Original article

Fish sensitization in a group of allergic Egyptian children

Background: *There are no published data on fish allergy in Egypt.*
Objective*: We sought to screen for the frequency of fish sensitization in a group of atopic Egyptian infants and children in relation to their demographic and phenotypic data.*
Methods: *We consecutively enrolled 87 allergic children; 1-15 years old (median 5.0 yr). The study measurements included clinical evaluation for the site and duration of allergy, possible precipitating factors, and family history of allergy as well as skin prick testing with a commercial fish extract, and serum fish specific and total IgE estimation.*
Results: *Twelve subjects (13.8%) were sensitized to fish as evidenced by positive skin prick test (SPT) results; five (41.7%) of them gave a history suggestive of fish allergy compared to two (2.7%) of the non-sensitized children (p=0.00). The SPT results did not vary significantly with age, gender, family history of atopy, or serum total or fish specific IgE (SpIgE).*
Conclusion: *Fish sensitization does not seem to be rare in atopic children in Egypt. It can be associated with any clinical form of allergy and the causal relationship needs meticulous evaluation. Wider scale population-based studies are needed to assess the prevalence of fish allergy and its clinical correlates in our country.*

Key Words: fish allergy; sensitization; skin prick test; specific IgE; children; Egypt

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INTRODUCTION

Fish is a major source of dietary protein, especially in coastal areas. A large share of fish is now consumed not longer as entity but as ingredient in highly processed food products. This development involves that the risk for food cross-contamination due to elaborate manufacturing processes and differently handled hygiene practices has likewise increased.[1, 2]

Fish and shellfish are two of the most common causes of IgE-mediated food-allergic reactions in both children and adults. The prevalence estimates of fish allergy range between 0% and 2%.[3-5] The edible fish include more than 20000 species; however, the most commonly consumed belong to only a few orders of the ray-finned fish (Actinopterygii). The major fish allergen, Gad c 1, has been extensively studied and is a 12-kDa calcium binding sarcoplasmic protein belonging to the protein family of parvalbumins.[6] Although species-specific epitopes have been reported, parvalbumin, in general, constitutes the major cross-reactive fish allergen.[7-9] It is estimated that 50% of individuals allergic to some type of fish are at risk for reacting to a second species. However, up to 40 % of patients sensitized to one or more fish do not present symptoms on consuming other species, the best tolerated of which belong to the Scombroidea family which includes tuna.[10] Cod parvalbumin shares IgE binding epitopes with frog parvalbumin. This in vitro cross-reactivity seems to be also clinically relevant.[11]

The age of the initial consumption may influence the age of the initial reaction.[12] In recent years, the number of new fish allergy cases during the first year of life appears to have diminished. This may reflect a parental tendency to delay the introduction of fish into the infant diet, although there is no public health advice to support this.[13] Until recently, the American Academy of Pediatrics had recommended that fish be avoided until the age of 3 years.[14] Paradoxically, early large exposure rather than conventional strict avoidance has induced immune tolerance.[15] After adjustment for potential confounders, late introduction of fish was significantly associated with increased risk of sensitization to fish and inhalant allergens.[16]

Sensitization to fish was not associated with the timing of fish introduction in a recent report from Sweden. However, the early introduction of fish into the child's diet was associated with less eczema development.[17] The early introduction of food allergens during infancy might thereby induce tolerance.[18]

Adverse reactions in infants or children at the first introduction of fish may result from the passage of fish allergens through breast milk or the presence of these allergens in the indoor air and dust of houses where fish is frequently cooked and/or processed. The sensitivity often appears at an early age, and in many patients, persists for life.[19] In a surveyed sample, only 3.5% of the subjects with fish allergy and 4% of the subjects with shellfish allergy reported loss of that allergy.[4] The duration of seafood avoidance until the attainment of tolerance is unknown but is longer than that for other foods such as cow's milk and egg.[20] It probably depends on several factors, such as age of onset, degree of sensitization, type of symptoms, severity of reaction, multiplicity of causative foods, and degree of avoidance. It is worth noting, however, that recurrence of intolerance might occur.[21]

The prevalence of fish sensitivity and allergy in Egypt is not sufficiently studied. Therefore, we were stimulated to study the frequency of sensitization to fish in a group of Egyptian allergic children suffering from various allergic disorders.

METHODS

Study Population

This cross sectional study comprised 87 children diagnosed clinically to have allergic diseases. They were enrolled consecutively from the Pediatric Allergy and Immunology Clinic, Children's Hospital, Ain Shams University, Cairo from July 1 to December 31, 2010. An informed consent was obtained from the parents or care-givers prior to enrollment. The study protocol was approved by the local ethics committee.

Inclusion criteria:

- Age at enrollment between one and 18 years.
- A physician made diagnosis of allergic diseases including asthma, allergic rhinitis, urticaria, and eczema.

Exclusion criteria:

- Patients who cannot stop antihistamine therapy.
- Extensive skin lesions, positive dermatographism, and very dark skin.
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Study Measurements

All patients included in the study were subjected to the following:

Clinical evaluation:

Detailed history was taken for the duration and severity of symptoms, possible precipitating factors including seafood, response to treatment, and family history of allergy. Patients were subjected to a general clinical examination, as well as chest, skin, and ENT examination to verify the diagnosis.

Skin prick test with fish extract:

This was performed using a commercial fish mix allergen extract, positive histamine control, and negative control (Omega Laboratories, Montréal, Canada). First generation short-acting antihistamines were avoided for at least 72 hours and second generation antihistamines were avoided for at least 5 days before testing. The test sites were marked and labeled at least 3 cm apart to avoid the overlapping of positive skin reactions. The marked site was dropped by the allergen and gently pricked by sterile skin test lancet. Positive and negative control solutions were similarly applied. The patient waited for at least 20 minutes before interpretation of the results. Largest and orthogonal diameters of any resultant wheal and flare were measured. A wheal of 3 mm or more above the negative control was taken as a positive result.[5,12,22]

Laboratory investigations:

Specific IgE (SpIgE) assay for fish mix was performed by Enzyme immunoassay (RIDASCREEN® Spezifisches IgE by Enzyme immunoassay for the determination of specific IgE in serum, R-Biopharm AG, Darmstadt, Germany). Levels of fish specific IgE $\geq$ 0.7 KIU/L were considered increased according to the manufacturer. Serum total IgE using the ELISA technique (Medix Biotech, Inc., Agenzyme Company, Industrial Road, San Carios, CA, USA). The value of IgE used for data analysis was the percentage calculated from the highest normal for age [23] as follows: Patient's normal value/highest normal for age multiplied by 100.

Statistical analysis:

Data were analyzed by a standard computer program using the statistical software package SPSS for windows v.13 Chicago IL, USA. The mean, standard deviation (SD), median, and interquartile (IQ) range presented the descriptive data. Groups were compared using the Kruskal-Wallis and Mann-Whitney Z tests for non-parametric data. Fisher's Exact and Chi square (X^2) tests were used for comparison of categorical data. Pearson and Spearman coefficient tests were used to correlate the numeric data. For all tests, p values

less than 0.05 were considered statistically significant.

RESULTS

The studied sample comprised 52 boys and 35 girls. Their ages ranged between one and 15 years [median (IQR) = 5.0 (6.0); mean (SD) = 6.0 (3.5) years]. The diagnoses included respiratory allergy (bronchial asthma and/or allergic rhinitis) in 44 children, skin allergy (atopic dermatitis and/or urticaria) in 14 children, and both respiratory and skin allergy in 29 children of the studied sample. The total IgE level was elevated for age in 33 subjects (37.9%). The fish SpIgE was elevated (above 0.7 kUA/L) in 87.4% (76/87) of the studied sample and the levels ranged between 0.4-18 kUA/L [mean (SD) = 4.5 (4.2); median (IQR) = 3.0 (6.0) kUA/L].

Skin prick testing with a fish allergen extract revealed positive results in 12 out of the 87 atopic children studied (13.8%). Their demographic, clinical, and laboratory data are displayed in table 1. The wheal and/or flare diameters did not significantly correlate to the SpIgE (p=0.69/p=0.82), total IgE (p=0.63/p=0.63), or age (p=0.41/p=0.48) of the fish sensitized children. The wheal and flare diameters were directly correlated (p=0.02).

Seven subjects in the studied sample (8%) gave a history suggestive of fish allergy. Of those, 5 (71.4%) had positive fish SPT results and the difference from cases with negative SPT was significant as two subjects only (2/75) gave such a history. Although 83.3% of patients with positive SPT in our series had positive family history of allergic diseases compared to 65.3% in children with negative SPT results, the difference did not reach statistical significance. The fish SPT results were not significantly influenced by age or gender and did not vary with the serum total IgE status whether elevated or not. Also, there was no significant relation between the fish SPT results and the fish SpIgE levels (Table 2).

The fish SPT results did not differ according to the site of allergy whether respiratory, skin or both (p=0.77) and the severity of bronchial asthma did not influence the fish SPT results of the asthmatic children (p=0.25). The fish SpIgE levels did not correlate with age (p=0.57) or total IgE levels (p=0.83).

Table 1. Clinical and laboratory data of the fish sensitized children

n	Sex	Age (yr)	History suggestive of fish allergy	FH of atopy	Allergy manifestation (target organs)	Wheal (mm)	Flare (mm)	Total IgE (kUA/l)	Specific IgE (kUA/l)
1	M	6	-	+	BA	8	15	80	4.2
2	F	12	-	+	BA - Urticaria	15	25	200*	1.0
3	M	3	+	+	BA - Urticaria	8	18	30	3.6
4	F	3	-	+	AD - BA	5	11	30	0.3
5	F	11	+	+	AR - BA	7	18	30	4.0
6	M	8	-	+	AD - BA	8	15	52	0.4
7	F	13	-	+	Urticaria	5	11	375*	6.0
8	M	2	+	-	BA	11	19	80*	9.0
9	M	11	-	-	AR - BA	7	15	43	1.2
10	M	7	+	+	BA	7	16	25	1.2
11	M	1	-	+	AD	8	12	10	2.0
12	M	7	+	+	BA - Urticaria	8	15	25	4.0

AD: atopic dermatitis; AR: allergic rhinitis; BA: bronchial asthma; F: female; FH: family history; M: male; *: elevated for age.

Table 2. Variation of some clinical and laboratory data according to fish sensitization*

Variable	Fish SPT+ (n = 12)	Fish SPT– (n = 75)	Test	p
Age (yr) - median (IQR)	7.0 (8.0)	5.0 (5.0)	Z = 0.96	0.34
Gender (male/female)	08/04	44/31	$X^2 = 0.28$	0.60
History suggestive of fish allergy (%)	41.7%	2.70%	$X^2 = 21.3$	0.00*
Positive family history of atopy (%)	83.3%	65.3%	$X^2 = 1.54$	0.22
Elevated total IgE (%)	25.0%	40.0%	$X^2 = 0.99$	0.32
Specific IgE to fish (kUA/L) – median (IQR)	2.8 (3.0)	3.0 (6.0)	Z = 1.27	0.20

IQR: interquartile range; *: significant.

DISCUSSION

The study demonstrated that 13.8% (12/87) of the enrolled allergy patients were sensitized to fish as indicated by a positive SPT to fish extract. The studied sample comprised children with physician-diagnosed allergy and therefore does not represent the general population.

Data from other parts of the world show different rates of sensitization. The marked heterogeneity could be a result of differences in study design or methodology, or differences between populations. Fish was the allergen detected, on skin prick and/or RAST testing, in 8% of a case series of children with anaphylaxis from the United Kingdom.[24] and in 30.8% in Ukrainian children with atopic dermatitis.[25] Studies on the general population reported that IgE sensitization to fish is rare in Swedish children at age 4; only 18 of 2614 tested children had specific IgE antibodies to fish, suggesting that fish is probably not an important allergen in this population. This was partly explained by the regular fish consumption before age one.[26] A low prevalence of sensitization to fish was also reported from Germany, where less than 1% of children up to 17 years of age had a positive skin prick test to herrings.[3] On the other hand, Fish sensitization was detected in 4.3% of a group of Swiss infants and children.[27]

A meta-analysis revealed that the allergy prevalence rate was ≤ 0.5% for fish and 1.4% for Shellfish.[5] The estimated rate of fish allergy in North America was about 0.1% in infants and children and 0.4% in adults.[12,28] Prevalence of fish allergy appears to relate to the amount of fish in the local diet. In Europe, the highest consumption occurs in Scandinavian countries, Spain and Portugal.[13] Seafood is a significant sensitizer in up to 40% and 33% of Asian children and adults respectively [2] but allergy on consumption of fish seems lower.[15] Worth mentioning is that all of our patients came from economically unprivileged families with poor consumption of fish and other animal proteins. The most commonly consumed fish was the Nile tilapia which is relatively less expensive in our country. Ebo et al [29] reported the clinical case of a fish allergic patient who was sensitized to tilapia and pangasius, but not to other types of fish. Notably, the patient was not sensitized to parvalbumin, as shown by enzyme-linked immunosorbent assay using purified allergens. It seems that prick-prick testing with a specific fish allergen may reveal higher rates of sensitization in a given community.

A wheal of at least 3 mm in diameter, or larger than the diluents control is considered positive.[30] In general, the larger the SPT response, the higher the likelihood of clinical relevance.[31] Fish SPT appears to have excellent sensitivity and negative predictive power, but poor specificity and positive predictive value. Some patients with wheals of 3 to 4 mm to fish had no history of reactivity to this food. False positive reactions caused by relatively high levels of histamine in fish extracts are one possible explanation.[30]

The relationship between fish SPT results and the history of possible fish allergy in our series was statistically significant (Table 2). However, 7 children in the current study had wheals that ranged between 5 and 15 mm in diameter and did not report clinical reactions on fish consumption. A study from Taiwan revealed that despite the varied positive skin tests and specific IgE levels in 11 atopic children, none of them had positive oral challenge tests.[19] In addition, some fish-hypersensitive patients are able to consume one or more other fish species without adverse allergic reactions.[32]

Concerning SpIgE, sensitization was set at 0.35 kUA/L for many food and inhalant allergens including fish.[16] According to the kit used in the current study, a level of 0.7 kUA/L was considered increased. The discrepancy between the SPT and SpIgE results could be due to the cut off level used for fish sensitization in the current study which is much below the diagnostic level which usually predicts clinical reactivity with greater than 95% certainty (>95% positive predictive value) This was estimated to be 20 kUA/L.[31,33-35]. None of the children in the current study demonstrated such level. Although, 4 children had a level of 18 kUA/L, none of them had a clinical history suggestive of fish allergy or a positive fish skin prick test result. Oral challenge tests would be worthwhile in those children.

The presence of a positive family history of allergic diseases in the present study did not seem to influence the rates of fish sensitization although ten (83.3%) of the fish sensitized children came from atopic families. This may be explained by the statistically comparable rate (65.3%) in the non-sensitized group. The limited sample size is also an influential factor. The heritability estimates indicate that food-specific IgE is likely influenced by both genetic and environmental factors.[35] A parent-completed questionnaire revealed that a history of food allergy in first-degree family members and a small sibship size were associated with a higher risk of FA in children.[36]

Out of the 12 subjects with positive SPT to fish, 10 had bronchial asthma of whom five had

concomitant skin allergy and two had concomitant allergic rhinitis. However, the cause-relationship between respiratory and fish allergy was not evaluated in the current study due to the limited sample size. It was hypothesized that children allergic to common food allergens in infancy are at increased risk of wheezing illness and bronchial hyperresponsiveness during school age.[37] Six (60%) of the fish sensitized asthmatic children concomitantly had moderate persistent asthma, two (20%) had mild persistent asthma, and two (20%) had intermittent asthma; the relationship to severity was statistically insignificant. The finding is also limited by the sample size.

This pilot study carries a number of limitations. First, the sample size does not allow for solid conclusions. Second, it is not population-based and it only traces fish sensitization in a group of physician diagnosed allergic children. In other words, it cannot give an idea about the rates of sensitization to fish in the general population. Also, there are no published data on fish intake in Egypt including the amount per person, species, or typical habits of fish ingestion. The current study was meant to explore the rate of fish sensitization among atopic Egyptian children. Details of sensitization in relation to intake patterns need to be outlined.

In conclusion, it seems that fish sensitization is not uncommon in atopic Egyptian children. Skin prick and specific IgE testing aided by history are good screening tools to determine candidates for oral fish challenging. Fish sensitivity can be associated with any clinical form of allergy and the causal relationship needs meticulous evaluation. Further wider-scale population-based studies are needed to be able to outline the real extent of fish sensitization and allergy in our country.

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