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Przeciwciała anty-*Anisakis* w ludzkiej krwi pępowinowej
Anti-*Anisakis* antibodies in human umbilical cord blood

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Streszczenie

Wstęp: Niewiele wiemy na temat wpływu seroreaktywności w kierunku antygenów nicieni pasożytujących na rybach na płód.
Cel pracy: Niniejsze badanie miało na celu stwierdzenie, czy seroreaktywność matki w kierunku *Anisakis* spp. wpływa na rozwój
dziecka w trakcie ciąży lub bezpośrednio po urodzeniu. **Metody:** W powyższym celu przeprowadzono retrospektywne badanie
analizujące dane pacjentek objętych opieką dwóch placówek położniczych w Niterói w Brazylii. Uwzględnione w badaniu
noworodki zostały wyłonione na podstawie obecności przeciwciał IgG anty-*Anisakis* w krwi pępowinowej i podzielone na dwie
grupy, jako charakteryzujące się seroreaktywnością bądź jej brakiem, na podstawie wyników badania krwi matki. W celu
sprawdzenia hipotezy dotyczącej zmiennych ciągłych posłużono się testem *U* Manna–Whitneya. W celu przeprowadzenia
binarnej regresji logistycznej zastosowano uogólniony model liniowy. **Wyniki:** Spośród 147 noworodków objętych badaniem
121 dzieci zaklasyfikowano jako niereaktywne, a 26 – jako reaktywne w kierunku anisakiozy. Nie stwierdzono występowania
statystycznie istotnych różnic pomiędzy grupami pod względem wieku matki ($p = 0,193$), liczby wizyt w okresie ciąży ($p = 0,362$),
występowania u płodu jednostek klinicznych w okresie prenatalnym ($p = 0,980$), sposobu rozwiązania ($p = 0,193$), wieku ciąży
w momencie rozwiązania ($p = 0,266$), masy urodzeniowej ($p = 0,294$), konieczności resuscytacji po urodzeniu ($p = 0,675$),
pojawienia się problemów zdrowotnych u dziecka w trackie pobytu w szpitalu ($p = 0,201$) ani też długości pobytu w szpitalu
($p = 0,528$). Nie stwierdzono również statystycznie istotnej korelacji pomiędzy obecnością przeciwciał klasy IgG w krwi
pępowinowej a obecnością surowych ryb w diecie matki ($p = 1,000$) ani też spożyciem surowych ryb częstszym niż dwa razy
w tygodniu ($p = 0,729$). **Wnioski:** W badanej przez nas populacji noworodków obecność przeciwciał anty-*Anisakis* klasy IgG
w krwi pępowinowej noworodków urodzonych przez matki charakteryzujące się seroreaktywnością w kierunku nicieni
z rodziny *Anisakis* nie stwarzała zagrożenia i nie miała wpływu na rozwój fizyczny płodu, występowanie u niego jednostek
chorobowych w momencie urodzenia ani ich pojawienie się w okresie pobytu w szpitalu po urodzeniu.

Słowa kluczowe: *Anisakis* spp., krew pępowinowa, ELISA, ryby, noworodki

Abstract

Introduction: Little is known about the influence of seroreactivity to fish nematode antigens on the unborn child. **Aim:**
The objective of this study was to ascertain whether infants born to women with *Anisakis* spp. seroreactivity had problems
with growth at birth, or during neonatal care. **Methods:** We conducted a retrospective database search of puerperal women
interviewed at two perinatal facilities in Niterói, Brazil. Neonates were selected by the presence of anti-*Anisakis* IgG antibodies
in cord blood and categorised, by analysis of maternal blood results, as reactive or nonreactive. The Mann–Whitney *U* test
was used for hypothesis testing in continuous variables. A generalised linear model was used for binary logistic regression.
Results: Of the 147 neonates studied, 121 were labelled as nonreactive and 26, as reactive. There were no significant between-
group differences in maternal age ($p = 0.193$), number of prenatal visits ($p = 0.362$), presence of prenatal conditions
($p = 0.980$), mode of delivery ($p = 0.193$), gestational age ($p = 0.266$), birth weight ($p = 0.294$), need for resuscitation
($p = 0.675$), development of conditions during hospital stay ($p = 0.201$), or length of hospital stay ($p = 0.528$). There was also
no significant association between IgG positivity in cord blood in raw fish intake ($p = 1.000$) or intake >2 times/week
($p = 0.729$). **Conclusions:** The presence of anti-*Anisakis* IgG in cord blood of infants born to women seroreactive to *Anisakis*
did not pose a hazard to this neonatal population and did not influence growth, conditions at birth, or development of any
clinical conditions before hospital discharge.

Keywords: *Anisakis* spp., cord blood, ELISA, fish, newborn

INTRODUCTION

During pregnancy, the intrauterine and extrauterine environment both influence individual growth. The ability of the foetus and neonate to respond to antigenic stimuli changes gradually as the immune system matures. Transplacental transfer of maternal antibodies is also a significant event[1]. *Anisakis* spp. is a nematode causing an infection that is often contracted through consumption of infected fish[2], particularly raw or undercooked. Given the continually increasing popularity of Asian eating habits in the rest of the world, this topic has become the focus of growing research interest in recent years.

Little is known about the presence of anti-*Anisakis* antibodies in healthy populations of pregnant women and their unborn children. Although recent work has demonstrated a non-association between seroreactivity and gestational risk[3], experimental research has concluded that *Anisakis simplex* somatic extract has a toxic effect on embryo chicken eggs and a great effect on the development of early embryos[4]. The objective of the present study was to ascertain whether neonates born to healthy women with *Anisakis* spp. seroreactivity had problems with growth or at birth, whether their development was adequate for the gestational age, and whether they developed any clinical conditions during their hospital stay.

METHODS

This retrospective study used a database from a previous research project approved by the Research Ethics Committee of the Federal Fluminense University (CAAE 0167.0.258.000-08). Information was obtained from a questionnaire, from medical records, and from blood samples collected from postpartum women at two perinatal centres in Rio de Janeiro, Brazil. All participants received information regarding anisakiasis and were enrolled from the Metropolitan2 area of Rio de Janeiro, Brazil (composed of 7 municipalities), between 2009 and 2010, after visits were scheduled randomly. The study was explained and informed consent was obtained before any subject participated in the study. The inclusion criteria used were the presence of anti-*Anisakis* serologies in both the newborn and the mother as well as the presence of neonatal data on birth, anthropometric data, and clinical course during their stay in the neonatal unit. It should be remembered that all the puerperae provided their free informed consent. Cases that were not complete (1) and those where the women claimed they did not eat fish (13) were excluded. The data were identified on the basis of *Anisakis* serology results in the database, and defined as reactive or nonreactive groups depending on the serum levels of anti-*Anisakis* antibodies in maternal blood. The following parameters from each newborn were assessed: maternal age; histories of any prior pregnancy and the recent pregnancy; any birth events; vitality of the newborn at birth; and intake frequencies of fresh, frozen, raw, or slightly cooked fish. Information on blood collection from participants[5], fish from which *Anisakis* specimens were collected and where they were purchased, identification of the nematode (Fisheries Laboratory at the Universidade Federal Fluminense School of Veterinary Medicine for taxonomic identification), larva and antigen handling[6], and a detailed description of the ELISA protocol with cutoff ranges for the analysis of results is available elsewhere[7]. Binary categorical variables were used to indicate serum reactivity to anti-*Anisakis* spp. antigens (0 for nonreactive, 1 for reactive), the number of prenatal visits (0 for >6, 1 for <6), prenatal problems (0 for not present, 1 for present), frequency of exposure to fish (0 for ≤2 times/week, 1 for ≥2 times/week), consumption of raw fish (0 for no, 1 for yes), mode of delivery (0 for vaginal, 1 for caesarean), infant sex (0 for female, 1 for male), need for resuscitation (0 for no, 1 for yes), and presence of problems during nursery stay (0 for no, 1 for yes). The principal continuous variables were maternal age, number of prenatal visits, gestational age, birth weight, 5-minute Apgar score of neonatal vitality (on a scale of 0 to 10, where 0 denotes severe asphyxia and 10 is good vitality) and optical density (OD) of anti-*Anisakis* IgG. Continuous variables were evaluated by the mean, median, standard deviation (*SD*), and 95% confidence interval (95% CI). The presence or absence of a normal distribution was verified and, on the basis of this analysis, the nonparametric Mann–Whitney *U* test was used. Contingency tables were constructed to evaluate the association between high fish intake (≥2 times/week)[8] (0/1), presence of reactive IgG anti-*Anisakis* responses (0/1), and intake of raw fish (0/1) as well as neonatal variables related to the mode of delivery, size and condition at birth, and hospital stay. The relationship between categorical variables was tested by chi-square analysis and adjusted by Fisher's exact test. After these preliminary tests, a generalised linear model (GzLM) was constructed for binary logistic regression, with reactivity as the dependent variable (0 for nonreactive, 1 for reactive) and the categorical and continuous variables related to mothers and their infants as predictors. The data were processed with SPSS® 22.0 statistical package (SPSS, IBM, USA). For all hypotheses, the tested significance level was set at 5%.

RESULTS

In the 147 neonates for whom data were available, the mean anti-*Anisakis* IgG OD on ELISA of cord blood samples was 0.22 (95% CI: 0.20–0.24; *SD*: 0.12). On the basis of maternal blood analysis, 121 neonates were classified as nonreactive (mean OD: 0.19; 95% CI: 0.17–0.21; *SD*: 0.10) and 26 as reactive (mean OD: 0.38; 95% CI: 0.36–0.41; *SD*: 0.06) (*p* < 0.001), in a manner consistent with the rise in maternal serologies. Most neonates classified as reactive were female (64%) and had been delivered vaginally (56%). The mean maternal age was 25.67 years (95% CI: 22.50–28.83; *SD*: 6.37), the mean number of prenatal visits was 6.56 (95% CI: 5.11–8.01; *SD*: 2.91), and the mean gestational age was 39.2 weeks (95% CI: 38.6–39.6; *SD*: 1.28). Birth weight was adequate (mean: 3,209.67 g; 95% CI: 3,015.76–3,403.57; *SD*: 389.92) and most neonates were free of foetal distress

or asphyxia at birth, with a median 5-minute Apgar score of 9. Most had no problems during their hospital stay (84%). Among those who did develop problems, the most common were suspected and/or confirmed infection (28%) and jaundice (28%). Nearly all infants received rooming-in care (89.9%), and the mean length of stay was 3.17 days (95% CI: 2.71–3.63; SD: 0.92). There were no significant differences between the reactive and nonreactive group in terms of maternal age ($p = 0.193$), presence or absence of prenatal care ($p = 0.213$), number of prenatal visits ($p = 0.362$), presence of prenatal problems ($p = 0.980$), mode of delivery ($p = 0.193$), infant sex ($p = 0.559$), gestational age ($p = 0.266$), birth weight ($p = 0.294$), need for resuscitation ($p = 0.675$), 5-minute Apgar ($p = 0.902$), presence of problems during hospital stay ($p = 0.201$), or length of hospital stay ($p = 0.528$). There were also no significant associations of IgG positivity with raw fish intake ($p = 1.00$) or fish intake >2 times/week ($p = 0.72$) in the reactive group (Tab. 1).

DISCUSSION

As demonstrated above, there were no significant differences between the study groups. Neonates whose serum levels of anti-Anisakis IgG were as high as their mothers

plasma levels did not have a substantial rate of problems during the prenatal period, during delivery, or before hospital discharge. A recent prospective study conducted among Italian children between the ages of 5 and 16 years reported an *Anisakis simplex* sensitisation rate around 4.5%, which is similar to rates reported in adults[9]. However, cases of sensitisation of children under 5, even in high-risk areas or settings, are scarce in the literature. Although there is an established correlation between maternal and neonatal blood when it comes to passive transfer of anti-Anisakis IgG[5], there have been no reports of anisakidosis in human pregnancy. Thus, the potential foetal effects of substances released by *Anisakis* spp. during its migration through human tissues are still unknown. According to Daschner et al., the insults caused by live *Anisakis* spp. larvae in the human host elicit early sensitisation after penetration of the gastrointestinal mucosa, and the resulting Th2 response would lead to production of specific IgE against excretory–secretory products and surface or somatic antigens. These IgE antibodies may be mast cell-bound to high-affinity IgE receptors in the submucosa or, from the circulation, might reach several target organs[10]. Despite the remote possibility of anisakidosis during pregnancy, a recent study revealed that fish consumption appears to confer a significant dietary benefit, as high

Parameter	B	Standard error	95% confidence limits (Wald)		Hypothesis test			Exp(B)	95% confidence limits (Wald) for Exp(B)	
			Upper	Lower	Chi-square (Wald)	gl	Sig.		Upper	Lower
Maternal age	0.087	0.0671	−0.044	0.219	1.697	1	0.193	1.091	0.957	1.245
Prenatal care = 0	−1.696	1.3622	−4.366	0.973	1.551	1	0.213	0.183	0.013	2.647
Prenatal care = 1	0[a]	.	.	.	.	.	.	1	.	.
Number of prenatal visits	−0.274	0.3014	−0.865	0.316	0.829	1	0.362	0.760	0.421	1.372
Prenatal problems = 0	0.024	0.9740	−1.885	1.933	0.001	1	0.980	1.024	0.152	6.912
Prenatal problems = 1	0[a]	.	.	.	.	.	.	1	.	.
Fish intake <2×/week	−0.390	1.1275	−2.600	1.820	0.120	1	0.729	0.677	0.074	6.170
Fish intake >2×/week	0[a]	.	.	.	.	.	.	1	.	.
Raw fish intake = 0	19.372	370.9251	−725.427	726.171	0.000	1	1.000	259.400	0.000	[b]
Raw fish intake = 1	0[a]	.	.	.	.	.	.	1	.	.
Vaginal/Caesarean delivery	1.053	0.8094	−0.533	2.639	1.692	1	0.193	2.866	0.587	14.003
Female sex	−0.484	0.8274	−2.105	1.138	0.342	1	0.559	0.617	0.122	3.121
Male sex	0[a]	.	.	.	.	.	.	1	.	.
Gestational age	0.349	0.3143	−0.267	0.965	1.235	1	0.266	1.418	0.766	2.626
Birth weight	−0.001	0.0011	−0.003	0.001	1.101	1	0.294	0.999	0.997	1.001
Resuscitation = 0	0.621	1.4805	−2.280	3.523	0.176	1	0.675	1.862	0.102	33.892
Resuscitation = 1	0[a]	.	.	.	.	.	.	1	.	.
Apgar, 5-min	0.076	0.6126	−1.125	1.276	0.015	1	0.902	1.079	0.325	3.583
Problems in hospital = 0	−2.285	1.7867	−5.787	1.217	1.636	1	0.201	0.102	0.003	3.375
Problems in hospital = 1	0[a]	.	.	.	.	.	.	1	.	.
Length of stay	−0.155	0.2466	−0.639	0.328	0.398	1	0.528	0.856	0.528	1.388
(Scale)	1[c]									

Dependent variable: Neonatal reactivity (1); Model: (Intercept), Prenatal care, Prenatal problems, Mode of delivery, Resuscitation, Sex, Problems during hospital stay, Raw fish intake, Fish intake >2×/week, Neonatal IgG, Maternal age, Prenatal visits, Gestational age, Birth weight, 5-minute Apgar, Length of stay; [a] Set to zero because this parameter is redundant. [b] Set to missing due to overflow. [c] Set at listed value.

Tab. 1. Parameter estimates (GzLM): maternal and neonatal variables in a binary model (reactive vs. nonreactive neonates, according to maternal reactivity)

levels of fish intake during gestation have been associated with a reduced rate of allergic phenomena in childhood[11]. It has been suggested that n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) contained in fish have anti-inflammatory properties and may reduce the risk of allergic disease, although the overall effect of fish in allergy remains controversial. This same review concluded that fish intake during childhood might reduce the risk of eczema and allergic rhinitis, while fish intake during pregnancy is not believed to have any effect on atopy[12]. One limitation of this study is the fact that, although 309 mother-infant pairs were sampled, only 147 met the inclusion criteria. Regardless of the impossibility of generalising these findings, further research should be planned, particularly in coastal populations constantly exposed to *Anisakis,* to investigate the possible consequences of its infection, including in utero and throughout childhood.

CONCLUSION

The presence of anti-*Anisakis* IgG in cord blood samples from infants born to *Anisakis*-reactive mothers was not associated with the presence of any problems during the prenatal period, at birth, or during hospital stay. There was no negative impact on intrauterine growth, even in women with high levels of fish intake.

What is already known on this topic

- Transplacental passage of anti-*Anisakis* antibodies to the foetus occurs during pregnancy.
- There are no reports of acute anisakidosis during pregnancy and potential foetal repercussions.
- Antibody behaviour in this setting and its potential impact on foetal development are unknown.

What this study adds

- Immunoglobulin levels in cord blood are increased in infants born to mothers seroreactive to anisakid antigens.
- Maternal anti-*Anisakis* reactivity was not associated with neonatal problems at birth or during hospital stay.
- Foetal development was normal in infants born to mothers carrying anti-*Anisakis* IgG.

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