Association of naturally acquired IgG antibodies against Plasmodium falciparum serine repeat antigen-5 with reduced placental parasitemia and normal birth weight in pregnant Ugandan women: A pilot study

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ABSTRACT

Plasmodium falciparum infection during pregnancy contributes substantially to malaria burden in both mothers and offspring. Analysis of naturally acquired immune responses that confer protection against parasitemia and clinical disease is important to guide vaccine evaluation as well as identify immune correlates. Unfortunately, few studies have addressed the relationship between immune responses to malaria vaccine candidate antigens and protection against adverse effects on pregnant women and newborn birth weight. This study examines the relationship of maternal antibody responses to serine repeat antigen-5 (SE36) and merozoite surface protein-1 (MSP119 and MSP142) with placental parasitemia and birth weight. In a peri-urban setting in Uganda, pregnant women without placental parasites have high median ODs for antibodies against SE36 (P<0.001). Naturally acquired anti-SE36 IgG was most prevalent in women without placental parasitemia (P<0.001). Furthermore, pregnant women with significantly high levels of anti-SE36 IgG delivered babies with normal birth weights (P<0.001). That antibody to SE36 was associated with both a reduced risk of placental parasitemia and resulting normal birth weight in newborns suggests some protective role. In contrast, although antibody to MSP142 was also associated with reduced placental parasitemia and immune responses to both MSP119 and MSP142 may be of importance, there was no association between anti-MSP119 antibodies and infant birth weight outcomes. This study highlights the need for conducting further studies to investigate the association of antibodies against SE36 and outcomes of malaria infection in pregnant women.

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In malaria endemic areas of Africa, pregnant women are a particular risk group for Plasmodium falciparum infection [1,2]. Although, the impact of malaria during pregnancy varies with epidemiological setting or pre-existing immunity [3], blood-stage parasites are significant contributors to maternal anemia and morbidity [4], low birth weight (LBW) [5], and infant morbidity and mortality [6]. The molecular basis for parasite adhesion and immune evasion in placental malaria has largely been clarified but other factors may still be involved in parasite sequestration in the placenta [7]. Serological clues are crucial for vaccine evaluation as well as for epidemiological studies to identify immune correlates [8]. Antibodies to the erythrocyte surface ligand that binds chondroitin sulfate A were associated with reduced placental malaria [7,9-11]. Antibodies that inhibit blood-stage replication may also be important in mediating immunity. However, the presence of antibodies to ring-infected erythrocyte surface antigen (RESA) [9], glutamate-rich protein (GLURP) [10], apical membrane antigen-1 (AMA-1), and merozoite surface protein-1(MSP-110) [11] were found not to correlate with pregnancy or parity of mothers.

P. falciparum serine repeat antigen-5 (SE36) is a blood-stage vaccine candidate currently in clinical trials [12]. SE36 antibody was found to correlate inversely with malaria symptoms and severe disease [13,14], inhibit cell proliferation by complement-mediated lysis of schizonts [15], agglutinate schizonts and merozoites blocking re-invasion to red blood cells [16,17], and exhibit antibody-dependent monocyte-mediated parasite growth inhibition [18]. Since a majority of asexual-stage parasites in the placenta consist of mature forms [7], the presence of anti-SE36 antibody that blocks merozoite release/invasion could reduce the number of infected erythrocytes both in the peripheral blood and in the intervillous spaces of the placenta. The association of anti-SE36 antibody with pregnancy-associated malaria, however, has not been investigated.

Here, we sought to determine if antibody levels against SE36 correlate with placental parasitemia and pregnancy outcome in a peri-urban


location in Kampala, a region characterized by low, seasonal malaria transmission.

Serum samples were obtained from a cross-sectional study designed to investigate the prevalence and effect of *P. falciparum* infections during pregnancy at St. Francis Nsambya Hospital in Makindye division, Kampala, during a 3-month dry spell from July to Oct 1998. Details of the study and demographic characteristics of the subjects have been published [19]. Pregnant women were recruited when they presented at the labor ward for delivery. The study was approved by the hospital ethical committee and the Uganda National Council for Science and Technology. Each participant donated 3 blood samples: peripheral blood (collected by venepuncture on admission to the labor ward), placental and cord blood (collected immediately upon uncomplicated delivery).

Prevalence of malaria was evaluated in each of the 3 samples using thick and thin smears and parasitemia, relative to 200 white blood cells, was estimated assuming 8000 white blood cells per microliter. All malaria infections observed were exclusively of *P. falciparum*. Samples were classified according to the following criteria for analysis: (a) *placental malaria-negative* or *positive*, depending on the absence or presence of parasites in the placenta; (b) *peripheral malaria-negative* or *positive*, depending on the absence or presence of parasites in the venous blood. The classification was based on the characteristic feature of infection during pregnancy which results in the accumulation of *P. falciparum* infected erythrocytes in the placental intervillous blood spaces even in the absence of detectable peripheral parasitemia [7].

Birth weights of live newborns were measured, with LBW defined as birth weight below 2500 g. For the present study, information on the pattern of infection and birth weights of live newborns were obtained using anonymized data and samples that cannot be linked to original personal identifiers.

For ELISA measurements, ELISA plates (Dynatech) were coated overnight at 4 °C with 100 μg/mL of antigens MSP142, MSP119 (100 μg/mL = 10 μg/well) and SE36 (1 μg/mL = 0.1 μg/well) in carbonate buffer. MSP142 and MSP119, which represent the amino acid sequence derived from the Wellcome strain of *P. falciparum* and expressed as GST fusion proteins in *E. coli*, were a kind gift from Dr. A. Holder (Department of Parasitology, National Institute of Medical Research, Mill Hill, London UK). Recombinant SE36, which represents the N-terminal 47-kDa domain without the serine repeat region of *P. falciparum* SERA-5 was prepared as described [14]. After washing 3 times in phosphate buffered saline (PBS) containing 0.05% v/v Tween 20 (PBS-T), plates were blocked (1% non-fat dry milk in PBS-T) for an hour at 37 °C and washed.
with prevalence in placental-negative vs placental-positive samples (89.5% vs 53.3%, respectively), but high ODs for MSP119 did not. High ODs for MSP119 may suggest that this antibody was produced in response to infection.

Women with peripheral blood parasitemia at delivery had significantly higher antibody levels (MSP142, P = 0.012) or antibody prevalence (MSP142, P = 0.028 and MSP141, P = 0.018) to MSP1 (Table 1). There was no statistical difference in median ODs or the prevalence of anti-SE36 antibody between women with or without peripheral parasitemia. There was no indication in these analyses if any of the three antibodies have significant protective effect against peripheral blood parasitemia. But, in endemic areas individuals can harbor circulating parasites asymptptomatically and thus, the antibodies and their prevalence may suggest that these were produced in response to infection rather than to have prevented its establishment. This was apparent from the significantly higher titers and prevalence of anti-MSP-1 antibodies in pregnant women who have peripheral blood parasitemia. We cannot rule out if the antibodies did reduce parasite burden (protecting against high parasitemia or clinical malaria) rather than infection per se, but in this study, placental parasitemia might be a more reliable measure of malaria burden in pregnant women. Earlier studies also observed that thick blood smears of intervillosal placental blood yield higher diagnostic sensitivity in comparison with peripheral blood smears. Caution is necessary at this time in our interpretation of the placental parasitemia data due to the small sample size.

To further investigate the possible association of antibody levels to SE36 in pregnancy outcomes due to malaria infection, we examined whether mean antibody levels in the placenta were correlated with prevalence in placental-negative or placental-positive samples (89.5% vs 53.3%, respectively). Although Uganda is a malaria-endemic region, malaria transmission levels vary considerably across the country depending on the geographical location [20]. The study subjects in the present cohort represent relatively privileged, lower-middle-class pregnant women in a peri-urban setting with low, seasonal malaria transmission.

**Table 2**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Normal birth weight (n = 63)</th>
<th>Low birth weight* (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE36</td>
<td>0.240</td>
<td>0.056</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(0.211–0.296)</td>
<td>(0.021–0.205)</td>
<td></td>
</tr>
<tr>
<td>MSP119</td>
<td>0.764</td>
<td>1.046</td>
<td>0.760</td>
</tr>
<tr>
<td></td>
<td>(0.366–1.495)</td>
<td>(0.163–1.657)</td>
<td></td>
</tr>
</tbody>
</table>

* Defined as birth weight <2500 g.

**Acknowledgement**

We acknowledge the contribution of Ruth Nabwoba who carried out data entry and statistical analyses. The study was supported by grants from Grant-in-Aid for Scientific Research on Priority Areas (13226058) (13225001) from the Japanese Ministry of Education, Science, Sports, Culture and Technology to T.H. and from the UNDP/WHO/ Special Program for Training and Research in Tropical Diseases (WHO/TDR) to T.G.E. The funding sources have no role in the study design, collection, analysis, interpretation and writing, or in the decision to submit the paper for publication.

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