

Is *Anisakis simplex* Responsible for Chronic Urticaria?

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ABSTRACT

The association of chronic urticaria (CU) to parasitic infestations has been poorly studied. Recently, sensitization to the parasite larva Anisakis simplex has been described as the cause of acute urticaria and anaphylaxis. The aim of this work was to study the relationship between sensitization to A. simplex and CU. One hundred one patients with CU were studied. Data of possible contacts with A. simplex were collected and the usual CU study was performed. Furthermore, total and specific immunoglobulin E (IgE; Pharmacia CAP system IGE fluorescence enzyme immunoassay: CAP) to A. simplex, Ascaris lumbricoides, Echinococcus granulosus, and Toxocara canis were determined as well as skin-prick test with A. simplex and serology to E. granulosus. In accordance with the results of the CAP to A. simplex, the patients were divided into two groups, positive and negative, and, subsequently, subdivided into two other subgroups that were alternatively told to stop eating fish or seafood in their diet or to continue with their normal diet. Checkups were performed at 6, 12, and 18 months. Thirty-five percent of the patients had positive skin tests to A. simplex, and CAP to A. simplex was positive in 55%. The fish-eating habits, acute or chronic gastrointestinal disease, and the background of abdominal surgery were not related to the results of the CAP and/or skin test to A. simplex. A total of 21.8% of all the patients had detectable CAP to A. lumbricoides, 91% of whom had positive CAP to A. simplex. Three patients had specific IgE to T. canis and five patients had specific IgE to E. granulosus in the absence of positive serology. All had specific IgE to A. simplex. Present infestation could not be proved in any of them. The clinical evolution and variations of CAP to A. simplex and of

total IgE were not statistically different among the groups during the 6, 12, and 18 months of the study. The percentage of sensitization to A. simplex in patients with CU is elevated and determines the sensitization to other parasites because of cross-reactivity. We have not found any causal relationship between the presence of specific IgE to A. simplex and CU. The clinical importance of this finding in this disease is still undetermined. (Allergy and Asthma Proc 24:339–345, 2003)

Acute urticaria is a highly prevalent disease that affects ~15–20% of the population at some time in their lives. Often, it is caused by an allergic reaction to foods or drugs that is more frequent in atopic children and young adults.¹ When the urticaria lasts >6 weeks, it is called chronic urticaria (CU), 50% of which persist for >6 months and 20% for >10 years.²

The etiology of most cases of CU (80–90%) is unknown (chronic idiopathic urticaria). Diagnosis should be made after excluding other possible causes such as food, drugs, physical agents, etc. Only recently, the importance of antibodies to the immunoglobulin E (IgE) mast cell receptor has been identified in this condition.^{3–5}

The association of a large number of helminthic parasites with CU (*Ascaris*, *Ancylostoma*, *Strongyloides*, *Filaria*, *Echinococcus*, *Schistosoma*, *Trichinella*, *Toxocara*, and *Fasciola*) has been described in the literature for some time;^{6,7} however, these associations have never been well established.

Recently, attention has been drawn especially to the larva of a parasite frequently found in the fish called *Anisakis simplex*, and an increase in the infestation of the north sea fishing grounds because of these larvae has been observed. This nematode parasitizes marine mammals and can infest humans with third-stage larvae when they eat raw or undercooked fish.⁸ Multiple cases of human infestation have been described, mainly in countries where eating raw fish is frequent; furthermore, a significant number of cases of allergic reactions caused by contact with this nematode

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(recurrent anaphylaxis, urticaria, and angioedema) are being reported.⁹⁻¹¹

Contrary to other antigens, it is not easy to suspect the relationship between contact with this parasite and the allergic clinical picture based on the patient responses because this fish contaminant generally goes unnoticed by the person eating it. Given its capacity to tolerate acidic conditions such as seasoning with vinegar or gastric juice for several days, it can cause gastric or cutaneous symptoms days after the fish is eaten and thus the cause-effect relationship is not so obvious.

The prevalence of patients who are sensitized to *A. simplex* in Spain is high. In our experience, "in vitro"-specific IgE (Pharmacia CAP system IGE fluorescence enzyme immunoassay: CAP) to this parasite is detected in 22% of the patients who attend the allergy outpatient clinic. This percentage increases to 45% in those who come for CU whose etiology is, in the beginning, undetermined.¹² Because of this finding, we wanted to study the possible cause-effect relationship between sensitization to *A. simplex* and the development or persistence of some cases of CU. The parasitic proteins in the body could be provoking intermittent hypersensitivity reactions with the appearance of urticaria.

In this study, we have found two important problems. The first arises from the limited information that we have on the time that the remains of this larva can persist in the human body. The human being is only an accidental host of this parasite, which does not reproduce in our body, and dies after a few days of remaining in the digestive tract. In most of the cases, this larva is eliminated without any problem; however, sometimes it remains and develops into a cyst in the intestinal mucosa, causing appendicitis, or it forms small granulomas (symptomatic or asymptomatic) in which the proteins of this parasite stay for an undefined period of time.¹³⁻¹⁵ On rare occasions, it has been described that they can cross through the mucosa of the digestive tract and cause extra gastrointestinal anisakiasis.¹⁶ This lack of understanding how long this larva or its fragments can remain in the body makes it difficult to define when the patients are free of contact with the parasite. The second problem refers to the high percentage of subclinical sensitization existing, which significantly make the diagnosis of allergy to this parasite difficult. The two diagnostic tests available (skin test and CAP) are not sufficient to evaluate the clinical risk of reaction when faced with future contact with the parasite. Oral challenge with antigens of this parasite could differentiate between the patients who tolerate or do not tolerate contact with *A. simplex*. However, oral challenges have been performed with the lyophilized parasite in patients with clearly positive clinical records, all of which, at present, have been negative.^{17,18} This problem in the diagnostic tests creates a large disparity of criteria in the subsequent treatments and dietary regimens that these patients should follow.

In this study, we aim to determine the prevalence of sensitization to *A. simplex* in patients with CU, the epide-

miological and clinical characteristics that the patients who are sensitized to *A. simplex* have, and to analyze their clinical and analytical evolution at 6, 12, and 18 months after their first visit in relationship to the presence or absence of fish in their daily diet.

PATIENTS AND METHODS

We studied 101 patients (35 men and 66 women) who came to our allergy outpatient clinic in the first 4 months of the year because of symptoms of CU whose etiology was unknown in the beginning. The mean age of the patients was 44 years (range, 14-72 years).

A complete clinical history and physical examination were performed in all patients. At first, none of them related the symptoms with drugs, foods, insect bites, or other diseases. Data that could show us there was possible contact with *A. simplex* were gathered: acute or chronic gastrointestinal disease (abdominal pain, nausea, vomiting, diarrhea, etc.), fish-eating habits (frequency and culinary preparation), and patient relationship with the urticaria symptoms.

The following complementary tests aimed at studying CU were performed in all the patients: complete blood count, general chemistry, erythrocyte sedimentation rate, urine and sediment analysis, complement study (C₃, C₄, and factor B), circulating immune complexes, and basic coagulation study. Other examinations aimed at studying allergy to any antigen suspected of being involved because of the clinical record as well as the data on sensitization to parasites also were included: total IgE and specific IgE (CAP) to *A. simplex*, *Ascaris lumbricoides*, *Echinococcus granulosus*, and *Toxocara canis*; skin-prick test (SPT) with *A. simplex* (IPI, International Pharmaceutical Immunology, S.A. Madrid); serology to *E. granulosus*; parasites in stools; SPT; and CAP with fish and seafood (to determine sensitization to these foods).

The SPTs were performed in patients who did not show dermatographism and who were not receiving treatment with antihistamines the week before the test was performed. An SPT was considered to be positive when there was a papule >3 mm of that found in the negative control, and the CAP was positive when there was a value >0.35 kU/L.

According to the result of the CAP to *A. simplex*, the patients were divided into two groups (positive, A; negative, B). Each group was subdivided into two other subgroups that were alternately advised to stop eating fish or seafood (1) or to continue with their normal diet (2). Clinical check-ups and analyses were performed at 6, 12, and 18 months, collecting data on the clinical condition and analyzing the evolution of the SPT to parasites and the total IgE.

No evaluation could be performed in the group of patients who did not demonstrate sensitization to *A.s* (SPT and CAP: negative) who had been instructed to stop eating fish for six months because none of them stopped eating it.

According to the clinical condition described in the different follow-up visits, we considered three situations: (1) patients who were clinically the same, presenting with ur-

TABLE I

Relation of Clinical Parameters to *A. simplex* Sensitization

	Positive Skin Test to <i>A. simplex</i>			Specific IgE to <i>A. simplex</i>		
	Positive	Negative	<i>p</i>	Positive	Negative	<i>p</i>
Patients with CU	31/88	57/88	—	55/101	46/101	—
Raw fish ingestion*	0.55	0.49	NS	0.53	0.46	NS
Fish ingestion*	2.8	2.2	NS	2.6	2.1	NS
Mean total IgE (SD)	528 (1136)	177 (230)	<0.005	447 (899)	149 (158)	<0.005
Atopy	7	19	NS	10	18	NS
Abdominal surgery	3	7	NS	6	5	NS
Gastrointestinal disease	7	15	NS	13	12	NS

Total IgE concentration is in kU/L. *p* is significant if *p* < 0.05.

NS = not significant.

*Times/week.

ticaria lesions with the same periodicity as before or those who had worsened; (2) patients who reported a clear improvement (with more time between the urticaria episodes); and (3) those who belonged to the group who had remained asymptomatic.

For patients reporting eating fish and experiencing acute urticaria, the onset had to be within 6 hours of eating the fish. The data were analyzed with the statistical software package SPSS 7.9 (SPSS, Inc., Chicago, IL).

RESULTS

Initial Results of Laboratory Tests

In the general examinations requested, the following alterations were observed.

Leukocytosis (leukocytes above $11.0 \times 1000/\text{mm}^3$) in 9 patients (8.9%), eosinophilia (above 450 eosinophils/ mm^3) in 29 patients (28.7%), elevated erythrocyte sedimentation rate in the 1st hour in 16 patients (15.8%), decrease in complement C3 fraction (below 83 mg/dL) in 9 patients (8.9%), decrease in C4 fraction (below 20 mg/dL) in 14 patients (13.9%), elevation of circulating immune complexes in 28 patients (27.7%), elevated total IgE (above 120 kU/L) in 57 patients (56.4%), and detection of parasites in stools in 14 patients (13.9%; 9 *Blastocystis hominis*, 3 *Giardia lamblia*, and 2 *Entamoeba coli*).

The general chemistry, urine, and sediment analyses; basic coagulation study; complement factor B; and serology to *E. granulosus* were normal in all patients. None of the alterations found, except from total IgE, was associated with the sensitization to *A. simplex* (positive SPT or CAP). Elevated eosinophil counts were observed in 14 patients with a positive CAP (25.5%) and in 6 patients with a positive SPT (19.4%).

Etiological Diagnosis of the Urticaria

Eighty-six patients (85%) were diagnosed with chronic idiopathic urticaria once other possible causes were considered. Sixteen of these patients (18.6%) reported their

symptoms with eating fish and/or seafood at some time, but they tolerated the same fish at other times.

Physical urticaria was diagnosed in 15 patients from the total group (11% of patients dermographism, 2% cholinergic urticaria, 1% because of pressure, and 1% because of heat).

Forty-eight patients included in the study (48%) presented with more than one allergic condition. In addition to CU, 16 patients (15.8%) were sensitized to some drug and 6 patients (5.9%) were sensitized to foods. However, the drug or food was not considered responsible for the CU symptoms in any of the cases.

Sensitization to *A. simplex*

Skin tests were performed with *A. simplex* extract in 88 of 101 patients (87.1%), being positive in 31 (35.2%), 15 women and 16 men (Table I). The mean size of the papules was 5.4×4 mm.

At the onset of the study, the specific IgE to *A. simplex* was positive (>0.35 kU/L) in 55 (23 men and 32 women) of the 101 patients (54.5%) with a mean of 21.7 kU/L (SD, 27.3 kU/L). The SPT and CAP were positive in 30 of the 88 patients (34%) in whom both tests were performed.

Fifteen of the 101 patients (14.9%) reported symptoms possibly compatible with clinical sensitization to *A. simplex* (urticaria after eating fish on some occasions and good tolerance in others). This finding occurred in 10 of those patients who had a positive skin test (32.2%), in 14 of those patients who were positive to CAP (25.4%), and in 10 of those patients who were positive for both tests (33.3%).

The mean total IgE concentrations were significantly greater in patients with positive CAP or skin tests to *A. simplex* (Table I). The specific IgE level correlated with the total IgE value ($r = 0.576$; $p < 0.01$).

The acute or chronic gastrointestinal disease and episodes of abdominal surgery that could hide evidence of parasitic infestation were not related to the results of the CAP and/or skin tests to *A. simplex* (Table I). At the onset of the study,

TABLE II

Relation Between Raw Fish Ingestion and *A. simplex* Sensitization

	Frequent Raw Fish Ingestion	No Raw Fish Ingestion
A (no. of patients)	29/55	26/55
B (no. of patients)	21/46	25/46
Mean total IgE	329 $\pm$ 890 kU/L	294 $\pm$ 402 kU/L
Mean IgE <i>A. simplex</i>	11.37 $\pm$ 21.9 kU/L	12.51 $\pm$ 23.67 kU/L

There were no statistical differences between groups.

A = CAP positive to *A. simplex*; B = CAP negative to *A. simplex*.

TABLE III

Clinical Follow-Up at the 6-Month Checkup

6-Month Checkup (n = 88)	A1 Group (n = 26)	A2 Group (n = 26)	B2 Group (n = 36)
Asymptomatic	16 (61.5%)	14 (53.84%)	13 (36.1%)
Better	8 (30.7%)	5 (19.23%)	17 (47.2%)
Same or worse	2 (7.69%)	7 (26.9%)	6 (16.6%)

Asymptomatic = without lesions of urticaria in the last 12 weeks; better = more time between the urticaria episodes or less lesions of urticaria; worse = less time between the urticaria episodes or more lesions of urticaria; A₁ = CAP positive to *A. simplex* who were advised to stop eating fish or seafood; A₂ = CAP positive to *A. simplex* who were advised to continue with their normal diet; B₂ = CAP negative to *A. simplex* who were advised to continue with their normal diet.

all the patients stopped eating raw fish on their own account, even in the group that continued to eat fish that was cooked first.

Relationship with Other Parasites

In the entire group, there were 22 patients (21.8%) who had detectable IgE specific to *A. lumbricoides* proteins (mean, 5.51 kU/L). No eggs were detected in the stools in any of them. Twenty of them (91%) also had evidence for specific IgE to *A. simplex* (mean, 35.7 kU/L).

Three of the 101 patients (3%) had specific IgE to *T. canis* (mean, 1.68 kU/L) and all of them had elevated values to *A. simplex* (mean, 53.83 kU/L).

E. granulosus was positive in five patients (mean IgE, 1.27 kU/L) in the absence of positive serology (IgM and IgG). The presence of hydatid cysts could not be observed in any of them. In addition, all patients had specific IgE to *A. simplex* (mean 68.7 kU/L).

Fish-Eating Habits

Fish-eating frequency, the common cooking degree, and the habit of eating raw fish in the forms of anchovy in vinegar, smoked salmon, or others were evaluated.

The weekly frequency of eating cooked or raw fish was statistically similar in the patients with the positive or negative skin test or in those who had or had not developed specific IgE to *A. simplex* (Table I). The percentage of patients who ate raw fish also was similar in the patient

group with positive or negative skin tests or specific IgE (Table II).

The mean values of specific and total IgE also were not statistically different between the group that ate raw fish and the group that avoided it. However, a positive relationship was found between the level of total IgE and the frequency of eating fish ($r = 0.28$; $p < 0.01$), but this relationship with the level of specific IgE did not exist ($r = 0.19$; $p = 0.06$).

The two groups that had positive CAP to *A. simplex* (A1 and A2) did not differ in their fish-eating habits or in the total and specific IgE value to *A. simplex* at the onset of the study.

Checkup at 6 Months

At 6 months of follow-up, 88 (88%) of the 101 patients, 52 belonging to group A (94.5%) and 36 belonging to group B (80%), were studied from the clinical point of view.

A total of 6.5% of the patients in group A1 reported being asymptomatic at 6 months, compared with 53.8 and 36.1% of the patients from group A2 and B2, respectively. However, these differences were not statistically significant (Table III).

We were able to gather data for evaluation from 48 patients (45.5%), 32 patients of whom presented as CAP⁺ for *A. simplex* (group A). The mean levels of CAP to *A. simplex* in patients who eliminated fish from their diet (A1) decreased 18% at 6 months, and 48.5% in those who con-

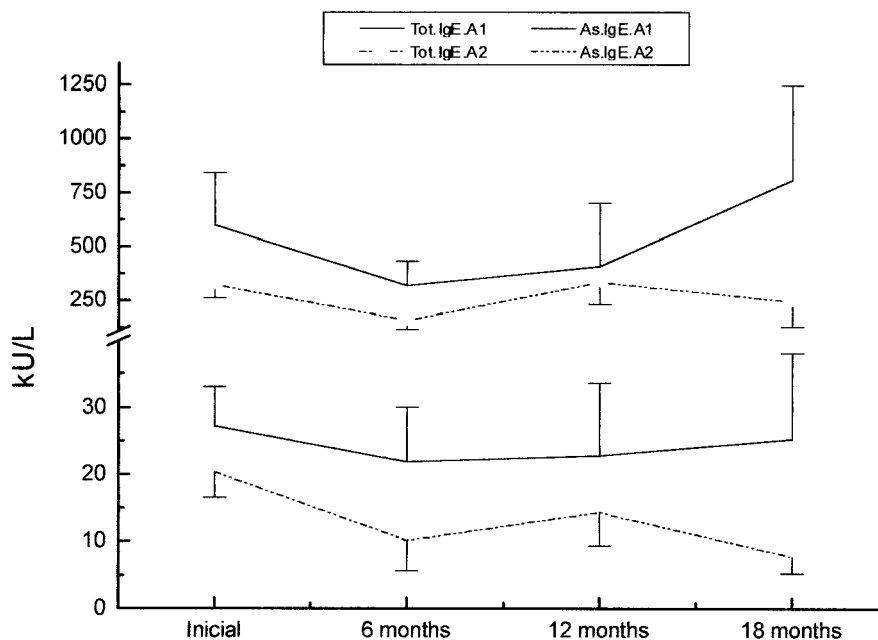


Figure 1. Total and specific IgE to *A. simplex* mean levels.

TABLE IV

Clinical Follow-Up at the 12-Month Checkup

12-Month Checkup (n = 84)	A1 Group (n = 12)	A2 Group (n = 37)	B2 Group (n = 35)
Asymptomatic	9 (75%)	27 (72.97%)	16 (45.71%)
Better	3 (25%)	7 (18.91%)	14 (40%)
Same or worse	0 (0%)	3 (8.1%)	5 (8.1%)

Asymptomatic = without lesions of urticaria in the last 12 weeks; *better* = more time between the urticaria episodes or less lesions or urticaria; *worse* = less time between the urticaria episodes or more lesions of urticaria.

tinued to eat it (A2), differences that are not statistically significant.

The decrease of the total IgE was greater in the group that avoided fish (Fig. 1), although the differences also were not significant.

The results of the general physical and laboratory examinations performed at 6 months were similar to those obtained at the beginning of the study.

Checkup at 12 Months

At 12 months, 86 (85.14%) of the 101 patients were evaluated. The clinical evolution was similar in all the groups, regardless of the diet followed (Table IV). Seventy-five percent of the patients from group A1 reported that they were asymptomatic, the same occurring in 73% in group A2 and 46% in group B2 (differences that are not statistically significant).

Laboratory tests were obtained in 58 of the 86 patients. There was an 8% decrease in the mean specific IgE to *A. simplex* in group A1 and 11.8% in group A2 from the onset of the study; these differences are not significant (Fig. 1).

The mean total IgE decreased by 51.3% in group A1 and increased ~7.5% in group A2.

Checkup at 18 Months

Seventy-four patients (75.2%) were seen. The clinical condition was similar between the groups, without significant differences (Table V).

Forty-three patients were assessed from the laboratory, and the CAP to *A. simplex* was positive in 26 patients. The CAP variations (initial and at 18 months) between groups A1 and A2 were not significant. These decreased from 17.8 to 25.3 kU/L in the first group and from 18.4 to 7.7 kU/L in the second group (Fig. 1).

DISCUSSION

Laboratory tests used in study of CU can not detect the etiology in most cases. In our study, ~85% of the patients were diagnosed with chronic idiopathic urticaria without knowing the cause of the disease despite the fact that many of these patients had peripheral eosinophilia.

TABLE V

Clinical Follow-Up at the 18-Month Checkup

18-Month Checkup (n = 84)	A1 Group (n = 6)	A2 Group (n = 37)	B2 Group (n = 26)
Asymptomatic	3 (50%)	28 (75.67%)	16 (61.53%)
Better	2 (33.33%)	5 (13.51%)	10 (38.46%)
Same or worse	1 (16.66%)	4 (10.81%)	0 (0%)

Asymptomatic = without lesions of urticaria in the last 12 weeks; *better* = more time between the urticaria episodes or less lesions of urticaria; *worse* = less time between the urticaria episodes or more lesions of urticaria.

The clinical significance of the specific IgE to parasites is a controversial subject. Approximately one-half of these patients showed specific IgE to proteins of the *A. simplex* larva, a number that is greater than that observed in the general Spanish population (22%).¹² That is why this parasite might be an important candidate for the etiological agent of CU.

The percentage of patients with specific IgE to other parasites also was elevated, but it seems to be caused by this same sensitization. Specific IgE to *A. lumbricoides* was detected in >20% of the patients in this study; however, >90% of them also had specific IgE to *A. simplex*. In addition, ascariasis is a rare infestation in the Spanish population and parasite eggs were not observed in any case in the stools of those affected. The five patients in whom specific IgE to *E. granulosus* was detected and the three in whom IgE to *T. canis* was detected also presented with specific IgE to *A. simplex* and no specific IgG or IgM were found in any of the cases. Thus, as in other cases that have already been published, it is explained by the cross-reactivity with these parasites.^{19–20} Therefore, the finding of specific IgE to these last three parasites should lead us to investigate a sensitization to *A. simplex*.

We have studied the significance that these antibodies have in chronic idiopathic urticaria. It is significant that only one-third of the patients with positive skin test and one fourth of those who have specific IgE to *A. simplex* could associate the symptoms at some time with fish ingestion. However, all the patients who reported an association of their symptoms with fish had a positive skin test or specific IgE to *A. simplex*.

The rate of 27.7% of atopic patients was not greater among the patients with specific IgE to *A. simplex*. No data were found in the clinical record that could be associated with this sensitization and more cases of gastrointestinal disease or abdominal surgeries were not observed. These patients also had similar habits regarding the frequency of eating fish and the cooking level.

The possible cause-effect relationship between sensitization to *A. simplex* and the development or persistence of CU was studied by *subjecting* the patients to diets with the inclusion/exclusion of fish. Because we disregarded the time that the possible remains of parasites can persist in the

human body, we studied these patients for 18 months, assessing that this would be a reasonable period for such proteins to be eliminated. However, we could not find any significant differences in the clinical course between patients who continued to eat fish and who possibly remained in contact with the *A. simplex* larvae and those who avoided fish. Most of the patients evolved favorably. The fact that all patients stopped eating raw fish during the study prevented some of the infestations by *A. simplex*, but many of the cases of allergic reactions to *A. simplex* have been confirmed after eating normally cooked fish.

It is important to emphasize that the total IgE value was much higher in the specific IgE or positive skin test to *A. simplex* group. This group of patients had been in contact with the parasite at some time. Thus, an elevated total IgE concentration in the study of CU should lead one to suspect the possible relationship with *A. simplex*.

The evaluation of the laboratory parameters also did not differ among the groups. In all of them, we observed a decrease in total and specific IgE over time and the latter decrease was not greater in those patients who did not eat fish.

In conclusion, although there was a high rate of sensitization to this parasite in our patients, the data of this study do not support that sensitization to *A. simplex* is a significant cause of CU or a perpetuating factor of the symptoms. It is likely that the finding of these antibodies is a purely concomitant effect of parasite exposure and sensitization.

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