Hypersensitivity to Chironomid Larvae in a Nonatopic Patient: Safe Diagnosis Tools to Identify a Potent Allergen

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Red midge (*Chironomus thummi thummi*), an insect belonging to the Diptera order, is found worldwide, especially in wetlands [1,2]. The use of red midge larvae as fish food in factories and in pastimes such as fishing and keeping pets has increased human exposure to this insect [1]. The allergenic potential of this nonstinging midge is well-known and more likely to be observed in patients who are allergic to dust mites, crustaceans, or other arthropods owing to cross-reactivity [1,3,4]. Inhalation of red midge protein can cause symptoms such as urticaria, bronchospasm, nephrotic syndrome, and anaphylaxis [1,2,5-7]. Adverse systemic reactions to skin prick tests (SPTs) with these larvae have been reported [7]. Chironomid hemoglobin has been identified as the major allergen in this species, with a molecular weight of 15 to 16.6 kDa (Chi t 1) [8].

A 27-year-old woman with no history of atopy reported rhinitis after 2 months of handling red midge larvae. Ten minutes after feeding her 2 puffer fish with *C thummi* larvae, she developed rhinorrhea, congestion, sneezing, and nasal itching. The symptoms improved after leaving the room. However, some weeks later, the rhinitis worsened and was accompanied by red, watery eyes and palpebral angioedema. Finally, the patient presented rhinoconjunctivitis symptoms when she entered the room where the fish had been fed 12 hours earlier by another person. The clinical symptoms did not appear if the fish were not fed with the larvae, and she became asymptomatic when she moved house.

The patient was asymptomatic when she came to our clinic, 1 month after her last exposure to the culprit larvae. Previously, SPT was performed with commercial extracts of different allergen sources that can cause cross reactivity with *C thummi* proteins [1,3,4], as follows: *Dermatophagoides pteronyssinus* (100 UB/mL, ALK-Abelló, S.A.), shrimp (1 mg/mL, Bial-Aristegui), mussel (1 mg/mL, Bial-Aristegui), *Anisakis simplex* (2 mg/mL, ALK-Abelló, S.A.), common mosquito (275 µg/ml, Laboratorios LETI), and cockroach (325 µg/mL, Laboratorios LETI). The results were negative (wheal size <3 mm)

Total IgE was 23.20 IU/L by ImmunoCAP (Thermo Fisher Scientific). Specific IgE levels (measured using the same technique) to the allergenic sources assayed in the SPT were negative (<0.35 kU_A/L).

Protein extract from freeze-dried pure *C thummi* larvae (commercial fish food used by the patient [3F Frozen Fish Food]) were prepared by delipidization with acetone (4% wt/vol), homogenization in PBS 100 mM and NaCl 100 mM (pH 7.5) (5% wt/vol) for 2 hours at 4°C, dialyzation, and lyophilization [1]. Specific IgE against *C thummi* larvae extract was 3.7 kU_A/L by Enzyme AllergoSorbent Test (EAST; Specific IgE EIA kit HYTEC HYCOR Biomedical Ltd [9]). SDS-PAGE IgE-immunoblotting was carried out under reducing electrophoretic conditions (with 2-mercaptoethanol) using extracts from *C thummi* larvae, *D pteronyssinus*, *Penaeus monodon*, *Blatella germanica*, and *Periplaneta americana*. IgE-binding bands were detected only with *C thummi* larvae extract. A faint IgE-binding area of

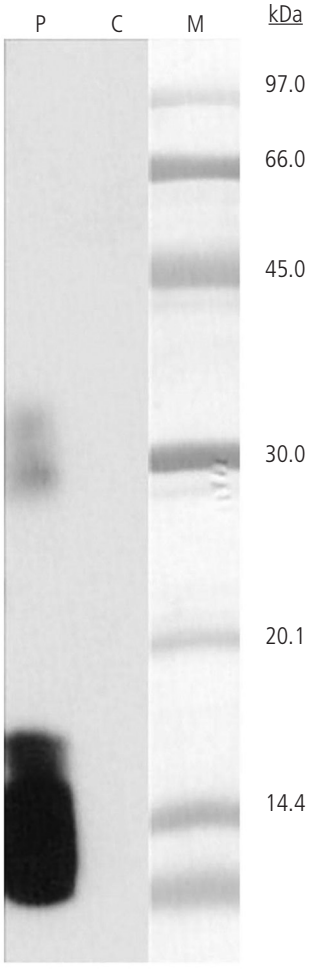

Figure. SDS-PAGE IgE-Immunoblotting with *Chironomus thummi* larvae extract under reducing electrophoretic conditions (with 2-mercaptoethanol). Lane P, patient serum; Lane C, control serum (pool of sera from nonatopic persons); Lane M, molecular mass marker.

approximately 30-32 kDa and an intense one of approximately 17-10 kDa were detected. The molecular mass of these areas matched those of the dimeric and monomeric forms of the chironomid hemoglobin, respectively (Figure) [1,8].

A flow cytometry assay to determine the percentage of activated blood basophils that expressed the CD63 marker (basophil activation test) after in vitro stimulation with *C thummi* larvae extract was performed as described in a previous publication [6]. Positive results (cut-off, 15% $CD63^+$ basophils) were obtained with 2 of the 3 concentrations tested (19.3% $CD63^+$ basophils at 0.25 mg/mL and 2.5 mg/mL and 12.1% at 0.025 mg/mL; baseline $CD63^+$ basophils, 1.7%; $CD63^+$ basophils with anti-IgE, 33.9%).

We report a case of allergy to *C thummi thummi* larvae in a nonatopic patient who was sensitized to these larvae after a short exposure to low amounts of the allergenic source. Because an anaphylactic reaction during skin testing with *C thummi* larvae extracts has been reported, the use of in vitro tests such as specific IgE and the basophil activation test [6] have been proposed as safe, useful, and valid diagnostic tools.

The diagnosis was based on the clinical history and in vitro tests (specific IgE determination and BAT). Only avoidance induced clinical remission of the symptoms. In spite of the widespread domestic use of these larvae and the scarce number of reports of *C thummi* allergy, our results highlight the high allergenic potential of this source, even in nonatopic individuals. Our results also support previously published evidence about the utility of in vitro testing to identify airborne allergenic sources. Given the high risk of airway involvement with skin testing and challenge testing [7], BAT and IgE-immunoblotting were shown to be reliable diagnosis tools.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentation

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