

Original Article

Chironomids and other causes of fish food allergy

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Background: Chironomids are a frequent cause of respiratory allergy in humid areas of Africa, Asia and North America, whereas in Europe clinical manifestations of this type are associated to exposure to fish foods. The cases of five patients who developed conjunctivitis, rhinitis with or without bronchial asthma or urticaria after handling fish foods are here reported. **Methods:** Skin prick tests were carried out with a testing battery of common respiratory allergens, an assortment of mites, various edible fishes, cereals, meats, different fish foods, common mosquito (*Aedes communis*), and a 1:20 (w/v) extract of *Chironomus thummi*. In addition, total serum IgE and specific IgE to *Chironomus thummi* and to the allergens yielding positive results in the skin tests (CAP) were investigated, and immunodetection was performed. **Results:** Two patients evidenced positive skin test results to common respiratory allergens. The prick tests and the specific IgE investigation were positive for *Chironomus thummi* in four patients; allergenic polypeptides with an approximate molecular weight of 15-16 kDa were detected in their sera and, in two of them, 45 kDa peptides were also found. No *Chironomus thummi* IgE was detected in the fifth patient (skin prick tests, specific IgE and immunodetection were negative), although this patient had positive specific IgE to shellfish/crustaceans (skin prick tests and CAP). No specific *Chironomus thummi* IgE was detected in five control subjects. **Conclusions:** We present five patients experiencing rhinitis with or without bronchial asthma after handling fish food. The aetiological agents were chironomid larvae in four cases, and in the fifth one the shellfish/crustaceans present in some fish foods. This sensitization may be diagnosed by both *in vivo* and *in vitro* methods. The possibility that these insects may induce bronchial asthma, rhinoconjunctivitis, angioedema and urticaria in anglers/fishermen and aquariophiles should be taken into account.

KEY WORDS: Allergy / Fish food / Shellfish / Chironomid larvae.

Quironómidos y otras causas de alergia a comida para peces

Fundamento: Los quironómidos son causa frecuente de alergia respiratoria en áreas acuáticas de África, Asia y el norte de América, mientras que en Europa este tipo de clínica se relaciona con la exposición a alimentos para peces. Se estudian cinco pacientes que presentaron conjuntivitis, rinitis con o sin asma bronquial o urticaria tras la manipulación de alimentos para peces. **Métodos:** Se practicaron pruebas cutáneas (*prick* test) con una batería de neuroalergenos habituales, grupo de ácaros, pescados, cereales, carnes, distintos alimentos para peces, mosquito común (*Aedes communis*) y un extracto de *Chironomus thummi* (1:20 p/v). Se determinaron la IgE sérica total y la específica (CAP) frente a *Chironomus thummi* y frente a los alergenitos positivos, así como inmunodetección. **Resultados:** Dos pacientes presentaron pruebas positivas frente a los neuroalergenos habituales. El *prick* test y la IgE específica fueron positivos para *Chironomus thummi* en cuatro pacientes, en los cuales se demostraron por inmunodetección polipéptidos alérgicos con un peso molecular aproximado de 15-16 kDa, y con un peso molecular de 45 kDa en dos de ellos. En el quinto paciente no se detectó IgE frente a *Chironomus thummi* (*prick*, IgE sérica específica e inmunodetección negativos), pero se detectó IgE sérica específica contra marisco/crustáceos por *prick* y CAP. Tampoco se encontró IgE sérica específica en cinco sujetos utilizados como controles. **Conclusiones:** Presentamos cinco pacientes con rinitis y diez con asma bronquial al manipular alimento para peces. El agente etiológico fueron las larvas de quironómidos en cuatro de los casos, y en el quinto los mariscos/crustáceos presentes en preparados de alimento para peces. Esta sensibilización puede diagnosticarse mediante la práctica de pruebas tanto *in vivo* como *in vitro*. Se debe pensar en estos insectos como inductores de asma bronquial, rinoconjuntivitis, angioedema y urticaria en pacientes aficionados a la pesca y en acuariófilos.

PALABRAS CLAVE: Alergia / Comida para peces / Mariscos / Larvas de quironómidos.

Chironomids are arthropods of the *Insecta* class, belonging to the order *Diptera* (Table I).

Adult chironomids closely resemble mosquitoes, and are often called "non-stinging mosquitoes". They proliferate in aquatic environments and in their surroundings, particularly when the watery areas have experienced an eutrophication process. Their larvae are mostly aquatic and they often have a red colouring because of the presence of haemoglobin in the extracellular fluid; this has led to their being popularly known as "blood worms"¹. Chironomids are responsible for type I hypersensitivity reactions (which cause rhinoconjunctivitis, bronchial asthma, urticaria and angioedema)²⁻⁵, and also for type IV reactions (giving rise to contact dermatitis)⁶.

These insects are a frequent cause of respiratory allergy in watery areas of Sudan, Egypt, Japan^{3,5} and the U.S.A. In Europe, however, allergy to these insects and/or their larvae has been described in persons who manipulate the larvae in fish foods. The larvae are the aetiological agent of occupational allergies in research workers and in workers in fish food factories and aquaria^{7,8}. On the other hand, Eriksson et al⁹ have found a high prevalence of sensitization in the western coastal areas of Sweden, without an apparent explanation.

Only a few cases of chironomid allergy have been reported in Spain^{8,10-13}. Galindo et al¹³ found a low prevalence of chironomid sensitization in patients with rhinitis and bronchial asthma in Ciudad Real and, in agreement with Eriksson, observed that some sensitized patients did not appear to be or have been exposed to chironomid larvae.

Fish foods are preparations composed of fish and fish subproducts, molluscs, crustaceans, vegetable protein extracts, algae, meats, cereals, animal subproducts and chironomid larvae. The number of aquariophiles is currently increasing, and fish foods may represent a further household allergen. Various crustaceans –such as *Daphnia*¹⁴– and different worms and larvae¹⁵⁻¹⁸ present in fish foods and fishing baits are some of the allergens hitherto described as inducing respiratory and/or skin allergy. We here report a series of five patients complaining of rhinoconjunctivitis, bronchial asthma, urticaria and/or anaphylaxis after manipulating fish foods.

CLINICAL CASE OBSERVATIONS

Case 1. A 16-year-old female, non-smoker and with no significant past personal history. She was asymptomatic until about one year prior to consultation. In the course of that last year she had experienced six episodes of cough and wheezing dyspnoea which remitted after bronchodilators. On one occasion, while preparing the food for the fishes in her aquarium, she developed facial urticaria, labial angioedema and bronchospasm requiring emergency treatment with systemic corticosteroids and bronchodilators.

Case 2. A 25-year-old female, smoker, with no further significant antecedents. She consulted because of rhino-ocular pruritus, sneezing, rhinorrhoea and conjunctival injection occurring since one year previously; in this time she had also suffered two episodes of severe bronchospasm requiring emergency treatment with systemic steroids and bronchodilators. These symptoms had occurred in relation with the manipulation of the food preparation she used daily for the fishes in her home aquarium.

Case 3. A 13-year-old male with a previous history of atopic rhinoconjunctivitis and bronchial asthma due to sensitization to *Dermatophagoides pteronyssinus* and cat epithelia. He had received immunotherapy over five years, with clinical improvement. Over the last eight months, and despite environmental control measures, drug therapy and immunotherapy his clinical evolution had not been satisfactory, with persisting nasal obstruction, rhinorrhoea, cough and episodes of wheezing dyspnoea. Since one year prior to consultation he had an aquarium in his home, and he fed the fish daily with Tetramin®. Two years previously, after the ingestion of seafood, he had developed an episode of pruritus, facial angioedema, skin rash, rhinorrhoea and wheezing dyspnoea.

Case 4. A 30-year-old male with no significant personal history came to consultation describing three episodes of conjunctival hyperaemia, epiphora, rhinorrhoea and wheezing dyspnoea in relation with the manipulation of larvae he used as live fishing bait and which he described as "blood worms". On one occasion, immediately after

Table I. Taxonomy: place of the genus *Chironomus* within the family *Diptera*

Class	<i>Insecta</i>
Division	<i>Endopterygota</i>
Order	<i>Diptera</i>
Suborder	<i>Nematocera</i>
Infraorder	<i>Culimorpha</i>
Superfamily	<i>Chironomoidea</i>
Family	<i>Chironomidae</i>
Subfamily	<i>Chironominae</i>
Genus	<i>Chironomus</i>
Species	<i>Ch. thummi thummi</i>

manipulating the food preparation for the fish in his home aquarium, he developed an episode of bronchospasm and urticaria in the exposed skin areas.

Case 5. A 25-year-old male, asymptomatic until three years prior to consultation. Since that time he complained of episodes of conjunctival hyperaemia, palpebral oedema, rhino-ocular pruritus, sneezing and watery rhinorrhoea. He commented that he had an aquarium at home and fed the fishes daily.

METHODS

Chironomid extracts. Extracts were prepared from (a) frozen chironomid larvae (37.4% w/v) in phosphate-saline buffer (PBS), pH 7.3, and (b) lyophilised chironomid larvae (Tetra Delica®) (5% w/v) in PBS. Both extracts were centrifuged at 4,500 g for 15 minutes. The supernatants were separated and filtered through a 0.45 µm pore size membrane.

Fish food extracts. Extracts were also prepared from four commercially acquired fish foods (JBL Gala®, Tetramin®, Nido Acuanid® and Warley®), all of them at a concentration of 20% w/v in PBS, following the same procedure as for the chironomid larvae extracts. The fish food extracts were then glycerinated (50%).

Skin prick tests. Skin prick tests were performed according to the EAACI recommendations¹⁹ and using standardised lancets (Prick Lancetter, Bayer, Leverkusen, Germany), with a panel of airborne allergens and foodstuffs common in our environ-

ment, and also with storage mites, cockroach, various fish, various seafoods, *Anisakis*, cereals, meats and common mosquito (*Aedes communis*) extracts. All these extracts were commercially acquired.

Skin prick tests were also performed with the chironomid and fish food extracts prepared in our laboratory (see above). In order to test the specificity of these extracts, they were also tested in five control subjects.

Total and specific IgE quantitation. The total serum IgE concentrations and the specific IgE concentrations to *Chironomus thummi thummi* and to those allergens that had elicited positive results in the skin prick tests were assayed by the CAP System (Pharmacia AB, Uppsala, Sweden). The results were expressed in kU/l.

SDS-PAGE. Different gels were prepared according to a discontinuous method, with a 16% concentration of acrylamide in the separator gel. The frozen chironomid larvae extract was electrophoretically run on to these gels. Coomassie blue was used for staining.

Immunodetection. After the electrophoresis, the gels were equilibrated for 15 minutes and the proteins were transferred to nitrocellulose membranes by passive diffusion at room temperature and under stirring. After the transfer and after several washings, the membranes were incubated with the sera of the five patients and with pooled sera from unsensitized subjects as the negative control, and immunodetection was carried out according to a previously described technique²⁰.

RESULTS

All the patients reported rhinoconjunctivitis; three of them also complained of bronchial asthma, one had had clinical features suggestive of anaphylaxis, and a further one contact urticaria (Table II). Two of the five patients studied were known atopics (Cases 3 and 4). The skin prick tests were positive to some of the fish food extracts in all five patients, with two different patterns: in Cases 1, 2, 4 and 5, the prick test was positive with JBL Gala®, while in Case 3 it was positive with Tetramin®. The skin prick tests with the remaining fish foods were negative in all the patients.

Table II. Results of the *in vivo* and *in vitro* test

Case	Symptoms	Positive <i>prick</i> tests (mm)	Total serum IgE (kU/L)	Specific serum IgE (kU/L)
1	Anaphylaxis	JBL Gala® 30x20 <i>Ch. thummi</i> 13x7	82,3	<i>Ch. thummi</i> 59,3
2	Rhinoconjunctivitis Bronchial asthma	JBL Gala® 25x20 <i>Ch. thummi</i> 20x10	134	<i>Ch. thummi</i> 60
3	Rhinoconjunctivitis Bronchial asthma	<i>D. pteronyssinus</i> 7x5 Tetramin® 6x6 <i>Ch. thummi</i> 4x4 Lobster 5x5 Prawn 4x4 <i>Aedes communis</i> 5x5	168	<i>D. pteronyssinus</i> 32 <i>Ch. thummi</i> <0,35 Lobster 6,12 Shrimp 6,56
4	Rhinoconjunctivitis Bronchial asthma Contact urticaria	<i>D. pteronyssinus</i> 10x8 J.B.L. Gala® 10x10 <i>Ch. thummi</i> 8x6	520	<i>D. pteronyssinus</i> 84,9 <i>Ch. thummi</i> >100
5	Rhinoconjunctivitis	J.B.L. Gala® 18x8 <i>Ch. thummi</i> 15x8	407	<i>Ch. thummi</i> 84,2

The skin prick tests with chironomid extracts were positive in Cases 1, 2, 4 and 5 and negative in Case 3 and in the five control subjects. Only Case 3 had a positive skin prick test with seafoods; this patient also had a positive test with *Aedes communis*, without clinical relevance.

The skin prick tests with storage mites, cereals, fish and meats were negative in all five patients.

The total IgE assessment yielded levels higher than the normal-for-age values in three patients.

High levels of specific *Chironomus thummi thummi* IgE were detected in four of the patients (Cases 1, 2, 4 and 5). The serum of Case 3 contained no specific *Chironomus thummi thummi* IgE, but it yielded positive results for lobster and shrimp.

The immunodetection study (Fig. 1) revealed that patients Nos. 1, 2, 4 and 5 had specific IgE against 15-16 kDa allergens; the sera of cases 2 and 4 also recognised allergens of about 45 kDa molecular weight. Patient No. 3 was again negative by this method.

DISCUSSION

The present study describes five patients who evidenced IgE-mediated allergic reactions in rela-

tion with manipulating fish foods. The aetiological agents were chironomid larvae in four of the cases, and in one the crustaceans present in fish foods.

All five patients could have become sensitized in their own household environments, where all of them had aquaria. One of the patients was also an amateur angler, and manipulated chironomid larvae as fishing bait.

In all five patients, the immunological *in vivo* and *in vitro* tests demonstrated the presence of an immediate hypersensitivity mechanism. In four of them both the skin prick tests and the CAP and immunodetection methods demonstrated the presence of specific IgE to chironomid larvae. Only one patient evidenced seafood allergy (both by prick test and by specific serum IgE assessment). This patient manipulated a certain brand of fish food, Tetramin®, which contains molluscs and crustaceans, but not chironomid larvae, and he also reported clinical features of alimentary allergy to seafoods.

The clinical features of the patients in the present series are similar to those already reported in the literature⁴; the most frequent symptoms were rhinoconjunctivitis (n = 4), bronchial asthma (n = 3) and urticaria (n = 2). Three of the patients had required emergency treatment because of bronchial asthma and/or anaphylaxis (Case 1).

Liebers et al⁴, in a study of 225 subjects exposed to chironomids, found specific IgE antibodies to Chi t 1 in 34% of them. These antibodies were associated with symptoms, and the highest levels were observed in patients who had bronchial asthma. The results of this study also indicated that the intensity of exposure is relevant for the development of specific IgE antibodies and for the apparition of allergic symptoms. The patients evidencing Chi t 1 sensitization were more frequently atopic and had higher total serum IgE levels. In the present series, three of the patients had high total serum IgE levels, and only one was atopic.

Considering the severity of the clinical manifestations, the use of a non-standardized extract and the risks implicit in the specific bronchial challenge tests, it would seem advisable to establish the diagnosis on the basis of the clinical history and of the (*in vitro*) immunological tests.

According to the immunodetection studies, the main allergen for the patients here studied has a molecular weight of approximately 16 kDa. The

major allergen Chi t 1 which is involved in type I hypersensitivity reactions has been extensively studied by Baur et al^{21,22}, and corresponds to several polypeptidic chains of an haemoglobin from the larval form of *Chironomus thummi thummi*. This allergen has been sequenced, and its molecular weight is 16 and 32 kDa for the monomeric and dimeric forms, respectively.

Using RAST inhibition techniques, cross reactivity has been described between chironomids and crustaceans⁹, and also between *D. pteronyssinus*, cockroach and chironomids²³. In the present study, the patients sensitized to chironomids did not evidence sensitization to seafoods or cockroach. Only one patient was sensitized to *D. pteronyssinus*.

Pascual et al²⁴ have recently reported cross reactivity between *Anisakis* (a nematode), the cockroach *B. germanica* and chironomids (both the latter are arthropods). In the present series we did not observe sensitization to this nematode in any of the patients.

Galindo et al¹³ have studied the prevalence of positive skin prick tests to chironomids in patients with a diagnosis of rhinoconjunctivitis and/or bronchial asthma in Ciudad Real (Spain). They found sensitization to chironomids in patients with no apparent exposure, and concluded that these patients might have become sensitized through inhalation of chironomid particles or of particles of chironomid-containing products used as fish foods, or through cross reactivity to other allergens such as mites, shrimp or mosquitoes.

In conclusion, we would like to point out the importance of the larvae of chironomids as a potent allergen present in fish foods and fish baits. This sensitization may be diagnosed by both *in vivo* and *in vitro* tests. These insects should be kept in mind as the possible causative agents of bronchial asthma, rhinoconjunctivitis, angioedema and urticaria in anglers and aquariophiles. However, chironomid larvae are not the only allergen in this context, and there are various species of arthropods (*Daphnia*, prawns and other crustaceans) as well as different larvae and worms which are present in fish food and fishing baits. All these components should be borne in mind as sensitizing agents able to induce allergic diseases among anglers, fishermen, exposed workers and aquariophiles.

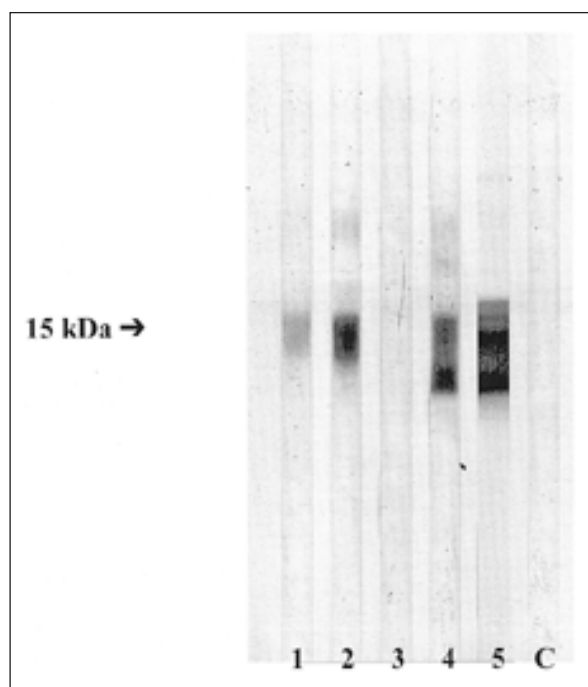


Fig. 1. Immunodetection of chironomid larvae IgE with the sera of patients 1,2,3,4 and 5 and with a negative control sera pool (C).

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