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Maternal vitamin A supplementation delays time to first episode of parasitaemia and reduces malaria parasite densities among infants in rural communities in Ebonyi State, Nigeria

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Abstract. The study was a randomized mother –infant dyad placebo controlled field trial that evaluated the effect of maternal vitamin A supplementation on time to first episode of parasitaemia and on malaria parasite densities among infants in three rural communities in Ebonyi State, Nigeria. One hundred and fifty-two (152) pregnant women with pregnancies that had mature to at least six (6) months were equally randomized into supplemented and placebo groups. Ten thousand international unit (10,000 IU) of vitamin A supplement was administered three (3) times per week to women of the supplemented group while placebo was given to the control group at equal frequencies. The regimen was continued until the participants delivered their babies. On delivery of their babies, 200,000 1U of vitamin A supplement was administered to the supplemented group while the other group also received placebo. The regimen was maintained every three (3) month until the infants were 12 months old. Follow-up was carried out monthly and 3 ml of venous blood was collected quarterly from the infants and used for determination of parasitaemia. The procedure was carried out following standard parasitological techniques. Data collected were analysed using analysis of variance (ANOVA), Fisher's least significant difference (FLSD), and T-test. Statistical significance was established at $p<0.05$. Infants of mothers from the placebo group were infected by malaria parasites at earlier months of their lives while their counterparts had parasitaemia much later. Malaria parasite densities significantly differed ($p<0.05$) among infants of the two groups, with infants of vitamin A supplemented women having lower parasite densities in comparison with their counterparts. Pre-partum and post- partum supplementation of vitamin A within the recommended dosages are required for improvement of nutritional status and well-being of both mothers and infants.

INTRODUCTION

Vitamin A plays essential roles in the development of healthy immune responses. Studies have indicated that vitamin A and some of its metabolites have essential roles in regulation of both innate and adaptive immune responses (Hall *et al.*, 2011). Vitamin A has been postulated to play a role in the development of thymus and in the maturation of thymocytes (Engedal, 2011) and it has been reported that its deficiency may result in thymic function impairment. Vitamin A deficiency (VAD) affected more than 19 million pregnant women and 190 million children within 5 years of age in Africa and South East Asia (WHO, 2009). Pre-partum administration of the recommended doses of vitamin A improves the health of the mother, the foetus and that of the infant. The transfer of vitamin A from mother to infant through breast milk has been reported to be 60% more efficient when it is compared with the placental transfer to the foetus during pregnancy (Stoltzfus, 1994). Administration of 200,000 or 400,000

international units of vitamin A within two (2) months of delivery has been reported to improve the vitamin A status of the mother and increase the breast milk concentration of vitamin A (Stoltzfus and Humphrey, 2002; Rice, 2007).

Malaria is a major public health problem in areas where humidity and temperature are favourable for breeding of the vectors and for growth of the parasites in the insects (Ichhpujani and Bhatia, 2005). The WHO (1996) has reported that the sub-Sahara African region had the greatest number of people exposed to malaria infections and the highest malaria morbidity and mortality rate in the world. In the year 2008, two hundred and forty three (243) million cases of malaria resulted to 863,000 deaths and greater than 80% of them occurred in children below 5 years of age in sub-Sahara Africa (WHO, 2009); where one in six death is caused by malaria (Ogunjimi *et al.*, 2012). Malaria in pregnancy is a common cause of severe maternal anaemia and low birth weight of babies (Taylor and Molyneux, 2003). Infants born with low birth weights suffer from high rates of morbidity and mortality from infectious diseases, and are underweight, stunted or wasted, beginning in the neonatal period through childhood (ACC/SCN, 2000).

Proper nutrition is very important in protecting the body against many parasitic diseases. An undernourished child may be unable to mount an appropriate immune response to malaria parasites due to the reduction in lymphocyte, impairment of antibody formation, and atrophy of thymus and other lymphoid tissues (Scrimshaw and SanGiovanni, 1997). VAD in malaria infection is associated with increased parasitaemia and mortality. Onset of early malaria infections has been reported to be common among infants in malaria endemic areas (D' Allessandro *et al.*, 2012). The protective effects of vitamin A supplementation have been documented in clinical trials with infants and young children (Shankar *et al.*, 1999; Zeba *et al.*, 2008). Some of the trials determined time to first clinical malaria episodes and mean parasite densities (Verandas *et al.*, 2001; Mwanga Amumpaire *et al.*, 2012). Post-partum vitamin A supplementation has been reported to be associated with reduced clinic visits for malaria infection (Rice, 2007). A randomized controlled trial using vitamin A supplementation in Papua New Guinea, indicated a reduction in risk of clinical malaria (Shankar *et al.*, 1999). Shankar *et al.* (1999) reported a 36% lower geometric mean parasite density with vitamin A supplementation. Parasite clearance efficacy of vitamin A has also been reported in in vitro studies (Davis *et al.*, 1998).

The increasing resistance of Anopheles mosquito species to many targeted insecticides and that of *Plasmodium* species to many anti-malaria drugs are fast becoming great public health concerns that need to be properly addressed. The problems have motivated researchers into seeking alternative prophylactic strategies of malaria prevention and control. In economic constrained settings of the tropics where the dietary patterns of the people are poor, vitamin A supplementation during pregnancy or after giving birth to babies seems a good practice for ameliorating vitamin A deficiency (VAD). Apart from supplementation, hypovitaminosis can also be controlled by food fortification, diet diversification, and genetic modification of crops. Vitamin A supplementation during gestation and lactation is gradually becoming adopted as adjuvant to other malaria control approaches in developing countries. This study was designed to assess the effect of maternal vitamin A supplementation on time to first episode of parasitaemia among infants and to determine its effect on malaria parasite densities of the young children in rural communities in Ebonyi State, Nigeria.

MATERIALS AND METHODS

The Study Area: The study was undertaken in three rural communities of Ebonyi State, Nigeria. The communities are Ndubia in Izzi Local Government Area, Agubia in Ikwo LGA and Oshiri in Onicha LGA. Ebonyi State

is one of the 36 states of Nigeria and one of the five (5) states in the eastern part of the country. The state lies approximately within longitudes 7°30[1] and 8°30[1] E and Latitude 5°40[1] and 6°45[1] N (Uneke and Ibeh, 2009). The state had a population of 2, 173,501 (NPC, 2006). The vegetation of the state is dominated by tropical rainforest, with two (2) distinct seasons (rainy and dry). The rains start from April and end in October while the dry season commences from November and stops in April.

Study Populations: The study populations were pregnant women who were 6 to 9 months pregnant and their infants when born. The inclusion criteria were that each woman must be pregnant up to 6 months and must be malaria parasitaemic. This is because dosage of vitamin A up to 10,000 international units is contraindicated in pregnancy below second trimester. The locality and socio-economic status of the participants were similar and the use of most of the malaria preventive methods, including insecticide- treated bed nets was not a common practice.

Design of the Study: The participants were randomised into two study groups – the supplemented and placebo groups. A total of 152 mother-infant pairs, 76 in each of the groups were followed up to the end of the study after 12 months.

Ethical Considerations: The study protocol was approved by the University of Nigeria Nsukka and by the Primary Health Care Unit of Ebonyi State Ministry of Health. Informed consents were obtained from the participating pregnant women, who also consented for their infants after birth before they were enrolled in the study. Before enrollment, the women were appropriately treated of the malaria infections with Mefloquine according to treatment guidelines (CDC, 2013; FMOH, 2015).

Supplement Administration and Monitoring: Ten thousand international units (10,000 IU) of vitamin A was administered to the women in the supplemented arm three times per week, starting from sixth months into pregnancy. Placebo was also given to the control group thrice weekly. The regimen was continued until the women delivered their babies. On delivery of the babies, women in the supplemented group received 200,000 IU of vitamin A while the control group received placebo. This was continued every other three (3) month (i.e. once at 3 month interval) with direct observed therapy until the study was over when the infants were 12 months old.

Infant Monitoring and Follow-up: Follow-up was carried out monthly to ascertain the health and breast feeding status of the infants during preceding periods. Blood films were obtained from the infants for examination for parasitaemia after every three month of follow-up. Malaria infected infants were treated appropriately, using artemisinin combined therapy.

Sampling Techniques and Laboratory Analysis: Collection, preparation and examination of blood smears were carried out following standard parasitological techniques as outlined by Cheesbrough (2010). One (1) ml of venous blood was collected from the infants every three month until the end of the study. Thick and thin blood smears prepared in situ were examined for malaria parasitaemia and parasite densities. Two independently prepared and examined slides by trained microscopists were used in determination of either positive or negative cases. The age (expressed in months) at which any infant was diagnosed with malaria parasites for the first time was noted and recorded appropriately.

Determination of Parasite Densities: Parasite density per microlitre of blood was estimated from the number of parasites per 200 leucocytes, with a standard leucocyte count of 8,000 (WHO, 1991; Cheesbrough, 2010; WHO, 2010).

Statistical Analysis: Data collected were analysed at $p<0.05$ level of significance, using analysis of variance (ANOVA) and Fisher's least significance difference (FLSD) for mean separation. T-test was used to compare the supplemented group with the placebo group, with statistical significance also determined at $p<0.05$.

RESULTS

A total of 195 women who had malaria infections and were 6 to 9 months pregnant were enrolled in the study. Ninety eight (98) of them (the supplemented group) received vitamin A while ninety seven (97) received placebo. There were no cases of twin pregnancies and preterm births but cases of relocations, voluntary withdrawals, still births, neonatal deaths, and infantile mortality reduced the mother- infant pairs to 76 in each of the groups. This number was followed up until the end of the study when the infants became 12 months old (Fig. 1).

The ages at which first episodes of malaria parasitaemia were observed among infants of women from the placebo group were generally lower when compared with those of the supplemented group. First episodes of malaria parasitaemia were generally delayed until older ages among infants of women in the supplemented group. When the two groups were compared, significant differences ($p<0.05$) were observed in some groups of gravidity except within the multigravids (Table 1).

There was a general statistical significant difference ($p<0.05$) in mean parasite densities between the supplemented and placebo groups, except among infants in the 6th and 7th month groups among the primigravids. In all cases, parasite densities were higher among the placebo group than in the supplemented group (Table 2).

DISCUSSION

Infants of mothers of the placebo groups were infected at early months of their lives while those from the supplemented arms were infected later. This finding agrees with Zeba *et al*. (2008). The prolonged period to first episodes of malaria parasitaemia observed in the supplemented group is a

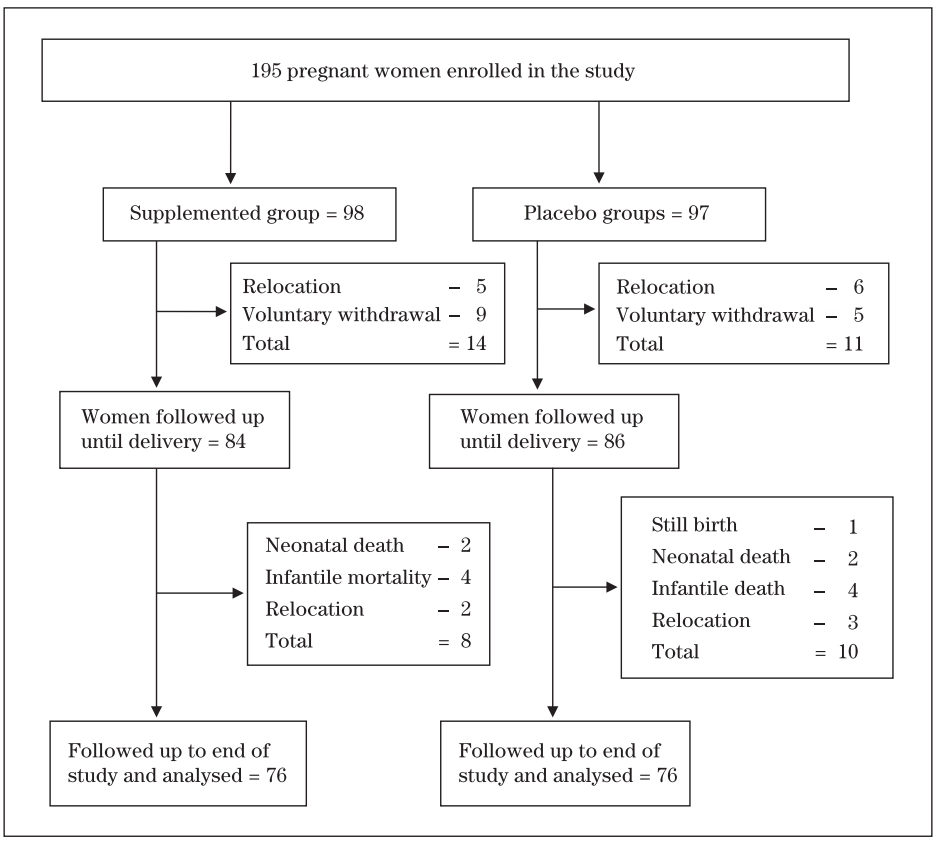

Figure 1. Flow chart of study participants.

Table 1. Effect of maternal vitamin A supplementation on infantile age (in months) at first episode of parasitaemia from supplemented and placebo group mothers in Ebonyi State, Nigeria

Month	Supplemented	Placebo
	Primigravids	
	(N = 21)	**(N = 21)**
6th	4.75±1.44^{a}	3.75±1.50$^{a\ ns}$
7th	5.50±2.50^{a}	4.60±1.52$^{a\ ns}$
8th	6.00±1.01^{a}	3.38±1.06$^{a\ *}$
9th	5.00±1.00^{a}	4.50±1.73$^{a\ ns}$
	Secundigravids	
	(N = 24)	**(N = 21)**
6th	7.00±0.00^{a}	3.67±1.15$^{a\ *}$
7th	6.71±1.70^{a}	4.50±1.38$^{a\ *}$
8th	6.29±1.89^{a}	4.83±2.40^{a}
9th	4.67±2.40^{a}	4.00±1.55^{a}
	Multigravids	
	(N = 31)	**(N = 34)**
6th	3.00±0.00^{a}	3.00±0.00^{a}
7th	6.00±2.52^{ab}	4.69±1.44^{ab}
8th	6.60±2.63^{b}	5.11±1.26^{b}
9th	5.57±2.70^{ab}	5.63±1.92^{b}
	Overall	
	(N = 76)	**(N = 76)**
6th	4.56±2.24^{a}	3.46±1.04^{a}
7th	6.20±1.94^{a}	4.61±1.41$^{b\ *}$
8th	6.32±2.60^{a}	4.44±1.70$^{ab\ *}$
9th	5.05±2.42^{a}	4.79±1.78^{b}

Key: values in a column with different alphabets as superscripts are significantly different. ($p<0.05$). * = significant difference ($p<0.05$), determined by t- test. Months from 6th to 9th stand for gestation age of pregnant women on commencement of vitamin A regimen.

Table 2. Effect of maternal vitamin A supplementation on parasite densities of infants from supplemented and placebo group mothers in Ebonyi State, Nigeria

Month	Supplemented group (N=76)	Placebo group (N=76)
	Primigravids	
6th	1293.33±293.63^{a}	1830.00±205.03^{a}
7th	1660.00±217.39^{a}	1892.00±171.95^{a}
8th	1271.30±111.33^{a}	2415.00±125.62^{b*}
9th	1308±152.74^{a}	2476±147.31^{b*}
	Secundigravids	
6th	900.00±140.00^{ab}	1996.67±224.42^{a*}
7th	1328±147.73^{b}	1936.92±139.42^{a*}
8th	897.14±135.79^{a}	2093.85±120.92^{a*}
9th	1104.00±104.51^{ab}	2296.67±129.50^{a*}
	Multigravids	
6th	708.24±94.54^{a}	1250.53±112.51^{a*}
7th	1232.31±122.84^{b}	1749.43±110.18^{b*}
8th	1237.14±91.34^{b}	2131.43±93.10^{c*}
9th	1065.26±123.54^{b}	2320.00±119.34^{c*}
	Overall	
6th	910.00±118.93^{a}	1645.60±114.14^{a*}
7th	1351.70±90.19^{ab}	1837.58±76.04^{a*}
8th	1447.38±196.91^{b}	2301.13±125.28^{b*}
9th	1154.86±72.30^{ab}	2350.54±75.03^{b*}

Key: values in a column with different alphabets as superscripts are significantly different. ($p<0.05$). * = significant difference ($p<0.05$), determined by t- test. Months from 6th to 9th stand for gestation age of pregnant women on commencement of vitamin A regimen.

clear indication of protective potentials of vitamin A supplementation that have been reported in many clinical trials (Shankar *et al.*, 1999; Zeba *et al.*, 2008). Some of the trials also determined the number of times of clinical episodes of malaria (Verandas *et al.*, 2001; Mwanga-Amumpaire *et al.*, 2012). Reported early parasitaemia that is common among infants under six (6) months of age (less than 6 month old) in malarious areas (D' Allessandro *et al.*, 2012) has been significantly reduced in this study by vitamin A supplementation.

With exception of the multigravids, all the groups in months of enrolment had significant differences in infantile age at first episode of parasitaemia. The non-significant difference observed among the multigravid group could be attributed to gravidity – dependent pattern of infant susceptibility to malaria as has been reported by Mutabingwa *et al.* (2005). The overall mean parasite densities in the placebo group were higher than in the supplemented group. Apart from the 6th and 7th month groups of the primigravidae, the mean parasite densities were statistically significant ($p<0.05$) in all the months across the gravidities. The findings of this study disagree with (Binka *et al.*, 1995).

However, the findings of the study are in line with Shankar *et al.* (1999). Reduction in parasite density could be attributed to the hypothesis that vitamin A acts by increasing phagocytosis of parasitized erythrocytes and by reducing the pro-inflammatory response to the malaria infection (Serghedes and Kain, 2002). This

is because vitamin A may assist in up-regulation of CD36 expression which facilitate phagocytosis. However, the mechanism of delay in episode of parasitaemia, attributed to vitamin A supplementation is not well understood.

The parasite clearance efficiency of vitamin A has been reported by Davis *et al.* (1998) and this is similar to the findings of (Fawzi *et al.*, 1999).

The increasing resistance of malaria vectors to many insecticides and the corresponding resistance of the human malaria parasites to various antimalarials have generated a public health concern in the control of malaria. These challenges have informed the need for an adjunct prophylactic measure in management and control of malaria. Maternal vitamin A supplementation in this study has proved to be effective in prolonging the time to first episode of malaria parasitaemia among infants and has also proved to be efficacious in the reduction of malaria parasite densities. The WHO recommended dosages of vitamin A could be very essential adjuncts in the management and control of malaria during pregnancy and infancy and is strongly recommended, especially in areas with high malaria transmission.

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