RESEARCH PAPER

Placental and Neonatal Outcome in Maternal Malaria

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Received: February 07, 2013; Initial review: March 18, 2013; Accepted: February 14, 2014.

Objective: *Primary*: To determine the incidence of congenital malaria in a cohort of pregnant women in a hyper-endemic area of central India. *Secondary*: (1) To find out the placental weight and placental malaria positivity, and to assess fetal and neonatal outcome in terms of survival, mean hemoglobin and mean birth weight.

Design: Prospective observational study.

Setting: Maternity and neonatal ward of a tertiary level hospital attached to a medical college located in Rewa, Madhya Pradesh, India.

Participants: Near term and term pregnant women admitted in the maternity ward with a singleton pregnancy, whose neonates were available for examination till at least 6 hours after birth.

Methods: Thick and thin blood smear were examined for malarial parasites from mothers prior to delivery. Based on the results of peripheral smear they were divided into 'exposed group' (peripheral smear positive for malaria parasite) and unexposed group' (smear negative for malaria parasite). These groups were then followed prospectively till delivery and subsequently till the mother and the neonates were discharged from the hospital.

Outcome variables: *Primary:* Presence of asexual parasite in neonate. *Secondary*: Placental weight, presence of asexual malarial parasite in placenta, still births, early neonatal deaths, mean birth weight and mean hemoglobin.

Results: Seventy-two (35.5%) of 203 blood smears of near term and term pregnant women were found positive for malaria parasite (60 *P. vivax* and 12 *P. falciparum*); rest 131 comprised the unexposed group. Six (2.95%) neonates had parasitemia (4 *P. vivax* and 2 *P. falciparum*). Of the 203 smears made from placental blood, 24 (11.8%) were positive for malaria parasite. The mean (SD) birth weight [2300 (472) g *vs* 2430 (322) g; *P*=0.98], proportion of preterm babies (6.9% *vs* 8.4%, *P*=0.71), incidence of still birth (4.2% *vs* 3.0%, *P*=1.0) and early neonatal death (2.8% *vs* 3.0%, *P*=1.0) were not significantly different between the exposed and unexposed group.

Conclusions: The incidence of congenital malaria is low despite high maternal smear positivity for malaria.

Keywords: *Low birth weight, Malaria, Placenta.*

Congenital malaria (CM), defined as 'the presence of *Plasmodium* parasites in the erythrocytes of newborns aged less than seven days', is an important consequence of malaria in pregnancy. In recent years, workers from endemic areas have reported the incidence of congenital malaria to be between 0-37 % [1,2]. Most of these studies were conducted in regions where the predominant species is *P. falciparum*, whereas in India it is *P. vivax* [3-7,9,10]. We decided to examine the contribution of maternal malaria to various neonatal outcomes in the Indian context and study peripartal malarial parasitemia in mothers, placentae and infants of a hyperendemic region in Central India. The effect of maternal, placental and neonatal malaria on identified neonatal parameters was also studied.

METHODS

This observational study was conducted over a 6-month period during the low malaria transmission region in the Maternity and Neonatal Wards of Gandhi Memorial Hospital, which is a tertiary level hospital attached to a Medical College in Rewa, Madhya Pradesh. Rewa falls in the hyperendemic zone for malaria with an annual parasite index (API) of 1.2. Most of the malarial infections (62-80%) of all positive smears are due to *P.vivax*.

Pregnant women with near-term and term singleton pregnancy, admitted in the maternity ward, were approached for enrolment. Women in active labor or those with any pre-existing medical disorder were excluded. We planned to include a convenience sample of 200 pregnant women in the study. Anticipating a refusal rate of around 10%, we planned to approach 225 subjects.

A written, informed consent was obtained from the pregnant woman and the husband/accompanying relative at the time of admission in the ward. Clearance from Departmental Ethics Committee was taken prior to the start of the study. All participants had the option to withdraw from the study anytime during their hospital stay.

The occurrence of any episodes of fever in the antenatal period and the treatment received for the same (especially antimalarials, confirmed either from prescription or from history) were recorded. Thick and thin blood smears were prepared and examined as per standard methods, at the time of admission of the mother to the hospital for child birth. The results of the peripheral smear examination were communicated to the obstetrician in-charge of the patient.

Based on the results of peripheral smear examination at admission, the women were divided into '*Exposed group'* (peripheral smear positive for malaria parasite) and *'Unexposed group'* (smear negative for malaria parasite). These groups were then followed prospectively till delivery, and subsequently till the mother and the neonates were discharged from the hospital.

After delivery, each placenta was weighed and grossly examined. Thick and thin smears were made from the blood withdrawn from intervillous spaces by placental prick**.** The placenta was placed on a raised sterile wire mesh stand, with the chorionic plate (fetal side) facing down to promote blood accumulation and intervillous space accessibility. A large-bore, 14-gauge needle attached to a syringe was directed approximately 0.5 cm deep through the wire mesh into the intervillous space, denoted as dark-purple regions, while avoiding puncture of the surrounding fetal vessels on the surface of the chorionic plate. The syringe was gently pulled to create a vacuum initiating blood flow, followed by withdrawal to allow for dripping blood, about 1 mL, to be collected into microcentrifuge tubes charged with 25 mL of a 1:4 heparin (stock concentration, 1000 units/mL) dilution in phosphate-buffered saline (PBS) and examined for malaria parasite.

All neonates were examined within 12 hours of birth (after initial stabilization). Gestational age assessment was done by history of last menstrual period and neonatal examination. Hematocrit was recorded in the labor room and a peripheral smear made for the presence of malaria parasite from the cord blood.

All the slides were coded immediately after fixing and evaluated microscopically by one of the authors (DS) and re-examined by a blinded hematopathologist (CBS). A minimum of 200 high power fields were examined for each thick and thin film before labelling a slide as negative. All slides examined by the author and found positive, were confirmed by hematopathologist. Parasite density was not ascertained. For the purpose of this study, Congenital malaria was defined as demonstration of asexual parasites on peripheral smear examination of a neonate within first 12 hours of birth.

Data from the structured proforma were entered in an Excel sheet that was updated weekly. Data entry of 10% of the forms was rechecked by the supervisor. Data were analyzed using SPSS 16.0. Differences between means were compared using student *t* test and differences between proportions were compared using the chi-squared test.

RESULTS

A total of 1019 near-term and term pregnant women were admitted in the maternity ward during the study period, out of which 225 were selected. Of these, 22 refused to participate and 203 were finally included in the study. Majority of mothers in both groups came from rural areas.

Of the 203 smears examined, 72 (35.5%) were positive for malaria parasite (83% *P. vivax*). None of the smears showed a mixed infection. The 72 mothers with a positive smear were labelled as 'Exposed' group and the remaining 131 comprised the 'Unexposed group'. Of the 203 placenta examined, 24 (12%) were found to be positive for malarial parasites. Out of the 24 positive smears, 21 (88%) were from the exposed group; *P. vivax* in 17 (81%) and *P. falciparum* in remaining four. All the three positive smears from placental blood in the unexposed group showed *P. falciparum*. Overall mean placental weight was 360g, and

TABLE I MATERNAL CHARACTERISTICS IN THE STUDY POPULATION

Characteristics	*Exposed group (n =72)*	*Unexposed group (n =131)*	*P value*
Demography			
Age (yr), mean (SD)	21.06 (3.03)	25.22 (4.37)	<0.001
Primigravida, n (%)	26 (36.1)	24 (18.3)	0.001
Place of residence: rural, *n* (%)	47 (65.4)	91 (69.4)	0.541
Clinical history			
History of antenatal fever, *n* (%)	26 (36.1)	19 (14.5)	<0.001
Past history of anti-malarial drugs, *n* (%)	13 (18.1)	9 (6.9)	0.014
Febrile during 72 hr preceding delivery, *n* (%)	10 (13.9)	17 (12.9)	0.957
Laboratory findings			
Hb <10g/dL, *n* (%)	21 (29.2)	20 (15.3)	0.018
Jaundice, *n* (%)	3 (4.2)	1 (0.8)	0.254
Physical examination			
Splenomegaly, *n* (%)	13 (18.1)	20 (15.3)	0.606
Hepatomegaly, *n* (%)	5 (6.9)	8 (6.1)	0.947

it was significantly less in the exposed group (294g) than in the unexposed group (397g) (P<0.001). Among the exposed group, the average placental weight in the placental smear-positive patients was significantly lower than that in placental smear-negative patients (232 g *vs* 321 g, P=0.011). However, the average placental weight between different species in the placental smear (240 g and 202 g for *falciparum* and *vivax* species, respectively) was not significantly different.

Of the 203 neonates, six (3%) had parasitemia, and all belonged to the exposed group (8 % of infected mothers) and placental blood smears of all these infants were positive. None of the enrolled neonates received any blood transfusion. A total of 13 newborns died in the perinatal period of which 7 were still births and 6 were early neonatal deaths. There was no significant difference in neonatal characteristics between exposed mothers and unexposed mothers (***Table* II**).

TABLE II PLACENTAL AND NEONATAL CHARACTERISTICS OF THE STUDY POPULATION

Characteristics	*Exposed group (n =72)*	*Unexposed group (n =131)*	*P value*
Placental positivity, *n* (%)	21 (29.2)	3 (2.3)	<0.001
Placental weight (g), Mean (SD)	294 (105)	396 (54)	<0.001
Birth weight (g) Mean (SD)	2300 (472)	2430 (322)	0.980
Preterm, *n* (%)	5 (6.9)	11 (8.4)	0.713
Still birth, *n* (%)	3 (4.2)	4 (3.0)	0.479
Early neonatal death, *n* (%)	2 (2.8)	4 (3.0)	0.639
Mean hemoglobin (g/dL)	15.0	17.2	0.863
Neonatal Malarial positivity, *n* (%)	6 (8.4)	0	

DISCUSSION

Studies examining the incidence of parasitemia in infants born in endemic regions have shown rates varying from 0-33% [11-12]. We demonstrated a lower incidence (29/1000) of congenital malaria. Low incidence of congenital malaria in the present study could be because of higher proportion of *P. vivax* infection which was not associated with decreased duration of pregnancy, stillbirth or miscarriage. Passive acquisition of maternal antibodies and fetal hemoglobin are reported to protect newborn from malaria. Also, we did not do histopathologic examination of placenta, which is a more reliable method of assessing placental infection than obtaining only blood smears. Most of the authors from India and a few from the United States and Asian countries have also reported *P. vivax* as the commonest organism causing congenital malaria [3-5, 8,13].

Maternal *P. falciparum* infection has been shown to be associated with low birth weight [14, 15]. In the present study, no significant association between low birth weight and maternal malarial infection was seen. In accordance with most recent reports, a significant effect of maternal parasitemia or placental malaria on the incidence of prematurity was also not found in this study. The results of this study are at variance with the data from Africa and the Far-East.

The major strength of the study was the near-complete follow-up of all the included mother-infant dyad, till initial smear examination of the neonate. Limitations were: small sample-size, non-inclusion of rapid diagnostic tests for malaria, no histopathological examination and non-documentation of parasite density or post-treatment response.

We conclude that despite a higher prevalence of maternal smear-positive malaria, the risk to neonate is not high. This variance may be due to the higher number of *P. vivax* infections in this area. There is a need to conduct more studies on maternal and fetal effect of maternal malaria in the Indian setup, where the relative proportion of various species are different from what is reported in most studies from Africa.

Contributors: DS: conducted the study under the supervision of JS and HPS, and helped in literature review; DM: planning, data analysis, and drafted the manuscript; JS and HPS: planning and conduct of the study, preparation of the manuscript. They will act as guarantors for the study; BS: data collection and analysis, and manuscript revision. All authors approved the final version.
Funding: None; *Competing interests*: None stated.
Acknowledgements: Dr CB Shukla and Dr. G Bannerjee, for administrative support and investigations, and Mr. GW Lanke for help in the statistical analysis.

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