Acta Tropica 109 (2009) 103–107

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Specific IgA and IgG antibodies in paired serum and breast milk samples in human strongyloidiasis

Daniela M.L. Mota-Ferreira[a], Maria do Rosário F. Gonçalves-Pires[b], Álvaro Ferreira Júnior[b], Mônica C. Sopelete[b], Vânia O.S. Abdallah[a], Julia M. Costa-Cruz[b,*]

[a] Departamento de Pediatria, Universidade Federal de Uberlândia, Av. Pará 1720, 38400-902 Uberlândia, MG, Brazil
[b] Departamento de Imunologia, Microbiologia e Parasitologia, Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Av. Pará 1720 38400-902 Uberlândia, MG, Brazil

ARTICLE INFO

Article history:
Received 13 October 2007
Received in revised form 27 August 2008
Accepted 2 September 2008
Available online 21 October 2008

Keywords:
Strongyloidiasis
Strongyloides stercoralis
IgA
IgG
Breastfeeding
Immunodiagnosis

ABSTRACT

Strongyloidiasis, caused by the nematode *Strongyloides stercoralis*, is one of the major worldwide parasitic infections in humans. Breastfeeding may offer a potential protection against this infection. Feces, serum and milk samples were obtained from 90 lactating women from Clinical Hospital of Universidade Federal de Uberlândia, Brazil. The fecal samples were collected for parasitological diagnosis and the serum and milk samples were examined for specific *S. stercoralis* IgA and IgG antibodies using the indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA). Fecal examination showed that the rate of prevalence of *S. stercoralis* infection in the lactating women was 4.4%. IFAT manifested a 16.7% positivity rate for specific IgA antibody in serum and a 28.9% rate in milk samples; specific IgG was 41.1% in serum and 25.5% in milk samples. According to ELISA the positivity rate for specific IgA antibody was 21.1% in serum and 42.2% in milk samples; specific IgG was 40% in serum and 18.9% in milk samples. In serum samples, these immunological tests showed a concurrence of 91.1% and 94.4%, respectively, in detecting specific IgA and IgG antibodies. In milk samples, they showed a concurrence of 70% and 78.9%, respectively, in detecting specific IgA and IgG antibodies. There was a statistically significant difference between concordant and discordant results of immunological tests ($P < 0.0001$). IFAT and ELISA highly concurred in their detection of specific *S. stercoralis* IgA and IgG antibodies in serum and in milk samples reconfirming prior studies that the serological method is a complement to the direct diagnosis of the parasite, and suggesting that immunological methods using milk samples can also be helpful. Furthermore, in endemic areas, infants may acquire antibodies to *S. stercoralis* from breast milk, possibly, contributing to the enhancement of specific mucosal immunity against this parasite.

1. Introduction

Strongyloidiasis, caused by *Strongyloides stercoralis*, is one of the major worldwide parasitic infections in humans in both tropical and subtropical regions (Dada-Adegbola and Bakare, 2004). This helminth has a complex parasitic life cycle and many chronically infected patients are asymptomatic, so their malady can persist for decades. However, in some situations, hyperinfection or disseminated infection has been found associated with host immunosuppression (Keiser and Nutman, 2004; Vadlamudi et al., 2006).

Strongyloidiasis is still diagnosed by the detection of its larvae in fecal samples by microscopic observation. This is a relatively insensitive method, which is rendered still more inefficient due to the fact that the shedding of larvae by this parasite is intermittent (Uparanukraw et al., 1999; Blatt and Cantos, 2003).

Maternal breastfeeding is the most natural and safe way to feed a small child (Hanson, 2007). There is a conclusive body of evidence that breastfeeding protects the infant against a wide range of infectious and other diseases. Efforts have been directed in the past few years to identifying in human breast milk the various immune-active substances which account for the observed protective effects. These include specific antibodies against microorganisms such as viruses, bacteria and parasites that can colonize the gut after delivery (Cleary, 2004; Paramasivam et al., 2006).

Due to the prevalence of strongyloidiasis and to the importance of breastfeeding in protecting the newborn against it, this study was conducted to detect and evaluate the presence of specific *S. stercoralis* IgA and IgG antibodies in paired serum and breast milk samples.

* Corresponding author. Tel.: +55 34 3218 2187; fax: +55 34 3218 2332.
E-mail address: costacruz@ufu.br (J.M. Costa-Cruz).

0001-706X/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2008.09.023

2. Materials and methods

2.1. Study population

All the lactating women of healthy term infants that were born in The Clinical Hospital of the Federal University of Uberlândia between November 2003 and October 2004 were invited to participate in the study. A total of 90 lactating women, mean age of 25.2 years (S.D. 5.8; range 14–40 years) with similar backgrounds and current socio-economic conditions, were enrolled in the study after giving their informed consent. Samples of feces, serum and breast milk were collected from all of them. This study was approved by the Federal University of Uberlândia's Ethical Committee.

2.2. Biological samples

Three fecal samples from each woman were collected in plastic vials without preservative in three consecutive days and stored at 4 °C. The Baermann (1917) method was used to perform parasitological diagnosis within the first 24 h after the third sample was collected. Furthermore, 10% formalin solution was added to the remaining samples for further analysis by the Lutz (1919) method. Fecal culture for *S. stercoralis*, which is commonly acknowledged as the most sensitive direct method was not accomplished due to delay in obtaining results and the risk of infection during manipulation of infective larvae.

A total of 10 ml of blood was collected from each subject by venipuncture, centrifuged at 350 *g* for 10 min and the serum samples were stored at −20 °C until their use in the serological assays. Serum samples from three lactating women that were eliminating larvae in their feces were used as positive controls, and serum samples from three lactating women with negative parasitological and serological tests for *S. stercoralis* were used as negative controls.

Milk samples (approximately 10 ml) were collected between the 15th and the 30th day after delivery, and stored at −20 °C. Immediately before the serological assays, human milk samples were slowly thawed centrifuged at 2500 *g* for 10 min at 4 °C and the lipid layer was removed and discarded. Positive and negative milk samples controls were obtained from the same lactating women of serum controls.

2.3. S. stercoralis larvae

Human strain of *S. stercoralis* larvae isolated from the feces of infected humans was maintained in dogs immunodepressed by administration of oral prednisone (2 mg/kg/day). Fifteen days after dogs were inoculated with 6000 *S. stercoralis* larvae via subcutaneous injection, their feces were collected and samples of these were mixed with an equal part of finely ground moistened wood charcoal, which was spread in a uniform layer in Petri dishes and incubated at 25 °C. After 7 days of fecal culture, filariform larvae were obtained by the Baermann (1917) method and concentrated by centrifugation for 5 min at 350 *g*. They were then stored at −20 °C until they were used for antigen preparation.

2.4. S. stercoralis antigen for an indirect fluorescent antibody test (IFAT)

S. stercoralis larvae were embedded in Tissue Tek (polyvinyl alcohol, polyethylene glycol, Miles, Elkahart, USA). Sections measuring 4 μm in thickness were prepared in cryo-microtome (International Cryostat IEC, CTI-Mass, USA) at −30 °C and placed on glass slides which were stored at −20 °C for future testing, as previously described by Costa-Cruz et al. (1997).

2.5. S. stercoralis saline extract for ELISA

A saline antigen was obtained from filariform larvae resuspended in 1 ml 0.01 M phosphate buffered saline (PBS) pH 7.2 and disrupted in tissue homogenizer (Omnith International, US) for five cycles of 1 min and then for eight ultra-sound cycles of 20 s at 40 khz in an ice bath. After overnight incubation at 4 °C under continuous agitation, the suspension was centrifuged at 12400 *g* for 30 min and the supernatant (larval extract) was analyzed for protein content by the Lowry et al. (1951) method and stored at −20 °C.

2.6. Immunological tests

2.6.1. IFAT

Preliminary experiments were carried out in order to determine the optimal conditions for IFAT through block titration of the reagents (antigen, specimens and conjugate). *S. stercoralis* larvae spread on microscopic slides were incubated with serum and milk samples diluted 1:10 (serum IgA), 1:20 (serum IgG), 1:2 (milk IgA) and 1:4 (milk IgG) in PBS for 30 min (serum samples) and 45 min (milk samples) at 37 °C. Positive and negative controls of serum or breast milk samples were included on each slide. Slides were washed three times for 5 min in PBS and fluorescent conjugates of anti-human IgA (Calbiochem®, Merck, Germany) or anti-human IgG (Laborclin, Brazil) were added at 1:10 (both serum and milk IgA) and 1:30 (both serum and milk IgG) in PBS containing Evan's blue at 3%. After incubation for 30 min at 37 °C, slides were again washed, mounted in buffered glycerine (pH 8.5) and examined by two different observers through a fluorescence microscope (ZEISS Axiolab, Germany) at 200× magnification. Results were expressed as antibody titers. Samples with serum IgA titers ≥ 10, serum IgG titers ≥ 20, milk IgA titers ≥ 2 and milk IgG titers ≥ 4 were considered positive reactions. Positive samples were retested in twofold serial dilutions until the end point titer was obtained.

2.6.2. ELISA

Preliminary experiments were carried out in order to determine the optimal conditions for ELISA through block titration of the reagents (antigen, specimens and conjugate). Polystyrenes microplates (DIFCO, Interlab, São Paulo, Brazil) were coated with 10 μg/ml of *S. stercoralis* saline extract (50 μl/well) in a 0.06 M carbonate-bicarbonate buffer (pH 9.6) overnight at 4 °C. Plates were washed three times in PBS plus 0.05% Tween 20 (PBS-T) and subsequently incubated (50 μl/well) with samples diluted 1:20 (serum IgA), 1:200 (serum IgG), 1:10 (breast milk IgA) and 1:5 (breast milk IgG) in PBS-T. After incubation for 45 min (serum samples) and 60 min (milk samples) at 37 °C, plates were washed as described and then incubated with peroxidase labeled anti-human IgA or anti-human IgG conjugates (Sigma, USA) diluted 1:2000 and 1:1000 for serum samples and milk samples, respectively. After incubation for 45 min at 37 °C, plates were again washed and the reaction was developed by adding enzyme substrate (50 μl/well)

Table 1
Comparison between specific *S. stercoralis* IgA and IgG antibodies by IFAT and ELISA in serum and in milk samples from lactating women ($n = 90$).

	Serum	Milk	*P*
	n (%)	*n* (%)	
IFAT			
IgA	15 (16.7)	26 (28.9)	0.0506
IgG	37 (41.1)	23 (25.5)	0.0269
ELISA			
IgA	19 (21.1)	38 (42.2)	0.0023
IgG	36 (40.0)	17 (18.9)	0.0019