



Low *Anisakis*-specific IgE prevalence in dyspeptic patients in Italy – a retrospective study.

Fabrizio Bruschi^{1*}, Barbara Castagna¹, Francesca Mori-Ubaldini^{1**}, Barbara Pinto¹, Francesco Costa³ and Ferruccio Bonino²

Abstract

The aims of this case-control study were to determine the prevalence of *Anisakis*-specific IgE in patients reporting chronic or acute gastrointestinal (GI) symptoms and to investigate the correlation with raw fish ingestion habits.

A group of patients undergoing gastric endoscopy and a control group of healthy subjects answered a self-administered questionnaire on their food habits, presence of symptoms (both allergic and not allergic), and general life style. The presence of anti-*Anisakis* IgE has been evaluated using a serum immunoCAP assay.

Our data show a low prevalence of IgE directed against *Anisakis* allergens in Italy in dyspeptic patients, despite the high consumption of poorly cooked fish. These findings does not correlate with the results of studies from other Mediterranean countries, such as Spain, for example.

The general prevalence of *Anisakis* allergens sensitization in Italy could be further investigated through screenings in the allergic population, especially on those patients who claim to have developed a fish allergy and with history of raw fish consumption. Moreover, the attention should be moved on recent allergic reactions associated with fishing ingestion. This could in fact indicate a recent encounter with the parasite.

Finally, we must underline that the evaluation of *Anisakis*-specific IgG would have probably shown a difference in terms of exposure between the two groups; thus, it might be useful to detect also this antibody class in future population-based studies.

Keywords: *Anisakis*, food allergy, raw fish, sensitization

Introduction

Anisakis simplex is a parasitic nematode belonging to the Anisakidae family. The life cycle of this worm involves crustaceans, fishes and marine mammals. Humans are infected as accidental hosts by eating raw or undercooked seafood that is contaminated with the parasite, causing a zoonosis called anisakidosis or anisakiasis. Within a few hours after the ingestion of a living third stage larvae (L3), the parasite causes an acute infection often associated with GI symptoms such as abdominal pain, nausea, vomiting, and/or diarrhea. The clinical picture ranges from being asymptomatic to requiring emergency room care. These clinical reactions correspond to the worm invasion of the gastrointestinal mucosae and submucosae, causing initially a hemorrhagic lesion, then inducing the formation of a pathognomonic eosinophilic phlegmon. In addition, IgE-mediated allergic reactions are often reported, as the result of a sensitization to a range of allergenic proteins. However, some patients develop simultaneously both the gastrointestinal and the allergic syndrome after ingesting living parasites. This condition would be termed “gastroallergic anisakiasis”. Allergic symptoms vary from angioedema or urticaria to anaphylactic shock. The allergic manifestations may occur also after the ingestion of dead worms present in cooked fish if the patient is already sensitized by *A. simplex* antigens (1).

¹Department of Translational Research, University of Pisa, Pisa, Italy

²Department of Clinical and Experimental Medicine, Università di Pisa, Pisa, Italy
Present position: Institute for Health, University of Pittsburgh Medical Center (UPMC) Chianciano Terme, Institute of Biostructure and Bioimaging (IBB), National Research Council (CNR), Naples, Fondazione Italiana Fegato (FIF), Area Science Park, Campus Basovizza, Trieste, Italy

³University Gastroenterology Unit, AOU Pisana, Pisa, Italy

**present position: Resident in the School of Infectious Diseases, University of Sassari, Sassari, Italy

*Corresponding author: F. Bruschi
E-mail: fabrizio.bruschi@med.unipi.it

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Sensitization is commonly diagnosed through a serological assay detecting specific anti-*Anisakis* IgE by the immunoCAP test. If the larva is not expelled through vomit or by surgical removal, a chronic granuloma surrounding the dead parasite is generated. Thus, disease may present with chronic GI symptoms (dyspepsia, colic pain, or complicate in acute abdomen) (2). The upper GI tract, such as stomach and duodenum, is the section more commonly affected by the infection (3). However, there have been reports of cases involving the ascending colon and the sigma (4). Infection of the sigmoid colon is considered a risk factor for the development of colorectal cancer (5).

The aims of this study were to determine the serum prevalence of anti-*Anisakis* IgE in patients reporting chronic or acute GI symptoms and to investigate the correlation with food habits, presence of symptoms (both allergic and not allergic), and life style. Participants to the study included patients undergoing gastric endoscopy and healthy subjects (control group). All participants answered a questionnaire about fish consumption habits and other risk factors for GI symptomatology. The presence of specific anti-*Anisakis* IgE has been evaluated using a serum immunoCAP assay.

Materials and Methods

Subjects: From May 2004 to January 2005 two groups of patients were enrolled in the study. The first group (G1) included 136 patients (60.2 % men and 39.8 % women, mean age 42 years, age range 21 to 78 years) suffering from dyspeptic symptoms, coming from a gastroenterological practice or undergoing a programmed gastric endoscopy. The second group (G2) included 49 healthy individuals (control group), (mean age 41 years, age range 19 to 62 years) randomly selected from the blood donors practice. Both patients and control subjects were interviewed by an anamnestic questionnaire, and anti-*Anisakis* allergen specific IgE were evaluated with an immunoCAP assay in the sera of both of the groups. Signed consent was obtained from each participant in the study.

Data collection: A self-administered questionnaire was fulfilled by each participant. The interview collected information about cigarette smoking habits, coffee and alcohol intake, food intolerance, poorly cooked fish consumption (raw fish or shellfish, smoked or marinate fish) and both allergic and GI symptoms. The smoking habit was categorized into current smokers and non-smokers. Cigarette consumption was expressed as cigarettes smoked per day and divided into two categories, <10 and ≥10. Subjects were also divided in categories according to the number of coffee cups consumed per day (<3, ≥3). Daily amount of consumed alcoholic beverages were obtained with the same method of coffee consumption. About the allergic symptoms, the presence of angioedema/urticaria after food ingestion, atopic status, previous episodes of anaphylactic shock, eczema, allergic asthma, allergic rhinitis, contact dermatitis, acute abdomen without certain cause, and rheumatologic manifestations were investigated, as well as the presence of hypereosinophilia, prior parasitic infections, and

family history of allergic reactions. As for the gastrointestinal symptoms patients were asked to indicate their manifestations choosing between nausea, vomit, dyspepsia, loss of weight, chest pain, retrosternal, epigastric or abdominal pain, anorexia, dysphagia, pyrosis, diarrhea or others.

ImmunoCAP® Specific IgE: allergen specific immunoglobulin E (IgE) to *Anisakis simplex* were evaluated by the ImmunoCAP® ISAC (*Phadia 1000*, reagent p4 , 14-4475-01, Thermo Scientific), made of mixed *Anisakis* antigens, also containing the antigen Ani s1. P4 is mixed with 150 µl of patient serum, and non-specific IgE are removed by a washing solution (code n° 10-9518-01). Anti-IgE antibodies (monoclonal from mice) combined with a hydrolytic enzyme (beta-galactosidase) are added. These bind to allergen specific IgE, if presents. After incubating for 1h, the sample undergoes a washing cycle, than it is incubated for further 30' with an enhancing solution (4-metilumbelliteril-beta-D-Galattoside). The result of the reaction is measured by fluorescence. A higher fluorescence value corresponds to a higher serum level of specific IgE. The instrument converts the fluorescence units in a concentration value based on a calibration curve. (Calibrator strip 0-100 0; 0.35 - 0.7; 3.5 - 17.5; 100 100KU/L 6 x 0,2 ml). Data are automatically double-read, and the average is calculated as the given result. A specific IgE concentration > 0.35 KUA/L was considered as a positive results.

Statistical analyses: The possible association of any of lifestyle factors (smoking habits, coffee and alcohol consumption) with seroconversion was analyzed using univariate analysis (Fisher exact test). Effects were considered to be statistically significant for $p<0.05$. We also calculated in each group the odds ratio (OR) of dyspeptic symptoms and risk factors as a measure of association between dyspepsia and consumption of uncooked or poorly cooked fish. p values are showed in the tables only when significant.

Results

No difference between the two groups as regards smoke, alcohol and coffee consumption was observed (Table 1). On the contrary, the number of patients affected by atopic status and history of allergic symptomatology (asthma or rhinitis) was significantly higher in patients, compared to healthy controls ($p<0.001$ and 0.028-0.0128, respectively)

Regarding the eating habits, we found a significant difference related to consumption of total poorly cooked fish (OR = 3.48, 1.61-7.52) whereas the consumption of raw fish is nearly the same in the two groups (Table 3). In particular, smoked fish and dry fish consumption showed the higher association with the first group (OR=5.32, 95%CI 2.63-10.76; OR=5.60, 95%CI 2.70-11.59, respectively). Also, the number of patients usually eating all the four different types of raw fish is higher in the first group (10%) compared to controls (2%). Even the number of patients consuming at least one type of raw fish is higher compared to healthy subjects (86.8% and 65.3%, respectively).

Among the 136 patients, 71 presented with a GI symptomatology at the moment the questionnaire was administered and the serum sample taken, see Table 4. The most common were pyrosis (74.6%) and dyspepsia (57.7%), followed by retrosternal and epigastric pain (38% and 35.2%). In 25 patients (35.2%) pyrosis and dyspepsia coexisted.

68 patients reported additional GI symptoms, such as regurgitation and other digestive alterations (Table 5).

Only five individuals resulted positive for the presence of anti-*Anisakis* IgE. Three of them belonged to the first group (patient A, B and C) whereas 2 of them belonged to the control group.

Table 1. Lifestyle habits concerning smoke, alcohol and coffee

	Patients n (%)	Healthy subjects n (%)
Smokers		
<10 cigarettes/die	34 (25.0)	10 (20.4)
>10 cigarettes/die	11 (8.0)	3 (6.1)
	23 (16.9)	7 (14.3)
Alcohol consumers	39 (28.7)	12 (24.5)
Coffee consumers	85 (62.5.)	34 (69.4)
<3 coffee/die	27 (19.8)	6 (12.3)
>3 coffee/die	58 (42.6)	28 (57.1)

Table 2. History of food intolerance and other allergic reactions

	Patients n (%)	Healthy subjects n (%)	P
Food intolerance	10 (7.3)	6 (12.2)	
History of food-related allergic reactions:			
• Angioedema	21 (15.4)	4 (8.2)	
• Urticaria	6 (4.4)	2 (4.1)	
• Both A/U	15 (11.0)	2 (4.1)	
	5 (3.7)	0 (0)	
Atopic status	43 (31.6)	4 (8.2)	0.001
Anaphylactic shock	5 (3.7)	3 (6.1)	
Eczema	27 (19.7)	4 (8.2)	
Allergic asthma	19 (14.0)	1 (2.0)	0.028
Allergic rhinitis	42 (30.9)	6 (12.2)	0.0128
Contact dermatitis	30 (22.1)	7 (14.4)	
Family history of allergic symptoms	37 (27.0)	11 (22.4)	

Table 3. Consumption of poorly or no cooked fish

	Patients (%)	Healthy subjects (%)	P	OR (95%CI)
Total poorly cooked fish consumers	118 (86.8)	32 (6.3)	0.0023	3.48 (1.61-7.52)
Raw fish	33 (24.2)	14 (28.6)		0.80 (0.38-1.67)
Smoked fish	98 (72.1)	16 (32.0)	<0.0001	5.32 (2.63-10.76)
Dry salted/pickled fish	91 (67.1)	13 (26.5)	<0.0001	5.60 (2.70-11.59)
Raw shellfish	33 (24)	6 (12.2)		2.29 (0.89-5.88)
2 among the above	21 (15.4)	9 (18.4)		-
3 among the above	14 (12.3)	4 (8.2)		-
4 among the above	17 (10.0)	1 (2.0)	0.0457	6.85 (0.88-52.97)

The level of immuncoCAP positivity of the patients from group one is respectively of 13.8 KUA/L, 0.98 KUA/L and 0.88 KUA/L. Patient A, the one with the highest IgE level, has an history of raw fish consumption involving all four type of poorly cooked fish, while patient B and C claims to consume only dry salted fish.

Presence of symptoms in positive patients vary from none to only GI to mainly allergic ones. Positive patients from the control group, instead, show an iCAP value of 0.51 KUA/L and 1.57 KUA/L and reported to eat respectively raw and smoked fish (C1) and raw fish (C2). They report to not suffer from any GI symptoms or allergic ones (Table 6).

Discussion

Positivity of IgE to *Anisakis* allergens showed no significant difference between the two groups.

These results totally differ from those obtained in a similar

study carried out on dyspeptic patients in Spain (6) where the prevalence of seropositivity to *Anisakis* 1 in those patients was found to be higher (13.8%), compared to results (0.42% of positivity) obtained in the population in Spain where a study was carried out with a random sample of 2801 healthy blood donors distributed in 53 geographic areas, proportional to the density of donors (7). This discrepancy cannot be ascribed to the sensitivity of our method which, conversely, lacks rather in specificity, compared to the method used by Spanish Authors (6).

Furthermore, in the paper cited above *Anisakis*-specific IgE resulted associated with the consumption of fish in vinegar, as well as raw and smoked fish (6). Our data suggest a low seropositivity to *Anisakis* allergens in Italy both in dyspeptic patients and in the general population, given that the 86.8 % of patients and the 65.3% in healthy controls claims to usually consume poorly or not cooked fish. Moreover, the level of

Table 4. Distribution of symptoms and other modifications in the two Groups

	Patients (%)	Healthy subjects (%)	P
Acute Abdomen	28 (20.6)	1 (2.0)	0.0011
Rheumatologic manifestations	37 (2.2)	4 (8.2)	0.0049
Eosinophilia	5 (3.7)	0	
GI symptoms	71 (52.2)	1 (2.0)	<0.0001

Table 5. Other common gastrointestinal symptoms reported by Group 1 patients

Others GI symptoms	N° of patients (Group 1) 68 pz (95,7%)
• Regurgitation	26 (36.6 %)
• Digestive alterations	17 (23.9%)
• Irritable Bowel Syndrome	19 (26.8%)
• Others	6 (8.4%)

Table 6. Positive patients to *Anisakis* sensitization

Patient (n°)	Raw fish consumption	GI symptoms	Allergic symptoms	Others	Food intolerance	Specific IgE KUA/L
Group 1 A	All 4 types	Pyrosis, Retrosternal pain, digestive alterations	None	Rheumatologic manifestations	No	13.8
Group 1 B	Dry salted fish	None	None	Polyp removal	No	0.98
Group 1 C	Dry salted fish	Pyrosis	Angioedema, Urticaria, Eczema	Eosinophilia, family history of allergic reactions	No	0.88
Group 2 C1	Raw and smoked fish	None	None	None	No	0.51
Group 2 C2	Smoked fish	None	None	None	No	1.57

specific IgE in four out of five positive patients is actually low, and for each of them the presence of anti Ani s 1 antibodies should be confirmed by Western Blot analysis , in order to rule out false positive results which may be obtained with a highly sensitive but not very specific method such as Immunocap, as shown recently (8).

Conclusions

Specific IgE production is not strictly related to infection, but it characterises the proneness to develop a hypersensitivity state. Evaluation of *Anisakis*-specific IgG might probably show significant differences between the two groups, in terms of exposition to *Anisakis* antigens.

The general seropositivity to *Anisakis* in Italy could be further investigated through population-wide screenings on raw fish consumption.

Finally, we think that particular attention should be given to patients who report recent allergic reactions . This could indicate a recent encounter with the parasite and, consequently, a higher level of specific anti-*Anisakis* IgE in the serum.

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