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Principal component analysis of factors for sensitization to *Anisakis* spp. in postpartum women

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ABSTRACT

Introduction: Immunoreactivity to *Anisakis* spp. is believed to be associated with frequency of fish intake. The objective of this study was to evaluate, using principal component analysis, the main factors potentially involved in reactivity to these nematodes in postpartum women.
Methods: Retrospective study conducted on a database of 309 postpartum women. All completed a structured questionnaire and had blood samples collected for ELISA analysis of specific immunoglobulins against total *Anisakis* spp. antigens and assessment of reactivity. Parametric and nonparametric tests were used to assess factors for sensitization in the reactive and nonreactive groups, and a principal component analysis was performed. A Pearson correlation matrix with varimax rotation was used to assess the variables of interest (place of residence, age, number of prenatal visits, type of birth facility, fish intake and frequency, raw fish intake, fish handling, history of allergies).
Results: After exclusions, samples from 203 women were assessed. Of these, 52 (25.6%) were reactive for anti-*Anisakis* IgG. Most women claimed not to handle fish (n = 121) and eat fish only sporadically (n = 71). Significant differences in age were seen between the reactive and nonreactive groups (p = 0.001). The first two components explained 32.55% and 38.94% of variances in the nonreactive and reactive groups respectively. The adjusted matrix assigned greater probabilistic weight to weekly intake frequency (0.804), followed by raw fish intake (0.759), with differences in relation to the nonreactive group.
Conclusion: Correlation matrices revealed a direct relationship between seroreactivity to *Anisakis* spp. and frequency of fish intake in a sample of postpartum women.

1. Introduction

Sensitization to *Anisakis* spp. usually occurs via the oral–intestinal route (Nieuwenhuizen and Lopata, 2014) after ingestion of fish infected with the third larval stage of nematodes from this genus (Klapper et al., 2015). Alongside toxins, parvalbumins, and other parasites, *Anisakis* is considered to be one of the key factors involved in fish allergy (Sharp and Lopata, 2014). Other possible

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routes of anisakid sensitization may be related to independent factors such as age and place of residence. Furthermore, the centuries-old habit of eating raw or barely cooked fish is a classic factor closely associated with sensitization.

There is substantial research interest in anisakiasis, the disease caused by intake or raw, uncooked, or poorly preserved (salted, acid-cured, or smoked) fish containing *Anisakis* spp. larvae (Baron et al., 2014). This condition is probably the result of a combination of two factors: direct action of the larvae during tissue invasion and interactions between the host immune system and substances released from or present on the surface of the parasite (Valls et al., 2003). These effects, often due to a breakdown in integrity of the intestinal mucosa (Polimeno et al., 2010), lead to intestinal symptoms, allergic manifestations, and potential injury to other organs and systems.

There have been few reports of anisakiasis in the Brazilian population, and, to the best of our knowledge, no description of factors potentially associated with seroreactivity in healthy adults.

Within this context, the present study sought to analyze the main factors associated with *Anisakis* sensitization in humans using a principal component analysis approach.

2. Methods

2.1. Study design and population

This retrospective study was performed on a database of volunteer postpartum women. The study was approved by the relevant Institutional Review Board and registered with the Brazilian National Research Ethics System (CAAE 0167.0.258.000-08), and all participants provided written informed consent.

2.2. Setting and participants

Two public birthing centers were randomly visited on all days of the week in the years 2009 and 2010. Data collection from records and questionnaires was always performed by the same investigator, on varying days of the week. A random list of numbers generated by statistical software was used to select medical records. Briefly, the last two numbers on the list were used to select hospital IDs, and the corresponding patients were then visited by the investigator.

The study questionnaire was initially administered to 309 postpartum women. Cases with missing data ($n = 89$) and participants who did not consume fish ($n = 17$) were excluded, for a final sample of 203 postpartum women whose laboratory test results were included for analysis.

2.3. Data sources/measurement

2.3.1. Questionnaire and blood sampling

After being informed of the objective of the study, women were administered a structured questionnaire designed to collect information on age, place of residence, variables related to pregnancy and delivery, the postpartum period, fish handling, fish and seafood intake frequency, and presence of allergic symptoms.

Using aseptic technique, blood (5 mL) was collected from the median cubital vein into anticoagulant-free tubes and centrifuged to obtain serum. Samples were stored at −20 °C until testing.

2.3.2. Larva and antigen sourcing and ELISA

Anisakis spp. larvae were obtained from pink cusk-eel (*Genypterus brasiliensis*), cutlassfish (*Trichiurus lepturus*), and red porgy (*Pagrus pagrus*) specimens acquired at the São Pedro Fish Market, in the municipality of Niterói, state of Rio de Janeiro, Brazil, and transported under refrigeration to the Fisheries Laboratory at the Universidade Federal Fluminense School of Veterinary Medicine for taxonomic identification. To obtain whole-body antigens, larvae were homogenized, centrifuged, and the supernatant collected and stored. Protein concentrations were determined (Lowry et al., 1951) and all antigens were stored at −70 °C until use. The presence of specific anti-*Anisakis* IgG antibody in the blood of the volunteers was determined by ELISA. Very briefly, Maxisorp 96-well ELISA plates (Nunc) were coated with 20 μg/mL of *Anisakis* crude extract per well in 50 μL of 0.05 M carbonate–bicarbonate buffer (pH 9.6) and incubated overnight at 4 °C. The plates were washed with PBS-T and blocked with PBS-1% gelatin (PBS-G) for 2 h at room temperature. Then, 50-μL aliquots of HRP-mouse anti-IgG (ε chain specific) (Invitrogen, Camarillo, CA, USA) were added and the optical density of each well was read with an automatic plate reader (Anthos 2010) at 490 nm. The results are expressed as a mean of each duplicate.

The cutoff was calculated as threefold the average optical densities of 20 wells of the ELISA reaction described, by substituting PBS-T for human sera (Figueiredo Junior et al., 2013).

2.4. Variables

The categorical variables of interest were place of residence, age, prenatal care, type of birth facility, fish intake and frequency, raw fish intake, fish handling, and history of allergies.

Fish intake frequency was categorized as follows: daily; six, five, four, three, or two days a week; once a week; every two weeks; three times a month; once a month; and sporadic. Participants were also asked whether they habitually ate raw fish.

All participants who reported having consumed fish two times a week or more frequently were categorized as having a high fish intake (Corres et al., 2001). The other categorical variables of interest behaved as dichotomous or binary variables. The only continuous variables of interest were maternal age and number of prenatal visits. For principal component analysis, participants were categorized dichotomously as reactive or nonreactive, depending on whether anti-*Anisakis* IgG antibodies were detected on ELISA (Johnson and Wichern, 2007).

2.5. Statistical methods

All tests were performed using IBM SPSS Statistics for Windows® Version 22.0 (IBM Corp, Armonk, NY, USA). The significance level was set at $p < 0.05$. Quantitative data are expressed as absolute values and means and standard deviations. Continuous variables were tested for normality with the Kolmogorov–Smirnov and Shapiro–Wilk tests to ascertain whether the *t*-test or Mann–Whitney *U* test would be more appropriate.

On multivariate analysis, principal component analysis was used to ascertain which of the variables of interest were most closely associated with sensitization. A correlation matrix was constructed to quantify intercorrelation among the variables (place of residence, number of prenatal visits, age, fish handling, fish intake frequency, raw fish intake, and presence of allergic symptoms).

The purpose of principal component analysis was to reduce the original set of eight variables of interest (dependent variables) into two sets of new variables (principal components). These calculations, based on the definition of only two components for analysis, allowed construction of two-dimensional plots to yield a quantitative ranking of the now-independent variables.

The statistical software was set up for descriptive analyses with calculation of Pearson correlation matrix coefficients. The principal component method was used to extract a fixed number of components (two) and a varimax adjustment was applied to the two groups (reactive and nonreactive) to define probabilistic weights for analysis of variables as if independent. Adjustments were also used to classify coefficients in descending order and suppress factor loadings <0.60. The eigenvalues and eigenvectors of the sample matrix were established to demonstrate the principal component differential. The contribution of each variable to the two principal components, as expressed by the correlation coefficient, represented the weight of the variable in each component. The higher the coefficient, the greater the discriminant capacity of the variable.

3. Results

Of the 203 women assessed, 52 (25.6%) were reactive for anti-*Anisakis* IgG. Mean age was 25.45 years. Most participants were from the municipalities of Niterói ($n = 132$) and São Gonçalo ($n = 41$). The majority ($n = 109$) had received antenatal care. Most claimed that they did not handle fish ($n = 121$) and ate fish only sporadically (weekly intake, $n = 71$). Significant differences in age were found between the reactive and nonreactive groups ($p = 0.001$); women in the former group were under age 30.

The first two components explained 32.55% and 38.94% of variance in the nonreactive and reactive groups respectively, whereas those with eigenvalues >1 (first five components) explained 70.70% and 78.14% of variance in the two groups.

The varimax-adjusted matrix assigned greater probabilistic weight to weekly intake frequency (0.804), followed by raw fish intake (0.759), with differences in relation to the nonreactive group (Fig. 1A and B). Analysis of the loadings of each variable for each component showed that intake frequency stood out among all variables (Fig. 1C and D).

4. Discussion

The current literature states that sensitization to *Anisakis* generally occurs in persons who eat raw fish or seafood (Shweiki et al., 2014). Interestingly, in the present study, the variable with the greatest weight to explain sensitization in the reactive group was fish intake frequency, not raw fish consumption.

Several routes of contact with *Anisakis* spp. have been studied, which has led to the characterization of specific population profiles. Patients who present with dermatitis (Nieuwenhuizen and Lopata, 2014), ophthalmic manifestations (Polimeno et al., 2010), and rhinitis (Lopata and Jeebhay, 2013) are usually fish processing plant workers, particularly those in close contact with fish (Sharp and Lopata, 2014; Mazzucco et al., 2012). Fishermen and their families would be the group most likely to develop a wide range of clinical manifestations, as any type of exposure is plausible in this population, from consumption of raw fish to aspiration of airborne particles in the working environment (Jeebhay and Cartier, 2010). No women in our sample reported contact with fish or seafood, and none reported symptoms that could be attributed to anisakiasis.

Although the sample was drawn from healthy women, ELISA revealed a sensitization rate of approximately 25%. Although total antigens are used more often and are easier to obtain, they may cross-react with some antigens (Lorenzo et al., 2001; Rodriguez-Perez et al., 2014; Weiler, 2007). Furthermore, during antenatal care in Brazil, women usually receive anthelmintic treatment. This leads us to assume there should be little influence of *Ascaris* or other nematodes in this group of puerperal women, reaffirming the possibility that putative reactivity on ELISA would be attributable to contact with *Anisakis* spp. The coefficient produced by principal component analysis allowed us to construct an interpretation model that specifically targeted possible sensitization factors in the study sample. When variables were analyzed in the same component in an attempt to identify outcomes shared by the reactive and nonreactive groups, the variables with the highest factor loadings in the first component showed that frequency of

A

Rotated Componente Matrix[a]

	Componente	
	1	2
Fish.Intake	,804	
Raw	,759	
Fish.Handling	-,695	
Age		
Perinatal.Points		,728
Allergy.History		,692
Prenatal.Counseling		
Municipality		

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 3 iterations.

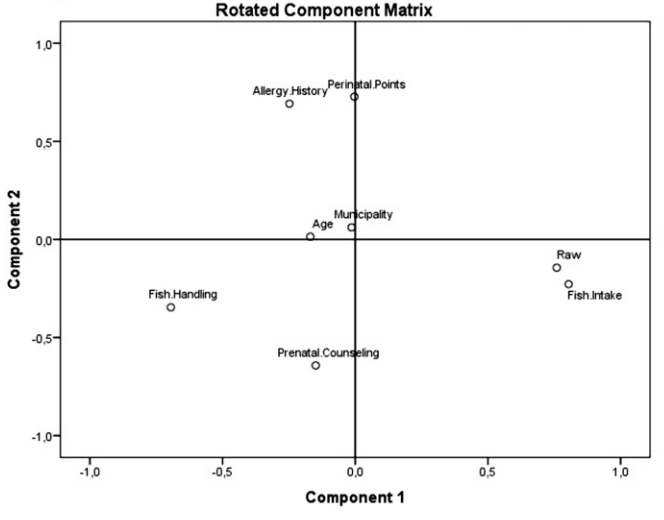
B

Rotated Component Matrix[a]

	Componente	
	1	2
Fish.Handling		
Fish.Intake		
Age		
Raw		
Municipality		
Prenatal.Counseling		,710
Perinatal.Points		-,703
Allergy.History		

a. Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

C



D

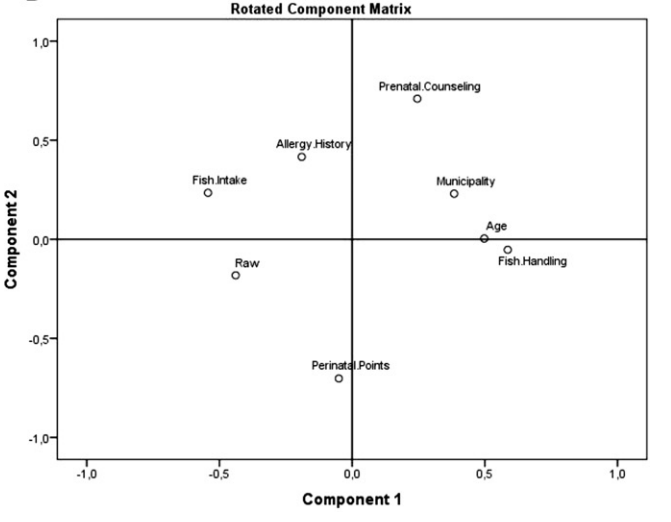


Fig. 1. First and second components, showing factor loadings between variables in the following groups: A and C, reactive; B and D, nonreactive.

fish intake, fish handling, and raw fish intake had significant weights in the reactive group, unlike in the nonreactive group. Age was not an explanatory variable in this study; its probabilistic weight had very little significance in the two principal components.

In conclusion, correlation matrices revealed a direct relationship between seroreactivity to *Anisakis* spp. and frequency of fish intake in a sample of postpartum women from the state of Rio de Janeiro, Brazil.

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