



Sensitization to *Anisakis simplex* s.l. in a healthy population

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Abstract

The main objective of this study was to determine the degree of sensitization to *Anisakis simplex* s.l. in healthy population. Using the determination of specific IgE, we studied the seroprevalence against *A. simplex* s.l. in blood donors selected at random in the region of Antequera (Southern Spain). We detected 22.1%. The immunoblotting technique revealed a band of approximately 60 kDa in the serum of individuals who also exhibited high values of specific IgE against *A. simplex* s.l. This band would be useful for diagnosis.

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1. Introduction

The Anisakidae include species of which the third-stage larvae (L₃) are present in a wide range of commercially important fish species (Huang, 1988; Adroher et al., 1996; Manfredi et al., 2000; Strommes and Andersen, 2000 and others). It is well-known that man can act as an accidental host when parasitized raw fish is consumed. In terms of public health, the most important species are *Anisakis simplex* complex. In man, illness appears mainly as gastrointestinal symptoms (Louredo Méndez et al., 1997; Del Rey Moreno et al., 1998).

However, in severe cases, symptoms are not limited to the gastrointestinal tract since larvae have sometimes been reported in heterological or ectopic locations, having passed through the wall of the digestive tract and reached the mesentery, liver, vesicle, pancreas, ovaries, pleura, lymphatic nodes, etc. In these locations, the larvae may encapsulate, remaining there until their gradual degradation. Thus, *A. simplex* antigens released during fixation, the passage of the larvae through body and in their encapsulation and/or disintegration sensitize the patient to future exposure and produce allergic reactions (Ishikura et al., 1992; Daschner et al., 1998; Domínguez Ortega et al., 2001). The number of allergic and gastro-allergic reactions to *A. simplex* reported in Spain has increased dramatically in the last decade. Since 1995, more than 150 cases of allergy to the parasite have been

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reported (López-Serrano et al., 2000; Domínguez Ortega et al., 2001; Audicana et al., 2002). In healthy populations, it is common to find subclinical sensitization to these parasites due to high consumption of parasitized fish or to cross-reactions with other nematodes (Kennedy et al., 1988; Iglesias et al., 1996; Lorenzo et al., 1999) or crustaceans, insects or mites (Pascual et al., 1997; Johansson et al., 2001).

2. Materials and methods

2.1. Subjects and serum samples

A total of 77 serum samples were randomly taken from blood donors in the region of Antequera (Alameda, Almogía, Antequera, Archidona y Campillos) during the first 4 months of 2000 with each participant consented to the extraction of a blood sample and participation in a telephone survey to obtain data such as age, sex and fish consumption, especially that of raw or lightly cooked fish. The participants were divided into four groups according to frequency of fish consumption (however, it was cooked): 1–3 days a month, 1–2 days a week, 3–5 days a week and 6–7 days a week.

2.2. Specific IgE determination

Specific IgE against *A. simplex* s.l. was determined using CAP-FEIA according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden) and was considered positive results greater than 0.7 kU/L.

2.3. Specific IgE detection by immunoblotting

The protein electrophoresis was carried out in gels of polyacrylamide (Laemmli, 1970). An *A. simplex* s.l. larval crude extract (CE) antigen (ALK-Abello, Madrid, Spain) were mixed 3:1 with dissociation buffer heated at 100 °C for 5 min and electrophoresed in 12% analytical SDS-polyacrylamide gel. Following the SDS-PAGE of the larval CE, the separated proteins were transferred onto a nitrocellulose membrane (Pharmacia, Uppsala, Sweden) in a Mini-Protean II (Bio-Rad, Richmond, CA, USA). After blocking, with 5% (w/v) fat-free milk powder, membranes were incubated overnight with 600 µl of sera from the patients 1:5 diluted. Following three washes, the membranes were incubated with peroxidase-labeled monoclonal anti-IgE (Sigma, St. Louis, MO) diluted 1:600. Finally, detection was carried out using an enhanced chemiluminescence method (ECL, Amersham Pharmacia Biotech).

2.4. Statistical analysis

We have used the Mann–Whitney test to evaluate the relationship between fish consumption and raw fish. To evaluate the relationship between dietary habits, sensitization to *A. simplex* and the immunoblot results we estimated odds ratios (OR) with 95% confidence limits, OR value were considered statistically significant if the 95% confidence interval did not include 1. The concordance of sensitization to *A. simplex* and the immunoblot results were determined using the kappa index.

3. Results

Monthly consumption of fresh anchovies in the Antequera region is around 15,000 kg for around 100,000 inhabitants (personal communication from Veterinary Coordinator from the Sanitary district of Antequera). Of the 77 samples chosen, 45 were men (58.1%) and 32 women (41.2%) with average ages of 39.4 and 39.0 years, respectively, being from 18 to 65 range. All were habitual consumers of fish. Of these, 26% habitually or sporadically consumed raw fish, nearly all of them fresh anchovies (*Engraulis encrasicolus*) pickled in vinegar (85%) and/or lightly grilled sardine (*Sardina pilchardus*) (15%). There were no significant differences regarding frequency of fish consumption between those who consumed raw fish and those who did not ($p = 0.1618$).

3.1. Determination of specific IgE

Positive results were obtained in 17 of the 77 sera of blood donors tested by CAP-FEIA, giving a seroprevalence of 22.1% (Table 1). IgE levels were very high in 3.9% of the subjects (>17.5 to 100 kU/L), high in 10.4% of the subjects (>3.5 to 17.5 kU/L) and moderate in 9.8% of the subjects (>0.7 to 3.5 kU/L), with a mean of 2.9 ± 12.0 kU/L, a median <0.35 kU/L and a 0 to 100 kU/L range. The ingestion of raw fish increased the risk of IgE sensitization to *A. simplex* (OR: 42.0, 95%CI: 9.3–189.3), although none of the seropositive

Table 1
Classes of specific IgE in the healthy population

Class of specific IgE (figure in kU/L)	Frequency	%
0 (<0.35)	59	76.6
1 (0.35 to <0.7)	1	1.3
2 (0.7 to <3.5)	6	7.8
3 (3.5 to <17.7)	8	10.4
4 (17.7 to <50)	2	2.6
6 (≥ 100)	1	1.3

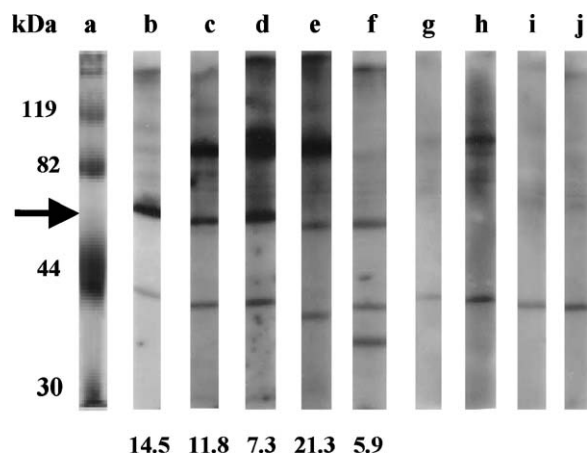


Fig. 1. Immunoblot of *Anisakis simplex* somatic antigen with sera from healthy donors (a) molecular weight markers (b–f) sera of blood donors with high specific IgE (g–j) sera of blood donors with negative specific IgE. The arrow shows the band of about 60 kDa. IgE values in kU/L in the bottom.

subjects remembered suffering abdominal pain and/or allergic symptoms.

3.2. Immunoblotting

Immunoblotting revealed that the serum of 52 blood donors (67.5%) recognized antigens of *A. simplex*. In the 60 and 119 kDa range between 1 and 6 antigenic bands were recognized. A band of approximately 60 kDa (Fig. 1) was detected in the serum of 21.15% individuals who also showed high or very high values of specific IgE (3–100 kU/L), with a concordance index: 0.693. Furthermore, a band of around 40 kDa appeared in the majority of the immunoblotting tests. The detection of the band of about 60 kDa was associated with the consumption of raw fish (OR: 34.0; 95% CI: 8.5–135.7).

4. Discussion

The prevalence of specific IgE antibodies against *A. simplex* s.l. in the epidemiological study of the healthy population was 22.1%. Other authors, in studies carried out on a healthy population group (Del Pozo et al., 1996; Fernández et al., 1997; Garcia et al., 1997; Purello-D'Ambrosio et al., 2000; Audicana et al., 2000), obtained positive results between 6.6 and 27.5%, in another study (Valiñas et al., 2001) only found 0.4% positive using a monoclonal antibody (UA3). Although none of the donors sensitized to *A. simplex* remembered having suffered digestive or allergic symptoms, we do not know whether the high seroprevalence found in this study had any clinical repercussions. We believe

that, as occurs in other infectious and parasitary diseases with a subclinical course, the disease only manifests itself through a positive result in the serology. The high percentage of sensitization obtained in our study (22.1%) may be due to a sensitization against other anisakids such as *Hysterothylacium aduncum* or *H. fabri*, which show high prevalence in commonly consumed fish from the Spanish coast (Adroher et al., 1996; Rello, 2003; Valero et al., 2004). These parasites shares antigens with *A. simplex* s.l. which can act as allergens. Using direct and inhibition ELISA techniques (Fernández-Caldas et al., 1998), and by immunoblotting (Valero et al., 2003), showed cross-reactivity between *A. simplex* s.l. and *Hysterothylacium* spp. In another study, by immunoblotting and using sera against somatic and excretory-secretory antigens of *A. simplex* s.l., revealed 11–12 and 14 bands, respectively, common to the three species (Lozano Maldonado et al., 2004). Thus, individuals sensitized to *Hysterothylacium* spp. can also show sensitization to *A. simplex* s.l. This may also be due to cross-reactions between *A. simplex* s.l. and arthropods (crustaceans, insects and mites) (Pascual et al., 1997 and Johansson et al., 2001).

There are no unanimous findings regarding the profiles of bands obtained by immunoblotting in anisakiosis (Akao et al., 1990; Pascual et al., 1999; Domínguez Ortega et al., 2000). In our case, the population studied also showed varied profiles. Bands were detected both in the serum of donors with high specific IgE and in that of some with low specific IgE. Different patterns of IgE recognition also were detected (Garcia et al., 1997) and identifying one band of about 40 kDa which was detected on immunoblotting of allergy cases and some nonallergy, doubtful and control subjects. In our study, although in 67.5% of the donors bands were detected by immunoblotting (cross-reactivity with other parasites or antigens), it should be noted that in only 21.15% one band of approximately 60 kDa was clearly observed. This appeared when the specific IgE against *A. simplex* s.l. was high or very high. We think that this band may have diagnostic value, a fact confirmed (Del Rey Moreno, 2003), who also detected this band in the serum of patients with anisakidosis. The difference in the molecular weights of the bands detected by immunoblotting between other authors (Garcia et al., 1997) and us, can be due to the lack of unification in the preparation of *A. simplex* antigenic extracts and the different blotting conditions, and therefore the number of obtained proteins could vary. In this study, we also detected a band of around 40 kDa in most of the immunoblotting, however, this was not considered since it is also observed in the sera of subjects with negative specific IgE. It should be

noted that the results of the sensitization to *A. simplex* s.l., measured as an increase in specific IgE or the presence in the immunoblotting test of the 60 kDa band, show a substantial concordance index (0.693). According to our data, the elevation of specific IgE against *A. simplex* s.l. and the presence of the band of around 60 kDa in the immunoblot are related to the consumption of raw fish (OR: 42 and 34, respectively).

In conclusion, the results obtained would support the high percentage of sensibilization against *A. simplex* s.l. and the use of immunoblot as diagnosis of this parasitization.

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