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Food allergy to shrimps and fish in patients suffering from atopic dermatitis, the results of ISAC Multiplex examination

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ABSTRACT

: The aim of this study is the evaluation of the occurrence of fish/shrimp allergy in atopic dermatitis patients. The occurrence of fish/shrimp allergy was confirmed in the challenge test or according to the history; the sensitisation to molecular components with ISAC Multiplex testing was analysed. Altogether 100 atopic dermatitis patients were examined. The food allergy to fish was confirmed in 13 patients (13%), while shrimp allergy occurred in 6 patients (6%). In patients with allergy to fish, the significantly higher occurrence of sensitisation to Asp f 1, Asp f 6, Alt a 6, Der f 1, Der f 2, Der p 1, Der p 2, Lep d 2 and Blo t 5 was confirmed. In patients with food allergy to shrimps, the significantly higher occurrence of sensitisation to Asp f 6, Alt a 6, Cla h 8, Bla g 1 and Bla g 5 was confirmed also.

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Introduction

Atopic dermatitis (AD) is a chronic intermittent inflammatory skin disease that may be in some cases associated with food allergies. Other numerous trigger factors for AD have been identified, including inhalable respiratory allergens, irritative substances and infectious microorganisms, such as *Staphylococcus aureus*. Psychogenic and climatic factors may also cause exacerbation of AD (Matricardi et al., 2016). Food allergy was considered to be an important factor in exacerbation of AD in only a subset of children (and a much smaller subset of adults) where non-IgE mediated mechanisms are sometimes implicated in AD flare-ups associated with ingestion of the foods in question (Matricardi et al., 2016). In patients with suspected food allergy, that is thought to be aggravating AD, it is therefore important that any food avoidance measures suggested are based on a detailed history and supportive evidence from objective allergy tests. Generally, three different clinical patterns are described after ingesting of food allergens: (a) immediate type symptoms such as erythema, angioedema, urticaria, but no eczema, bronchial,

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gastrointestinal or cardiovascular symptoms of anaphylaxis, which appear within minutes up to two hours after ingesting the relevant food; (b) isolated, eczematous, late-type reactions, which normally appear as flare-ups of typical eczematous lesions occurring within hours to two days after ingestion and (c) combined reaction pattern of non-eczematous, immediate type reactions and eczematous, late-type reactions (Matricardi et al., 2016). *As animal – derived allergens among the eight most common food allergens, fish, egg, milk and shellfish are the chief allergens in food allergy* (Flammarion et al., 2011; Matricardi et al., 2016; Soares-Weiser et al., 2014). As fish is both an important food component and a potent source of food allergens, fish has been included in the European Union regulation of food labeling (Kremmyda et al., 2011; Taylor & Baumert, 2015).

Fish allergy is generally IgE-mediated, and affected patients present with immediate clinical signs, which are usually severe. The classic clinical signs include oral allergy syndrome, cutaneous symptoms (diffuse urticaria, angioedema), gastrointestinal manifestations (abdominal pain, diarrhea, and sudden-onset vomiting), respiratory symptoms (rhinitis, asthma), and, in the most severe cases, anaphylactic shock. Respiratory symptoms can occur due to inhalation of vapor while cooking fish (Kremmyda et al., 2011; Sharp & Lopata, 2014; Taylor & Baumert, 2015). Non – IgE-mediated reactions have mainly been reported in pediatric cases as food protein-induced enterocolitis syndrome (FPIES) (Sharp & Lopata, 2014). Despite the broad biodiversity among fishes, the most frequently consumed species belong to a limited number of orders. Fish allergens have been described in around 40 species but detailed analysis of the allergy-eliciting molecules was performed mainly for fish, which are commonly consumed in Europe such as carp, cod, salmon, trout, and tuna (Sharp & Lopata, 2014). Food processing does not seem to affect the allergenic potency of the fish but rather the allergen content which varies in different species (Kuehn et al., 2010). An increasing number of studies reports on IgE-mediated allergy which is caused by the parasite *Anisakis simplex* found in contaminated raw or undercooked fish. *Anisakis* is a parasitic nematode which mainly infects fish, but has been found in fish or shellfish and can induce severe allergic reaction in fish or shellfish (Nieuwenhuizen & Lopata, 2014). Parvalbumins are the major fish allergens, other fish allergens present in the muscle are enolases and aldolases. A strong and clinically relevant crossreactivity seems to exist between parvalbumins of different fish species. Major and relevant minor fish allergenic molecules are recorded in Table 1 (Griesmeier et al., 2010; Kuehn et al., 2013; Radauer et al., 2008; Sicherer et al., 2004; Worm et al., 2015). Shellfish (shrimps), along with fish are commonly termed as seafood, but these two groups are very distinct in evolutionary terms and contain different molecular repertoires of food allergens. All shellfish species are invertebrate animals, in comparison with fish, which are lower vertebrates. Comparing evolutionary distance, crustaceans are placed closer to insects and arachnids, and this seems to be the major factor for molecular cross-sensitization and clinical cross-reactivity between crustacean, house dust mites, and insects (Chokshi et al., 2015; Kamath et al., 2014a; Yu et al., 2011). In general, 2% of the population are affected by food allergy to shellfish, including the crustacean and mollusk groups. Shellfish allergy, particularly to prawns, has one of the highest rates of food-induced anaphylaxis with nearly 42% among affected adults and 12% in children (Chokshi et al., 2015). The tropomyosin family represent the major heatstable allergen present in all shellfish species, and may

Table 1. Major and relevant minor fish allergenic molecules (Griesmeier et al., 2010; Kuehn et al., 2013; Matricardi et al., 2016; Radauer et al., 2008; Sicherer et al., 2004; Worm et al., 2015). Molecular components in ISAC Multiplex testing are shown extra bold*.

	Allergen	Molecular component	Biochemical name Protein family
Clupeiformes	Herring (<i>Clupea harengus</i>)	Clu h 1	Parvalbumin
	Pilchard (<i>Sardinops sagax</i>)	Sar sa 1	Parvalbumin
Cypriniformes	Carp (<i>Cyprinus carpio</i>)	Cyp c 1	Parvalbumin
Gadiformes	Atlantic cod (<i>Gadus callarias</i>)	Gad c 1*	Parvalbumin
	Atlantic cod (<i>Gadus morhua</i>)	Gad m 1	Parvalbumin
		Gad m 2	Enolase
		Gad m 3	Aldolase
Perciformes	Tuna (<i>Thunnus albacares</i>)	Thu a 1	Parvalbumin
		Thu a 2	Enolase
		Thu a 3	Aldolase
Pleuronectiformes	Megrim, whiff (<i>Lepidorhombus whiffiagonis</i>)	Lep w 1	Parvalbumin
Salmoniformes	Pacific salmon (<i>Oncorhynchus keta</i>)	Onc k 5	Vitellogenin
	Rainbow trout (<i>Oncorhynchus mykiss</i>)	Onc m 1	Parvalbumin
	Salmon (<i>Salmo salar</i>)	Sal s 1	Parvalbumin
		Sal s 2	Enolase
		Sal s 3	Aldolase
Scorpaeniformes	Redfish (<i>Sebastes marinus</i>)	Seb m 1	Parvalbumin

constitute up to 20% of the total protein content (Koeberl et al., 2014). In the seafood industry, workers are constantly exposed to airborne shellfish particulate matter arising from different processing activities that results in the inhalation of airborne allergens and cooking fumes (Kamath et al., 2014b; Lopata & Jeebhay, 2013). The foodborne parasite *Anisakis* or herring worm is an important food allergen also (Nieuwenhuizen & Lopata, 2013). More importantly, the tropomyosin family of allergens are thought to be primarily responsible for cross-reactivity between *Anisakis* and other invertebrates such as insects, mites, and crustaceans. However, it is difficult to estimate the prevalence data of *Anisakis* and shellfish cross-reactivity due to the lack of population-based studies. At least 6 shellfish allergens have been identified displaying mainly metabolic or structural functions. The major allergen, tropomyosin was first identified in 1993 as the major shrimps allergen (Shanti et al., 1993). Subsequently, arginine kinase (Yu et al., 2003), myosin light chain (Ayuso et al., 2008), and sarcoplasmic calcium-binding protein (Fernandes et al., 2015) were identified as minor crustacean allergens. Previous sensitization rates were mainly based on skin or IgE testing to whole shellfish extracts (Abramovitch et al., 2013; Osborne et al., 2011; Pascal et al., 2015; Rona et al., 2007; Sicherer et al., 2004; Vidal et al., 2015). Allergenic molecules present in crustacean species are shown in Table 2. Registered allergen names are stated in accordance with WHO/IUIS Allergen Nomenclature (Matricardi et al., 2016). There are only rare studies investigating the sensitisation to molecular components of fish/ shrimp allergy in atopic dermatitis patients.

The initial laboratory approach in the diagnosis of allergies (such as atopic dermatitis, rhinitis and wheezing disorders) is to detect the type of allergic reaction, *i.e.* whether the patient's allergy is mediated by immunoglobulin E (IgE) or not. Using traditional forms of allergen-extract-based diagnosis such as skin testing (*i.e.* skin prick testing, atopy patch testing), it is often challenging and sometimes impossible to identify the disease-triggering allergens.

Progress in laboratory diagnostics of IgE-mediated allergy is the use of component-resolved diagnosis (CRD) or molecular diagnosis of allergies. The CRD approach has been developed when highly purified or recombinant allergen molecules have become

Table 2. Allergenic molecules present in crustacean species. Registered allergen names are stated in accordance with WHO/UIIS Allergen Nomenclature (Matricardi et al., 2016). Molecular components in ISAC Multiplex testing are shown extra bold*.

Molecular components	Biochemical name Protein family
Cha f 1, Cra c 1, Hom a 1, Lit v 1	Tropomyosin
Mel l 1, Met e 1, Pan b 1, Pen m 1* , Por p 1, Cra c 2, Lit v 2, Pen m 2*	Arginin kinase
Hom a 3, Lit v 3, Pen m 3	Myosin light chain
Cra c 4, Lit v 4, Pen m 4* , Pon l 4	Sarcoplasmic calcium-binding protein
Cra c 6, Horn a 6, Pen m 6	Troponin C
Arc s 8, Cra c 8	Triose Phosphate isomerase

available. These molecules are the allergenic proteins toward which the specific and clinically relevant IgE immune response is directed (Matricardi et al., 2016; Melioli et al., 2011; van Hage et al., 2017). Molecular allergy diagnosis using singleplex allergens or multiplex allergen microarrays are typical methods of precision medicine (Matricardi et al., 2016) and they enhance the specificity of IgE-diagnosis in polysensitized respiratory allergies, can be applied in food allergies and atopic dermatitis (Hanifin & Rajka, 1980) and may even reveal unexplained anaphylaxis (Matricardi et al., 2016). ImmunoCAP ISAC is an in vitro assay for the measurement of allergen specific IgE antibodies in human plasma (Melioli et al., 2011; van Hage et al., 2017). It is intended to aid in the diagnosis of IgE-mediated allergic disorders (Melioli et al., 2011; van Hage et al., 2017). ImmunoCAP ISAC (Thermo Fisher), based on 112 different molecular components (both extracted and recombinant), is the most studied and most frequently used molecular diagnostic tool based on a microarray (Melioli et al., 2011; van Hage et al., 2017). The major advantage of ISAC is the comprehensive IgE pattern obtained with a minute amount of serum (Melioli et al., 2011).

Aim

The aim of our study is to show the occurrence of fish/shrimp allergy in atopic dermatitis patients, to evaluate the sensitivity of ISAC Multiplex testing and to analyse the results of ISAC Multiplex testing. We evaluate also, if there is a significant relation between the occurrence of fish/shrimp allergy and the sensitisation to molecular components of *Alternaria* Alt a 1 (Acidic glycoprotein), Alt a 6 (Enolasa), molecular components of *Aspergillus* such as Asp f 1 (Mitogillin family), Asp f 3 (Peroxisomal protein), Asp f 6 (Mn superoxide dismutase), molecular component of *Cladosporium* Cla h 8 (Mannitol dehydrogenase), molecular components of House dust mites Der f 1 (Cysteine protease), Der f 2 (NPC2 protease), Der p 1 (Cysteine protease), Der p 2 (NPC2 family), Der p 10 (Tropomyosin), molecular components of Cockroach such as Bla g 1 (Cockroach group 1), Bla g 2 (Aspartic protease), Bla g 5 (Glutathione S-transferase), Bla g 7 (Tropomyosin), and Lep d 2 (Storage mite, NPC2 family), Blo t 5 (Mite group 5).

Method

Patients and methods

In the period of 2018–2019, 100 patients suffering from atopic dermatitis at the age of 14 years and older were examined. All these patients were examined in the Department of

Dermatology, Faculty Hospital Hradec Králové, Charles University, Czech Republic. The diagnosis of atopic dermatitis was made with the Hanifin-Rajka criteria (Hanifin & Rajka, 1980). Exclusion criteria were long term therapy with cyclosporin or systemic corticoids, pregnancy, breastfeeding. Patients with atopic dermatitis having other systemic diseases were excluded from the study as well. Complete dermatological and allergological examination was performed in patients included in the study. This study was approved by Ethics committee of Faculty Hospital Hradec Králové, Charles University in Prague, Czech Republic.

Examination of specific IgE to molecular components

The sIgE level of the serum was measured by the component-resolved diagnosis microarray-based sIgE detection assay ImmunoCAP ISAC (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). ImmunoCAP ISAC is a solid-phase multiple immunoassay which enables to determine 112 different components from 51 allergen sources (Melioli et al., 2011; van Hage et al., 2017). The allergens are applied in triplicates to ensure the test reproducibility. The specific IgE values are presented in arbitrary units called ISAC Standardized Units (measuring range of 0.3 – 100 ISU-E). The level of specific IgE higher than 0.3 ISU-E was assessed as positive. The level of molecular components in ISU – E was evaluated: < 0.3 – negative, 0.3–0.9 low positivity, 0.9–15 moderate positivity, above 15 ISU – E very high positivity (Melioli et al., 2011; van Hage et al., 2017).

Confirmation of food allergy

The fish/shrimp allergy was confirmed in these cases: (1) In patients with the positive result in the open exposure test (early and/or late reactions) with examined food allergens. (2) In patients with the clinical allergic reaction after the ingestion of fish and/or shrimps in their history; the open exposure test was not performed in these patients because of anaphylactic reaction danger. (3) Sensitisation to examined food allergens was confirmed in patients with positive results in ISAC Multiplex testing and with the negative results in the open exposure test or history.

The elimination diet

In patients without previous history about fish/shrimp allergy and with the positive results in ISAC Multiplex testing we recommended the elimination diet with the elimination of suspected food allergens for the period of 5 weeks (Čelakovská et al., 2015a, 2015b).

Open exposure test

Open exposure test was performed after this diet in intervals without symptoms or during a consistent period with regard to atopic dermatitis (not in pollen season in patients with pollen allergy). In generally, the open exposure test (OET) was recommended to performe with three doses of examined foods given during two days. One dose of examined food was given in incremental dosages in 10 min intervals

during one hour. The food challenge results was scored as positive if one or more of the following objective and subjective clinical reactions were noted: itching, rash, urticaria, angioedema, vomiting, wheezing, abdominal pain, diarrhea, pruritus, or worsening of atopic dermatitis. Early reactions were defined as clinical symptoms within 2 h after the ingestion of food and late symptoms if occurring after more than 2 h. In the case the physician or the patient recorded worsening of the atopic dermatitis or some other reactions during the open exposure test, the patient continued in the elimination of suspected food and the severity of atopic dermatitis was evaluated every 3 month during 1 year. If the open exposure test was negative, the patient introduced this food in the diet regimen. The severity of atopic dermatitis was evaluated during the ingestion of food over a period of 3 months during 1 year. If there is a suspicion to food allergy to more than one food, the next open exposure test was performed in 2–3 weeks after the first exposure test (Čelakovská et al., 2015a, 2015b).

Statistical analysis

The comparison of the results in the examination of ISAC Multiplex testing and really confirmed fish/shrimp allergy in the open exposure (patient 's history) test was performed; according to these results, we calculated the sensitivity of the ISAC Multiplex testing.

Sensitivity was calculated as proportion of positive IgE antibody tests in patients with allergic reaction. We tested if there is the significant relation between the really confirmed fish/shrimp allergy and the sensitisation to some molecular components of *Alternaria*, *Aspergillus*, *Cladosporium*, molecular components of House dust mites and molecular components of Cockroach. Such a table was evaluated by the usual Chi – squared test. The *p* – value is displayed in the right hand column. Unfortunately, zeroes appeared in some of the tables and one has to be careful in making conclusions.

Results

Patients

One-hundred patients suffering from atopic dermatitis were included in the study – 48 men and 52 women with the average age 40.9 years and with the average SCORAD 39 points (s.d.13.1 points). The mild form of AD was recorded in 14 patients (14%), moderate form in 58 patients (58%), severe form in 28 patients (28%); 55 patients (55%) suffer from asthma bronchiale and 78 patients (78%) suffer from rhinitis. The characteristics of patients is shown in Table 3. ISAC Multiplex examination was performed in all included patients. The highest observed sensitization rate was 61.0% to grass specific molecule Phl p 1, the second most frequent sensitization was 57. 0% to Betulaceae-specific molecule Bet v 1. Frequently observed sensitizations were those to PR-10 proteins, NPC2 proteins family, Uteroglobulin and Lipocalin. *The food allergy to fish was confirmed in 13 patients (13%), while shrimp allergy occurred in 6 patients (6%).* The results of the open exposure test (history) are shown in Table 4; gastrointestinal symptoms (vomiting), pruritus, and worsening of AD were recorded in patients suffering from fish/shrimp allergy. In 9 patients (9%) with fish allergy, gastrointestinal symptoms such as abdominal pain, diarrhea,

Table 3. The characteristic of patients.

- 100 patients examined – 48 men, 52 women, the average age 40.9 years
- the average SCORAD 39 s.d. 13.1 points
- the positive results in sensitisation to molecular components – 93 patients (93%)
- mild form of AD patients 14 (14%)
- moderate form of AD patients 58 (58%)
- severe form of AD patients 28 (28%)
- number of patients with asthma bronchiale – 55 (55%)
- number of patients with allergic rhinitis – 78 (78%)

Table 4. The results of open exposure test (or history) with the symptoms of food allergy (pruritus, gastrointestinal symptoms, worsening of atopic dermatitis (AD) 100 patients = 100%).

	Clinical symptoms of fish/shrimp allergy			
	Number of patients with confirmed FA	GIT symptoms	Pruritus	Worsening of AD
Fish	13 (13%)	9 (9%)	3 (3%)	4 (4%)
Shrimps	6 (6%)	4 (4%)	2 (2%)	2 (2%)

One patient suffers simultaneously with food allergy to shrimp and fish. GIT = gastrointestinal symptoms (diarrhea, vomiting, cramps)

vomiting, cramps were recorded according to the history; the pruritus and new lesions of atopic dermatitis were observed after the open exposure test in another 4 patients with fish allergy. In 4 patients (4%) with shrimp allergy, gastrointestinal symptoms such as abdominal pain, diarrhea, vomiting, cramps were recorded according to the history; the pruritus and new lesions of atopic dermatitis were observed after the open exposure test in another 2 patients (2%) with shrimp allergy. One patient suffers simultaneously with shrimp/fish allergy, (Table 4). The positivity of specific IgE to fish/shrimp molecular components was evaluated in the whole group of 100 patients. We confirmed the positivity of specific IgE to Pen m 1 (Tropomyosin) in 8 patients (8%), to Pen m 2 (Arginin kinase) in 22 patients (22%), to Pen m 4 (Sarcoplasmic calcium binding protein) in 2 patients (2%), to Gad c 1 (Parvalbumin) in 6 patients (6%), to Ani s 1 (Serine protease inhibitor) in 1 patient, to Ani s 3 in 4 patients (4%). The sensitivity of the ISAC Multiplex testing examination is recorded in Table 5; the sensitivity of Pen m 1 is – 33.3%, Pen m 2 – 50.0%. Pen m 4 – 0%, Ani s 1 – 0%, Ani s 3 – 16.7%. In patients without clinical symptoms of food allergy to shrimp, the positive results of specific IgE were recorded to Pen m 1 in 6 patients (6%), to Pen m 2 in 19 patients (19%), to Pen m 4 in 2 patients (2%), to Ani s 1 in 1 patient (1%) and to Ani s 3 in 3 patients (3%). The sensitivity was calculated in patients with confirmed allergy to fish; the sensitivity of Gad c 1 is 46.2%, Ani s 1 – 7.7%, Ani s 3 – 7.7%. The positivity to Gad c 1 and to Ani s 1 was confirmed only in patients with clinical reactions to fish; we did not confirm the positive results to these molecular components in patients without clinical symptoms of food allergy. On the other hand, in patients without confirmed food allergy to fish, we recorded the sensitisation to Ani s 3 in 3 patients (3%), Table 5. In the **Complement to Table 5**, we show the positive results to Gad c 1 in patients suffering from food allergy to shrimps, the positive results to Pen m 1, Pen m 2 and Pen m 4 in patients with food allergy to fish.

We evaluated the mean level of specific IgE to molecular components in patients with clinical symptoms of fish/shrimp allergy and in patients *without* clinical symptoms of fish/shrimp allergy – with only sensitisation to fish/shrimp. In patients with confirmed

Table 5. The positivity to fish/shrimp molecular components.

Food allergen, number of patients with confirmed FA	Molecular component	Protein family	Number of positive results to molecular components – in patients			Sensitivity of molecular components %
			1) in the whole group	2) FA yes	3) FA no	
Shrimps, 6 patients	Pen m 1	Tropomyosin	8 (8%)	2 (2%)	6 (6%)	33.3%
	Pen m 2	Arginine kinase	22 (22%)	3 (3%)	19 (19%)	50.0%
	Pen m 4	Sarcoplasmic calcium binding protein	2 (2%)	0	2 (2%)	0
	Ani s 1	Serine protease inhibitor	1 (1%)	0	1 (1%)	0
	Ani s 3	Tropomyosin	4 (4%)	1 (1%)	3 (3%)	16.7%
Fish, 13 patients	Gad c 1	Parvalbumin	6 (6%)	6 (6%)	0	46.2%
	Ani s 1	Serine protease inhibitor	1 (1%)	1 (1%)	0	7.7%
	Ani s 3	Tropomyosin	4 (4%)	1 (1%)	3(3%)	7.7%

(1) in the whole group of patients (100 patients = 100%)

(2) in patients with confirmed food allergy to fish (13 patients) and shrimps (6 patients)

(3) in patients without clinical symptoms of food allergy to fish and shrimps. Sensitivity of ISAC Multiplex testing in patients with food allergy to fish, shrimps. FA = food allergy.

shrimp allergy, the mean level of specific IgE to Pen m 1 is 50.2 ISU – E, to Pen m 2 is 56.3 ISU – E, to Ani s 3 is 100. 0 (only 1 patient has the positive level of sIgE), on the other hand, *the mean levels of specific IgE in patients without clinical symptoms of food allergy are lower* (to Pen m 1 30.38 ISU – E, to Pen m 2 is 15.87 ISU – E, to Ani s 1 is 47.0 ISU – E (only in 1 patient), and to Ani s 3 is 17.56 ISU – E), (Table 6). In patients with confirmed food allergy to fish, the mean level of specific IgE to Gad c 1 is 11.93 ISU – E. The level of specific IgE to Ani s 1 is in 1 patient 47.0 ISU – E and the level to Ani s 3 is 100.0 ISU – E in 1 patient with confirmed fish allergy. No patients without clinical symptoms of fish allergy are sensitized to Gad c 1 and Ani s 1. On the other hand, in 3 patients without clinical symptoms of fish allergy, the mean level to Ani s 3 is 17.56 ISU – E, (Table 7).

We recorded the significant relation between the occurrence of fish/shrimp allergy and the sensitisation to molecular components of *Alternaria*, *Aspergillus*, *Cladosporium*, molecular components of House dust mites, molecular components of Cockroach. In patients suffering from fish allergy, the significantly higher occurrence of sensitisation

Table 5. Complement to Table 5. The positive results to Gad c 1 in patients suffering from food allergy to shrimps, the positive results to Pen m 1, Pen m 2 and Pen m 4 in patients with food allergy to fish. The positivity to Gad c 1 was confirmed in 1 patient suffering from food allergy to shrimps. In patients suffering from food allergy to fish (13 patients = 100%), the positivity to Pen m 1 was confirmed in 3 patients (23.07%), to Pen m 2 in 9 patients (69.2%) and to Pen m 4 in no patient.

Number of patients with FA	Molecular component	Number of patients	
		positive results in the whole group of patients	positive results in patients with FA
Shrimps, 6 patients (= 100%)	Gad c 1	6	1 (16.7%)
Fish, 13 patients (= 100%)	Pen m 1	8	3 (23.1%)
	Pen m 2	22	9 (69.2%)
	Pen m 4	2	0

Table 6. The levels of specific IgE to molecular components of shrimps in ISAC Multiplex testing in ISU – E.

Number of patients with positive results to molecular components and mean level of specific IgE in ISU – E						
Molecular component	1) the whole group of patients		2) shrimp FA yes		3) FA no (sensitisation only)	
	number of patients	mean level of specific IgE in ISU – E	number of patients	mean level of specific IgE in ISU – E	number of patients	mean level of specific IgE in ISU – E
Pen m 1	8 (8%)	35.33	2 (2%)	50.2	6 (6%)	30.38
Pen m 2	22 (22%)	21.38	3 (3%)	56.3	19 (19%)	15.87
Pen m 4	2 (2%)	3.25	0	0	2 (2%)	3.25
Ani s 1	1 (1%)	47	0	0	1 (1%)	47.0
Ani s 3	4 (4%)	38.175	1 (1%)	100.0	3 (3%)	17.56

1) in the whole group of 100 patients (= 100%)

2) in patients with confirmed food allergy (FA) to shrimps

3) in patients without clinical symptoms of food allergy to shrimps

to molecular components Alt a 6 (Alternaria, Enolase), Asp f 1 (Aspergillus, Mitogillin family), Asp f 6 (Aspergillus, Mn superoxide dismutase), Der f 1 (House dust mite, Cysteine protease), Der f 2 (House dust mite, NPC2 protease), Der p 1 (House dust mite, Cysteine protease), Der p 2 (House dust mite, NPC2 protease), Lep d 2 (NPC2 family) and Blo t 5 (Mite group 5) was confirmed, [Table 8](#). In patients suffering from shrimp allergy, the significantly higher occurrence of sensitisation to molecular components Alt a 6 (Alternaria, Enolase), Asp f 6 (Aspergillus, Mn superoxide dismutase), Cla h 8 (Cladosporium, Mannitol dehydrogenase), Bla g 1 (Cockroach, Cockroach group 1) Bla g 5 (Cockroach, Glutathione S-transferase) was confirmed, [Table 9](#). The list of molecular components with the significantly higher occurrence of sensitisation in patients suffering from fish/shrimp allergy is shown in the summary [Table 10](#).

Discussion

The sens of our study was to show the occurrence of fish/shrimp allergy in atopic dermatitis patients and to analyze the data of ISAC Multiplex examination. The examination of sensitisation to food and inhalant allergens (skin prick test, examination of extract specific IgE, atopy patch test) is a common part of examination in atopic dermatitis, because these allergens may play the role in exacerbations of AD (Matricardi et al.,

Table 7. The levels of specific IgE to molecular components of fish in ISAC Multiplex testing in ISU – E.

Number of patients with positive results to molecular components and mean level of specific IgE in ISU – E						
Molecular component	1) the whole group of patients (100 patients = 100%)		2) fish FA yes		3) FA no (sensitisation only)	
	number of patients	mean level of specific IgE in ISU – E	number of patients	mean level of specific IgE in ISU – E	number of patients	mean level of specific IgE in ISU – E
Gad c 1	6 (6%)	11.93	6 (6%)	11.93	0	0
Ani s 1	1 (1%)	47	1 (1%)	47.0	0	0
Ani s 3	4 (4%)	38.175	1 (1%)	100.0	3 (3%)	17.56

1) in the whole group of 100 patients (= 100%)

2) in patients with confirmed food allergy (FA) to fish

3) in patients without clinical symptoms of food allergy to fish.

Table 8. The relation between the occurrence of fish allergy and the sensitisation to molecular components. The significant relation is marked extra bold.*

Molecular components	positive results in the whole group (100 patients)	Number of positive results to molecular components in 100 patients		<i>p</i> – value
		Fish allergy no 87 patients = 100%	Fish allergy yes 13 patients = 100%	
Alt a 1	29	25(28.7%)	4(30.8%)	0.880
Alt a 6	16	11(12.6%)	5(38.5%)	0.018*
Asp f 1	6	3(3.4%)	3(23.1%)	0.005*
Asp f 3	6	6(6.9%)	0(0.0%)	0.329
Asp f 6	16	9(10.3%)	7(53.8%)	0.000*
Cla h 8	4	3(3.4%)	1(7.7%)	0.466
Der f 1	34	25(28.7%)	9(69.2%)	0.004*
Der f 2	45	34(39.1%)	11(84.6%)	0.002*
Der p 1	36	27(31.0%)	9(69.2%)	0.007*
Der p 2	46	35(40.2%)	11(84.6%)	0.003*
Der p 10	5	3(3.4%)	2(15.4%)	0.065
Bla g 1	2	2(2.3%)	0(0.0%)	0.581
Bla g 2	3	3(3.4%)	0(0.0%)	0.497
Bla g 5	3	2(2.3%)	1(7.7%)	0.288
Bla g 7	5	3(3.4%)	2(15.4%)	0.065
Lep d 2	13	9(10.3%)	4(30.8%)	0.041*
Blo t 5	9	4(4.6%)	5(38.5%)	0.000*

2016). Molecular allergy diagnosis is a typical method of precision medicine (Matricardi et al., 2016) and it enhances the specificity of IgE-diagnosis in polysensitized respiratory allergies, can be applied in food allergies and atopic dermatitis (Hanifin & Rajka, 1980). The results of our study may be valuable, because we show the sensitivity of ISAC Multiplex testing in atopic dermatitis patients suffering from fish/shrimp allergy and the relation between the fish/shrimp allergy and the sensitisation to other molecular components.

Table 9. The relation between the occurrence of shrimp allergy and the sensitisation to molecular components. The significant relation is marked extra bold.*

Molecular components	positive results in the whole group (100 patients)	Number of positive results to molecular components		<i>p</i> – values
		Shrimps allergy no 94 patients = 100%	Shrimp allergy yes 6 patients = 100%	
Alt a 1	29	28(29.8%)	1(16.7%)	0.492
Alt a 6	16	13(13.8%)	3(50.0%)	0.019*
Asp f 1	6	5(5.3%)	1(16.7%)	0.256
Asp f 3	6	5(5.3%)	1(16.7%)	0.256
Asp f 6	16	13(13.8%)	3(50.0%)	0.019*
Cla h 8	4	2(2.1%)	2(33.3%)	0.000*
Der f 1	34	32(34.0%)	2(33.3%)	0.972
Der f 2	45	42(44.7%)	3(50.0%)	0.800
Der p 1	36	33(35.1%)	3(50.0%)	0.461
Der p 2	46	43(45.7%)	3(50.0%)	0.839
Der p 10	5	4(4.3%)	1(16.7%)	0.176
Bla g 1	2	1(1.1%)	1(16.7%)	0.008*
Bla g 2	3	3(3.2%)	0(0.0%)	0.657
Bla g 5	3	2(2.1%)	1(16.7%)	0.043*
Bla g 7	5	4(4.3%)	1(16.7%)	0.176
Lep d 2	13	11(11.7%)	2(33.3%)	0.127
Blo t 5	9	8(8.5%)	1(16.7%)	0.499

Table 10. Summary table – the list of molecular components with the significantly higher occurrence of sensitisation in patients suffering from fish/shrimp allergy.

Allergen	Molecular component	Protein family	Food allergens with the significantly higher occurrence of sensitisation to molecular components.
Alternaria	Alt a 6	Enolase	fish*, shrimps
Aspergillus	Asp f 1	Mitogillin family	fish*
Aspergillus	Asp f 6	Mn superoxide dismutase	fish*, shrimps
House dust mite	Der f 1	Cysteine protease	fish*
House dust mite	Der f 2	NPC2 protease	fish*
House dust mite	Der p 1	Cysteine protease	fish*
House dust mite	Der p 2	NPC2 protease	fish*
Storage mite	Lep d 2	NPC2 family	fish*
House dust mite	Blo t 5	Mite group 5	fish*
Cladosporium	Cla h 8	Mannitol dehydrogenase	shrimps
Cockroach	Bla g 1	Cockroach group 1	shrimps
Cockroach	Bla g 5	Glutathione S-transferase	shrimps

*According to our results, patients suffering from food allergy to fish are sensitised to Pen m 1 in 23.1%, to Pen m 2 in 69.2%; patients suffering from food allergy to shrimp are sensitised to Gad c 1 in 16.7%.

The purpose of the study is to show, that the occurrence of fish/shrimp allergy in atopic dermatitis is high (fish allergy in 13% and shrimp allergy in 6%). As for diagnosis of fish/shrimp allergic patients, most people are aware of ingesting fish, and thus, the patient history is often quite reliable as for the exposure. We also observed, that patients suffering from food allergy to fish eliminated shrimps from their diet also. The fish/shrimp allergy was confirmed in majority of patients according to the history with clinical reactions such as gastrointestinal symptoms (diarrhea, vomiting, cramps, abdominal pain). According to the challenge test (history), fish allergy can lead to the exacerbation and worsening of the skin finding in 4% of atopic dermatitis patients and shrimp allergy in 2% of atopic dermatitis patients. All these patients eliminate fish and shrimp from their diet and they are always carefully monitored. Regarding the sensitivity of ISAC Multiplex testing, the sensitivity of Pen m 1 is 33%, higher sensitivity shows molecular component Pen m 2 (50.0%). High sensitisation rate was recorded in patients without clinical symptoms of food allergy to shrimp – to Pen m 2 in 19 patients (19%). In patients with confirmed shrimp allergy, the higher level of specific IgE to Pen m 1 and Pen m 2 was recorded in comparison to patients without the clinical symptoms of shrimp allergy, but with the positive results to molecular components of shrimps. The sensitivity of Gad c 1 is 46.2%; in patients without confirmed clinical reaction to fish, we recorded no sensitisation to Gad c 1 and to Ani s 1. The level of specific IgE to Ani s 3 was recorded 100.0 ISU – E in one patient with simultaneously occurrence of fish/shrimp allergy; 3 patients (3%) are sensitized to Ani s 3 and have no symptoms of fish/shrimp allergy.

Epidemiological studies on fish allergy are missing to present consistent data of sensitization to fish and fish allergens. Prevalence rates to fish have been determined in studies of variable design and methodology (Sicherer et al., 2004; Worm et al., 2015). In general, the main clinical manifestations of fish/shrimp allergy include vomiting and diarrhea, whereas the extreme form of reaction is life-threatening anaphylactic shock (Magnusson et al., 2013; Matricardi et al., 2016; Mazzucco et al., 2018). Occupationally exposed workers (fishermen, fishmongers and workers of fish-processing industries) documented specific IgE between 11.7% and 50% of cases. Symptomatic allergic patients to any kind of allergen were found to be positive to Anisakis specific IgE

detection between 0.0% (in children with mastocytosis) to 81.3% (among adults with shellfish allergy) (Mazzucco et al., 2018). Sensitization rates for parvalbumins were determined based on studies during allergen characterization (Sharp & Lopata, 2014; Sten et al., 2004). It was concluded that 90%–95% of the patients had specific IgE to these muscle proteins. Recent studies showed that the fish-allergic population might be subdivided into the following clinical clusters: (i) highly sensitized patients reacting to all fish, (ii) oligo-sensitized patients reacting to several, specific fishes, and (iii) patients with “selective reactions” to individual fish species only (Kuehn et al., 2014a, 2014b). Patients of these clinical clusters vary by their IgE recognition profiles. It was shown that the prevalence of IgE binding to parvalbumin was lower than assumed. The sensitization rate to this major allergen might be rather around 70%, which would need to be confirmed in future studies. A single study demonstrated that fish-allergic patients with specific IgE to cod parvalbumin might be cosensitized to cod enolases (81%) and aldolases (58%). The clinical origin and relevance of this cosensitization is not yet resolved. However, specific parvalbumin-negative patients seem to develop IgE antibodies to fish enolase (47%) and aldolase (41%) which is rather linked to species-specific fish allergies (Kuehn et al., 2014b).

In a US population, a diagnostic serum IgE level of 20 kUA/l to cod (ImmunoCAP, ThermoFisher) has been established to predict an allergy to this fish with 95% certainty (Sampson & Ho, 1997). However, clinical reactions have been indicated for patients with much lower IgE-titers. The availability of individual allergens for IgE testing is still limited and, thus, not of much help in predicting whether the patient is allergic to other fish species (Sampson & Ho, 1997). According to our results, in patients with confirmed fish/shrimp allergy, the mean level of specific IgE to molecular components was higher in comparison to patients with the positive results to molecular components, but without clinical reactions to these food allergens. While probably extremely rare, there are examples of patients reacting to fish allergens that are hidden in foods (Aníbarro et al., 2007); the lowest provoking dose of fish is in the low milligram range (Ballmer-Weber et al., 2015; Helbling et al., 1999; Poulsen et al., 2001; Untersmayr et al., 2005; Untersmayr et al., 2007).

Regarding the allergy to shrimps, previous sensitization rates were mainly based on skin or IgE testing to whole shellfish extracts (Rona et al., 2007) and some studies have identified the prevalence of shellfish allergy to be 2% for the general population and 0.1–0.9% for children (Nwaru et al., 2014); 60% of individuals with confirmed allergy to shellfish elicit specific IgE binding to tropomyosin. More importantly, it has been demonstrated that serum specific IgE to tropomyosin is a better predictor of shrimp allergy than shrimp skin prick test or IgE to whole shrimp extract. Tropomyosin (Pen m 1) and sarcoplasmic calcium-binding protein (Pen m 4) sensitization has been associated with clinical reactivity to shrimp allergy (Abramovitch et al., 2013). In our previous study we evaluated the occurrence of food hypersensitivity reactions to seafish in AD patients; the reactions to fish were recorded as mild OAS, vomiting, pruritus, and worsening of AD altogether in 11% of AD patients, (Čelakovská et al., 2020). In our previous studies we evaluated the occurrence of sensitisation to food and inhalant allergens in patients suffering from AD also. The challenge test was performed according to the results of examinations sIgE and atopy patch tests with suspected foods. Our previous studies demonstrate that there is a significant association between the severity of AD

and the incidence of perennial rhinitis, asthma bronchiale, and the worsening of atopic dermatitis in relation to food (Čelakovská & Bukač, 2017; Čelakovská et al., 2015a, 2015b; Magnusson et al., 2013).

At this study, we also evaluated, if there is some significant relation between the occurrence of fish/shrimp allergy and the sensitisation to molecular components of some inhalant allergens in ISAC Multiplex testing. In patients with fish allergy, the significantly higher occurrence of sensitisation to Asp f 1 (Mitogillin family), Asp f 6 (Mn superoxide dismutase), Alt a 6 (Enolase), Der f 1 (Cysteine protease), Der f 2 (NPC2 protease), Der p 1 (Cysteine protease), Der p 2 (NPC2 protease), Lep d 2 (NPC2 family) and Blo t 5 (Mite group 5) was confirmed. The explanation can be in the fact that patients suffering from food fish allergy are sensitised to molecular components of shrimps also (in fish allergic patients we recorded the positivity to Pen m 1 in 23.1%, to Pen m 2 in 69.2%).

In patients with shrimp allergy, the significantly higher occurrence of sensitisation to Asp f 6 (Mn superoxide dismutase), Alt a 6 (Enolase), Cla h 8 (Mannitol dehydrogenase), Bla g 1 (Cockroach group 1) and Bla g 5 (Glutathione S-transferase) was confirmed. Allergenic tropomyosins are highly conserved muscle proteins found in invertebrates, such as arachnids (house dust mites), insects (cockroaches), crustaceans (shrimp, prawn, lobster, crawfish, crab), and mollusks (mussel, oyster, squid, cuttlefish, octopus, abalone, limpet, snail), therefore being considered panallergens (Becker et al., 2012; Pedrosa et al., 2015; Witteman et al., 1994). Tropomyosin was the first identified allergen involved in cross-reactivity between *Dermatophagoides pteronyssinus* mite, shrimp and insects (Witteman et al., 1994) and it is still considered a major shellfish allergen frequently responsible for clinical cross-reactivity with inhaled house dust mites (Becker et al., 2012). The house dust mites-crustaceans-mollusks syndrome is a relatively rare variant of food allergy in which the house dust mites are the primary IgE sensitising agents, while shellfish can induce food allergy, up to anaphylaxis, even at first ingestion (Pedrosa et al., 2015). In the more usual mite-shrimp syndrome, the typical allergen component mentioned is tropomyosin Der p 10 (Pedrosa et al., 2015). In our study we did not confirm the significant relation between the allergy to shrimps and significantly higher occurrence of sensitisation to Der p 10.

Arginine kinase, a 40-kDa enzyme involved in the storage of excess energy as arginine phosphate, is a potential new class of invertebrate panallergens, identified mainly in crustaceans, such as black tiger shrimp *Penaeus monodon* (Pen m 2). These are cross-reactive with arginin kinase allergens from house dust mites (Der p 20, Der f 20, Blo t 20), cockroaches (Bla g 9, Per a 9), (Čelakovská et al., 2015a). Myosin light chain is a 20 kDa crustacean allergen identified in common shrimp *Crangon crangon* (Cra c 5), brine shrimp *Artemia franciscana* (Art fr 5), black tiger shrimp *Penaeus monodon* (Pen m 3), whiteleg shrimp *Litopenaeus vannamei* (Lit v 3), American lobster *Homarus americanus* (Hom a 3), with potential cross-reactivity with aeroallergens from dust mite *Dermatophagoides farinae* (Der f 26) and German cockroach (Bla g 8), (Ayuso et al., 2008).

According to the literature, the IgE-sensitivity to shrimp tropomyosin in a Moroccan population from Fez region was evaluated. This work was conducted with a questionnaire completed by a sera-bank, obtained from 500 patients recruited from Fez Hospitals. Their sera were analyzed for specific IgE-sensitivity to shrimp tropomyosin. From questionnaire, 9.8% of people were allergic to fish and shellfish where shrimp was one of the

most common species causing allergy in patients. Evaluation of specific IgE showed that 10.2% of patients present higher values. Further indirect ELISA and Dot-blot results indicated that shrimp tropomyosin showed a decrease in the human IgE binding under heating or pepsin hydrolysis. These results demonstrate that this population was sensitive to shrimp tropomyosin and the sensitivity could be reduced by heating and more where it was digested by pepsin (Mejrhit et al., 2017).

Another study attempted to identify and purify the major allergen implicated in the allergic response to largemouth bass (*Micropterus salmoides*), a freshwater fish widely consumed in China. Fifteen patients with a positive history of type I allergy to fish were recruited from skin-prick test and the allergy screen. Total protein extracts and purified allergenic protein from bass were tested for their immunoglobulin E – binding properties. Immunoblot assay resulted in strong reactivity with the 17-kDa protein in all patients. In summary, nucleoside diphosphate kinase B was identified as a novel fish allergen in largemouth bass. This finding is important for allergy diagnoses and the treatment of freshwater fish–allergic disorders (Liu et al., 2014).

Conclusion

In atopic dermatitis patients, the food allergy to fish was confirmed in 13% of patients, the food allergy to shrimps in 6% of patients. Gastrointestinal symptoms (diarrhea, vomiting, cramps, abdominal pain) and worsening of atopic dermatitis were recorded in patients suffering from food allergy to fish and /or shrimps. *Fish allergy can lead to the exacerbation and worsening of the skin lesions in 4% of atopic dermatitis patients and shrimp allergy in 2% of atopic dermatitis patients.* In patients without clinical symptoms of food allergy to shrimps, we recorded the sensitisation rate to Pen m 2 in 19% patients, but the levels of specific IgE to Pen m 2 were lower in comparison to the levels of specific IgE in patients with clinical symptoms of food allergy to shrimps.

The sensitivity of ISAC Multiplex testing is for Pen m 1 – 33.3%, Pen m 2 – 50.0%, and for Gad c 1 46.2%. In patients with food allergy to shrimps and fish, the significantly higher occurrence of sensitisation to Alt a 6 (*Alternaria*, Enolase) and to Asp f 6 (*Aspergillus*, Mn superoxide dismutase) was confirmed. Moreover, in patients with allergy to fish, the significantly higher occurrence of sensitisation to Asp f 1, Der f 1, Der f 2, Der p 1, Der p 2, Lep d 2 and Blo t 5 was confirmed; in patients with food allergy to shrimps the significantly higher occurrence of sensitisation to Cla h 8, Bla g 1 and Bla g 5 was confirmed also. Patients suffering from food allergy to fish are sensitised to molecular components of shrimps also (to Pen m 1 in 23.1%, to Pen m 2 in 69.2%).

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