

## Malaria specific-IgG, inter-pregnancy intervals, birth weights and body mass index in cases of asymptomatic malaria parasitaemia

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*Background & objectives* : Considerations of both inter-pregnancy intervals and malaria parasitaemia may help in understanding some aspects of susceptibility and pregnancy outcomes in malaria endemic areas.

*Methods* : Pregnant women with asymptomatic malaria parasitaemia were recruited and divided into groups based on their inter-pregnancy intervals and malaria specific-IgG, body mass index, and birth weights were studied in the groups.

*Results* : The results showed that the *P. falciparum* specific-IgG concentration ( $f = 3.52, p < 0.02$ ), malaria parasites density ( $f = 6.44, p < 0.001$ ) and birth weights ( $f = 7.36, p < 0.001$ ) were significantly different amongst the groups with varying inter-pregnancy intervals. In addition, different levels of associations between variables such as 'inter-pregnancy intervals vs *P. falciparum* specific-IgG concentration' ( $r = 0.23, p < 0.05$ ); 'malaria parasites density vs birth weight' ( $r = -0.84, p < 0.01$ ) was observed.

*Interpretations & conclusion* : This study suggests that inter-pregnancy intervals could be one of the factors influencing dynamic serum concentrations of *P. falciparum* specific-IgG while malaria parasitaemia could be one of the factors affecting birth weights. Hence, observance of inter-pregnancy intervals has its own implications in malaria endemic areas.

**Key words** Asymptomatic malaria – inter-pregnancy interval – neonatal birth weight – parasitaemia

Inter-pregnancy intervals have been shown to affect pregnancy and neonatal outcomes. Long (>24 months) and short (<6 months) intervals have been associated with high morbidity and mortality rates<sup>1–6</sup>. Similarly, high morbidity and mortality rates have been reported in cases of malaria infection especially amongst pregnant women and children less than two years of age<sup>7–12</sup>. Hence, considerations of both inter-pregnancy intervals and malaria parasitaemia may help in un-

derstanding some aspects of susceptibility in malaria endemic areas. Therefore, understanding the implications of inter-pregnancy intervals on certain parameters in malaria endemic area is necessary and will aid the clinical management of such women.

### Material & Methods

*Subjects* : Pregnant women with no signs and symptoms of malaria infection reporting for routine antena-

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tal care at the antenatal clinic were screened for malaria parasitaemia. Ninety-one of the pregnant women with consistent detectable malaria parasitaemia during the current pregnancy were eventually recruited for the study based on their inter-pregnancy intervals. The pregnant women were divided into groups—Group A < 6 months (n=31), Group B >6<12 months (n=20), Group C >12<24 months (n=20), and Group D >24<48 months (n=20). The pregnant women gave informed consent and the study design received approval from the UI/UCH, Board of Ethical Committee PIMRAT, Ibadan.

*Sampling and methods of analysis* : Blood sample was collected close to term from each of the pregnant women during pregnancy for malaria parasite density and analyses of *P. falciparum* specific-IgG. The body mass index (BMI) of the pregnant women and birth weights were also recorded.

*Determination of inter-pregnancy intervals* : This is the interval beginning from the date of last birth to the date of conception of present pregnancy.

*Malaria parasite detection* : This was performed by microscopic examination of Giemsa stained thin and thick blood films. The method was as described by Rooth and Bjorkman<sup>13</sup> and the parasite density was expressed in  $\log_{10}$ . *P. falciparum* malaria parasite species was considered.

*Determination of P. falciparum specific-IgG* : Standard indirect enzyme-linked immunosorbent assay technique was used for the detection of *P. falciparum* specific-IgG (Cellabs Pty Australia). 100  $\mu$ l of pre-diluted (1:100) test samples, positive and negative reference sera were added to wells on plates pre-coated with anti-*P. falciparum* IgG antibody respectively. This was incubated for 60 min at 37°C in a humid chamber and the wells were washed with PBS-Tween solution. Subsequently, 100  $\mu$ l of diluted conjugate of enzyme-labeled anti-human globulin (Cellabs Pty Australia) was added to wells on the plates and incubated for 60 min at 37°C in a humid chamber. After wash-

ing, 100  $\mu$ l of TMB substrate chromogen solution was added to the wells on the plate and kept in the dark for colour development at room temperature for 15 min. The reaction was stopped by addition of 50  $\mu$ l HCL (2.5 M) and absorbance of the wells was read at 450 nm using Dynatech MR 250 microplate reader against unreacted well containing only the substrate and stopping solution. The cut-off absorbance for positive samples was mean (+3 SD) reference negative serum absorbance.

*Anthropometric measurements* : The body mass index was calculated (weight/height<sup>2</sup>) for the pregnant women but birth weight was measured in kilograms.

*Statistical analysis* : Analysis of variance (ANOVA) was used to determine the levels of significance of variables, while Pearson correlation analysis was used to assess levels of associations between variables. Significant level was regarded as  $p < 0.05$ .

## Results

The mean (+SD) serum absorbances of *P. falciparum* specific-IgG concentration were : < 6 months 0.593+0.163; >6<12 months 0.512+0.171; >12<24 months 0.649+0.163; >24<48 months 0.648 + 0.104; (f =3.52,  $p < 0.02$ ). In addition, the mean (+SD)  $\log_{10}$  parasite density (per  $\mu$ l of blood) was : < 6 months 2.532+0.222; >6<12 months 2.470 + 0.273; >12<24 months 2.407+0.252; >24<48 months 2.722+0.218; (f=6.44,  $p < 0.01$ ). The mean (+SD) birth weight (kg) was : < 6 months 3.01 + 0.42; >12<24 months 3.41+0.35; > 24 < 48 months 2.98+0.44; (f =7.36,  $p < 0.001$ ). These parameters were significantly different amongst different inter-pregnancy intervals.

However, the mean (+SD) BMI was < 6 months 25.1+5.43; >6<12 months 24.8+3.5; >12<24 months 25.7+3.6; >24<48 months 25.8+4.7; (f = 0.23,  $p > 0.05$ ) was not significantly different amongst the different inter-pregnancy intervals.

Strong positive association was observed between inter-pregnancy intervals and *P. falciparum* specific-IgG ( $r = 0.23$ ,  $p < 0.05$ ) while strong negative association was observed between malaria parasite density and neonatal birth-weight ( $r = -0.84$ ,  $p < 0.01$ ). However, there was lack of association between *P. falciparum* specific-IgG and log parasite density ( $r = 0.01$ ,  $p > 0.2$ ), and between inter-pregnancy intervals and log parasite density ( $r = 0.13$ ,  $p > 0.1$ ).

### Discussion

The incidence of malaria parasitaemia in apparently healthy individuals has been observed<sup>14–15</sup>. Considerations of both inter-pregnancy intervals and malaria parasites density showed different levels of parasitaemia with different intervals. However, the length of intervals is not proportional to degree of parasitaemia. This means that other factors may also be influencing parasitaemia.

Different values of *P. falciparum* specific-IgG were also observed for different inter-pregnancy intervals. However, the length of intervals seem to influence proportionally the concentrations of the specific antibody. This possibly suggests that inter-pregnancy intervals may play a role in achieving desired level of protection against malaria in pregnant women. The maintenance of *P. falciparum* specific-IgG concentration in any patient is subject to time, frequency of exposure to parasites and immune competence of the host<sup>16,17</sup>. In this study it is assumed that the patients are in constant exposure to the parasites and are immunologically competent, however, time allowed between pregnancies varied. This might explain the reason why the study did observe significant association between inter-pregnancy intervals and *P. falciparum* specific-IgG concentration. Therefore, the lack of association between the *P. falciparum* specific-IgG concentration and malaria parasite density suggests that malaria parasitaemia is not the only factor that affects blood concentrations of the protective antibodies.

For pregnant women with different inter-pregnancy intervals, the beginning of pregnancy might be an interception of the immune recovery period post-partum. It has been suggested that for each post-partum mother there seem to exist a latent period within which the effect of pregnancy on the immune system gradually wanes before full immunological recovery occurs<sup>18</sup>. It is therefore necessary that such periods of time be determined for women of childbearing age for subsequent pregnancies.

None of the groups had low birth weights although there was significant difference in birth weights amongst the different inter-pregnancy intervals. Birth weight was most favourable for women within 12 < 24 months interval groups. This finding is consistent with other reports of impact of inter-pregnancy intervals on birth weight and neonatal outcomes<sup>1,2</sup>. However, it is important to note that during pregnancy these women had malaria parasitaemia and this might have also contributed to the observed birth weights. Studies have shown that malaria<sup>19,20</sup> or short and long inter-pregnancy intervals<sup>2,5,6</sup> could independently result in intra uterine growth retardation, low birth weight, poor pregnancy and neonatal outcomes.

Lack of change in body mass index suggest no immediate change in nutritional states of the pregnant women despite differences in inter-pregnancy intervals. Therefore, this study suggests that considerations of both inter-pregnancy intervals and malaria parasitaemia are necessary for acquiring information that will enable proper management of pregnant women with asymptomatic malaria and allow for good pregnancy and neonatal outcomes. Hence observance of inter-pregnancy intervals has its own implications in malaria endemic areas.

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