

Allergy to chironomid larvae (red midge larvae) in non professional handlers of fish food

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Summary. Chironomids are insects which inhabit wetlands. In countries such as Sudan, The United States, Egypt and Japan they are the cause of serious environmental allergy.

In Europe, and particularly in Spain, allergy to Chironomids is infrequent and has only been described in patients who handle Chironomid larvae which form part of certain fish foods.

Materials and methods: We report a case of hypersensitivity to the Chironomid Midge (*Chironomus thummi thummi*) in a 23-year-old patient who on two occasions, after being in contact with fishfood, suffered rash, rhinoconjunctivitis, dyspnea and dysphagia.

A Prick test was carried out with the habitual pneumoallergens, Chironomid Midge extract (PBS: 1.3 mg/ml), Common Mosquito (*Culex pipiens*), Squid, Mussel, Prawn and Anisakis. Conjunctival provocation was also carried out with Chironomid Midge extract; detection of specific IgE for Chironomid Midge, Common Mosquito (*Aedes communis*), Mussel, Squid, Shrimp, Anisakis, house dust and house mites by means of the CAP technique; detection of IgE by means of ELISA in response to Chironomid Midge, *Aedes mosquito*, Squid, Prawn, Mussel and Anisakis; ELISA-inhibition and Immunoblot-inhibition.

Results: The positive results of the cutaneous tests, the detection of specific IgE and conjunctival provocation confirmed the existence of an IgE-mediated mechanism. In our patient, the *in vitro* techniques demonstrated cross reactivity with the Common Mosquito.

Conclusions: We report on a patient with a case history of rhinoconjunctivitis, rash, dyspnea, and *dysphagia* after handling fish food. The etiological agent was the Chironomid larvae. The sensitization of our patient has been demonstrated by means of *in vivo* and *in vitro* techniques.

Key words: Chironomids, allergy to insects, chironomid midge, fish food, cutaneous test.

Resumen. Los quironómidos son insectos que habitan en los humedales. En países como el Sudán, los Estados Unidos, Egipto y Japón, son la causa de una grave alergia ambiental. En Europa, y especialmente en España, la alergia a quironómidos es poco frecuente y sólo se ha descrito en pacientes que manipulan larvas de quironómidos que forman parte de determinado tipo de comida para peces.

Materiales y métodos:

Se notificó un caso de hipersensibilidad al mosquito quironómico (*Chironomus thummi thummi*) en un paciente de 23 años que, en dos ocasiones, tras haber manipulado comida para peces, experimentó sarpullido, rinoconjuntivitis, disnea y disfagia. Se realizó una prueba de punción con los neumoalérgenos habituales, extracto de mosquito quironómico (PBS: 1,3 mg/ml), mosquito común (*Culex pipiens*), calamar, mejillón, gamba y anisakis. También se llevó a cabo una provocación conjuntival con extracto de mosquito quironómico; detección de IgE específica para mosquito quironómico, mosquito común (*Aedes comunis*), calamar, mejillón, gamba, anisakis, polvo doméstico y ácaros domésticos mediante la técnica CAP; detección de IgE mediante ELISA como respuesta al mosquito quironómico, mosquito *Aedes*, calamar, gamba, mejillón y anisakis; inhibición por ELISA e inhibición por inmunotransferencia.

Resultados: Los resultados positivos de las pruebas cutáneas, la detección de IgE específica y la provocación conjuntival confirmaron la existencia de un mecanismo mediado por IgE. En nuestro paciente, las técnicas *in vitro* demostraron una reactividad cruzada con el mosquito común.

Conclusiones: Se notificó el caso de un paciente con historia clínica de rinoconjuntivitis, urticaria, disnea y disfagia tras la manipulación de comida para peces. El agente etiológico fue la larva de quironómido. La sensibilización de nuestro paciente se ha demostrado mediante técnicas *in vivo* e *in vitro*.

Palabras clave: Quironómidos, alergia a los insectos, comida para peces, prueba cutánea.

Introduction

Chironomids are non-stinging insects which belong to the Diptera order in the Nematocera suborder (Table 1) [1, 2]. The adult members have many segmented antennae, often longer than the head and thorax. Their body is soft and they are easily confused with the mosquit, given their similarity. They are known as non-biting mosquitoes.

Approximately 10,000 species exist worldwide. Their natural habitat is wetlands. The larvae are mostly red in color due to the presence of hemoglobin in extra-cellular medium, and as they develop into adults their bodies fragment and the particles which fall can produce conjunctivitis, rhinitis and asthma by inhalation in settlements near lakes or rivers [1,2].

Depending on the source of exposure, the allergy to Chironomids can be of different origins.

- 1) Environmental: In settlements near lakes such as in Sudan, Venice and certain regions of Japan [3].
 - 2) Occupational: in handlers of fish food in the work place (fish food factories, fishermen, pet shops...) as in some European countries [4-5].
 - 3) Related to hobbies: in countries where Chironomid Midge larvae are commercialized (*Ch. Thummi thummi*) as fish food [2,6-9]. Fish food is prepared with fish, mollusks, crustaceans, algae, meat and cereal products and Chironomid Midge larvae [10].
- In 1985, Eriksson and collaborators demonstrated that

simultaneous sensitizations to Chironomids and the Aedes Mosquito are common. In a later study (1989), by means of RAST inhibition techniques, they concluded that cross reactivity existed between Chironomids (inhalant allergen) and prawns (allergen by consumption) since the two extracts inhibited with each other. It is also possible that cross reactivity exists between Chironomids, crustaceans and mollusks. The *in vitro* studies have not demonstrated cross reactivity between Chironomids and mites. However, there is a high prevalence of sensitization to Chironomids among those allergic to mites [11-13].

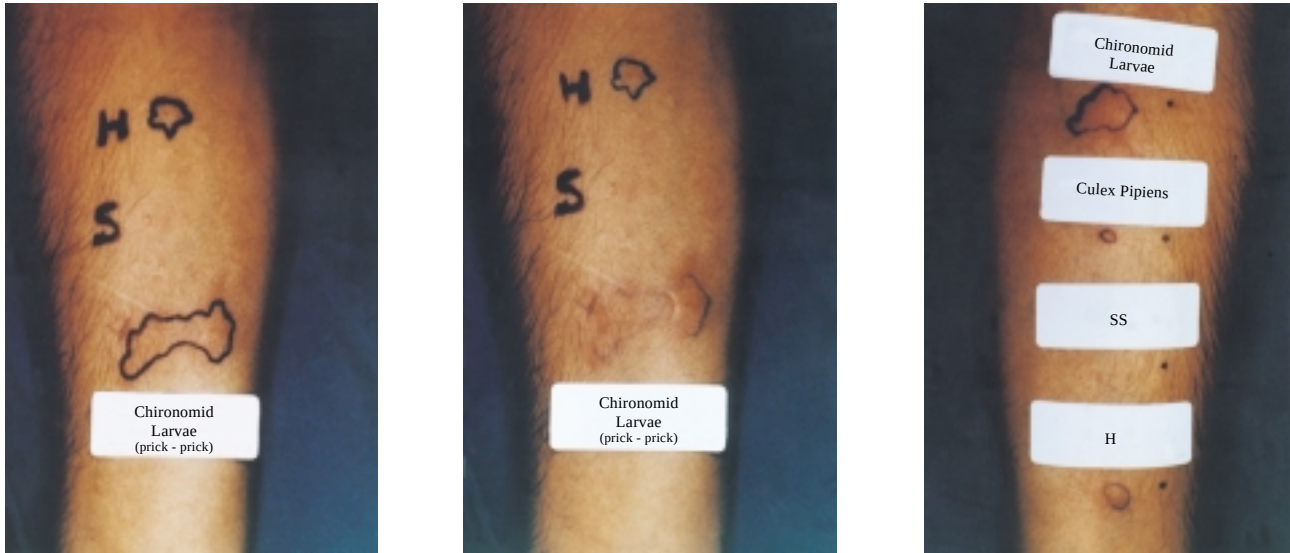
We report a case of allergy to Chironomid Midge (*Ch. Thummi thummi*) in a patient who handled fish food on a non-professional basis, in which we ascertained sensitization mediated by IgE and possible cross reactivity with the Common Mosquito.

Clinical case

A 23-year-old man, a student of Agricultural Engineering with a personal case history of atopic dermatitis and pollen-provoked rhinoconjunctivitis. He came to us because three months after installing an aquarium in his house he began to suffer symptoms immediately after handling the fish food which consisted of dried Chironomid Midge larvae. There were two attacks of rhinocojunctivitis, frontal exanthematous rash and on the second occasion a feeling of something in the throat,

Table 1. Taxonomy of *Chironomus thummi thummi* and Mosquito *Aedes*.

SPECIES:	Ch. Thummi thummi	Aedes comunis / Culex pipiens
SUBFAMILY:	Chironominae	Culicinae
FAMILY:	Chironomidae	Culicidae
SUPERFAMILY	Chironomoidea	Culicoidea
SUBORDER:	culicomorpha	culicomorfa
ORDER:	Nematocera	
DIVISION:	Diptera	
CLASS:	Endopterygota	
PHYLLUM	Insect	
	Arthropod	



Figures 1, 2 and 3. Prick test with extract of chironomid midge and aedes mosquito.

a dry cough, dyspnea and dysphagia. On both occasions he required treatment at hospital.

Materials, methods and results

Cutaneous Prick tests were carried out with an extract of dried Chironomid Midge larvae prepared in our laboratory. The larvae were dissolved in PBS at a final proteic concentration of 1.3 mg./ml and with commercial extracts of Common Mosquito (Alyostal), pollens (ALK-Abelló), mites (ALK-Abelló, Aristegui and Alyostal), Squid (LETI), Mussel (Bencard), Prawn (Alyostal) and Anisakis (IPI). The tests were measured after 15 minutes. Histamine at 10 mg/ml (ALK-Abelló) was used as a positive control and saline solution at 0.9% was used as a negative control. Tests with a papular rash 3 mm larger than the negative control were considered positive. We chose 9 atopic and 5 non-atopic patients for the control group. The cutaneous tests were positive in the patient for Olive, Graminaceous plants, Artemisia, Taraxacum, Chrysanthemum and Chironomid Midge (*Ch. thummi thummi*); less than Histamine for the Common Mosquito (*Culex pipiens*), with a papular rash of 3x4 mm and negative for Mites, Fungi, Dog, Cat, Squid, Mussel, Prawn and Anisakis. The cutaneous tests with extract of Chironomid Midge (*Ch. thummi thummi*) were negative in the control group (Figures 1, 2, 3).

A conjunctival provocation was carried out by using the Chironomid Midge extract at a proteic concentration of 0.13 microgr/ml and a drop of said extract was placed in one of the patient's conjunctival sacs and a drop of control solution in the other (saline physiological solution). The same test was carried out on two control patients, one atopic and one non-atopic. The reaction was read at 15 minutes. Our patient suffered conjunctival erythema, ocular pruritus and homolateral rhinorrhoea.

The reaction in both atopic and non-atopic controls was negative. The repetition of the test after giving the patient cromolyn sodium was negative.

By means of the CAP Pharmacy technique, specific IgE was determined for Chironomid Midge (34.8 KU/L), Common Mosquito (*Aedes communis*) (4.20 KU/L) and <0.35 KU/L for Mussel, Squid, Shrimp, Anisakis, house dust and mites.

An ELISA was developed to detect specific IgE to Chironomid Midge extract and Common Mosquito, Squid, Prawn, Mussel and Anisakis. The Chironomid Midge extract dissolved in PBS was applied to a microtitration plate for 18 hours (13 microgr of protein/cup), as were the rest of the commercial extracts. After blocking with Casein for an hour at 37°C it was extensively washed and then incubated with serum from the patient, serum from a patient who was allergic to the Common mosquito and serum from a healthy control. It remained at room temperature for two hours and was rewashed. Human anti-IgE was applied, combined with Peroxidase for another hour at 37°C and after washing the chromogen

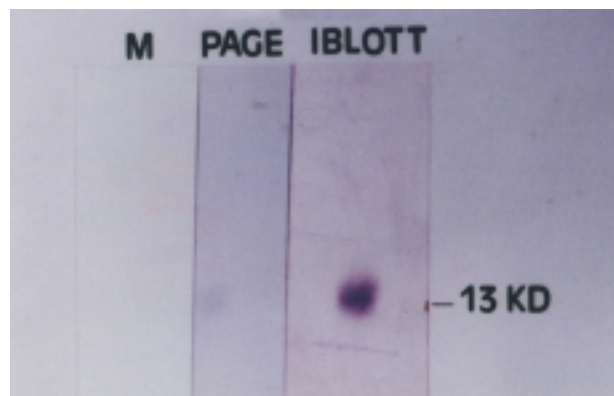


Figure 4. 13 kd band detected by patient serum in the Chironomid Midge extract.

Table 2. ELISA.

	Patient allergic to Chironomid	Patient allergic to Mosquito Aedes	Healthy control
ELISA to Chironomid (OD 490 nM)	1.648	0.301	0.07

substrate was applied. The reaction was detained with sulphuric acid. The reaction was positive with the serum of the patient and with the serum of the patient who was allergic to the Common Mosquito (Table 2).

ELISA did not detect specific IgE for the Common Mosquito, Squid, Prawn, Mussel and Anisakis.

For the study of crossed antigenicity between the Chironomid Midge and the Common Mosquito an Elisa-Inhibition was carried out. The serum of the patient was pre-incubated with Chironomid Midge and Common Mosquito following the previously described steps. The Chironomid Midge-Chironomid Midge inhibition was 97%, between Chironomid Midge and Common Mosquito at 1:1 it was 56% and at 1:10 it was 41% (Table 3).

We carried out an SDS-PAGE at 12% with Chironomid Midge (13.5 microgr/cup), and with Aedes Mosquito (5 microgr/cup). The proteins were transferred to a membrane of Immobilon P. After being blocked, the membrane was incubated with the patient's serum and afterwards with anti IgE combined with alkaline phosphate and substrate. The patient's serum detected a single antigenic band of 13 KD in the Chironomid Midge

Table 3. Percentage of patients allocating each item in the first three importance positions.

Chironomid / Chironomid	Chironomid / Aedes Mosquito	Chironomid / Aedes Mosquito
97%	1:1 56%	1:10 41%

extract (Figure 4) and another less intense band of approximately 30 KD in the extract of Aedes Mosquito.

The band of this latter extract was totally inhibited by the Chironomid Midge extract. We did not detect a clear inhibition of the 13 KD band of the Chironomid Midge for the Aedes Mosquito, probably due to differences in the proteic concentration (Figure 5).

Discussion

Since the 1950s reactions of hypersensitivity to Chironomids have been reported, primarily in Japan, Sudan, The United States and Egypt. These are countries where, in certain areas, Chironomids are one of the most common allergens by inhalation, often causing rhinocconjunctivitis and asthma [3].

In Europe, allergy to Chironomids has occasionally been observed in people who handle fish food because they have aquariums, or in the workplace, although recently Erikson et al. have described cases of environmental allergy in Sweden in rural areas near lakes and rivers where this species is abundant [2,4-9,14].

In Spain, a case of urticaria-angioedema has been reported due to contact in the workplace with larvae of Chironomid Midge (*Ch. thummi thummi*) in a patient with a history of pollen allergy which became worse six months after the patient started work in a pet shop [7] and another seven cases, of sensitization at home [10,15-16]. In all the cases, the Prick test and the CAP for Chironomid Midge were positive.

The antigenic determiners responsible for sensitization have been identified as hemoglobins [2,17,18] low molecular weight (erythroquorins). These hemoglobins, and in particular component III, are powerful human allergens. The hemoglobins demonstrate great polymorphism depending on the environment and the state of development of the insect [19, 20]; the maximum number is found at the larval stage and progressively diminishes, reaching the minimum in the adult state.

Studies have been carried out to characterize the major

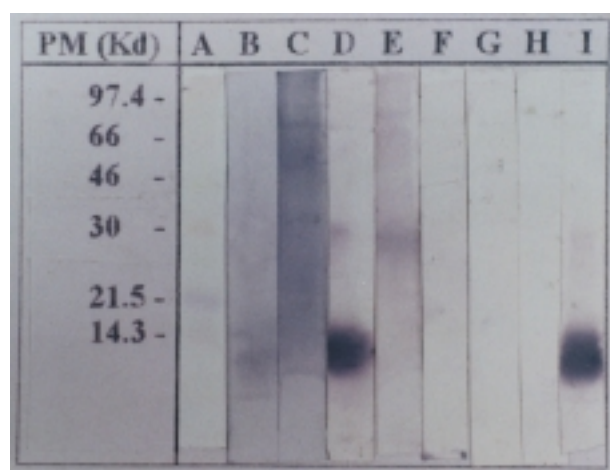


Figure 5. A: PM markers. B: Proteic tinct of Chironomid Midge extract (amidoblack). C: Proteic tinct of Culex Mosquito extract (amidoblack). D: Inhibition of Chironomid Midge extract with patient's serum. E: Inhibition of Culex Mosquito extract with patient's serum. F: Inhibition of Culex Mosquito extract inhibited by Culex Mosquito. G: Inhibition of Culex Mosquito extract inhibited by Chironomid Midge. H: Inhibition of Chironomid Midge extract by Chironomid Midge. I: Chironomid Midge inhibited by Culex Mosquito.

allergens from the chemical point of view. These studies indicate that the major allergens belong to a group of peptide acids with a strong interrelation and with molecular weights which are situated between approximately 15 and 20 KD. In addition, allergenic material of a greater molecular weight was detected, from which we deduce that it is unlikely that hemoglobins are responsible for all the allergenicity in all the sensitive individuals.

In 1985, Erikson and his collaborators observed, by means of RAST-Inhibition, cross reactivity between Chironomids, Crustaceans and Mollusks, as well as simultaneous sensitizations to Chironomids and the Common Mosquito (2,11,21-24). Witterman [25] also found cross reactivity between Chironomids and house dust mites. However, Komase and Yamashita [7,26] did not find this cross reactivity. Recently, Pascual CY et al have demonstrated the cross reactivity between Chironomids (arthropods), Anisakis (nematode) and *Blatella germanica* (arthropod) [27].

The case which we have presented is related to a non professional sensitization. The positive cutaneous tests, the detection of specific IgE, the positive conjunctival provocation and its inhibition after the instillation of cromolyn sodium, demonstrate the existence of a mechanism of immediate hypersensitivity. The clinical characteristics of this patient are similar to those described in the literature. The most frequent symptoms are usually rhinoconjunctivitis and rash.

We have not been able to demonstrate sensitization of our patient to crustaceans, mollusks or mites. Therefore, there does not appear to be cross reactivity with these allergens in this case. However, despite the lack of clinical evidence, our patient has specific IgE against the Common mosquito especially directed against antigens similar to those of the Chironomid Midge.

By means of Immunoblotting, we detected a single allergenic band of apparent PM of 13 KD which was responsible for the hypersensitivity of our patient to the Chironomid Midge. This band is close to that detected by other authors (between 15 and 32 KD) and identified as the major allergen of Chironomid insects which could correspond to hemoglobins (or erythroquorins) which they possess dissolved in their blood.

In conclusion, our attention is drawn to the importance of Chironomid larvae as a powerful allergen hidden in commercialized fish foods. This sensitization can be demonstrated by *in vivo* and *in vitro* methods. We should not forget that this agent could well be the cause of sensitization in people with aquariums or whose hobby is fishing.

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