Sensitization profile to related animal proteins (crocodile, frog, and chicken) among fish-allergic patients

Haroun-Díaz E[1], Blanca-López N[1], Martín-Pedraza L[1], Ruano FJ[1], Somoza ML[1], Vázquez de la Torre M[1], Pérez-Alzate D[1], López-González P[1], Prieto-Moreno A[1], Bartolomé B[2], Blanca M[1], Canto G[1]

[1]Allergy Service, Infanta Leonor University Hospital, Madrid, Spain.

[2]R+D Department, Roxall, 48170 Bilbao, Spain.

Correspondence:

Elisa Haroun-Díaz

E-mail address: elisaharoun@hotmail.com

Accepted Article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0705

© 2021 Esmon Publicidad

Key words: Fish allergy, Crocodile, Frog, Chicken, Cross reactivity.

Palabras clave: Alergia a pescado, Cocodrilo, Rana, Pollo, Reactividad cruzada.

Accepted Article

Fish allergy is one of the most common food allergies, particularly in children [1, 2]. Parvalbumins are major allergens present in the white muscle of lower vertebrates, and cod parvalbumin (Gad c 1) was the first parvalbumin studied [3]. Tropomyosin, collagen, aldehyde-phosphate-dehydrogenase, enolase and aldolase are other fish allergens [1, 4]. Cross-reactivity among fish parvalbumins, due to their high amino-acid sequence identity, is responsible for allergy to different fish species [5]. However, some patients have selective specific IgE antibody (sIgE) reactivity to single parvalbumins and develop symptoms to a particular species [6-8].

Cross-reactivity between fish and chicken due to parvalbumin and other allergens has been reported [9]. Published findings describe allergy to fish and other animals such as frog [10], chicken [9, 11] and crocodile [12], and to crocodile and chicken [13], also due to parvalbumins cross-reactivity. Two crocodile parvalbumins (*nCro p 1, Cro p 2)* have been identified (WHO/IUIS; www.allergen.org).

We aimed to study the pattern of IgE-reactivity in extracts from crocodile, frog, and chicken with sera from fish-allergic patients.

© 2021 Esmon Publicidad
doi: 10.18176/jiaci.0705

Subjects with a clear history of fish allergy, positive skin prick test (SPT), and/or positive sIgE to fish extract were evaluated. Oral challenge was performed in case of negative SPT and/or sIgE [14].

Crocodile, frog, cod and chicken were purchased fresh and extracts were prepared from raw and cooked foods as described [9]. SPT was performed with raw and boiled crocodile extract (10 mg/ml) and 7 fish extracts (*Roxall, Spain®).* Prick-by-prick were performed with raw and boiled crocodile, frog and chicken meats. Non-atopic patients were included as negative controls.

We included 27 patients with fish allergy; their median age was 8 years (2–46, IQR=5–17), being 18 male. In 59.2%, hake was the culprit allergy, most frequently triggering urticaria (48.1%). Three cases did not remember the species involved. Two also had allergy to chicken (12 and 22) and one to crocodile (9). Atopic dermatitis and rhino-conjunctivitis were present in 55.5%, 40.7% presented asthma, 74% nut allergy, and 44.4% shellfish allergy.

Results of SPT to commercial fish extracts and specific IgE (*Siemens Immulite 2000/Xpi®;* Enzyme AllergoSorbent Test (EAST)) to different fish species are shown in Tables E1 and E2, (Online Repository).

SDS-PAGE IgE-immunoblotting using the sera of all patients and raw food extracts was carried out as described [12], detecting a low-molecular-mass IgE-binding protein of ~12 and/or 15-kDa in cod, crocodile, frog, and chicken extracts, revealing a similar molecular mass to those detected by a rabbit anti-cod parvalbumin serum (Figures E1 and E2, Online Repository). Twenty-five sera samples recognized a protein of ~12-kDa in cod extract (Figure 1A, Online Repository). Eighteen and 14 samples detected both proteins

Accepted Article

© 2021 Esmon Publicidad

of 12 and 15-kDa in crocodile and frog extracts, and only the 12-kDa band was detected with 2 and 4 sera in crocodile and frog extracts, respectively (Figure E1B,C Online Repository).

A different IgE pattern was observed with chicken meat (Figure E1D, Online Repository). In 2 cases (12 and 22), the 12 and 15-kDa bands were detected, and the 15-kDa band was recognized in 14 cases. Other higher molecular weight bands were recognized in all meat extracts. Some of these are parvalbumin aggregates as could be observed in Figure 2, online repository, being reported elsewhere [12].

Patient 24 recognized a ~50-kDa IgE-binding band in crocodile, frog and chicken that was not detected in cod extract. Patients 12, 15, 22 and 25 detected an ~18-kDa protein in cod extract, and in patient 15 the band was recognized in crocodile, frog and chicken extract.

Pools of sera selected with high IgE levels were used to carried out the immunoblotting assays with cooked cod extract (pool of sera: 3, 7, 8, 9, 13, 20, 21), cooked frog extract (pool of sera: 3, 10, 13, 14, 17, 18, 21), cooked crocodile (pool of sera: 3, 9, 10, 12, 13, 14, 16, 17, 19, 21) and cooked chicken (pool of sera: 5, 9, 10, 13, 14, 17, 18). SDS-PAGE immunoblotting with cooked food extracts revealed the same bands profiles between ~16 and 12-kDa with pools of sera and rabbit anti-cod parvalbumin serum: a band of ~12-kDa in cod, a couple of bands of ~16 and 12-kDa in frog, three bands of 16, 13and 12-kDa in crocodile, and a band of 15-kDa in chicken (Figure E3, Online Repository).

SDS-PAGE Immunoblotting-inhibition with the described pools of sera and cooked frog, chicken and crocodile extracts showed a total IgE-binding inhibition with cooked cod extract confirming the existence of cross-reactivity (Figure E4, Online Repository).

Accepted Article

© 2021 Esmon Publicidad

We present a group of subjects allergic to fish, some of whom also developed allergy to crocodile and chicken, with a variable IgE reactivity allergen recognition.

On immunoblotting assay, most sera detected IgE-reactive protein bands of 12 and/or 15-kDa. The band of 12-kDa was the most frequently detected in cod extract, and in crocodile, frog and chicken extracts bands of 12 and/or 15-kDa.; these may correspond to beta and alpha parvalbumin as indicated by the rabbit anti-cod-parvalbumin results. In addition, bands of 24, 31 and 50-kDa were recognized in cod, crocodile, frog and chicken extracts by most patient sera.

Parvalbumin can be detected as a 12-kDa monomer, dimer (24-kDa), trimer (36-kDa), or polypeptide of more than 40-kDa, showing remarkable IgE-reactivity [12]. In our study, the same band profile found in these extracts was observed with a cod parvalbumin-rabbit-antiserum, revealing the parvalbumin origin of the bands observed, including parvalbumin monomer and aggregates (Figure E2, Online repository).

Other bands of higher molecular mass (~18 and 50-kDa) were detected in all meat extracts. The protein of ~50-kDa may belong to the family of enolases, described as allergens in fish and chicken meat [9]. All these proteins could play an important role in the induction of allergic reactions in these patients.

Summarizing, crocodile and frog should be considered relevant sources of animal proteins that can act as allergens, having clinical relevance due to their *in vitro* cross-reactivity and potentially involving other wild exotic foods. Further research is needed to identify and characterize other new allergens.

Accepted Article

© 2021 Esmon Publicidad

FINANCIAL DISCLOSURE:

This work was not supported by any funding source. All authors declare that they don´t have any commercial and/or organizational associations.

CONFLICTS OF INTEREST: All authors declare that there is no conflict of interest.

Accepted Article

REFERENCES

1. Kourani E, Corazza F, Michel O, Doyen V. What Do We Know About Fish Allergy at the End of the Decade? J Investig Allergol Clin Immunol. 2019;29(6):414-21.

2. Grabenhenrich LB, Dölle S, Moneret-Vautrin A, Köhli A, Lange L, Spindler T, et al. Anaphylaxis in children and adolescents: The European Anaphylaxis Registry. J Allergy Clin Immunol. 2016;137(4):1128-37.e1.

3. Van Do T, Hordvik I, Endresen C, Elsayed S. Characterization of parvalbumin, the major allergen in Alaska pollack, and comparison with codfish Allergen M. Molecular immunology. 2005;42(3):345-53.

4. Kuehn A, Hilger C, Lehners-Weber C, Codreanu-Morel F, Morisset M, Metz-Favre C, et al. Identification of enolases and aldolases as important fish allergens in cod, salmon and tuna: component resolved diagnosis using parvalbumin and the new allergens. Clin Exp Allergy. 2013;43(7):811-22.

© 2021 Esmon Publicidad
doi: 10.18176/jiaci.0705

5. Carvalho S, Marcelino J, Cabral Duarte MF, Costa C, Barbosa MA, Pereira Dos Santos MC. Role of Recombinant Parvalbumin Gad c 1 in the Diagnosis and Prognosis of Fish Allergy. J Investig Allergol Clin Immunol. 2020;30(5):340-5.

6. Asero R, Mistrello G, Roncarolo D, Casarini M, Falagiani P. True monosensitivity to a tropical sole. Allergy. 1999;54(11):1228-9.

7. Ebo DG, Kuehn A, Bridts CH, Hilger C, Hentges F, Stevens WJ. Monosensitivity to pangasius and tilapia caused by allergens other than parvalbumin. J Investig Allergol Clin Immunol. 2010;20(1):84-8.

8. Kuehn A, Hutt-Kempf E, Hilger C, Hentges F. Clinical monosensitivity to salmonid fish linked to specific IgE-epitopes on salmon and trout beta-parvalbumins. Allergy. 2011;66(2):299-301.

9. Kuehn A, Codreanu-Morel F, Lehners-Weber C, Doyen V, Gomez-Andre SA, Bienvenu F, et al. Cross-reactivity to fish and chicken meat - a new clinical syndrome. Allergy. 2016;71(12):1772-81.

10. Hilger C, Thill L, Grigioni F, Lehners C, Falagiani P, Ferrara A, et al. IgE antibodies of fish allergic patients cross-react with frog parvalbumin. Allergy. 2004;59(6):653-60.

11. Gonzalez-de-Olano D, Bartolome B, Maroto AS, Vivanco F, Pastor-Vargas C. Asthma after chicken consumption due to cross-reactivity between fish and chicken parvalbumin. J Investig Allergol Clin Immunol. 2012;22(3):227-8.

12. Haroun-Diaz E, Blanca-Lopez N, Vazquez de la Torre M, Ruano FJ, Somoza Alvarez ML, Labrador Horrillo M, et al. Severe anaphylaxis due to crocodile-meat allergy exhibiting wide cross-reactivity with fish allergens. J Allergy Clin Immunol Pract. 2017.

Accepted Article

© 2021 Esmon Publicidad
doi: 10.18176/jiaci.0705

13. Ballardini N, Nopp A, Hamsten C, Vetander M, Melen E, Nilsson C, et al. Anaphylactic Reactions to Novel Foods: Case Report of a Child With Severe Crocodile Meat Allergy. Pediatrics. 2017.

14. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. Allergy. 2014;69(8):1008-25.

Accepted Article

© 2021 Esmon Publicidad
doi: 10.18176/jiaci.0705

Table 1. Skin test and specific IgE to crocodile, frog, and chicken extracts data (grouped in percentages)

	CROCODILE					FROG			CHICKEN		
	Prick test		Prick-prick		EAST* kU_A/L	Prick-prick		EAST kU_A/L	Prick-prick		CAP* kU_A/L
%	**RCrme**	**BCrme**	**RCrPP**	**BCrPP**	**Cr**	**RFPP**	**BFPP**	**F**	**RChPP**	**BChPP**	**Ch**
Positive	78.2	66.6	80.7	61.5	85.2	69.5	59	77.7	78.2	40.9	64
Negative	21.8	33.4	19.3	38.5	14.8	30.5	41	22.3	21.8	59.1	36

Cr**:** crocodile**;** F: frog**;** Ch**:** chicken; RCrme: raw crocodile meat extract; BCrme: boiled crocodile meat extract; RCrPP: raw crocodile prick-prick; BCrPP: boiled crocodile prick-prick; RFPP: raw frog prick-prick; BFPP: boiled frog prick-prick; RChPP: raw chicken prick-prick; BChPP: boiled chicken prick-prick; ND: not done.

* Positive values were those ≥0.1 kU_A/L (*Siemens Immulite 2000/Xpi*®)), and ≥0.35 kU_A/L (EAST)

Accepted Article

© 2021 Esmon Publicidad
doi: 10.18176/jiaci.0705