing anti-protease cocktail (Promega, Fitchburg, USA). The mixture was stirred overnight at 4°C, the homogenate was then centrifuged at 14000 rpm for 30 minutes at 4°C, and the clear supernatant was collected as raw extract. For thermal treatment, we heated samples from the raw extract at 50, 60, 70, 80, 90, and 100°C for one hour. Following another centrifugation, the protein concentration of each sample was determined by the Bradford method.

SDS-PAGE and Western Blotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in reducing conditions as previously described.[18] In brief, the extracts were electrophoresed on 15% polyacrylamide gels and stained with Coomassie Brilliant Blue G-250, or alternatively were subjected to western blotting. For western blotting, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Amersham Biosciences, Uppsala, Sweden) using a semidry system as previously described.[18] Then

Table 1. Demographic data of fish-allergic patients.

Patient	Age	Gender	Specific IgE (IU/ml)
1	48m	F	10.8
2	6m	M	3.2
3	8m	M	9.3
4	7m	M	13.1
5	6m	F	2.8
6	6m	M	7.7
7	9m	F	12.9
8	8m	F	4.8
9	14m	F	2.9
10	24m	M	2.5
11	7m	F	4.2
12	10m	F	2.2
13	12m	M	7.8
14	8m	M	9.4
15	6m	M	4.9
16	15m	M	10
17	5m	F	3.4
18	20m	M	2.9
19	27m	F	4.2
20	9m	F	3.6
21	4m	M	10.4
22	9m	F	9.7
23	12m	M	3.9
24	29m	M	2.8
25	7m	F	9.8

m: month, F: female, M: male

the membranes were cut into strips and blocked with 2% bovine serum albumin (BSA) for 4 hours at 37°C.

After washing 3 times for 5 minutes each with phosphate-buffered saline containing 0.05% Tween 20 (PBS-T), the strips were incubated with patients' sera diluted 1:3 overnight at 4°C on a rocker. After a washing step, the strips were incubated with 1:2000 diluted biotin-labeled anti-human IgE (Abcam, USA) for 2 hours at room temperature (RT). After another washing step, blots were incubated with 1:30000 diluted horseradish peroxidase (HRP)-conjugated streptavidin (Sigma, USA) for 1 hour at RT. Finally, the reactive proteins were visualized by DAB/H_2O_2 substrate, as previously described.[19]

Protein Purification

After determination of the allergenic proteins with western blotting, we fractionated the protein contents of the heated extract. For this, the 100°C-treated extract was lyophilized and dialyzed overnight in 20 mM Tris-HCl pH 8.6. Proteins were resolved on an AKTA prime plus fast protein liquid chromatography (FPLC) system (GE Healthcare, Life Science, USA) using a diethylaminoethyl (DEAE) Sepharose CL-6B column as the anion exchange matrix. Proteins were eluted by a linear gradient mixture of starting buffer (20 mM Tris-HCl pH 8.6) supplemented with 1 M NaCl solution. The fractionation was performed at a 1 ml/min flow rate and the fractions were collected in 5 ml volumes. After adding 3% sucrose as a stabilizer, fractions were freeze-dried. The purity of the fractions was evaluated by SDS-PAGE and Coomassie blue staining.

Identification of Desired Proteins by Mass Spectrometry

Following purification of the IgE-reactive bands

and re-confirmation of their IgE-reactivity with fish-allergic patients' sera by western blotting, comparable bands were excised from the slabs and their identities determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). In brief, the fractions were subjected to SDS-PAGE and the resolved proteins visualized by mass spectrometry-compatible colloidal Coomassie blue staining. After a mild destaining in distilled water, proteins of interest were cut from the polyacrylamide gels. Mass spectrometry was performed using an ABI 4800 MALDI TOF/TOF analyzer (Applied Biosystems, Foster City, CA, USA). Mass spectra were recorded in reflector-positive mode with a scanning range of 900-4000 Da. Five monoisotopic precursors from each purified fraction with signal/noise (S/N) ratios greater than 200 were selected for mass spectrometry (MS/MS) analysis. The observed peptide mass list was used to search the National Center for Biotechnology Information non-redundant (NCBInr) and Mascot database search engines (http://www.matrixscience.com). The well-matched results with high total protein scores and high confidence intervals were considered to comprise parts of amino acid sequences of the unknown allergens.

Disk ELISA and IgE-Reactivity

IgE-reactivities of the total extract and collected fractions were evaluated by disk ELISA as previously described.[20] In brief, we compared the IgE-reactivity of purified protein-coated nitrocellulose disks with crude extract-coated disks using sera from fish-allergic patients and healthy individuals. Allergen-coated disks were prepared using routine methods. Five mm diameter nitrocellulose disks were incubated with 50 µl of a 100 µg/ml solution of total extract or 10 µg/ml solutions of each concentrated FPLC fraction for 3 hours. After washing for 15 minutes with PBS-T, disks were blocked for 2 hours with 2% BSA. After another washing step, disks were incubated for 3 hours with 1:5 diluted patients' sera. Then the disks were washed and incubated for 2 hours with 1:2000 diluted biotin-labeled anti-human IgE (Abcam, USA). After another washing step, disks were incubated for 1 hour with 1:30000 diluted HRP-conjugated streptavidin (Sigma, USA). Then TMB/H_2O_2 chromogen/substrate was added and the plates were incubated in the dark for 15 minutes. The reaction was stopped by adding 100 µl of 2 N H_2SO_4 solution. Finally, disks were removed and the optical density (OD) of the wells was measured at 450/630 nm. All steps were performed with constant shaking on an orbital shaker at RT.

RESULTS

Preparation of Crude Extract and Purification of Thermoresistant Allergens

Crude Extract Analysis

The protein concentration of the unheated extract was approximately 1 mg/ml. SDS-PAGE and Coomassie blue staining revealed a smear of proteins with some distinct bands ranging from 11 to 200 kDa (Figure 1A, lane A).

Heated Extract Analysis

After heating, few of the bands seen on SDS-PAGE of the unheated extract were visible, and only the heat-stable proteins remained in the 100°C sample. Five distinct bands of 12, 18, 24, 38, and 51 kDa could be seen on electrophoresis of the 100°C heated extract (Figure 1A, lane G). After dialysis, the protein concentration of the 100°C heated extract was approximately 200 µg/ml, equivalent to 1:5 dilution of the initial content.

Protein Purification from the Heated Extract

The contents of the 100°C-heated extract were lyophilized and the heat-stable proteins were separated by anion exchange chromatography. Four protein bands with apparent molecular weights of 12, 24, 38, and 51 kDa were eluted in the second main peak containing fractions 17-19 (Figure 1A, lane H).

Western Blotting

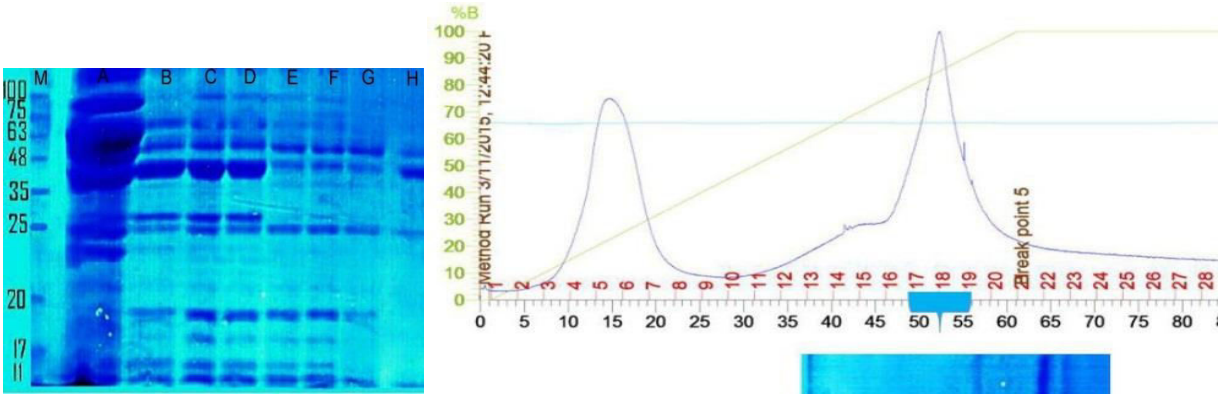
The IgE-reactive proteins of the heated extract were determined by western blotting. Proteins with apparent molecular weights of 12, 24, 38, and 51 kDa showed significant IgE-reactivity with fish-allergic patients' sera (Figure 2). Sera from 8 out of 9 patients (88%) reacted with the 12 and 51 kDa proteins. Moreover, 5 out of 9 (55.5%) and 3 out of 9 (33.3%) patients' sera reacted with the 38 and 24 kDa allergens, respectively (Figure 3).

Identification of Proteins by Mass Spectrometry

The identities of the fractionated allergens were determined by mass spectrometry. The IgE-reactive proteins by western blotting were matched with their counterparts in Coomassie blue stained polyacrylamide gels, and the selected bands were cut out of the gels for further characterization by mass spectrometry. Based on the computed results, the 12, 38, and 51 kDa proteins showed acceptable sequence coverage with parvalbumin (Table 2).

Disk ELISA

By disk ELISA, mean ODs±SD of the wells that contained patient sera and the crude extract- and parvalbumin-coated disks were 0.341±0.280 and 0.550±0.309, respectively; significantly greater than the ODs±SD from healthy individual's sera (0.060±0.036 and 0.082±0.026, respectively). The mean OD of the parvalbumin-coated disk wells was greater than that of the crude extract-coated disk wells (Figure 4).



A B

Figure 1. Purification of heat-resistant allergens of wolf herring fish.

A: SDS-PAGE of fish total and heated extracts on 15% polyacrylamide gel. M: Protein marker; Lane A: crude extract of fish muscle; Lane B-G: crude extracts of heated extracts at 50-100°C; H: elutes of the main peak (fractions 17-19). *B*: purification of wolf herring parvalbumin by DEAE anion exchange chromatography. Fractions 3-8 contained an 18 kDa protein and lesser amounts of other proteins, while fractions 17-19 contained 12, 24, 38, and 51 kDa protein bands.

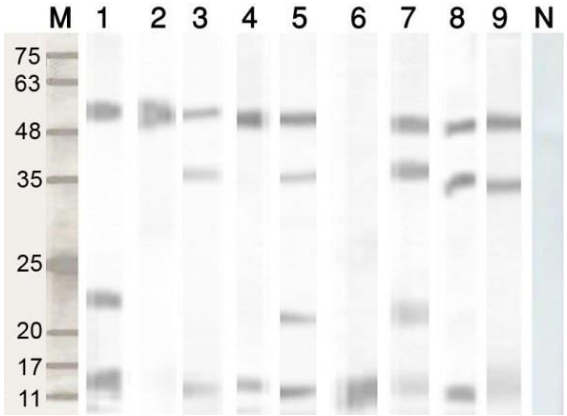


Figure 2. IgE-western blot of heated fish extract with fish-allergic patients' sera. M: protein molecular weight marker; Lane 1-9: fish extracts from unheated and heated samples; N: negative control (pooled sera of five healthy individuals).

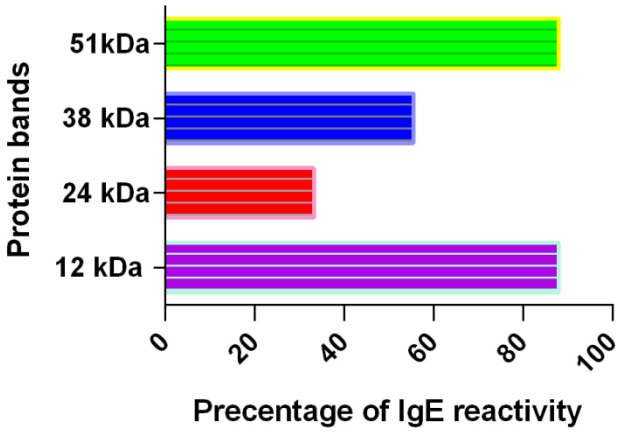


Figure 3. The IgE-reactivity percentage of 12, 24, 38, and 51 kDa protein bands. Y-axis and X-axis represent IgE-reactive protein bands and the IgE-reactivity percentage of 12, 24, and 38 kDa proteins purified from wolf herring fish with sera from fish allergic patients, respectively.

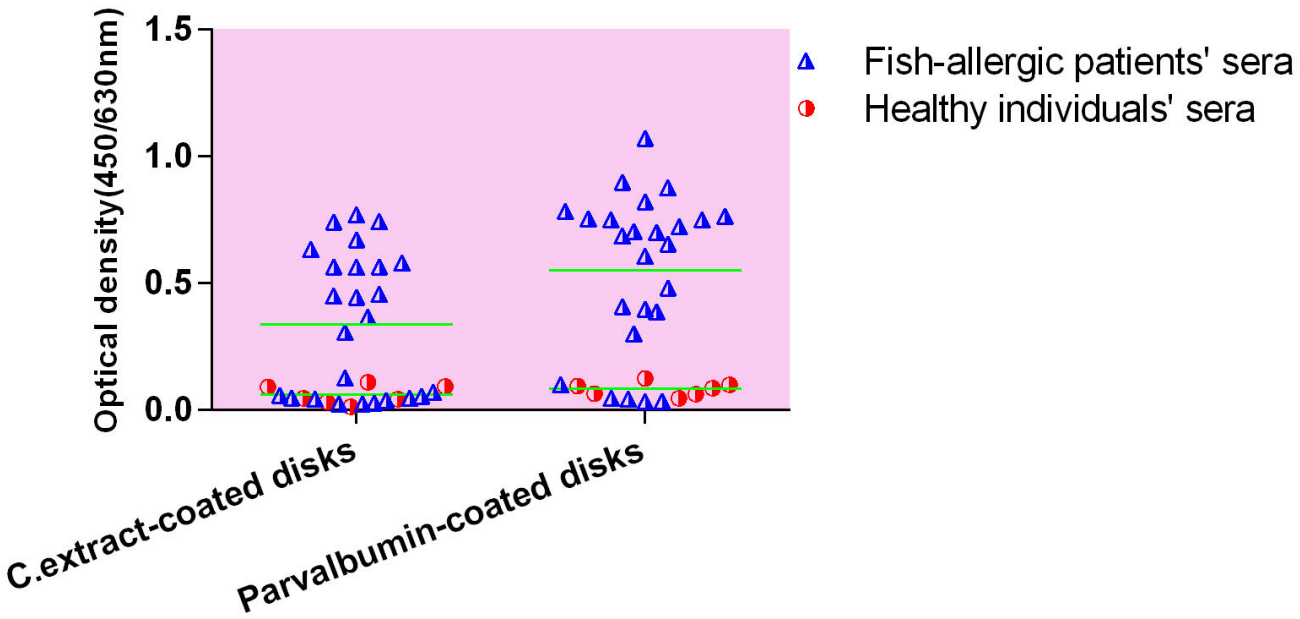


Figure 4. IgE-reactivity of oligomeric parvalbumin- and crude extract- coated disks. IgE levels to the oligomeric parvalbumin- and crude extract- coated disks in 25 fish-allergic patients' sera and 7 healthy individuals' sera. Optical densities were recorded by ELISA reader.

Table 2. MALDI-TOF-TOF results for heat-stable fish allergens

Protein bands	Protein name/ species	Protein MW(Da)/PI	Protein score	Accession key	Observed mass	Peptide sequence
12 kDa allergen	β-parvalbumin/ Stegastes partitus	11518/4.46	433	gi|657565876	1020.5386	^{48}KAFAIIDQDKS^{56}
					2168.0723	^{48}KAFAIIDQDKSGFIEEDELKL^{66}
					2371.2168	^{55}KSGFIEEDELKLFLQNFSAGARA^{77}
					1223.6759	^{64}KLFLQNFSAGARA^{77}
38 kDa allergen	β-parvalbumin/ Gadus morhua callarias	12157/4.37	68	gi|131112	2065.0120	^{88}KAGDSDGDGKIGVDEFGALVDKW^{109}
					692.59	^{88}KAGDSDGDG.K94
51 kDa allergen	β-parvalbumin/ Cyprinus carpio	11486/4.67	288	gi|131110	2057.0715	^{55}KSGFIEEDELKLFLQNFKA^{73}
					1020.5386	^{49}KAFAIIDQDKS^{57}
					2168.0723	^{49}KAFAIIDQDKSGFIEEDELKL^{67}

DISCUSSION

Improvement of presently employed laboratory methods is an essential requirement for more specific diagnosis and management of allergic patients. Here, we described disk ELISA-based diagnosis of fish allergy to discriminate between patients who were sensitive to thermoresistant allergens and those who were sensitive to thermolabile allergens.

In the present study, we characterized and identified allergic proteins from cooked wolf herring fish using serum from patients who showed allergic reactions to fish. Thermal treatment of raw fish extracts revealed that proteins of 12, 18, 24, 38, and 51 kDa, are highly thermoresistant (Figure 1). Moreover, MALDI-TOF-MS analysis revealed that three of those thermoresistant protein bands with apparent molecular weights of 12, 38, 51 kDa, are different oligomers of parvalbumin.

The comparison of the IgE-reactivity of the purified parvalbumin with the crude extract revealed that the purified parvalbumin-coated ELISA disks had greater IgE-reactivity than total extract-coated disks which could be due to parvalbumin enrichment and lack of non-allergic substances on parvalbumin-coated ELISA disks.[21,22] The application of parvalbumin may improve diagnosis and management of fish allergy in cooked-fish consumer regions due to its role as the sole allergen in heated fish and the better management of parvalbumin in fish-specific IgE tests. As expected, parvalbumin abundance and the absence and presence of unrelated proteins differs between fish species.[23] In addition, development of new in vivo and in vitro assays, based on detection by parvalbumin-specific IgE, could be a forward step in fish allergy diagnoses. It is likely that this protein can help to identify patients who are allergic to thermolabile proteins but can tolerate cooked fish, and physicians can guide them to use this nutrient-rich food source in their diets.[24,25]

Despite the increased prevalence of food allergies in recent decades, there is still considerable gaps in diagnostic methods.[26] Allergy diagnostic tests are categorized into in vivo assays, such as skin prick test, and in vitro assays. Both are based on detection of allergen-specific IgE,[26,27,28] and usually employs raw crude extract.[29] It was previously shown that food processes such as cooking may decrease allergenicity by altering allergen structure or eliminating target proteins.[30] Therefore, crude extracts are less sensitive than heated extracts for the serodiagnosis of allergies to thermostable proteins, especially in the case of cooked foods.[31] Fish allergy inducing proteins are usually categorized as either thermoresistant or thermolabile.[32] Generally, the management of food-allergic patients is based on avoidance, without consideration of patient tolerance to processed forms of the food.[33] In many previous studies parvalbumin was identified as the major allergen in various fish species.[7-12,34] Moreover, due to its thermoresistance, it was demonstrated to be the exclusive allergen in cooked fish.[12,13,23] However, in some recent studies, a 151 kDa protein was characterized as a main allergen in cooked tuna fish, and proteins with molecular weights of 42 and 51 kDa were demonstrated as major thermolabile allergens.[3] In another study collagen was identified as a

heat-resistant allergen in various fish species.[35] Therefore, scarce and contradictory data exists regarding fish allergens. The described thermolabile allergens could considerably affect the allergic manifestations in raw-fish consumers, while only heat-resistant allergens result in allergic reactions in cooked-fish consumers. In fact, the allergen content differs between raw and cooked fish.[13]

Our results agree with those of Das Dores et al., who showed that the main immunoreactive proteins could be monomeric or polymeric forms of parvalbumin.[9] In other studies, Rosmilah et al. showed that the 51 kDa protein could be enolase.[3,12]

In our study, 12 kDa and 51 kDa protein bands were more immunoreactive with fish-allergic patients' sera than 24 kDa and 38 kDa ones. We recommend that further research be undertaken to demonstrate possible oligomeric forms of parvalbumin and also molecular mechanism of oligomerization. In conclusion, our study shows that thermal treatment followed by anion exchange chromatography is a rapid, straightforward, and efficient method for purification of parvalbumin oligomeric forms which could be applied for serodiagnosis and treatment of fish allergy.

ACKNOWLEDGEMENTS

This work was supported by Grant 25863 from Tehran University of medical sciences (TUMS), Tehran, Iran. The authors thank Professor Iraj Dayhimi and Miss. Aghili from Day general hospital for providing us with the patients' sera.

REFERENCES

1. Lopata AL, Lehrer SB. New insights into seafood allergy. Curr Opin Allergy Clin Immunol 2009; 9(3):270-7.
2. Chiang WC, Kidon MI, Liew WK, Goh A, Tang JPL, Chay OM. The changing face of food hypersensitivity in an Asian community. Clin Exp Allergy 2007; 37(7) :1055-1061.
3. Rosmilah M, Shahnaz M, Meinir J, Masita A, Noormalin A, Jamaluddin M. Identification of parvalbumin and two new thermolabile major allergens of Thunnus tonggol using a proteomics approach. Int Arch Allergy Immunol 2012;162(4):299-309.
4. Das Dores S, Chopin C, Villaume C, Fleurence J, Guéant JL. A new oligomeric parvalbumin allergen of Atlantic cod (Gad mI) encoded by a gene distinct from that of Gad cI. Allergy 2002; 57(s72):79-83.
5. Adams A, Murrell K, Cross J. Parasites of fish and risks to public health. Rev Sci Tech Oie 1997; 16(2):652-60.
6. Valenta R, Twaroch T, Swoboda I. Component-Resolved Diagnosis to Optimize Allergen-Specific Immunotherapy in the Mediterranean Area. J Investig Allergol Clin Immunol 2007; 17(1):88-92
7. Swoboda I, Bugajska-Schretter A, Verdino P, Keller W, Sperr WR, Valent P, et al. Recombinant carp parvalbumin, the major cross-reactive fish allergen: a tool for diagnosis and therapy of fish allergy. J Immunol 2002; 168(9):4576-84.
8. Do V. Expression and analysis of recombinant salmon parvalbumin, the major allergen in Atlantic salmon (Salmo salar). Scand J Immunol 1999; 50(6):619-25.
9. Das Dores S, Chopin C, Villaume C, Fleurence J, Guéant JL. A new oligomeric parvalbumin allergen of Atlantic cod (Gad mI) encoded by a gene distinct from that of Gad cI. Allergy 2002; 57(s72):79-83.
10. Bugajska-Schretter A, Grote M, Vangelista L, Valent P, Sperr W, Rumpold H, et al. Purification, biochemical and immunological characterisation of a major food allergen: different immunoglobulin E recognition of the apo-and calcium-bound forms of carp parvalbumin. Gut 2000; 46(5):661-9.
11. Van Do T, Elsayed S, Florvaag E, Hordvik I, Endresen C. Allergy to fish parvalbumins: studies on the cross-reactivity of allergens from 9 commonly consumed fish. J Allergy Clin Immunol 2005; 116(6):1314-20.
12. Rosmilah M, Shahnaz M, Masita A, Noormalin A and Jamaludin M. Identification of major allergens of two species of localsnappers: Lutjanus argentimaculatus (merah/ redsnapper) and Lutjanus johnii (jenahak/ golden snapper). Trop Biomed 2005; 22(2):171-7.
13. Arif SH, Hasnain A-u. A major cross-reactive fish allergen with exceptional stability: parvalbumin. Afr J Food Sci 2010; 4(3):109-14.
14. Tomm JM, Van Do T, Jende C, Simon JC, Treudler R, von Bergen M, Averbeck M. Identification of New Potential Allergens from Nile Perch (Lates niloticus) and Cod (Gadus morhua). J Investig Allergol Clin Immunol 2013; 23(3):159-67.
15. Das Dores S, Chopin C, Villaume C, Fleurence J, Gueant JL. IgE-binding and cross-reactivity of a new 41kDa allergen of codfish. *Allergy* 2002; 57 (72):79-83.

16. Bernhisel-Broadbent J, Strause D, Sampson HA. Fish hypersensitivity: clinical prevalence of altered fish allergenicity caused by various preparation methods. J Allergy Clin Immunol 1992; 90:622-9.
17. Whitehead PJP, Nelson GJ, Wongratana T. FAO species catalogue: an annotated and illustrated catalogue of the Herrings, Sardines, Pilchards, Sprats, Shads, Anchovies and Wolf-Herrings. 1988 FAO species catalogue, Vol 7, Clupeoid fishes of the world; prepared by Peter J.P. Whitehead**,** British Museum (Natural History), Cromwell Road, London SW7 5BD, UK.
18. Soukhtanloo M, Falak R, Sankian M, Varasteh A R. Generation and Characterization of anti-chitinase monoclonal antibodies. Hybridoma 2011; 30:145-51.
19. Falak R, Sankian M, Noorbakhsh R, Tehrani M, Assarehzadegan M A, Jabbari Azad F , Abolhasani A, Varasteh AR. Identification and characterisation of main allergic proteins in Vitis vinifera vitis. Food and Agricultural Immunology 2013; 24(3):255-68.
20. Pourpak Z, Mostafaie A, Hasan Z, Kardar GA, Mahmoudi M. A laboratory method for purification of major cow's milk allergens. J Immunoassay Immunochem 2004; 25(4):385-97.
21. Coughlin DK, Solomon S, Wilwert JL. Parvalbumin expression in trout swimming muscle correlates with relaxation rate. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 2007; 14:1074-82.
22. Esch RE. Allergen source materials and quality control of allergenic extracts. Methods 1997; 13(1):2-13.
23. Coughlin DJ, Solomon S, Wilwert JL. Parvalbumin expression in trout swimming muscle correlates with relaxation rate." Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 2007; 147(4): 1074-82.
24. Lewit BA, Réty S. EF-hand calcium-binding proteins. Current opinion in structural biology 2000; 10(6):637-43.
25. Griesmeier U, Vázquez-Cortés S, Bublin M, Radauer C, Ma Y, Briza P, et al. Expression levels of parvalbumins determine allergenicity of fish species. Allergy 2010; 65(2):191-8.
26. Andrew WK, De Schryver S, Mill J, Mill C, Dery A. Diagnosis and management of food allergies: new and emerging options: a systematic review. J Asthma Allergy 2013; 7:141-64.
27. Bellini F, Ricci G, Dondi A, Piccinno V, Angelini F, Pession A. End point prick test: could this new test be used to predict the outcome of oral food challenge in children with cow's milk allergy? Ital J Pediatr 2011; 37:52.
28. Mehl A, Niggemann B, Keil T, Wahn U, Beyer K. Skin prick test and specific serum IgE in the diagnostic evaluation of suspected cow's milk and hen's egg allergy in children: does one replace the other? Clin Exp Allergy 2012; 42(8):1266–72.
29. Turnbull JL, Adams HN, Gorard DA. Review article: the diagnosis and management of food allergy and food intolerances. Aliment Pharmacol Ther 2015; 41(1):3-25.
30. Clark AT, Skypala I, Leech SC, Ewan PW, Dugué P, Brathwaite N, Huber PAJ, Nasser SM. British society for allergy and clinical immunology guidelines for the management of egg allergy. Clin Exp Allergy 2010; 40:1116–29.
31. Valenta R, Twaroch T, Swoboda .Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area. J Investig Allergol Clin Immunol 2007; 17(1): 36-40.
32. Arif SH, Jabeen M, Hasnan AU. Biochemical characterization and thermostable capacity of parvalbumin: the major fish-food allergens. J food biochem 2007; 31(1):121-37.
33. Allen CW, Campbell DE, Kemp AS. Food allergy: Is strict avoidance the only answer? Pediat Allergy Immunol 2008; 20:415–22.
34. Rosmilah M, Shahnaz M, Meinir J, Graham T, Dinah R, Masita A, et al. Identification of the Major Allergens of Indian Scad (Decapterus russelli). Asian Pac J Allergy Immunol 2008; 26:191-8.
35. Maki K, Hiroyuki T, Yuki H, Yuji N, Kazuo S. New extraction method suitable for immunoblotting analysis of fish allergens. Eur Food Res Technol 2011; 233:991-7.