Transplacental transfer of filarial antigens from *Wuchereria bancrofti*-infected mothers to their offspring

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SUMMARY

Objective. Maternal infection has been considered to be a risk factor for filarial infection in offspring. In order to examine the influence of maternal infection in neonates, we have determined the prevalence of circulating filarial antigen (CFA) and anti-filarial antibodies in 119 maternal and corresponding cord blood samples collected from an area endemic for bancroftian filariasis. **Method.** Prevalence of antigenaemia was detected using Og4C3 circulating filarial antigen enzyme-linked immunosorbent assay. The presence of microfilariae was determined by filtration of a 1 ml sample through a Nuclepore membrane. Antibody isotypes (IgG, IgM, and IgE) to filarial antigen (Setaria *digitata* antigenic extract) were determined by enzyme linked immunosorbent assay (ELISA). **Results.** Microfilariae were detected in 14 cases (11·8%), whereas the Og4C3 assay could detect filarial antigen in 44·5% of pregnant mothers. Interestingly, 24·5% of samples born from CFA-positive mothers were found positive for CFA. None of the cord samples from CFA-negative mothers were found positive for CFA. No significant difference was observed in prevalence of filarial-specific IgG, IgM and IgE antibodies in CFA-positive and negative mothers. IgG antibody was detected in 60·5% of maternal and 21·8% of cord samples. IgG antibody in the cord does not differ with the antigen status of the mother. In contrast IgM and IgE antibody prevalence was significantly higher in cord from infected mothers than non-infected mothers (11·3% *vs* 0 for IgM, 24·5% *vs* 3·03% for IgE). **Conclusion.** Our study demonstrates the transplacental transfer of circulating filarial antigen from mother to cord. Filaria-specific IgM and IgE antibodies were higher in cord blood from infected mothers than from non-infected mothers. The findings of the study provide additional circumstantial evidence for pre-natal sensitization to filarial antigens developed *in utero*.

Key words: lymphatic filariasis, *Wuchereria bancrofi*, cord blood, circulating filarial antigen, India.

INTRODUCTION

Lymphatic filariasis is caused by the filarial nematodes *Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori* and continues to be a major public health problem in many tropical countries. Infection is initiated by the infective larvae (L3) stage of the parasite during the bite of infected mosquitoes. Infective larvae then develop into male and female worms in the lymphatics and, after mating, release microfilaria that appear in the blood circulation. The manifestation varies from asymptomatic infection to severe pathology. It is also unknown whether clinical symptoms are always preceded by an asymptomatic microfilaraemic stage. Although host genetic polymorphism and other environmental factors may influence susceptibility to infection and disease, maternal filarial infection has been considered a risk factor for increased susceptibility and facilitated parasite persistence in offspring. A number of studies have shown that children whose mothers were microfilaraemics during gestation were more likely to be microfilaraemics compared to children whose mothers were amicrofilaraemics during gestation (Lammie *et al*. 1991; Hightower *et al*. 1993; Malhotra *et al*. 2003, 2006; Rajan, 2007). Children born of filarial infected mothers have been shown to impair filarial Ag-specific T cell responses (Steel *et al*. 1994). Children of infection-free mothers have been shown to respond vigorously to filarial antigen with lymphocyte proliferation, production of IL-2 and IFN-γ. It has been shown in animal models of helminthic infections that offspring of infected pregnant rodents are immunologically less responsive to parasite antigens, have less pathology, and are more susceptible than the offspring of uninfected mothers (Storey *et al*. 1988; Haque *et al*. 1988). These observations support the hypothesis that pre-natal exposure to filarial antigens may affect development of subsequent immune responses. The exposure of the fetus to filarial antigens might be beneficial, increasing resistance to infection, or detrimental by

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inducing at least partial tolerance. Definite demonstration of filarial antigens in cord blood is lacking. Only one study, using the Og4C3 assay, reported circulating filarial antigen in cord blood samples from newborns born to mothers with filariasis (Hitch *et al*. 1997). In the present study, in order to examine the influence of maternal infection in neonates, the prevalence of circulating filarial antigen (CFA) and anti-filarial antibodies was investigated in paired maternal and cord blood samples collected from an area endemic for bancroftian filariasis.

MATERIALS AND METHODS

Study population

The study was performed at the O & G Department of Khurda Hospital in the Khurda district of Orissa, India. Pregnant mothers come for delivery from neighboring villages which are known to be highly endemic for bancroftian filariasis, having a microfilaria rate of about 12% (Beuria *et al*. 2003). Informed consent for participation was obtained before subjects were included in the study. The age of the mothers ranged from 18 to 35 years with a mean age of 25 years. None of the mothers had symptoms of clinical filariasis at the time of admission. Paired cord and maternal blood samples ($n=119$) were collected at the time of uncomplicated delivery. Venous blood samples were collected from mothers before delivery. Venous umbilical cord blood samples from neonates were collected immediately after birth. The collection of cord blood involved direct aspiration via puncture of the ethanol-sterilized umbilical vein at a site distal to the placenta, to reduce minimum cross-contamination. Maternal and cord samples were collected in different sized tubes to avoid the chance of mislabelling. Sera collected from individuals from non-filarial regions of Orissa served as non-endemic controls. Sera were stored at -70 °C till further use. The presence of microfilarial status of all pregnant women admitted to the hospital was checked before delivery by examining Giemsa-stained smears of 60 μl of blood collected between 20:30 and 22:30. The microfilarial status of cord blood samples of offspring was checked by filtration of a 1 ml sample through a Nuclepore membrane. Women were informed about their infection status and offered treatment through the doctor of the government hospital. The study was approved by the Indian Council of Medical Research and the review boards of the Human Ethical Committee of the Regional Medical Research Centre.

Circulating filarial antigen assay

Detection of CFA was carried out in serum samples using an Og4C3 enzyme linked immunosorbent assay test kit (JCU Tropical Biotechnology, Queensland, Australia) according to the manufacturer's instructions. A serum sample from each individual was tested and the optical density values were used to determine the antigen concentration in units from the standard curve prepared using 7 standard antigens supplied in the kit. Serum samples with an antigen unit of 128 (>titre of standard no. 3) were considered as antigen positive.

Determination of antibody isotypes

Antibody isotypes (IgG, IgM, IgE) to filarial antigen (Setaria *digitata* antigenic extract) were determined by enzyme linked immunosorbent assay (ELISA) following a published procedure (Das *et al*. 1992). Briefly, polystyrene microtitre plates were coated overnight with *S. digitata* antigens (2 μg/ml) in alkaline buffer pH 9·2. Plates were saturated with 0·4% bovine serum albumin in phosphate-buffered saline for 1 h at room temperature.

Sera for the detection of IgG and IgM were diluted 200-fold and for IgE sera were diluted to 100-fold, then added to the plate and kept at 37 °C for 3 h. Following incubation with test sera, 1000-fold diluted anti-human IgG peroxidase (Sigma) or 2000-fold diluted anti-human IgM peroxidase (Sigma) or 1000-fold diluted anti-human IgE peroxidase (Sigma) were used for detection of filarial-specific antibodies. The incubation continued for 3 h and, after washing, the presence of antibodies was detected with OPD substrate (Sigma, O-phenylene diamine containing H_2O_2). The enzymatic reaction was stopped by adding a drop of 4N sulphuric acid. The absorbance was read at 492 nm using an EIA reader (Bio-Rad, Richmond, USA).

The positivity of each isotype was calculated by taking the high-titred chronic serum pool as positive control (100 antibody unit). A serum was considered seropositive if its absorbance exceeded a threshold value of the mean O.D.+3 S.D. of 14 non-endemic normal sera.

Statistical analysis

The significance of difference between groups in the prevalence of responses was determined using Fisher's exact test.

RESULTS

The prevalence of microfilaraemia and antigenaemia in paired maternal and cord samples is shown in Table 1. Fourteen mothers (11·8%) out of 119 studied were observed to be microfilaraemic. Mf counts among microfilaraemic mothers varied from 3 to 210 per 60 μl blood. All the cord sera were found to be negative for microfilariae. The presence of circulating filarial antigen (CFA) was determined in both maternal and corresponding cord blood samples

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Table 1. Prevalence of microfilaraemia and antigenaemia in maternal and cord blood samples

Maternal infection status		No. tested	Cord blood infection status: Microfilaraemia *N*	(%)	Antigenaemia *N*	(%)
Microfilaraemic	CFA +ve	14	0	(0)	11	(78·6)
Amicrofilaraemic	CFA +ve	39	0	(0)	2	(5·1)
	CFA −ve	66	0	(0)	0	(0)

Table 2. Filaria-specific antibody isotype (IgG, IgM, IgE) seropositivities in maternal and cord serum samples

(Prevalence of seropositivity was determined by the percentage of individuals showing antibody units above the mean + 3 S.D. of 14 non-endemic normal sera.)

Maternal infection status	*N*	IgG seropositive: Maternal sera No.	(%)	Cord sera No.	(%)	IgM seropositive: Maternal sera No.	(%)	Cord sera No.	(%)	IgE seropositive: Maternal sera No.	(%)	Cord sera No.	(%)
CFA +ve	53	32	(60·4)	8	(15·1)	34	(64·2)	6	(11·3)	29	(54·7)	13	(24·5)
CFA −ve	66	40	(60·6)	18	(27·3)	40	(60·6)	0	(0)	47	(71·2)	2	(3·03)

using an Og4C3 enzyme linked immunosorbent assay kit. All the microfilaraemic mothers tested were antigen positive. Interestingly, 39 of 105 amicrofilaraemic mothers were also found to be positive for CFA, indicating the presence of adult worms within them. An overall CFA positivity among mothers was noted to be 44·5% (53/119). None of the cord samples from CFA-negative mothers were CFA positive, whereas 24·5% of neonates from CFA-positive mothers tested CFA positive suggesting placental transfer of CFA from the mother. An overall prevalence of antigenaemia among all the cord samples was observed to be 10·9% (13/119). Out of 13 mothers who have transferred CFA to their respective cords, 11 were microfilaraemics and 2 were amicrofilaraemics.

Antibody isotype to filarial antigen was determined in paired maternal and cord blood samples (Table 2). No significant difference was observed in positivity of anti-filarial isotype (IgG, IgM and IgE) in CFA-positive and negative mothers. Anti-filarial IgG positivity in cord blood did not vary with the antigen status of the mothers ($P = 0{\cdot}1239$). IgG positivity was determined in 21·8% (26/119) of cord samples. All of these 26 IgG-positive infants except 1 were CFA negative. In contrast, IgM and IgE isotypes were significantly higher in cord blood ($P = 0{\cdot}006$ for IgM and $P = 0{\cdot}0005$ for IgE) of infected mothers than uninfected mothers. IgM only could be detected in infants, who were CFA negative, but born from CFA-positive mothers. IgE antibody was detected in 15 cord blood samples of which 13 were from CFA-positive mothers. Of these 13 IgE-positive neonates, 8 were CFA positive and 5 were CFA negative.

DISCUSSION

The present study demonstrated the influence of maternal infection in the cord blood samples of offspring born from infected mothers living in an area endemic for bancroftian filariasis. Microfilariae were detected in only 14 cases, whereas the Og4C3 enzyme linked immunosorbent assay could detect filarial infection in 53 pregnant mothers indicating the advantage of the circulating filarial antigen assay for detecting genuine filarial infection among pregnant women. The development of a more sensitive assay has provided the opportunity to diagnose filarial infection by determining circulating filarial antigen independent of microfilariae (More and Copeman, 1990). Microfilariae were not detected in any of the cord samples from microfilaraemic mothers which is in contrast to the studies reported by others (Eberhard *et al.* 1993; Campello *et al.* 1993). Circulating filarial antigen was detected in 13 cord blood samples and they were from 11 microfilaraemic and 2 amicrofilaraemic CFA-positive mothers, suggesting that transplacental transfer of filarial antigens takes place from mothers to offspring. The reason for why circulating filarial antigen was detected in a proportion of cord samples is not known. Similar observations were also reported from Haiti, where filarial antigen was detected in 10% of cord blood

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samples from newborns born to mothers with filariasis (Hitch *et al*. 1997). But CFA was not detected in cord blood by the Og4C3 assay in other investigations (Ramzy *et al*. 1991). Studies have been reported suggesting that maternal schistosome antigens are transferred through the placenta. Carlier *et al*. (1980), using polyclonal antibodies, detected a circulating *S. mansoni* antigen in sera from umbilical cords of newborns of *S. mansoni*-infected mothers. In another study, a 63 kDa schistosoma antigen was detected in cord blood samples from mothers with schistosomiasis (Attallah *et al*. 2003).

Immunological evaluation of humoral responses (IgG, IgM and IgE) to filarial antigen was determined in paired maternal and cord samples to evaluate the influence of maternal infection on the development of anti-filarial immunity in offspring. No significant difference in the antibody isotypes among the infected versus uninfected mothers was observed, emphasizing the extensive exposure to infective larvae in a high filarial endemic region. IgG positivity was determined in 21·8% (26/119) of cord samples compared to 60% in maternal samples. Such a high prevalence of IgG antibodies in cord blood samples may probably be due to the passage of maternal IgG across the placenta (Desowitz *et al*. 1993). A study in Haiti has demonstrated that cord and maternal IgG levels were directly correlated and independent of maternal infection status (Hitch *et al*. 1997). Filaria-specific IgM and IgE isotypes that do not cross the placenta have been detected to be significantly higher in cord blood from infants born to infected than uninfected mothers, suggesting that sensitization to filarial antigens developed *in utero*, which is in agreement with the other studies (Dissanayake *et al*. 1980; Weil *et al*. 1983; Malhotra *et al*. 1997). All 6 cord samples from infected mothers (CFA +ve) showing as IgM positive were CFA negative. The absence of CFA (but IgM positive) in cord blood gives indirect evidence that no significant admixture of maternal blood and cord blood had occurred at the time of birth. Direct proof that there was no cross-contamination of maternal blood to cord blood is lacking in this study. Two cord blood samples from CFA-negative mothers were also positive for IgE antibody isotype. In the absence of filarial antigen, maternal antibody may directly stimulate antibody production *in utero* through idiotypic interactions (Lammie, 2002). It has been reported that chronic helminth parasite infection during pregnancy can stimulate Ag-specific B cell immunity as well as T cell memory *in utero* (King *et al*. 1998). In an attempt to study the cord blood response from infected mothers in Leogane, Haiti, exposure to filarial antigen *in utero* resulted in limited induction of parasite-specific immune responsiveness (Hitch *et al*. 1997). In onchocerciasis, maternal filarial infection has been shown to sensitize *in utero* parasite-specific cellular immune responsiveness in neonates and activate O.v.Ag-specific production of several Th-1 and Th-2 type cytokines (Soboslay *et al*. 1999). Our laboratory is engaged in elucidation of the anti-filarial immune reactivity in neonates following exposure to filarial antigens.

The present findings provide additional circumstantial evidence for pre-natal sensitization but lack some of the rigor provided in earlier studies by others (Weil *et al*. 1983; King *et al*. 1998). Longitudinal follow-up of circulating filarial antigen-positive and negative infants born to infected mothers will provide insight into the development of anti-filarial immune reactivity and susceptibility or resistance to infection in the later part of life. This finding also emphasizes that women of child-bearing age should be the target for treatment in lymphatic filariasis elimination programmes.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest concerning the work reported in this paper.

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