TRANSPLACENTAL PASSAGE OF *PLASMODIUM FALCIPARUM* AND SEROEVALUATION OF NEWBORNS IN NORTHERN NIGERIA

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Abstract. The findings of a prospective study of 656 near-term pregnant women, and of the cord and peripheral blood of newborns of positive mothers are reported. 292 (44.51%) of the pregnant women were infected with *Plasmodium falciparum*. Further microscopic screening of the cord blood of newborns of the 292 positive cases at delivery showed a parasite rate of 10.95%. Transplacental passage of *P. falciparum* was confirmed by detection of parasitemia in the peripheral blood of 2.82% of newborns within 7 days of birth. Serological investigation of sera of 284 newborns by indirect fluorescent technic (IFA) with *P. falciparum* IgM specific conjugate indicated that 72 (24.66%) had IgM antibodies of *P. falciparum* in their blood. The average birthweight of seropositive newborns was 400 g less than seronegative ones. There was no significant difference in the rate of neonatal infection regardless of whether or not the mothers had taken chloroquine prophylaxis.

INTRODUCTION

Reports have shown that malaria parasitemia is both more prevalent and more intense in pregnant than non-pregnant African women (Walton, 1949). Malaria in pregnancy has been recorded to be associated with such complications as low birthweight, preterm delivery, fetal mortality and congenital malaria (McGregor, 1983; Brabin, 1991).

Although congenital transmission has been reportedly rare in endemic areas (Covell 1950), reports have been made from Nigeria Ezeoke (1985), India (Singh and Tandon, 1966), Malaysia (Thomas, 1980), Papua New Guinea (Lehner and Andrews, 1988) and Zambia (Larkin and Thuma, 1991). These studies refer principally to *Plasmodium falciparum* which is the commonest cause of malaria infection in pregnancy.

The low frequency of transplacental passage of *Plasmodium* has been explained on the basis of the placental barrier in general, and on the enhanced immunity of the endemic population in particular (McGregor 1970; Reinhardt *et al*, 1978). Although early studies in endemic areas indicate that placentas of infected mothers may harbor large numbers of malaria parasites without apparent transmission into the fetal circulation (Blacklock and Gordon, 1925; Cannon, 1958; Galbraith *et al*, 1980), contemporary reports however reveal transplacental passage of parasites (Marshall 1982; Lehner and Andrews, 1988; Larkin and Thuma, 1991). Thomas (1980) had earlier establishd the value of the detection of specific IgM antibodies in new-borns in the diagnosis of congenital transmission in newborns.

This study investigates the occurrence of transplacental passage of malaria parasite and active *Plasmodium* antibodies in newborns in order to provide baseline data on the status of congenital malaria transmission in hyperendemic Northern Nigeria.

MATERIALS AND METHODS

Study area

The survey was carried out between July and October 1992 in Plateau and Bauochi States of Northern Nigeria. The area is situated between latitudes 9-12°N with tropical climate and two marked periods; rainy and dry seasons. The subjects investigated are pregnant women who attend antenatal clinics at the University of Jos Teaching Hospital and Bauchi specialist Hospital, which are the two main health institutions in the area. The hospitals are situated in urban areas where 80% of the subjects live.

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Parasitological technics

Blood samples were obtained from 656 near-term pregnant women and 600 non-pregnant women who served as controls. Cord blood and peripheral blood from 284 newborns (of pregnant women with parasitemia) after delivery were also examined. Thick and thin smears of the blood samples were air-dried, fixed and stained using methods described by Cheesbrough and Prescott (1974). Thin smears were examined only if thick smears were positive. Microscopic examination was under an 100 x oil immersion objective.

Trophozoites were counted concomitantly with white blood cells in each field and positive smears were recorded as the ratio of trophozoites per 200 wbc. Parasite densities were calculated by multiplying the number of trophozoites per 200 wbc by average white blood cell count of the maternal and newborn population. Observations made by hospital staff on whether or not the pregnant women were given chloroquine prophylaxis in the last two trimesters of gestation were recorded.

Serological diagnosis was carried out using the indirect fluorescent antibody (IFA) technic. Blood samples from newborns were tested within one week of birth against *Plasmodium falciparum* antigens using IgM specific conjugates. Fluorescence at a dilution of serum of 1:20 or higher was regarded as positive test. The test were performed at the University Teaching Hospital, Parasitology Laboratory.

RESULTS

Prevalence of malaria infection in blood smears from near-term pregnant mothers, cold, newborns and non-pregnant women is shown in Table 1. Only *Plasmdium falciparum* was encountered in course of the study. Of a total of 656 near-term pregnant

Table 1

Prevalence of falciparum malaria in blood from near-term pregnant women, newborn-cord and peripheral blood, and non-pregnant women.

Blood	Overall			Primigravidae			Multigravidae		
	No. examined	No. positive	%	No. examined	No. positive	%	examined	No. positive	%
Near-term pregnant women	656	292	44.51	360	208	57.78	296	84	28.4%
Newborn-cord	292	32	10.95	208	32	15.38	84	0	0
Newborn-peripheral blood	284	8	2.82	156	8	5.12	128	0	0
Non-pregnant women (control)	600	208	34.67	-	-	-	-	-	-

Table 2

Serological reaction of peripheral blood of newborns after tests by indirect fluorescent antibody (IFA) technic.*

	n	Males	Females	LBw (2,500g)	Av Bw ± SD	MaCOPx	PG	MG
S P+	72	24	48	40	2,650 ± 240	56	28	44
S P-	212	80	132	8	3,060 ± 120	160	60	152

* SP+ = Seropositive newborns; SP- = Seronegative newborns; LBw = Low birthweight; Av Bw = Average birthweight of newborns; MaCOPx = newborns with mothers who received chloroquine prophylaxis during pregnancy; PG = newborns with primigravida mothers; MG = newborns with multigravida mothers.

women screened, 292 representing 44.57% had *P. falciparum* in their peripheral blood. This is against a parasite rate of 34.67% recorded among non-pregnant women. The rate of infection was significantly higher in primigravidae (57.78%) than in multigravidae (28.4%) ($p < 0.05$).

Examination of cord blood of newborns from all 292 positive maternal women, showed that only 32 representing 10.95% had the parasites. All 32 positive cases were from primigravid mothers.

Eight of the parasitemic pregnant women had still births, hence only 284 newborns were examined; of these only 8 (2.82%) were infected with *P. falciparum*. The 8 were also from primigravid mothers.

The average parasite densities of those infected were 8,450 ± 200 (mean ± SD) parasites/ml for maternal population and 6,820 ± 160 parasites/ml for the newborn population. There was no correlation between the densities of parasites in maternal blood samples and the numbers of parasites found in corresponding newborn peripheral blood samples.

Results of serological investigation of blood samples from the 284 newborns of parasitemic mothers by IFA technic indicated that 72 had IgM antibodies specific for *P. falciparum* in their blood (Table 2). The fraction of males in seropositive and seronegative groups were 24 out of 72 and 80 out of 212 respectively. Chi square analysis confirmed that there was no significant difference in congenital infection between sexes ($p \geq 0.05$).

Of the 72 seropositive newborns, 40 had low birthweight (< 2,500 g), while 8 out of 212 newborns with seronegative blood had low birthweight. Further comparisons of newborns with and without IgM antibodies in their blood, revealed that seropositive newborns had an 400 g lower average birthweight. Statistical analysis using the two tailed *t*-test indicated that this lower average birthweight among newborns was significant ($p \leq 0.05$).

Of 216 newborns born from mothers on chloroquine prophylaxis (300 mg base/week) during the last two trimesters of gestation, 56 about 26% were found to be seropositive for falciparum malaria. These 56 account for about 78% (56/72) of all seropositive newborns (Table 2). About 75% (80/212) of newborns who were seronegative were born from mothers taking chloroquine prophylaxis. Chi-square analysis showed that there was no significant difference in the rate of newborn infection notminding whether or not the mothers had taken prophylaxis.

DISCUSSION

Our findings on the prevalence of falciparum malaria in pregnancy conforms with earlier reports in other parts of Africa which showed that pregnant women are more prone to malaria than non-pregnant women. This has been attributed to the fact that pregnancy depresses pre-existing acquired immunity and so reactivates malaria (McGregor, 1984). The significantly higher infection rates in primigravidae than multigravidae also agrees with the findings of other workers (Archibald, 1956; Cannon, 1958; McGregor, 1978; Bay and Anderson, 1979). This is a reflection of a differential host susceptibility between primigravidae and multigravidae.

The detection of parasitemia in 10.95% of cord blood of newborns from infected mothers suggests and evidence of transplacental passage of *Plasmodium falciparum*. This agrees with earlier findings by Kortman (1972) who recorded low density cord blood infection in 3.8% of newborns in Tanzania and Reinhardt *et al* (1979) who recorded an incidence of 21% in Ivory Coast. Infection of the placenta in endemic areas is relatively frequent and infection rates of 20-30% is fairly representative in tropical Africa (Bruce-Chwatt, 1952; Archibald, 1958; Jellife, 1968; Reinhardt *et al*, 1978). The increasing frequency of cord blood parasitemia in endemic areas in the 1st ten years is indicative of active transplacental passage of *P. falciparum* during pregnancy as against accidental passage during labour as suggested by Marshall (1982) in Solomon Islands. Schwetz and Peel (1934) had earlier attributed transplacental passage of *Plasmodium* to the chronicity of infection during pregnancy which causes greater friability and permeability of the placenta. Further studies on the histopathology of the placenta and cord are required to establish definite reasons for transplacental passage of malaria parasites.

Of newborns 2.82% had the parasite in their peripheral blood. The blood samples were taken in less than seven days after birth hence infection is traceable to parasitemic mothers. An earlier study in Zambia recorded peripheral parasitemia in 19 (29%) of 65 newborns (Larkin and Thuma, 1991).

These findings represent a new trend, since parasitemia in the peripheral blood of newborns even in heavy infection of maternal placenta was considered very rare in highly endemic areas (Covell, 1950; Bruce-Chwatt, 1952).

The detection of IgM *P. falciparum* specific antibodies in 72 (25%) of 284 newborns shows a higher level of congenital transmission than revealed by microscopic examination. It seems that *P. falciparum* present in the blood of some newborns were not detected by routine microscopy. McGregor (1984) noted that low level parasitemia hardly detectable by microscopy in newborns may occur for several weeks until some immunological or biochemical changes in their new environment permit parasite multiplication. IgM antibodies from pregnant mothers do not normally cross the placental barrier (Thomas and Chit, 1980), therefore the *P. falciparum* specific antibody in the peripheral blood of newborns is a primary response by the newborns to the presence of the parasite. This confirms intrauterine infection since the human fetus has been proved to be immunologically competent and may start IgM synthesis during gestation (Eichenwald and Shinefeld, 1963). The lower average birthweight of seropositive newborns is consistent with other findings on the relationship between maternal malaria and birthweight (Jilly, 1969; Korthman, 1972; Larkin and Thuma, 1991). Premature births associated with mothers with high parasitemia (Brabin, 1983), or retardation of growth can be advanced as a cause of lower average birthweight of seropositive newborns.

This study has shown that compliance with maternal chemoprophylaxis did not significantly affect infection rates of newborns. Similar finding was also obtained by Kaseje *et al* (1987) in Kenya, which showed that, although infants whose mothers were taking chemoprophylaxis had lower prevalence of parasitemia than those from other infants, the difference was not statistically signficant. The fact that maternal chemoprophylaxis only reduces parasite density (intensity of infection) without affecting the chronicity of infection and possible chloroquine resistance (Peters, 1990) may be responsible for congenital transmission regardless of maternal chemoprophylaxis.

A final deduction from this study is the appreciation of the increasing prevalence of congenitally acquired parasitemia among newborns in hyperendemic areas and its potential capacity to initiate primary clinical malaria among infants.

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REFERENCES

Archibald HM. The influence of malarial infection of the placenta on the incidence of prematurity. *Bull WHO* 1956; 15 : 842-5.

Archibald HM. Influence of maternal malaria on newborn infants *Br Med J* 1958; 2 : 1512-4.

Bay RS, Anderson MJ. Falciparum malaria and pregnancy. *Trans R Soc Trop Med Hyg* 1979; 73 : 427-31.

Blacklock DB, Gordon RM. Malaria infection as it occurs in late pregnancy; its relationship to labor and early infancy. *Ann Trop Med Parasitol* 1925; 19 : 327-65.

Brabin BJ. An analysis of malaria in pregnancy in Africa. *Bull WHO* 1983; 61 : 1005-16.

Brabin BJ. The risks and severity of malaria in pregnancy. Applied Field Research in Malaria Reports No. 1 Tropical Disease Research, 1991.

Bruce-Chwatt LJ. Malaria in African infants and children in Southern Nigeria. *Ann Trop Med Parasitol* 1952; 46 : 173-200.

Cannon DS. Malaria and prematurity in the western region of Nigeria. *Br Med J* 1958; 2 : 877-8.

Cheesbrough M, Prescott LM. Manual of basic technics for a health laboratory. *WHO* 1974: 20-5.

Covell G. Congenital malaria. *Trop Dis Bull* 1950; 47 : 1147-67.

Eichenwald HF, Shinefield HR. Antibody production by the human fetus *J Pediatr* 1963; 63 : 870.

Ezeoke AC. Congenital malaria at Universitv of Calabar Teaching Hospital with reference to haemoglobin and immunoglobin *Cen Afri J Med* 1985; 31 : 241-3.

Galbraith RM, Faulk WP, Galbraith MP, Holbrook TW. The human maternofoetal relationship in malaria: 1. Identification of pigment and parasites in the pla-

centa. *Trans R Soc Trop Med Hyg* 1980; 73 : 427-31.

Jelliffe EF. Placental malaria and foetal growth failure. In: Wostenhelme GEW, Ed. Nutrition and infection. London, Churchill: CIBA. Foundation Study Group 1967; No. 31.

Jilly P. Anaemia in parturient women, with special reference to malaria infection of the placenta. *Ann Trop Med Parasitol* 1969; 63 : 109-16.

Kaseje DC, Sempebwa EK, Spencer HC. Malaria chemoprophylaxis to pregnant women provided by community health workers in Saradidi, Kenya I. Reasons for non-compliance. *Ann Trop Med Parasitol* 1987; 81 : (Suppl 1) : 77-82.

Kortmann HF. Malaria and pregnancy. Utrecht: University of Amsterdam, 1972. MD Thesis.

Larkin GL, Thuma PE. Congenital malaria in a hyperendemic area. *Am J Trop Med Hyg* 1991; 45 : 587-92.

Lehner PJ, Andrews JA. Congenital malaria in Papua New Guinea. *Trans R Soc Trop Med Hyg* 1988; 82: 822-6.

Marshall DE. The transplacental passage of malaria parasites in Solomon Islands. *Trans R Soc Trop Med Hyg* 1982; 77 : 470-3.

McGregor IA. Placental malaria. In: Sterky, G, Mellander L, eds. Birth weight distribution an indicator of social development. Uppsala, Swedish agency for research co-operation with developing countries. 1978; 17-18 (Sarec report, NOR2).

McGregor IA, Rowe DA, Wilson ME, Billewicz WZ. Plasma immunoglobulin concentrations in an African (Gambian) community in relation to season, malaria and other infections and pregnancy. *Clin Exp Immunol* 1970; 7 : 51-74.

McGregor IA, Wilson ME, Billewicz WZ. Malaria infection of the placenta in The Gambia, West Africa; its incidence and relationship to still birth, Birth weight and placental weight. *Trans R Soc Trop Med Hyg* 1983; 77 : 232-44.

McGregor IA. Epidemiology, malaria and pregnancy. *Am J Trop Med Hyg* 1984; 33 : 517-25.

Peters W. *Plasmodium*: Resistance to antimalarial drugs. *Ann Parasitol Hum Comp* 1990; (suppl 1): 103-6.

Reinhardt MC, Ambroise-Thomas P, Cavallo-Serra R, Mylan C, Gautier R. Malaria at delivery in Abidjan. *Helv Paed Acta* 1978; 33 (suppl 41) : 65-84.

Schwetz J, Peel M. Congenital malaria and placental infections amongst the negroes of Central Africa. *Trans R Soc Trop Med Hyg* 1934; 28 : 167-74.

Singh SD, Tandon PL. Congenital malaria (Brief review with a case report). *Indian Practitioner* 1966; 19 : 359-62.

Thomas V, Chit CW. A case of congenital malaria in Malaysia with IgM malaria antibodies. *Trans R Soc Trop Med Hyg* 1980; 74 : 73-6.

Walton GA. On the control of malaria in Freetown, Sierra Leone. II. Control methods and the effects upon the transmission of *Plasmodium falciparum* resulting from the reduced abundance of *Anopheles gambiae*. *Ann Trop Med Parasitol* 1949; 43 : 117-39.