High Prevalence of Seropositivity to a Major Allergen of *Anisakis simplex*, Ani s 1, in Dyspeptic Patients

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Finding evidence of anisakidosis requires invasive methods. We have developed a serological assay based on the detection of an immunoglobulin E (IgE) specifically directed against Ani s 1 protein, a major parasite allergen of *Anisakis simplex*, which has shown a high level of accuracy in the diagnosis of anisakidosis. We used this tool to determine the prevalence of anti-Ani s 1 IgE in dyspeptic patients and to investigate if its seropositivity could be related to epidemiological factors other than raw fish consumption. A total of 174 dyspeptic patients who submitted to upper digestive tract endoscopy were studied. Specific IgE against Ani s 1 was determined by immunoblotting. Quantitative information on smoking, alcohol consumption, and fish consumption as well as a history of gastric surgery was recorded. Twenty-four (13.8%) patients were seropositive for Ani s 1 protein. The seroprevalence of anti-Ani s 1 IgE increased with age in patients who were less than 62 years old (P = 0.047). Seropositivity to Ani s 1 was associated with the consumption of fish in vinegar (P < 0.001), raw fish (P = 0.001), and smoked fish (P = 0.007). There was no relationship between anti-Ani s 1 IgE seropositivity and the number of cigarettes smoked (P = 0.098) or alcohol intake (P = 0.179). Five patients had undergone previous gastric surgery, and three of those patients were seropositive for Ani s 1 (P = 0.019). In multivariate analysis, the consumption of fish in vinegar (P = 0.006), raw fish (P = 0.017), and smoked fish (P = 0.002) and a history of gastric surgery (P = 0.005) were independent factors associated with anti-Ani s 1 IgE detection. In conclusion, at present, anisakidosis might frequently be underdiagnosed, and it might have a clinical role in patients with upper dyspeptic symptoms. Uncooked-fish ingestion and previous gastric surgery were associated with seropositivity for Ani s 1 protein.

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**Anisakis simplex** is a nematode parasite that belongs in the *Anisakidae* family. Its life cycle involves larval stages with several intermediary hosts and the adult stage, during which the worm parasitizes the stomachs of marine mammals. Humans can be accidental hosts by eating raw or undercooked fish or seafood that contains the third-stage larvae of *A. simplex*. After ingestion, larvae can be invasive, penetrating the digestive tract and producing a disease, known as anisakidosis or anisakiasis, that can mimic other gastrointestinal disturbances (12).

The finding of evidence of anisakidosis is based on visualization of larvae by upper digestive tract endoscopy within the first 24 h after fish ingestion or by surgical findings (12, 18). For these reasons, it is probable that only the most severe cases that require invasive techniques are diagnosed and that many of the mild cases remain underdiagnosed (21). Although serologic techniques have been developed for the detection of *A. simplex* infection, cross-reactivities with other helminths have been a serious limitation (8, 10, 11, 17). In an attempt to improve the diagnosis, we purified and characterized a major immunoglobulin E (IgE)-binding protein from the parasite. This major parasite allergen, named Ani s 1, is a protein from the excretory gland that shows no homology to a previously described protein (14). Recently, we have demonstrated that the detection of IgE specifically directed against Ani s 1 has a high accuracy in the diagnosis of anisakidosis (sensitivity, 86%; specificity, 90%) (6).

Up to now, the use of invasive methods for anisakidosis diagnosis has restricted the study of this disease, and, therefore, the true prevalence of the disease in dyspeptic patients is unknown. On the other hand, although risk factors, such as the consumption of raw or undercooked fish, are well known, other studies have suggested that host factors could be involved. However, those series published about anisakidosis are retrospective studies with a limited epidemiological value (13, 16, 19). The aims of this study were to determine the prevalence of *A. simplex* infection in dyspeptic patients and to investigate whether anisakidosis is related to epidemiological factors other than the consumption of raw fish in this population. We carried out a cross-sectional study of a group of dyspeptic patients who were investigated to determine the prevalence of primary drug resistance to *Helicobacter pylori*.

MATERIALS AND METHODS

Patients. From February to December 1998, 174 patients (91 men and 83 women; mean age, 49.3 ± 15.1 years; age range, 21 to 80 years) who underwent a programmed upper gastric endoscopy because of dyspeptic symptoms were studied. Dyspepsia was defined as intermittent or persistent pain or discomfort in the upper abdominal region or the lower part of the chest, heartburn, nausea, a feeling of postprandial fullness, or any other symptoms thought to be related to the upper gastrointestinal tract that lasted at least 3 weeks (4). Patients who had previously been treated for eradication of *H. pylori*, had received antimicrobial or antacids therapies during the previous 3 months, and/or had taken nonsteroidal
RESULTS

No cases of active parasitism were diagnosed during endoscopic procedures. However, 24 (13.8%) of 174 patients were reactive for Ani s 1 protein. Table 1 shows epidemiological results for the 174 patients. There was no association between gender distribution and seroreactivity to Ani s 1 protein. Overall, we did not observe any association of anti-Ani s 1 IgE detection with age. However, when we examined the patients who were less than 62 years of age, the seroprevalence of anti-Ani s 1 IgE increased with age (P = 0.047). Seropositivity to Ani s 1 protein decreased in the group of patients over 61 years, but the difference did not achieve statistical significance (P = 0.358).

Sixty-six (37.9%) patients were smokers and 103 (59.2%) reported alcohol consumption. We found no evidence of a dose-dependent association between Anti-Ani s 1 IgE seropositivity and the number of cigarettes smoked (P = 0.098) or alcohol intake (P = 0.179).

With regard to fish consumption, nonconsumers and subjects with sporadic ingestion were regrouped into one category. Data obtained in the interviews revealed that the habitual consumption of fish in vinegar sauce, namely, anchovies (*Engraulis encrasicolus*), was frequent in our population (32 subjects; 18.4%) and was associated with seroreactivity to Ani s 1 protein (P < 0.001). Only five subjects (2.9%) usually ate other raw fish dishes (sardines [*Sardina pilchardus*] and hake [*Merluccius merluccius*]). All but one of these subjects were seropositive to Ani s 1 protein (P = 0.001). With regard the consumption of smoked fish, seven (4%) patients were habitual consumers and four of those patients were anti-Ani s 1 IgE positive (P = 0.007). As expected, we did not find a relationship between the consumption of cooked fish and reactivity to Ani s 1 protein.

One hundred thirty-eight subjects (79.3%) were infected by *H. pylori*. No relationship was established between *H. pylori* infection and the detection of anti-Ani s 1 IgE. Five subjects had past histories of nonresective gastric surgery (pyloroplasty and vagotomy in all cases). Three of the patients were seropositive for Ani s 1 (P = 0.019).

To enable multivariate analysis, subjects were regrouped as under 62 years of age or 62 years of age and older, and the following variables were considered independent variables: tobacco consumption (smokers or nonsmokers); alcohol intake (drinkers or nondrinkers); consumption of raw fish, fish in vinegar sauce, and smoked fish (habitual consumers or sporadic consumers and nonconsumers); and previous gastric surgery (yes or no). Table 2 shows the results for multivariate analysis. Ingestion of fish in vinegar, raw fish, and smoked fish and a history of gastric operation were associated independently with anti-Ani s 1 IgE positivity.

DISCUSSION

In the present study, we found that 24 patients (13.8%) were seropositive to Ani s 1 protein. Therefore, these patients probably suffered previous episodes of *A. simplex* infection that had not been diagnosed. It is noteworthy that this work was carried out with dyspeptic patients in whom anisakidosis was not suspected. This finding highlights the observation that *A. simplex*...
infection might be more frequent than expected, since only the most severe cases with alarming gastric or allergic symptoms that require urgent upper endoscopy examination are being diagnosed at present. Moreover, we suggest that many cases of anisakidosis may be misdiagnosed and confounded with other conditions. In this context, studies about the clinical significance of seropositivity to Anisakis in dyspeptic patients are in progress.

This relevant seroprevalence of Anisakis IgE in our study can be due to several factors. First, Spain has a very elevated level of consumption of fish (85 g per person per day) (3). Second, a high level of fish parasitism of up to 45% has been detected in the market, depending on the species examined (1). Last, in our population, the habitual consumption of uncooked fish was very frequent, especially for anchovies in vinegar sauce (18.4%). In contrast, other studies have reported a very low seroprevalence of anti-Anisakis IgE in the north of Spain (20). This low seroprevalence can be explained, aside from the methodology used, by the culinary habits among different regions of Spain. Our study was performed in the central area of the country (Madrid), where the consumption of uncooked fish is more frequent than in the north (9).

In our work, the seroprevalence of Anisakis IgE increased with age in patients younger than 62 years. However, in older patients, the rates decreased slightly. Previous studies have reported that cases of anisakidosis are uncommon in elderly patients. This finding has been related to a decrease in gastric acid secretion with age (15). The possible influence of gastric pH on anisakidosis may be related to an increase of larval activity (12). In fact, nematode parasites of warm-blooded hosts use chemical and thermal signs in their hosts in the subsequent resumption of development after a metabolic arrest (2).

Our data revealed a strong association between anti-Anisakis IgE seroreactivity and the consumption of uncooked fish. Therefore, the disease is completely preventable by the appropriate preparation of fish or by general prophylactic measures, such as freezing before marketing. In this sense, current European Community regulations require that species intended for marinating or salting at temperatures of $\geq 60^\circ C$ must be stored at $-20^\circ C$ for 24 h. The U.S. Food and Drug Adminis-

| Characteristic                                      | Total | Positive for IgE against Anisakis | Negative for IgE against Anisakis | $P$  |
|----------------------------------------------------|-------|-----------------------------------|-----------------------------------|------|
| Sex                                                |       |                                   |                                   |      |
| Male                                               | 91 (52.3) | 10 (11)                          | 81 (89)                           | 0.366 |
| Female                                             | 83 (47.7) | 14 (16.9)                        | 69 (83.1)                         |      |
| Age (yr)$^a$                                       |       |                                   |                                   |      |
| 21–37                                              | 43 (24.7) | 3 (7)                            | 40 (93)                           | 0.535  |
| 38–49                                              | 44 (25.3) | 7 (15.9)                         | 37 (84.1)                         |      |
| 50–61                                              | 45 (25.9) | 10 (22.2)                        | 35 (77.8)                         |      |
| 62–80                                              | 42 (24.1) | 4 (9.5)                          | 38 (90.5)                         |      |
| Tobacco consumption                                |       |                                   |                                   |      |
| Nonsmokers                                         | 108 (62.1) | 12 (11.1)                        | 96 (88.9)                         | 0.098  |
| Smokers (no. of cigarettes/day)$^b$                | 66 (37.9) | 12 (18.2)                        | 54 (81.8)                         |      |
| 1–14                                               | 31 (17.8) | 4 (12.9)                         | 27 (87.1)                         |      |
| 15–50                                              | 35 (20.1) | 8 (22.9)                         | 27 (77.1)                         |      |
| Alcohol consumption                                |       |                                   |                                   |      |
| Nondrinkers                                        | 71 (40.8) | 8 (11.3)                         | 63 (88.7)                         | 0.179  |
| Drinkers (g of ethanol/wk)$^b$                     | 103 (59.2) | 16 (15.5)                        | 87 (84.5)                         |      |
| 1–40                                               | 54 (31) | 6 (11.1)                         | 48 (88.9)                         |      |
| 44–402                                             | 49 (28.2) | 10 (20.4)                        | 39 (79.6)                         |      |
| Consumption of fish                                |       |                                   |                                   |      |
| In vinegar                                         |       |                                   |                                   |      |
| Sporadic or nonconsumers                           | 142 (81.6) | 13 (9.2)                         | 129 (90.8)                        | <0.001 |
| Habitual consumers                                 | 32 (18.4) | 11 (34.4)                        | 21 (65.6)                         |      |
| Raw                                                |       |                                   |                                   |      |
| Sporadic or nonconsumers                           | 169 (97.1) | 20 (11.8)                        | 149 (88.2)                        | 0.001  |
| Habitual consumers                                 | 5 (2.9) | 4 (80)                           | 1 (20)                            |      |
| Smoked                                             |       |                                   |                                   |      |
| Sporadic or nonconsumers                           | 167 (96) | 20 (12)                          | 147 (88)                          | 0.007  |
| Habitual consumers                                 | 7 (4) | 4 (57.1)                         | 3 (42.9)                          |      |
| H. pylori infection                                 | 138 (79.3) | 20 (14.5)                        | 118 (85.5)                        | 0.788  |
| History of gastric operation                       | 5 (2.9) | 3 (60)                           | 2 (40)                            | 0.019  |

$^a$ Percentile, 25th.

$^b$ Percentile, 50th.

$^c$ Value was subjected to chi-square analysis to determine the linear trend.
tration demands that all fish products not intended for cooking or processing at temperatures of >60°C should be deep-frozen at −35°C for 15 h or at −23°C for a minimum period of 7 days (7). These measures should reduce infection rates.

Anisakidosis has rarely been reported to occur in patients following gastrectomy. The decrease of gastric acid and/or the shortened length of a resected stomach might be the factors responsible (2). In contrast, we found an association between patients with previous nonresective gastric surgery and seropositivity to Anis1i protein. Although the number of subjects was small and this finding could be casual, we cannot rule out the possibility that the persistence of dyspeptic symptoms in these patients is due to misdiagnosed episodes of A. simplex infection.

In conclusion, anisakidosis is at present frequently underdiagnosed. It may play a clinical role in patients with dyspeptic symptoms. Further studies are necessary to determine the clinical significance of seropositivity to Anis1i in this clinical setting. Consumption of different preparations of uncooked fish is a risk factor for A. simplex seropositivity.

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