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Anisakis simplex as a risk factor for relapsing acute urticaria: a case–control study

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ABSTRACT

Background: IgE-mediated hypersensitivity reactions to *Anisakis simplex* have been described after ingestion of fish, suggesting that sensitisation to this parasite may induce acute urticaria and anaphylaxis. *Anisakis simplex* allergens are highly resistant to heat and freezing, and sensitisation may occur even in populations with low consumption of raw/undercooked fish. This study aimed to quantify the association between sensitisation to *A. simplex* and relapsing acute urticaria.

Methods: This is the first case–control study on this topic. Cases ($n = 200$) were patients with a clinical diagnosis of relapsing acute urticaria, consecutively approached at the immunoallergology unit of Porto's largest paediatric hospital. Controls ($n = 200$) were consecutively selected at the surgery department of the same hospital, from subjects undergoing programmed orthopaedic, maxillofacial or general surgical procedures. Specific IgE measurements (*Anisakis* and *Ascaris*) were taken, and skin-prick tests (*A. simplex*, common aero-allergens, fish, and *Ascaris*) were done.

Results: Sensitisation to *A. simplex* (OR 3.86, 95% CI 2.04% to 7.29%), *Ascaris* (OR 3.37, 95% CI 1.89% to 6.02%), fish (OR 4.62, 95% CI 1.85% to 11.52%), and at least one aeroallergen (OR 4.59, 95% CI 2.99% to 7.05%) were associated with increased risk of acute urticaria. Regarding the sensitisation to *A. simplex*, the aeroallergen sensitisation-adjusted OR was 2.61 (95% CI 1.33% to 5.12%) for the whole sample, and 2.72 (95% CI 0.99% to 7.47%) for those not sensitised to *Ascaris* or fish.

Conclusion: Sensitisation to *A. simplex* increases the risk of relapsing acute urticaria in subjects not sensitised to *Ascaris* or fish, and this is independent of aeroallergen sensitisation.

Anisakis simplex is a parasitic nematode of the *Anisakidae* family. The life cycle involves larval stages, as intermediate hosts, in fish and cephalopods, such as cod, sardine, tuna, mackerel and squid, and an adult stage, during which the worm parasitises the stomach of marine mammals. Humans can become accidental hosts after 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.¹ However, the *A. simplex* allergens are highly resistant to heat and freezing; though cooking is expected to kill the parasites it may not result in loss of their allergenicity,² and sensitisation may occur even in populations with low consumption of raw or undercooked fish,^{3,4} or after the ingestion of other foods contaminated with *Anisakis* species

allergens.⁵ Immunoglobulin E-mediated hypersensitivity reactions to *Anisakis simplex* have been described after the ingestion of fish,^{6–9} suggesting that sensitisation to this parasite may induce acute urticaria and anaphylaxis.

Considering the worldwide increase in fish consumption, and that a large proportion of the commercial fish is infected,¹⁰ *A. simplex* has the potential to become an important determinant of allergic reactions otherwise classified as food allergies.

It may be hypothesised that parasitic allergens, and specifically *A. simplex*, are responsible for cases of chronic and relapsing acute urticaria frequently labelled as idiopathic. Therefore, we conducted the first case–control study to quantify the association between sensitisation to *A. simplex* and relapsing acute urticaria.

METHODS

We performed a hospital-based case–control study in children and adolescents aged 6 to 18 years, comprising 200 cases and 200 controls. Cases were consecutively approached during a visit to the immunoallergology unit of the largest paediatric hospital in Porto. Patients with a clinical diagnosis of acute urticaria, having at least three episodes in the previous two years were eligible. Controls were consecutively selected at the surgery department of the same hospital, among subjects admitted for programmed orthopaedic, maxillofacial, or general surgical procedures. Children undergoing otorhinolaryngologic surgery were not eligible. No refusals were observed.

Sample size was estimated for a 95% confidence level and a power of 80%, assuming a 1:1 ratio of controls to cases, and a proportion of participation between 95% and 100%. It allows for the detection of a difference in the prevalence of sensitisation between cases and controls corresponding to an odds ratio (OR) of 3, if the prevalence of exposure among the controls is 5%, or corresponding to an OR of 2, if 20% of the controls are sensitised. The local ethics committee approved this study, and the parents of all participants signed an informed consent form.

Skin-prick tests were performed and interpreted by the same experienced allergist (HF) according to standard procedures,¹¹ using the following allergen extracts: *A. simplex* (2 mg/ml), *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat dander, dog dander, mixed grass, *Sebastes marinus*, blue fish, and white fish, all from ALK-Abelló; and *Ascaris*, whiting, codfish, tuna, salmon, and sardine from BIAL-Aristegui.

Anisakis- and *Ascaris*-specific IgE determinations (CAP-FEIA system, UniCAP-Pharmacia) were performed for cases and controls simultaneously.

Several criteria for *Anisakis simplex* sensitisation were considered: 1) prick-test positive; 2) IgE titre ≥ 0.70 kU/litre; 3) IgE titre ≥ 0.35 kU/litre; 4) prick-test positive and IgE titre ≥ 0.70 kU/litre; 5) prick-test positive or IgE titre ≥ 0.70 kU/litre; 6) prick-test positive and IgE titre ≥ 0.35 kU/litre; 7) prick-test positive or IgE titre ≥ 0.35 kU/litre. The latter was used to compute adjusted risk estimates.

Sensitisation to *Ascaris* was considered present when either the prick test was positive or IgE titre ≥ 0.35 kU/litre, but results are also presented using other criteria. Subjects were classified as sensitised to mites when prick tests were positive for *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae*, were classified as sensitised to common aeroallergens when prick tests were positive for at least one of cat dander, dog dander, mixed grass or mites (*Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae*), and as sensitised to fish when prick tests were positive for one or more types of fish.

Statistical analysis

Crude and adjusted ORs and 95% CI were computed through unconditional logistic regression using STATA version 9.2 (Stata, College Station, Texas).

The association between sociodemographic characteristics, sensitisation to *Anisakis*, *Ascaris*, fish, mites or aeroallergens, and urticaria was firstly assessed in a univariate analysis. The association between *Anisakis* and relapsing acute urticaria was also quantified for the different levels of the exposures significantly associated with urticaria in the univariate analysis. Variables yielding stratum-specific OR estimates similar between each other and different from the overall measure of

association by at least 10% were considered as confounders, and adjusted OR estimates were also computed. The statistical significance of potential interactions was addressed, including the respective interaction term in the logistic regression models.

RESULTS

The risk of acute urticaria was higher in subjects sensitised to *Anisakis* (table 1).

The OR ranged from 2.03 to 5.10, depending on the criteria used to define sensitisation. The most precise estimates were obtained when we defined as sensitised to *Anisakis* the subjects having positive prick test responses or specific IgE ≥ 0.70 kU/litre (OR 3.60, 95% CI 1.86% to 6.96%), or those with positive prick tests or specific IgE ≥ 0.35 kU/L (OR 3.86, 95% CI 2.04% to 7.29%).

Also, sensitisation to *Ascaris* (OR 3.37, 95% CI 1.89 to 6.02), fish (OR 4.62, 95% CI 1.85 to 11.52), mites (OR 4.33, 95% CI 2.80 to 6.70), or to at least one aeroallergen (OR 4.59, 95% CI 2.99 to 7.05) was associated with a significantly increased risk of acute urticaria, and the risk was lower in males (OR 0.58, 95% CI 0.38 to 0.87) (table 2).

The OR for sensitisation to *A. simplex* was similar in subjects sensitised to at least one aeroallergen (OR 2.60; 95% CI 1.07 to 6.33) and in those not sensitised to aeroallergens (OR 2.63; 95% CI 0.94 to 7.34), and similar results were obtained when the analysis was performed considering sensitisation to mites (OR 2.71; 95% CI 1.05 to 7.04 for subjects sensitised to mites; OR 2.85; 95% CI 1.12 to 7.25 for subjects not sensitised to mites). However, the point estimates were different according to test results for *Ascaris* (OR 4.30, 95% CI 1.78 to 10.36 for those not sensitised; OR 1.34, 95% CI 0.45 to 4.02 for the sensitised; P for interaction = 0.105) or fish sensitisation (OR 3.66, 95% CI 1.83 to 7.32 for those not

Table 1 Association between *Anisakis* sensitisation and relapsing acute urticaria

Sensitisation to <i>Anisakis</i>	Relapsing acute urticaria		
	Controls n (%)	Cases n (%)	Crude OR (95% CI)
Prick-test positive			
No	189 (94.5)	167 (83.5)	[reference]
Yes	11 (5.5)	33 (16.5)	3.40 (1.66 to 6.93)
Specific IgE ≥ 0.70 kU/litre			
No	197 (98.5)	188 (94.0)	[reference]
Yes	3 (1.5)	12 (6.0)	4.19 (1.16 to 15.09)
Specific IgE ≥ 0.35 kU/litre			
No	194 (97.0)	182 (91.0)	[reference]
Yes	6 (3.0)	18 (9.0)	3.20 (1.24 to 8.23)
Prick-test positive and specific IgE ≥ 0.70 kU/litre			
No	199 (99.5)	195 (97.5)	[reference]
Yes	1 (0.5)	5 (2.5)	5.10 (0.59 to 44.07)
Prick-test positive or specific IgE ≥ 0.70 kU/litre			
No	187 (93.5)	160 (80.0)	[reference]
Yes	13 (6.5)	40 (20.0)	3.60 (1.86 to 6.96)
Prick-test positive and specific IgE ≥ 0.35 kU/litre			
No	197 (98.5)	194 (97.0)	[reference]
Yes	3 (1.5)	6 (3.0)	2.03 (0.50 to 8.24)
Prick-test positive or specific IgE ≥ 0.35 kU/litre			
No	186 (93.0)	155 (77.5)	[reference]
Yes	14 (7.0)	45 (22.5)	3.86 (2.04 to 7.29)

Table 2 Risk factors for relapsing acute urticaria

	Relapsing acute urticaria		
	Controls n (%)	Cases n (%)	Crude OR (95% CI)
Sex			
Female	66 (33.0)	92 (46.0)	[reference]
Male	134 (67.0)	108 (54.0)	0.58 (0.38 to 0.87)
Age (years)			
<9	97 (48.5)	100 (50.0)	[reference]
≥ 9	103 (51.5)	100 (50.0)	0.94 (0.64 to 1.39)
Paternal education (years)*			
≤ 4	91 (46.0)	80 (40.0)	[reference]
4–9	77 (38.9)	88 (44.0)	1.30 (0.85 to 2.00)
≥ 10	30 (15.1)	32 (16.0)	1.21 (0.68 to 2.17)
Sensitisation to <i>Anisakis</i>			
No	186 (93.0)	155 (77.5)	[reference]
Yes	14 (7.0)	45 (22.5)	3.86 (2.04 to 7.29)
Sensitisation to <i>Ascaris</i>			
No	182 (91.0)	150 (75.0)	[reference]
Yes	18 (9.0)	50 (25.0)	3.37 (1.89 to 6.02)
Sensitisation to fish			
No	194 (97.0)	175 (87.5)	[reference]
Yes	6 (3.0)	25 (12.5)	4.62 (1.85 to 11.52)
Sensitisation to mites			
No	156 (78.0)	90 (45.0)	[reference]
Yes	44 (22.0)	110 (55.0)	4.33 (2.80 to 6.70)
Sensitisation to aeroallergens			
No	150 (75.5)	79 (39.5)	[reference]
Yes	50 (25.0)	121 (60.5)	4.59 (2.99 to 7.05)

*Three-hundred and ninety-eight participants as information is missing for two subjects.

sensitised; OR 1.57, 95% CI 0.24 to 10.22 for the sensitised; P for interaction = 0.407).

The aeroallergen sensitisation-adjusted OR for the association between *A simplex* and urticaria was 2.61 (95% CI 1.33 to 5.12) for the whole sample, 2.84 (95% CI 1.13 to 7.15) for those not sensitised to *Ascaris*, 2.63 (95% CI 1.27 to 5.46) for those not sensitised to fish, and 2.72 (95% CI 0.99 to 7.47) for those not sensitised to *Ascaris* or fish (table 3).

DISCUSSION

The risk of relapsing acute urticaria is three times higher in subjects sensitised to *A simplex*. This association is independent from sensitisation to aeroallergens, and is apparently weaker in subjects sensitised to *Ascaris* or fish.

Previous studies on this topic involved only cases,^{7 12 13} and this is the first case-control study on the association between *A simplex* and relapsing acute urticaria. The present investigation was specifically designed to address this topic, and information on potentially the most important confounders or effect modifiers was collected through objective measurements. However, some of the limitations of the study need to be discussed.

A hospital based case-control study is more prone to bias than population-based case-control or cohort designs, but both the definition of the control group and the evaluation of participants were conducted in order to minimise selection and information biases. Controls were selected in the surgery department of a general paediatric hospital, from patients admitted for minor surgical procedures, excluding those admitted for otorhinolaryngologic surgery to avoid a biased prevalence of allergic diseases (those admitted for otorhinolaryngologic surgery are more likely to have allergic diseases than the general population). Orthopaedic and inguinal hernia surgeries are more frequent in males and this may explain the higher proportion of boys among controls.

The same researcher evaluated all cases and controls, minimising the occurrence of differential systematic errors in technical execution of prick tests, but no blinding was possible in this evaluation and biased measurements of the wheals cannot be ruled out. However, laboratory IgE evaluations were performed for cases and controls simultaneously and the

operator was blinded to the case-control status of the samples, yielding results similar to those obtained from the prick tests.

Anisakis simplex sensitisation was considered present in subjects with positive prick test or specific IgE ≥ 0.35 kUI/litre. As shown in table 1, the magnitude of the association was similar across different definitions of sensitisation, with strong associations being observed (statistically significant in most cases) regardless of the methods/criteria used. Moreover, misclassification resulting from considering subjects sensitised when one of two tests was positive is expectedly non-differential, conservatively driving the OR towards unity. The robustness of our findings to these assumptions shows that our conclusions do not spuriously arise from an exercise in data dredging.

The patterns of fish consumption, preservation and cooking may be associated with the risk of sensitisation to *A simplex*, and it is likely that the magnitude of the association between sensitisation to *A simplex* and urticaria increases with the degree of sensitisation, as suggested by our results (the point estimates presented in table 1 tend to be higher when the cut point used to define *Anisakis* sensitisation according to specific IgE was ≥ 0.70 kUI/litre). Therefore, it is possible that the magnitude of the association between *A simplex* sensitisation and urticaria differs across settings with different patterns of consumption of fish, being even higher in populations with higher consumptions of raw and undercooked fish than in Portugal, where it is not frequent, especially in children.

In our study, the ORs for the association between *A simplex* and relapsing acute urticaria were clearly different for subjects sensitised to *Ascaris* (OR 1.38) and for those not sensitised (OR 2.84), suggesting that this association is modified by sensitisation to *Ascaris*. Our study has a limited power to assess the role of potential effect modifiers from a statistical point of view, but the biological plausibility of this interaction strengthens our interpretation based on the differences in the point estimates obtained from the stratified analysis.

This could be expected due to cross-reactivity between allergens of these two nematode species, as previously described.¹ The association of sensitisation to nematodes and arthropods also has been studied by some authors, and it was found that German cockroach, chironomids, dust mite and *A simplex* share allergens.¹⁴⁻¹⁶ Although skin-prick testing to

Table 3 Risk factors for relapsing acute urticaria (stratified analysis)

Strata	Subjects sensitised to <i>Anisakis</i>		Association between sensitisation to <i>Anisakis</i> and relapsing acute urticaria			
	Controls n (%)	Cases n (%)	Crude OR*(95% CI)	p for interaction	Adjusted OR $\dagger$ † (95% CI)	p for interaction
Females	4 (6.1)	19 (20.6)	4.03 (1.30–12.49)		2.82 (0.87–9.18)	
Males	10 (7.5)	26 (24.1)	3.93 (1.80–8.58)	0.971	2.58 (1.13–5.89)	0.901
Not sensitised to aeroallergens	7 (4.7)	9 (11.4)	2.63 (0.94–7.34)		–	
Sensitised to aeroallergens	7 (14.0)	36 (29.8)	2.60 (1.07–6.33)	0.989	–	–
Not sensitised to mites	8 (5.1)	12 (13.3)	2.85 (1.12–7.25)		– $\ddagger$	
Sensitised to mites	6 (13.6)	33 (30.0)	2.71 (1.05–7.04)	0.944	– $\ddagger$	–
Not sensitised to <i>Ascaris</i>	7 (3.8)	22 (14.7)	4.30 (1.78–10.36)		2.84 (1.13–7.15)	
Sensitised to <i>Ascaris</i>	7 (38.9)	23 (46.0)	1.34 (0.45–4.02)	0.105	1.38 (0.44–4.32)	0.334
Not sensitised to fish	12 (6.2)	34 (19.4)	3.66 (1.83–7.32)		2.63 (1.27–5.46)	
Sensitised to fish	2 (33.3)	11 (44.0)	1.57 (0.24–10.22)	0.407	1.37 (0.20–9.28)	0.532

*Reference category is the group of participants not sensitised to *Anisakis*.

†Adjusted for sensitisation to aeroallergens.

‡Sensitisation to mites is included in the definition of sensitisation to aeroallergens.

What is already known on this subject

- ▶ *Anisakis simplex* infects a large proportion of commercial fish.
- ▶ *Anisakis simplex* allergens are highly resistant to heat and freezing, and sensitisation may occur even in populations with low consumption of raw or undercooked fish.

What this study adds

- ▶ This is the first case–control study quantifying the association between sensitisation to *A simplex* and relapsing acute urticaria.
- ▶ Sensitisation to *A simplex* increases the risk of relapsing acute urticaria threefold in subjects not sensitised to *Ascaris* or fish.

Policy implications

- ▶ The recognition of sensitisation to *A simplex* as a cause of relapsing acute urticaria will warrant a stricter sanitary control of fish for human consumption.

Anisakis may show a false-positive in patients with reactions to the homologous antigens, the fact that the OR is similar in participants not sensitised to common allergens (largely due to the sensitisation to mites) strengthens our conclusion and makes cross-reactivity an unlikely explanation for these findings.

Sensitisation to fish could be a confounder, as humans have contact with *Anisakis* through the consumption of fish, which is known to be associated with the occurrence of urticaria. However, the explanation of these results appears more difficult because the precision of risk estimates in participants sensitised to fish is lower than that observed for *Ascaris*, requiring an even larger sample.

Confounding by sensitisation to aeroallergens was also expected, as those patients have a higher capacity of producing

specific IgE, which is clearly associated with the sensitisation to both *A. simplex* and other allergens able to trigger urticaria.

In conclusion, sensitisation to *A. simplex* is independently associated with the occurrence of relapsing acute urticaria, increasing the risk by threefold in subjects not sensitised to *Ascaris* or fish.

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