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Anisakiasis: The Importance of Prevention and the Role of Diet Therapy in Allergic Patients

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Anisakiasis · Diet · Prevention · Awareness · Cytokines · Polyphenols

Abstract
Introduction: Anisakiasis is a zoonosis of parasitic origin whose diffusion seems to be continuously increasing. ***Objective:*** The aim of this study was to evaluate the benefits of a fish-free diet in patients allergic to *Anisakis simplex* as well as underlining the importance of awareness and prevention. Furthermore, we aimed to investigate the critical issues related to the spread of anisakiasis in relation to eating habits. ***Methods:*** Patients were assessed by means of skin prick tests (SPTs) and targeted laboratory testing, with an 18-month-long fish-free diet being recommended in cases of severe sensitization. The degree of awareness about anisakiasis was evaluated from interviews. Patients were subjected to follow-up visits after 18 months. ***Results:*** A total of 70 cases of sensitization to *A. simplex* were evaluated. The Interview answers highlighted a general state of misinformation among patients and healthy subjects along with a remarkable underestimation of anisakiasis-related risks. An overall lack of care regarding eating habits and diet plans also emerged. In 21 patients affected by severe sensitization, clinical and laboratory evaluations were repeated after 18 months of the subjects being on a fish-free diet. There was a remarkable improvement in serum IgE levels and clinical symptoms. ***Conclusion:*** Data analysis proved the need to implement new and more effective awareness-raising and prevention campaigns in order to reduce the incidence of anisakiasis. It is crucial to establish an adequate diet therapy for sensitized patients. Evaluation of cytokine patterns suggests how a polyphenol-rich regime can activate regulatory T cell function and possibly reduce the allergic and inflammatory components of the disease.

Introduction

Anisakiasis is a parasitic zoonosis transmitted by the accidental ingestion of *Anisakis simplex* larvae, typically found in raw/undercooked fish or any food preparation where the allergenic power has not been deactivated [1]. Anisakiasis can be considered an endemic disease [2] due

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to the widespread and ever-increasing consumption of raw fish-based dishes (sushi, sashimi, etc.), as well as the traditional use of marinated/smoked fish in coastal areas of Apulia, Italy. Larvae are usually eliminated via vomiting within a few hours of ingestion; should this not occur, acute gastroenteritis with vomiting and diarrhea follows, and even chronic subocclusive manifestations due to the formation of eosinophilic granulomas [3]. While gastrointestinal manifestations are more common, ectopic localization of parasites may cause symptoms related to the colonized organ [4].

Due to the nonspecificity of symptoms during the acute phase [5], direct diagnosis based on observation of the parasite rarely occurs. Indirect diagnosis via serological tests [6] is more common, following allergic manifestations of varying degrees. Common anisakiasis-related IgE-mediated symptoms are rhinitis, asthma, and urticaria, which can lead to a decrease in the patient's quality of life. More severe manifestations include angioedema or anaphylaxis; these possibly life-threatening cases of sensitization call for greater attention to prevention and treatment. An appropriate diet therapy is the only viable option to contain symptoms in chronic anisakiasis [7].

High-temperature cooking alone is insufficient to contain the risk of allergic manifestation since thermoresistant antigens remain unaffected and capable of triggering the host's immune response, while the larvae are killed. Anisakiasis can only be reliably prevented by freezing fish at temperatures lower than –20 °C for at least 24 h before preparation [8], or at –35 °C for 15 h [9]. Literature data suggest that total avoidance of fish ingestion represents the sole effective dietary intervention, particularly in patients with severe hypersensitivity. It should be noted that this, in turn, can expose the patient to the effects of a poor omega-3 fatty acids intake [10–12].

With this study, we aim to assess the effects of a fish-free dietary regimen in patients sensitized to *A. simplex* as well as the role of awareness and prevention in high-risk subjects.

Materials and Methods

Patients

This observational prospective study was carried out by the Allergy and Clinical Immunology Unit of Andria Hospital, Andria, Italy, on consecutive subjects accessing the allergology outpatient ambulatory between November 2016 and November 2017. Patients were considered for inclusion if they fulfilled the following criteria: (1) a history of allergic or gastrointestinal symptoms within 24 h of fish ingestion, (2) positive skin prick tests (SPTs) for *A. simplex*, (3) an *A. simplex*-specific IgE serum level >0.35 kUA/L, and (4) negative SPTs for other, possibly *A. simplex* cross-reacting antigens. Individuals with specific IgE levels of 0.35–0.70 kUA/L were classified as "sensitized", whereas those showing values >0.70 kUA/L were considered "allergic" to *A. simplex* [13, 14]. A total of 70 patients were enrolled, ranging in age between 14 and 83 years (mean ± SD 51.94 ± 16.02 years); 23 were males (32.85%). Urticaria was the most common clinical presentation in the study population (41.43%), followed by respiratory disorders (rhinitis, cough, and asthma [30%]), gastrointestinal symptoms (5.71%), and angioedema (2.86%). Fourteen patients (20%) exhibited ≥2 concurrent classes of symptoms. An isolated case of anaphylactic shock was recorded in a patient affected by gastrointestinal symptoms. Demographic and clinical characteristics are presented in Table 1. A control group of 71 age- and sex-matched subjects with no clinical history of *A. simplex* infection was randomly selected to further investigate dietary habits and correlated risks as well as awareness of preventive measures.

Table 1. Demographic and clinical characteristics of the 70 enrolled patients

Age, years (range)	51.94±16.02 (14–83)
Males/females	23/47
Sensitization	
Sensitized, *n* (%)	7 (10)
Allergic, *n* (%)	63 (90)
Total IgE, kUA/L	4.874776±1.636226
Specific IgE, kUA/L	1.092391±1.410711
Symptoms	
Urticaria	29
Angioedema	2
Respiratory	21
Gastrointestinal	4
Multiple (≥2)	14

Values are presented as *n* or log-mean ± SD, unless otherwise indicated.

Diagnostic Tests

Skin Prick Tests. We utilized a standardized panel of allergens (ALK-Abelló, Milan, Italy) including *A. simplex*, hazelnuts, peanuts, celery, soy, banana, peach, wheat, rice, corn, tomato, cod, tuna, salmon, sardines, shrimp, egg whites, dust mites, and cockroach. Tests were performed and analyzed according to the EAACI guidelines [15]. Negative (saline) and positive (0.1% histamine) controls were applied.

Radioallergosorbent Tests. The UniCAP system (Pharmacia, Uppsala, Sweden) was used to measure the concentration of total and specific IgE in serum. Recombinant Ani s 1 antigen was used for the determination of specific IgE.

Personal Interviews and Related Measurements

In order to investigate the awareness of risks and prevention measures in both healthy subjects and sensitized patients, 2 different questionnaires were administered, both via phone and direct interview. Awareness of the risks related to *A. simplex* infection (7 items) was ranked as "none" (0–3 correct answers), "limited" (4–5 correct answers), or "good" (≥6 correct answers); awareness of correct preventive measures (5 items) was ranked as "none" (0–2

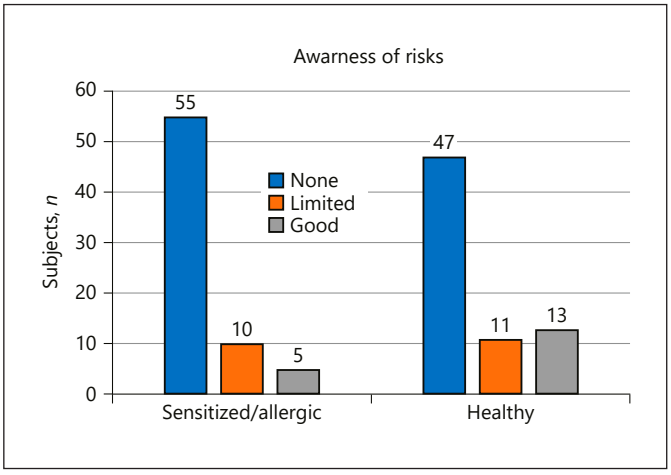

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Fig. 1. Awareness of risks related to *A. simplex* infection in healthy and sensitized patients.

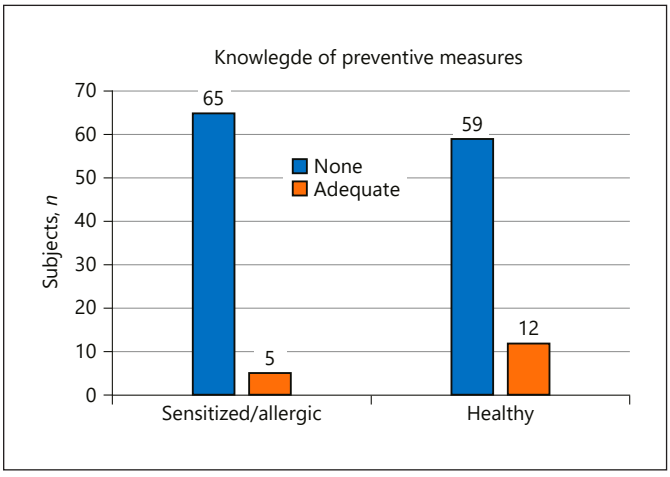

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Fig. 2. Awareness of correct measures to prevent anisakiasis.

Table 2. Serum IgE levels in sensitized patients subjected to a fish-free diet

IgE type	T_0	T_1
Total ($p = 0.00233$)	5.742±1.769	5.000±1.648
Specific ($p = 0.000726$)	2.730±1.057	1.697±0.332

Values are expressed as log-means ± SD. T_0, at presentation; T_1, at 18 months.

correct answers), or "adequate" (≥3 correct answers). Dietary habits in both groups and disease-specific diet therapy in sensitized and allergic patients was investigated, when applicable.

Dietary Intervention

Patients with a history of chronic symptoms, SPT positivity for *A. simplex*, and specific IgE values ≥3.5 kUA/L were recommended a fish-free dietary regimen for the next 18 months. IgE values were reevaluated after 6 months. On follow-up, complete and persistent disappearance of allergic symptoms for the entire time was considered a positive response. Patients were subsequently allowed to freely consume fish, as long as it had been properly frozen and cooked.

Statistical Tests

Data on IgE levels were corrected for nonnormality by log transformation, tested with quantile-comparison and Shapiro-Wilk's test ($p = 0.05$), and subsequently used for analysis. Patients were grouped according to clinical symptoms (urticaria, angioedema, gastrointestinal disorders, respiratory symptoms, or polysymptomatic). Student's *t* test for paired samples was subsequently used to compare total and *A. simplex*-specific IgE levels before and after the required dietary regimen.

Statistical tests were performed using EZR™ v1.41 [16]; questionnaire results were processed using Microsoft Excel 2019.

Results

Twenty-five patients met the criteria for dietary intervention, and 21 of these followed the recommendations for the full 18 months (i.e., a 16% drop-out rate). Laboratory testing showed a statistically significant reduction in total and specific IgE counts (Table 2), along with the complete remission of clinical symptoms. A second clinical follow-up after 6 months, reintroducing properly handled and cooked fish into the diet, recorded a reappearance of urticaria in 2 subjects, possibly due to exposure to unprocessed raw fish.

Answers given at interviews denoted a low awareness of the risks related to *A. simplex* infection (Fig. 1), which are grossly underestimated by both healthy and affected subjects (72%); likewise, knowledge of appropriate preventive measures appeared inadequate (87%) (Fig. 2). Worthy of note is the largely domestic preparation and consumption of raw fish (62% of pooled answers), with both groups exhibiting incorrect behavioral choices that actually increase exposure to *A. simplex* infection.

Discussion

According to available literature data, patients sensitized to *A. simplex* had peculiarities in their cytokine profile, more specifically an allergic-inflammatory pattern with an increase of IFN-γ and IL-17 levels during gastroenteric involvement [17], while urticaria was more closely related to higher levels of IL-4 and IL-5 [18]. Increased

IL-4 levels induce the switching of the cellular B response towards IgE production, while increased IL-5 contributes to the proliferation of eosinophils and the formation of granulomas [19]. Daschner et al. [20] showed that patients with previous episodes of gastroallergic anisakiasis were positive on SPT for *A. simplex*-specific IgE; specificity appears to be highly variable among patients, as well as the amount of response. Other authors have demonstrated the presence of specific IgE in gastrointestinal anisakiasis even without allergic symptoms [21, 22]. An appropriate fish-free diet appears to be beneficial for patients affected by severe sensitization to *A. simplex*, with a decrease in serum IgE counts and a remarkable improvement in symptoms. The said benefits are retained over time as long as proper preventive measures are followed.

The inclusion of polyphenols in the diet of sensitized subjects affected by urticaria has been evaluated [23]. Research by Magrone et al. [24] showed that cultures of peripheral blood lymphomonocytes showed an increase in IFN-γ and IL-7 levels along with a decrease in IL-10 level after 18 months on a fish-free diet (subsequently stimulated with 4-phorbol 12-myristate 13-acetate). This led to a switch of T helper cell pattern from type 2 (allergic) to type 1; these patterns have also been correlated with the disappearance of urticaria. Patients could further benefit from the role of polyphenols as activators of the regulatory T cell response [25]. Notable among flavonoids is turmeric, which has been proven to reduce the formation of hepatic granulomas induced by *Schistosoma* [26]. Turmeric's usage in cases of anisakiasis should be considered, in accordance with the studies of Nieuwenhuizen [3] that pointed out the nematode's ability to induce massive eosinophil infiltrations and granuloma formation in the gastrointestinal tract. There are also literature findings of the ability of turmeric to inhibit the release of histamine by mast cells, which leads to improvements in skin symptoms [26].

Our findings support the necessity of greater education of both healthy and affected subjects regarding the risks of *A. simplex* infection as well as the knowledge of adequate prophylactic measures, particularly in view of the primarily domestic preparation and consumption of raw fish-based dishes. Campaigns targeted at raising awareness in the general population may be helpful in containing the spread of this zoonosis.

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Statement of Ethics

Oral informed consent was obtained for each patient before inclusion in the study. The study was approved by the Independent Ethics Committee of the University of Bari Medical School in agreement with all participating centers.

Disclosure Statement

The authors have no conflicts of interest to declare.

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This study did not receive funding.

Author Contributions

A.F.M.G.: data tabulation and statistical analysis, and manuscript review. R.B.: literature research and manuscript drafting. V.G.: clinical data collection and patient interviews. S.N.: clinical evaluation of enrolled patients. M.T.V.: study coordinator.

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