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Malaria parasitaemia and *Plasmodium falciparum* specific-IgG in maternal peripheral, placental and cord circulaton

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Clincial cases of malaria infection during pregnancy may result in placental[1-5] and/or congenital malaria infections. Emerging evidence has also shown that pregnant women could remain asymptomatic with malaria parasitaemia[6]. Hence, this study was designed to assess the possible impact of asymptomatic maternal peripheral malaria parasitaemia and *Plasmodium falciparum* specific-IgG during pregnancy on placental and cord circulations.

Twenty-one pregnant women were recruited for this study and blood was collected from maternal peripheral, placental and cord circulations after delivery for serological screening of *P. falciparum* histidine rich protein (hrp-2), detection of malaria parasites and malaria specific antibody. Informed consent was obtained from the subjects and the study design received ethical approval.

The detection of *P. falciparum* hrp-2 antigen in blood samples from peripheral, placental and cord circulation of the pregnant women was carried out using precoated monoclonal anti-*P. falciparum* hrp-2 wells (Cellabs Pty, Australia). The procedure was followed as described by the manufacturer. In brief, blood sampels collected from the pregnant women stored in ethylene-diamine-tetra acetic acid (EDTA) tubes were frozen and thawed before analysis. Thawed whole blood samples 100 ?l reference positive and negative controls (Cellabs Pty, Australia) were added to the appropriately labelled pre-coated wells and incubated at room temperature for 60 min. This allows the fixing of hrp-2 antigen in the blood or reference positive control to the anti-hrp-2 coated wells. The wells were washed with 0.01 M phosphate buffered saline (PBS)-tween solution thrice. Then 100 ?l of (1 : 200) diluted conjugate of enzyme labelled anti-human globulin was added to all the wells and incubated for 60 min at room temperature. After washing as described above, 50 ?l of (1 : 20) diluted 3,3', 5,5'-tetramethylbenzidine (TMB) substrate chromogen was added to the wells and placed in the dark room for 15 min for colour development. The 50 ?l of HCl (2.5 M) was added to stop the reaction and the colour developed was read using a Dynatech MR 250 microplate reader at 450 nm. The absorbance of this test is not pro-

portional to the level of parasitaemia. The cut-off point for samples positive for *P. falciparum* hrp-2 antigen was determined as mean absorbance (+3 standard deviation) reference negative control.

The *P. falciparum* specific-IgG concentration in samples of the pregnant women was performed using precoated *P. falciparum* antigen wells (Cellabs Pty, Australia). The procedure followed as described by the manufacturer. In brief, 100 ?l of diluted (1 : 100) pregnant women sera, reference positive and negative sera were added to appropriately labelled *P. falciparum* antigen coated wells and incubated in a humid chamber for 60 min at 37°C. The washing, addition of conjugate antibody and substrate chromogen and stopping of the reaction and reading of absorbance of colours developed and determination of cut-off point was as described for *P. falciparum* hrp-2 antigen. However, the absorbance of this test is proportional to the concentration of the *P. falciparum* specific-IgG in the samples.

The malaria parasite density was reported as number of parasites per microlitre of blood as previously described by Rooth[7]. Statistical anaylsis was performed on data using ANOVA, student t-test, likelihood ratio and p-value < 0.05 was considered significant.

The result showed that 15 (71%) of the maternal peripheral, 18 (86%) of the placental and 9 (43%) of the cord samples were sero-reactive to anti-*P. falciparum* hrp-2 antigen; while 6 (29%), 3 (24%) and 12 (57%) of the maternal peripheral, placental and cord samples were non-reactive with anti-*P. falciparum* hrp-2 antigen respectively. There was no strong likelihood that maternal peripheral malaria parasitaemia in asymptomatic pregnant women will result in parasitaemia of the cord circulation (likelihood ratio: 0.704, p >0.1), or placental circulation (likelihood ratio: 0.038, p > 0.1). However, there was a strong likelihood that malaria parasitaemia of the placental circulation will result in malaria parasitaemia of the cord circulation (likelihood ratio: 4.357, p<0.05) (Table 1).

The mean (± 2 SD) malaria parasites density expressed as number of parasites per microlitre of blood was not significantly different amongst the different circulations (p>0.l) (Table 2). However, the mean (± 2 SD) *P. falciparum* specific-IgG concentration was significantly different amongst the malaria parasitaemia maternal peripheral, placental and cord circulations (p<0.01) (Table 2).

Table 1. The likelihood ratio for malaria parasitaemia between the maternal peripheral, placental and cord circulations (n = 21)

Circulations	Likelihood ratio	p-value
Maternal peripheral vs. cord	0.704	0.1 ns
Placental vs. cord	4.357	< 0.05
Maternal peripheral vs. placental	0.038	0.1 ns

n—Number of subjects; ns—Not significant.

Table 2. *P. falciparum* specific-IgG and malaria parasites density in maternal peripheral, placental and cord circulations with malaria parasitaemia

Circulations	Maternal peripheral	Placental	Cord	ANOVA
Pf-IgG	0.547 ± 0.133 (n =15)	0.605 ± 0.102 (n =18)	0.422 ± 0.198 (n = 9)	< 0.01
MPD	483 ± 274 (n =15)	356 ± 230 (n =18)	386 ± 273 (n = 9)	> 0.1

n — Number of subjects; MPD — Malaria parasite density.

The finding of the present study shows high incidence of malaria parasitaemia in various circulations in asymptomatic individuals during pregnancy. This finding is consistent with our earlier report of high prevalence of asymptomatic malaria parasitaemia amongst pregnant women[6].

Similarly, the present study observed that malaria parasitaemia in the peripheral circulation of asymptomatic pregnant women might not necessarily result in placental and or congenital malaria parasitaemia in all instances. However, in most instances, malaria parasitaemia of the placental circulation resulted in congenital malaria parasitaemia of the neonates. Thus, over-reliance on maternal peripheral circulation for diagnosing and excluding possible congenital malaria infections may conspicuously encourage high rates of congenital malaria infection of the neonates because clearance of the malaria parasites from the maternal peripheral circulation does not exclude congenital malaria. Hence, placental malaria parasitaemia should be prevented in pregnant women due to the risk associated with congenital malaria infection.

However, the most effective way of controlling or avoiding placental malaria parasitaemia is by preventing exposure of pregnant women to infective bites of mosquitoes or by administration of effective antimalaria drugs which helps to limit and/or clear the parasitaemia in pregnant women. It may not be easy to identify such asymptomatic pregnant women with malaria parasitaemia except by compulsory routine antenatal malaria screening. This is because such patients rarely present with signs and symptoms of malaria[8]. The lack of clinical evidence based on signs and symptoms, excludes these subjects from early drug intervention and thus exposes them to possible risk of congenital malaria infection of the neonates.

It has been reported that malaria in pregnancy especially during the last trimester may result in alteration of placental function[1,2] and could adversely affect the feto-placental unit irrespective of the parity[3,9-11]. Hence, extent of damage to the placental integrity might be a strong indicator that determines the frequency of parasites transmission to cord circulations in an asymptomatic state as is likely in the present study.

The *P. falciparum* specific-IgG concentration was significantly different amongst the different malaria parasitaemia circulations. The concentration was least in malaria parasitaemia cord circulations. However, infection of cord circulations with malaria parasites seemed to result in blood reduction in *P. falciparum* specific-IgG concentration. This was clearly reflected in this finding in which the concentration of the specific antibody was significantly higher in un-infected cord circulations compared with infected circulations, both circulations having similar history of maternal peripheral malaria parasitaemia. This will imply possible earlier exhaustion of protective specific antibody during neonatal life by neonates with congenital malaria infections.

In conclusion, this study showed that malaria parasitaemia of the placental calculations has strong likelihood to result in congenital malaria infection. Therefore, over-reliance on maternal peripheral circulation for high-risk congenital malaria infection should be avoided.

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