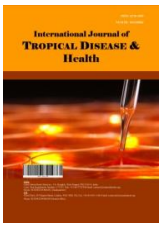

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Transplacental Transmission of Filarial Infections and Its Impact on Oxidative Stress and Immune Responses during Foetal Life in Bamenda, North West Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Author LB conceived the study and designed the experiments, collected field data, analysed the data and drafted the manuscript. Author OM conceived the study and designed the experiments, supervised and coordinated the study and helped to draft the manuscript. Author NHN conceived the study, designed the experiments and helped to draft the manuscript. Author NAD collected field data and contributed to the preparation of the manuscript. Author TNA conceived the study, designed the experiments, supervised and coordinated the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Women commonly harbour filarial infections during their childbearing years, raising the possibility that the developing foetus may be exposed to filarial antigens in the uterus and thereby have altered immunity and susceptibility to infection during early childhood. However, there are no concrete proofs to justify the risk of infections in infants born from mothers having filarial infections during pregnancy.
Aim: The purpose of this study was to assess the prevalence of microfilariae in umbilical cord blood and respective mothers and to evaluate the relationship between the cord blood filarial infection and

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the oxidative stress status and concentration of IL-2, IL-10, IL-13, INF-γ and IgG in umbilical cord blood.

Methods: This was a nested case–control study of cords and mothers of normal gestational age (>250 days of gestation). A total of 316 pairs of umbilical cords and mothers were examined. The presence of microfilariae was assessed by microscopy in mothers and cords. Oxidative stress status (total oxidative stress and total oxidative defence) and nitric oxide of umbilical cord and mother's blood were investigated by the colorimetric method. ELISA was carried out for IL-2, IL-10, IL-13, INF-γ in umbilical cord and mother's blood. Equally, umbilical cords were subjected to ELISA for total IgG.

Results: Results obtained showed that microfilariae had a prevalence of 32.9% and 29.7% in umbilical cord blood and women at time of delivery respectively. High levels of total oxidative stress (TOS) with low total oxidative defence (TAD) was found in filarial infected (Mf +ve) umbilical cord and mother's blood compared to controls or uninfected (Mf -ve) cords and mothers blood. IL-2 was lower in the blood of microfilariae infected cords and mothers, while INF-γ, IL-13 and IL-10 were higher as compared to microfilariae negative cords and mothers. Equally, plasma total IgG concentration was higher in microfilariae positive cords compared to the negative cords and positively correlated with IL-10.

Conclusions: There is high frequency of transplacental transmission of microfilariae. Cord blood filarial infections were associated with a high TOS, a protective immune response with low IL-2 and high INF-γ, and a typical Th2 immune response that was associated with higher concentration of immune total IgG regulatory cytokine IL-10 and IL-13 in neonate.

Keywords: Microfilariae; cord blood; oxidative stress; immune response.

1. INTRODUCTION

Helminth infections are a major public health problem, especially in the tropics. They are regarded as master manipulators of the host immune response, being associated with chronic but generally asymptomatic infections. Infected individuals have an altered immune response with evidence that helminth infections impaired the immune response in the foetus and infant if a mother has an infection during pregnancy [1,2]. This immunological change can influence the way a child responds to the same infection when exposed in later life [3,4,5]. They are preventable causes of maternal and neonatal morbidity and mortality [6]. Even though helminth infections induce strong Th2 responses, parasitic worms may survive in their mammalian hosts by switching off inflammatory immune responses and inducing a tolerant response to parasite antigens.

Filarial nematode infections are still noted as a major cause of morbidity in the tropics [7]. Cameroon is known to be endemic for onchocerciasis [8,9] and bancroftian filariasis [10]. Research carried out in eight regions of Cameroon revealed a prevalence of 0.11% [10] while work done in the far North and North regions reported a prevalence of 0.4% with 80.95% positive cases found in children between 6-7 years old, the North West Region 6.7% antigen positive and 0.08% microfilariae positive cases [11].

Previous studies demonstrated that children born of filarial infected mothers have been shown to have an impaired filarial antigen (Ag)-specific T cell response [12]. These observations support the hypothesis that pre-natal exposure to filarial antigens may affect the development of subsequent immune responses and transplacental transfer. This can be attributed to a multitude of factors, amongst which are: the inflammation of placenta and production of immunoregulatory cytokines which are still to be elucidated.

In the present study, we determined the prevalence of vertical transmission of filarial infections and compared the concentration of IL-2, IL-13, INF-y and IL-10 and IgG in cord and mother blood serums among mothers with filarial infection and counterpart mothers free of filarial infections.

2. MATERIALS AND METHODS

2.1 Description of Study Site

This study was carried out at the Regional Hospital Bamenda and at the Tubah Health District Hospital both in Mezam Division of the North West Region of Cameroon. The Bamenda

Regional Hospital serves as a reference hospital in the North West Region. Bamenda is located between latitude $5^{o}56^{'}$ and $5^{o}58^{'}$ North of the Equator and longitude $10^{0}08^{'}$ and $10^{o}10'$ East of the Greenwich Meridian [13]. It occupies a surface area of 3,125 hectares [14].

2.2 Inclusion Criteria

Pregnant mothers admitted in the hospital for delivery without any complications, free from other chronic diseases were selected for the study. Other factors were; participants presenting to the labour wards at or above 32 weeks of gestation, uncomplicated pregnancy and vaginal delivery.

2.3 Exclusion Criteria

Women with complicated pregnancies (pre eclampsia and poorly controlled diabetes) and premature deliveries (<32 weeks' gestation) were not selected for the study. Malaria cases (as declared in their medical books three months before or during the study) were excluded. Additional reasons for dropout and exclusion from analysis were; cases with induction of delivery, failure to collect corresponding blood pairs in both mothers and cord and withdrawal of some participants.

2.4 Study Participants

This was a cohort study conducted at the Regional Hospital Bamenda and Tubah Health District Hospital Bambui from August to October 2018. A total of 316 preterm pregnant mothers of different age groups were recruited. A well simplified open ended questionnaire (S3 File) was administered to assess the demographic parameters of participants. The demographic part consisted of questions on; age and village of residence while the knowledge part assessed knowledge on filariasis and its mode of transmission. Participants were prospectively followed through the prepartum period, and blood samples were collected from the women upon admission to the delivery unit during labour or upon admission to the ward prior to delivery.

2.5 Experimental Design

In all, 316 women were examined for the prevalence of filarial infections, but only the first 158 women were considered for the immunological analysis. Their delivery records were used to get the medical information of each participant including the gestational age and to randomly select a normal pregnancy.

2.6 Collection of Plasma Samples

Mother's venous blood was collected by trained personnel into ethylenediethylamine (EDTA) tubes. From the cord, blood samples were collected immediately after cutting the neonate from the placenta. Thereafter, plasma was obtained by centrifuging blood samples at 3000 revolutions per minute for 10 minutes. The samples were aliquoted and stored at -80°C till the date of analysis.

2.7 Parasitological Diagnosis

Assessment of microfilariae status of maternal and cord blood was based on the tube centrifugation lysed blood technique [15]. Five microliters of blood collected in EDTA tubes were gently mixed with 1 ml of distilled water and allowed to sit for 10 minutes. After lysis of red cells, the haemolysate was centrifuged for 10 minutes at 300 –500 g using a horizon model 653V centrifuge. The supernatant was discarded. After mixing the sediment, 5 µl were transferred to a slide. The slide was covered with a cover slip and examined microscopically for motile microfilariae using the 10X objective.

2.8 Assessment of Cytokines

ELISA assay kit (Sigma Aldrich, St. Louis, MO) were used to quantitatively assess the levels of cytokines [16]. The assay was conducted according to the manufacturer's instructions. The limits of detection for the cytokine ELISAs were: 38pg/ml for IL-2, 20 pg/ml for IL-5, 10 pg/ml for IFN-γ, 32 pg/mL for IL-13 and 16 pg/ml for IL-10.

2.9 Assessment of Serum Total IgG

Total IgG was measured using high-binding-level microassay plates (Costar, Cambridge, MA) coated with 4 µg/ml of an anti-human antibody (IgG) overnight at 4°C. Plates were blocked with 0.15 M PBS, pH 7.2, containing 10% FCS and 0.05% Tween 20 (Sigma, St. Louis, MO) overnight at 4°C. Samples were diluted 1:10 in PBS containing 5% FCS and 0.05% Tween 20 and incubated overnight at 4°C. Plates were incubated with biotinylated anti-human (IgG) (Sigma Chemical Co., St. Louis, MO), followed by streptavidin-peroxidase (Pharmingen, San

Jose, CA) and H_2O_2-OPD substrate (Merck and Co., Inc., White House Station, NJ), and were read using a 480-nm filter. A pool of parasite-infected patient plasma was used as a positive control. Umbilical cord serum from a new born of a non-atopic and non-parasitized mother was used as a negative control [16,17].

2.10 Data Analysis

Data was analysed using Prism version 3. Categorical data for cases and controls were summarized as counts and proportions while continuous variables were summarized using means, and standard deviations. To assess risk factors for cord infection, the presence of Mf among the cases and controls were compared using odds ratios, 95 % CIs and their p values. The Sperman's rank Order correlation test was used to assess the correlation between total IgG and the cytokines. P values ≤ 0.05 were considered statistically significant.

3. RESULTS

3.1 Characteristics of the Studied Population

A total number of 374 pregnant women admitted to the Bamenda regional hospital and Bambili district hospital for delivery from August 2018 to October 2018 were enrolled for the study. Out of this lot, 54 (15.5%) mothers were excluded because of complications during delivery or infant death or unwillingness. Finally only 316 mother-new born pairs were enrolled and examined for the presence of Mf, but the serum cytokines and total IgG were analysed in 50% (158) of them. The 316 mothers were in different age groups which included 200 ((63.29%), in the 16-29 years age group, 102 (32.27%) in the 30 – 39 years age group and 14 (4.43%) in the ≥ 40 years age group respectively. Results obtained showed that out of the 316 mothers examined, 94 of them were infected giving a prevalence of 29.74% with the ≥40 years age group having the highest prevalence of 54.14% as shown on Table 1 below. Equally 104 cords were infected giving a prevalence of 32.91% with 10 (3.16%) cords from Mf-negative mothers testing positive. The overview of the enrolment and prevalence of microfilariae (Mf) is presented in Table 1.

3.2 Influence of Mother and Cord Blood Filarial Infections on the Gestational Ages of Mothers and Body Weights of Neonates

The results of this study showed that the birth weights and gestational ages of babies were not significantly affected by the filarial status of the mother or cord. There was no significant difference (P≥0.05) in the birth weights and also gestational ages when the mother or cord was infected or uninfected (Table 2).

3.3 Impact of Mother and Cord Blood Filarial Infections on the Total Oxidative Stress and Antioxidant Defence of Mother and Cord Blood

The TOS of Mf +ve mothers were significantly higher (P<0.05) than those of Mf –ve mothers, but there was no significant difference between TOS of Mf +ve cords and Mf –ve cords. In contrast, there was no significant difference between TAD in Mf +ve and Mf –ve mothers as well as Mf +ve cords and Mf –ve cords. When TOS and TAD in mothers was compared to that of cord blood, there was no difference between Mf +ve mothers and Mf +ve cords as well as between Mf –ve mothers and Mf –ve cords (Table 3).

Table 1. Prevalence of filariasis in cord blood and mother's peripheral blood with respect to the mother's age at the date of birth

Variable	No Examined	No Infected	Prevalence (%)
Age group (years)			
16 - 29	200	50	25.00
30 - 39	102	36	35.29
≥40	14	8	57.14
Total	316	94	29.74
Cord filarial status			
Cord	316	104	32.91

Mf +ve, Microfilariae positive; Mf -ve, Microfilariae negative

Table 2. Gestational age and baby's body weight with respect to the presence of microfilariae in peripheral blood and cord blood

Variables	Infected Cord Mean ± SD	Uninfected Cord Mean ± SD	P value
Weights of babies at birth (Kg)			
Infected mothers	3.30±0.40	3.53±0.58	0.16
Uninfected mothers	3.19±0.38	3.36±0.43	0.18
P-value	0.40	0.28	
Gestational age (Days)			
Infected mother	272.76±13.91	274.52±7.18	0.59
Uninfected mother	273.03±6.97	275.40±13.28	0.53
P-value	*0.91*	*0.75*	

*Values in bracket indicate the number of samples. *P value indicates statistically significant differences between the two groups (P≤0.05)*

Table 3. Plasma level of radical compounds and antioxidant compounds in Mf +ve and Mf -ve mothers and their cord at the time of delivery

Variables	Mf +ve	Mf -ve	P value
Total oxidative stress (in millimol of H_2O_2/l)			
Cord mean ± SD	10.78±6.64 (17)	10.48±4.17 (20)	0.86
Mother mean ± SD	10.32±1.80 (20)	8.5±1.10 (17)	0.04
*P value	0.62	0.38	
Total antioxidant defense (Optical density, 540 nm)			
Cord mean ± SD	0.30±0.3 (17)	0.31±0.03 (20)	0.75
Mother mean ± SD	0.32±0.13 (20)	0.29±0.07 (17)	0.35
*P value	0.61	0.29	

*Values in bracket indicate the number of samples. P-value as compared Mf +ve mother and cord to Mf –ve mother and cord groups. *P value as compared Mf +ve or Mf -ve mother to counterpart Mf +ve or Mf -ve cord groups. P values in bold are statistically significant (P≤0.05)*

Total oxidative stress in Mf +ve and Mf –ve cord blood of Mf +ve mothers was not significantly different (P>0.05), but the TAD was significantly different, with high TAD materialized by low optical in Mf +ve cords. In Mf –ve mothers, Mf +ve cords had a significantly (P<0.05) low TOS compared to Mf –ve cords, while there was no difference in TAD (P>0.05). Otherwise, there was no difference between the TOS of Mf +ve cords blood plasma of Mf +ve mothers and Mf –ve mothers, with significantly (P<0.05) high TAD indicated by low optical density in Mf +ve cords of Mf +ve. In contrast, Mf -ve cords blood plasma of Mf +ve mothers showed a significant (P<0.05) low TOS than Mf –ve of Mf –ve mothers, with no significant difference between TAD (P>0.05; Table 4).

3.4 Impact of Mother and Cord Blood Filarial Infections on the Nitric Oxide (NO) in Cord Blood Plasma

To evaluate the probably role of maternal infection and transplacental transmission of filarial infections on development of inflammatory responses in neonates during their foetal development, we analysed the quantity of NO in cord and mother's blood plasma. Irrespective of the Mf status, NO were not significantly different (p>0.05) in cords and mothers who were Mf+ve compared to Mf-ive cord mothers (Table 5). There was no significant difference (P>0.05) in NO among Mf +ve mothers and Mf +ve cords, but a significant difference (P=0.002) in NO was observed among mothers and cords of Mf -ve status.

3.5 Impact of Mother and Cord Blood Filarial Infections on IL-2 and INF-Γ Levels of Mother and Cord Blood Plasma

Infected women and cords had a lower IL-2 than uninfected groups and these values were statistically significant at P≤0.05. In contrast, infected women and cords had a higher INF-γ than uninfected women and cords respectively as shown on Table 6. When infected or uninfected mothers and cords were compared, there were no significant differences (P>0.05) regarding these cytokines.

Table 4. Plasma levels of total oxidative stress and antioxidant defence in Mf +ve and Mf-ve cords with respect to Mf status of mothers

Variables	Mf +ve cord	Mf -ve cord	P value
Total oxidative stress (in millimol of H_2O_2/l)			
Mf +ve mother	13±7.90 (10)	8.86±4.94 (10)	0.17
Mf -ve mother	7.61±1.99 (7)	12.1±2.55 (10)	0.001
*P value	0.10	0.08	
Total antioxidant defense (Optical density, 540 nm)			
Mf +ve mother	0.29±0.03	0.32±0.03	< 0.0001
Mf -ve mother	0.33±0.02	0.30±0.03	< 0.08
*P value	0.03	0.30	

*Values in bracket indicate the number of samples. P-value as compared Mf +ve cords of Mf +ve or Mf -ve mother to Mf -ve cords of Mf +ve or Mf -ve mother. *P-value as compared Mf +ve or Mf -ve cord of Mf +ve mother to Mf +ve or Mf -ve cords of Mf -ve mother groups. P values in bold are statistically significant at P≤0.05*

Table 5. Plasma level of nitric oxide in Mf +ve and Mf -ve mothers and their cords at the time of delivery

Variables	Plasma level of nitric oxide		P value
	Mf +ve status	Mf -ve status	
Cord mean ± SD	0.71±0.81 (17)	0.63±0.39 (20)	0.69
Mother mean ± SD	1.34±1.07 (20)	1.26±0.77 (17)	0.79
*P value	0.054	0.002	

*Data represent mean ± SD (µM). In bracket are the number of samples. P-value as compared Mf +ve cords or Mf +ve mother with Mf -ve cords or Mf -ve mother. *P-value as compared Mf +ve or Mf -ve cord with Mf +ve or Mf -ve mother groups. P values in bold are statistically significant differences (P≤0.05)*

Table 6. Plasma level of IL-2 and INF-γ in Mf +ve and Mf-ve mothers and their cord at the time of delivery

	Mf +ve status	Mf -ve status	P value
IL-2 (in pg/ml)			
Cord mean ± SD	0.99±0.55 (17)	2.50±1.83 (19)	0.0023
Mother mean ± SD	0.90±0.63 (20)	2.50±2.02 (16)	0.0022
*P value	0.73	0.99	
INF-γ (in pg/ml)			
Cord mean ± SD	101.24±26.89 (17	69.32±17.05 (19)	0.002
Mother mean ± SD	129.16±53.98 (20)	67.11±39.08 (16)	0.005
*P value	0.06	0.82	

Data represent mean ± SD. In bracket are the number of samples

Irrespective of the Mf status of mother at the time of birth, IL-2 were significantly high ($p < 0.05$) in cords that were microfilaria positive and negative with Mf +ve mother compared to the controls of microfilaria negative mother. IL-2 were not significantly different in Mf +ve and -ve cords when both were from Mf +ve mothers, while it was high in Mf +ve cords of Mf-ve mothers compared to Mf -ve cords of Mf -ve mothers. INF-γ was significantly ($p \leq 0.05$) high in Mf +ve cords compared to Mf –ve cords when the mothers were Mf positive or negative. But no significant difference ($P \geq 0.05$) in INF-γ was observed among Mf +ve and Mf -ve cords of Mf +ve mothers or Mf -ve mothers as shown on Table 7.

3.6 Impact of Mother and Cord Blood Filarial Infections on IL-13 and IL-10 Level of Mother and Cord Blood Plasma

We quantitatively assessed the level of IL-10 (the hallmark cytokine for regulatory response) and IL-13 in the plasma of cord blood of infected and uninfected mothers to evaluate the level of Th2 during filarial infection. (Table 8). IL-13 and IL-10 were significantly higher ($p < 0.05$) in Mf positive cords as compared to Mf negative cords. But, no significant difference ($p > 0.05$) was recorded in Il-13 or IL-10 of Mf +ve and Mf -ve mothers. With respect to Mf status, the plasma levels of IL-13 and IL-10

were not significantly different in mothers and cords.

We equally examined the effect of maternal infection on IL-10 and IL-13 secretion in infected and uninfected cords. As shown on Table 9, the concentration of IL-10 and IL-13 were significantly (P<0.05) higher in infected cords than uninfected cords independent of maternal status. Concentration of IL-10 and IL-13 in Mf +ve cords of Mf +ve and Mf –ve mothers had no significant differences (P>0.05). In contrast, only IL-10 in the uninfected cord, was found higher in infected than uninfected women (P<0.05).

3.7 Impact of Mother and Cord Blood Filarial Infections on Igg Levels of Mother and Cord Blood Plasma

At the time of birth, the level of IgG was significantly higher in Mf +ve mothers as compared to Mf +ve cords (p=0.006) as shown on Table 10. Similarly, a significantly higher level of IgG was observed in Mf +ve mothesr in comparison to Mf –ve mothers (p=0.01). However, there was no significant difference in IgG between cords of Mf +ve and Mf –ve mothers as well as between Mf –ve cords and mothers (p>0.05).

Total IgG concentration was not different (P>0.05) in Mf +ve cords of Mf +ve mothers and that of Mf-ve mothers as well as in Mf +ve cords and Mf -ve cords of Mf +ve mothers. However, the IgG concentration was significantly higher (P<0.05) in Mf +ve cords of Mf -ve than Mf –ve cords of Mf -ve as well as in Mf +ve cords of Mf –ve mothers than Mf –ve cords of Mf –ve mothers (Table 11).

4. DISCUSSION

A total of 316 mothers and cord pairs were involved in this study out of which 158 pairs (mothers and cords) were used to examine the impact of infection on TOS, TAD, NO, IL-2, IL-10, IL-13, IgG and INF-γ in mothers and cords blood plasma. Results showed that, 29.74 % of the

Table 7. Plasma levels of IL-2 and INF-γ in Mf +ve and Mf -ve cords with respect to Mf status of mothers

	Mf +ve cord	Mf -ve cord	P- value
IL-2 (in pg/ml)			
Mf +ve mother	1.23±0.51 (10)	1.74±0.67 (10)	0.07
Mf -ve mother	0.65±0.43 (7)	3.35±2.30 (9)	0.009
*P value	0.02	0.048*	
INF-γ (in pg/ml)			
Mf +ve mother	107.18±31.82 (10)	72.13±17.20 (10)	0.006
Mf -ve mother	92.76±16.34 (7)	66.21±18.53 (9)	0.009
*P value	0.29	0.48	

*Data represent mean ± SD of IL-2 and INF-γ. P-value as compared Mf +ve cords of Mf +ve or Mf -ve mothers to Mf -ve cords of Mf +ve or Mf -ve mothers. *P-value as compared Mf +ve or Mf -ve cord of Mf +ve mother to Mf +ve or Mf -ve cords of Mf -ve mother groups*

Table 8. Plasma level of IL-10 and IL-13 in Mf +ve and Mf -ve mothers and their cords at the time of delivery

	Mf+ve status	Mf-ve status	P value
IL-10 (in pg/ml)			
Cord	12.69±3.59 (17)	7.70±3.21 (19)	0.0001
Mother	3.71±3.27 (20)	5.61±3.55 (16)	0.10
*P value	< 0.0001	0.07	
IL-13 (in pg/ml)			
Cord	2.40±0.66 (17)	1.37±0.35 (19)	< 0.0001
Mother	1.97±0.88 (20)	1.67±0.79 (16)	0.29
*P value	0.11	0.14	

*Data represent mean ± SD. In bracket are the number of samples. P-value as compared Mf +ve cords or Mf +ve mother with Mf -ve cords or Mf -ve mother. *P-value as compared Mf +ve or Mf -ve cord with Mf +ve or Mf -ve mother groups. P values in bold are statistically significant differences (P≤0.05)*

Table 9. Plasma levels of IL-10 and IL-13 in Mf +ve and Mf -ve cords with respect to Mf status of mothers

	Mf +ve cord	Mf -ve cord	P value
IL-10 (in pg/ml)			
Mf +ve mothers	12.64±4.17 (10)	9.09±3.15 (10)	0.04*
Mf -ve mothers	12.78±2.86 (7)	6.16±2.64 (9)	0.0003*
P value	0.93	0.04*	
IL-13 (in pg/ml)			
Mf +ve motherss	2.39±0.67 (10)	1.40±0.23 (10)	0.0003*
Mf -ve mothers	2.41±0.70 (7)	1.35±0.47 (9)	0.002*
P value	0.94	0.76	

*Data represent mean ± SD of IL-10 and IL-13. P-value as compared Mf +ve cords of Mf +ve or Mf -ve mother to Mf -ve cords of Mf +ve or Mf -ve mother. *P-value as compared Mf +ve or Mf -ve cord of Mf +ve mother to Mf +ve or Mf -ve cords of Mf -ve mother groups. P values in bold are statistically significant differences (P≤0.05)*

Table 10. Plasma total IgG concentration following the filarial status of mother and cord

	Mf +ve	Mf -ve	P value
Cords	1753.43±219.30 (17)	1696.62±432.10 (19)	0.62
Mothers	2079.73±419.98 (20)	1761.55±326.83 (16)	0.01
*P value	0.006	0.62	

*Data represent mean ± SD of total IgG concentration (in AU/ml). In bracket are the number of samples. P-value as compared Mf +ve cords or Mf +ve mother with Mf -ve cords or Mf -ve mother. *P-value as compared Mf +ve or Mf -ve cord with Mf +ve or Mf -ve mother groups. P values in bold are statistically significant differences (P≤0.05)*

Table 11. Comparison of total IgG concentration between infected and uninfected cord plasma from infected and uninfected mothers

	Mf +ve Cord	Mf -ve Cord	P value
Mf +ve Mother	1782.29±241.10 (10)	1954.39±399.47 (10)	0.25
Mf -ve Mother	1712.21±194.12 (7)	1410.21±255.39 (9)	0.02
*P value	0.53	0.002	

*Data represent mean ± SD of IgG concentration (in AU/ml). P-value as compared Mf +ve cords of Mf +ve or Mf -ve mother to Mf -ve cords of Mf +ve or Mf -ve mother. *P-value as compared Mf +ve or Mf -ve cord of Mf +ve mother to Mf +ve or Mf -ve cords of Mf -ve mother groups. P values in bold are statistically significant differences (P≤0.05)*

mothers were Mf +ve while Mf +ve cords were 32.91%. The difference of filarial prevalence showed in mothers and cords may be due to the immune responses of the mothers which can succeed to clear the microfilariae in contrast to the weak immune responses of cords. Similar findings were reported by Bal et al., [18], Mpairwe et al., [19] and Jennewein et al., [20]. A recent study showed that damage to the lymphatic system by the parasites makes it subclinical for years before the manifestation of clinical features [21]. Also, studies have proven that some individuals have active infections but are microfilariae negative [22] which could be the reason for having infected cords from uninfected mothers. This high presence of Mf in cord blood is in line with a similar study conducted by Eberhard and others in 1993 [17]. Otherwise, this particular result demonstrated that treatment of Mf +ve mothers would not totally prevent the child from intrauterine filarial transmission. Moreover, this high prevalence of Mf in pregnant women may be due to the fact that pregnant women are not included in the massive drug administration programmes [23].

In the present study, the prevalence of filarial infections was highest in the maternal age group 40-50 years and least in the group 16-29 years. A possible reason for this difference is that these mothers must have acquired these parasites in the active years of their lives either as farmers or were involved in activities that exposed them to the vectors of these parasites. Their immune system was able to produce anti-inflammatory factors that helped the parasite to survive, These findings are in line with those of (Garraud et al., [24] and Jaoko et al., [25] who equally observed the 40-50 years age group to be the most infected.

The results of the present study indicate that the presence of microfilariae in cords or in mothers has no significant effect on the baby's body weight at birth and the duration of gestation. However, the body weight of babies of Mf +ve mothers was a little higher than that of babies of Mf –ve mothers. This may suggest that filarial infections have a way of surviving in pregnant women such that they are capable of increasing the birth weights of the resulting neonates as reported by Jessica et al. [26] in a similar study in Kenya. Therefore, filarial infections may be risky to women in complicated delivery. Similarly, the mean gestational age was higher in Mf -ve mothers compared to Mf +ve mothers, suggesting that filarial parasites may reduce the gestational age which can lead to premature delivery as equally suggested by some previous studies [27,28].

Pregnancy favours oxidative stress probably because of the mitochondria rich placenta during pregnancy [29]. Apart from pregnancy, oxidative stress is common among parasites infected patients such as in malaria [30] as a result of the activation of the immune responses by the parasites, thereby causing release of reactive oxygen species (ROS) [31,32]. Results of the present study showed that TOS as well as TAD were not significantly different in Mf +ve and Mf –ve cords. However, TOS was high in Mf +ve cord plasma. High TOS indicates that transplacental filariasis may induce an oxidative stress. These findings are in line with those of other researchers who observed that free radicals play a major role in the etio-pathogenesis of filariasis [33,34]. Otherwise, Mf -ve cords (from infected or uninfected women) had a high TAD than Mf +ve cords. These findings demonstrate that filarial infection in mothers may increase the total oxidative stress of cords Increase of TOS in cords as it was observed in Mf +ve cords in the present study signifies that infection of cords can compromise the immune system of the neonate.

Nitric oxide (NO) plays a major role in changes of vascular arterial tone [35]. In addition to regulating vascular tone, nitric oxide is an important signalling molecule in the nervous system and the immune system [36]. Results showed a high level of NO in Mf +ve cords and mothers than Mf -ve cords and mothers. This suggests that filarial infections may stimulate the production of NO. This increase of NO may result from a high production of INF-γ. Filarial parasites may stimulate a high production of iNOS as a result of high INF-γ, which result in a high release of nitric oxide [37]. Filarial infection may increase shear stress, therefore stimulating the activity of the endothelial nitric oxide synthase (eNOS), which is specifically important in the placenta [38]. NO is one of the important mediators of vasodilation. An increase of its concentration may be favourable to transplacental transmission of microfilariae.

The presence of parasitic infections during pregnancy is known to impact the immune system of an unborn child directly, through transfer of parasites or antigens across the placenta. Assessing the impact of cords and maternal infection on Th1 responses, we observed that transplacental transfer of microfilariae or in-utero sensitization of neonates modulates immune responses in neonatal stages, as a decreased level of IL-2 and increased levels of interferon gamma (IFN-γ) were observed in Mf +ve cord blood. The presence of microfilaria in cords or in-utero sensitization may induce anergy, consequently leading to a decrease in IL-2 production. Otherwise, the high level of INF-γ observed in this study may be transferred from mothers as it was significantly high in Mf +ve mothers. IL-2 promotes antigen-activated T cells clonal expansion, activates NK cells, promotes B cells proliferation and antibody production, and potentiates activated T cells apoptotic death. Therefore, a low concentration of IL-2 suggests that transplacental transfer of microfilariae and exposure to filarial infections. Similar findings by Bal et al, [17] showed that prenatal filarial specific immune tolerance as a consequence of active maternal filariasis increased the risk of infection during the first 7 years after birth. With respect to maternal infection, we realized that Mf +ve cords of Mf +ve mothers had a higher IL-2 than Mf +ve cords of Mf -ve mothers, while Mf -ve cords of Mf +ve mothers had low IL-2 concentration than Mf -ve cords of Mf -ve mothers. This suggests that the concentration of IL-2 in cord may be affected by the presence of Mf in cords instead of the maternal positivity as it was demonstrated that the production of Th1-type cytokine at early age has an independent association with maternal cytokine [33,34].

In various epidemiological studies it has been demonstrated that in-utero sensitization down-regulate responses among offspring which may be due to bias in the foetal and neonatal immune response towards development of T regulatory cells. In the present study, a significantly high level of IL-10 and IL-13 was observed in Mf +ve

cords. IL-10 is known to be a major inhibitor of activated macrophages, down-regulates and terminates many reactions of innate immunity that involves macrophages. It inhibits the production of macrophage-derived TNF-α and IL-12, thereby suppressing inflammatory reactions as well as the Th1 pathway of T helper cell differentiation. High IL-10 as found in Mf +ve cords justifies the low level of IL-2 and signifies that transplacental transfer of microfilariae promotes immunological tolerance. Besides, IL-13 produced by Th2 cells and some epithelial cells is well known to suppress macrophage activation and antagonize INF-γ. Therefore, it is quite clear that the passage of Mf to the cord could induce immunological tolerance [38]. These results are supported by previous researches which demonstrated the development of strong Th2 cell responses in children born in areas endemic for helminthic infections during early childhood [34].

Furthermore, we evaluated the level of IgG in Mf +ve and Mf –ve cords of the two groups of mothers to establish the impact of filarial infection on IgG and draw the functional relationship with IL-10. Results showed that Mf +ve cord blood had a significantly high level of total IgG as compared to Mf –ve cords suggesting that in utero transfer of microfilariae antigens from Mf +ve mothers to cords may lead to high IgG in cords. This elevated level of IgG in Mf +ve cord blood of Mf +ve and Mf -ve mothers in an environment of high level of IL-10 signifies the IgG concentration in Mf +ve cords may be associated with the production of IL-10. IgG4 and IgG1 are elevated in chronic filarial infections [16]. These isotypes are being mostly dependent on both IL-4 and IL-10, therefore the results may indicate a modulation of immune responses in cord bloods of sensitized foetus. In parasitic infections, IL-10 is almost strongly associated with total IgG levels [39,40,17,21]. In this study, we observed a weak and statistically not significant positive correlation between total IgG and IL-10 in Mf +ve mothers and cord blood plasma. Similar observations were made by Wammes et al*.,* [33] and Djuardi et al., [34].

5. CONCLUSIONS

In conclusion, there is transplacental transmission of microfilariae. Cord blood filarial infections were associated with an increase of total oxidative stress and a decrease in the total antioxidant defence of cord plasma. Furthermore, this compromised the immune response in neonates characterized by a low IL-2 and a typical Th2 immune response associated with higher concentration of IL-10 and IL-13 in cord blood.

6. AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included in this published article (and its supplementary information files).

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

CONSENT AND ETHICAL APPROVAL

This study was approved by the Institutional Review Board of the Faculty of Health Sciences Ethical Review Committee at the University of Bamenda (No. 2018/0099/UBa/IRB) An authorization was also obtained from the Regional Delegation of Public Health and the different hospitals, All blood and placental samples were collected after the subjects had provided written informed consents. No incentives were given to participants and results of each participant were made known to them. Participants were enlightened on the need to receive preventive drugs or treatments from the local MDA team and also on how to protect themselves from filarial infections.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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