Submicroscopic Plasmodium falciparum infections during pregnancy, in an area of Sudan with a low intensity of malaria transmission
Submicroscopic *Plasmodium falciparum* infections during pregnancy, in an area of Sudan with a low intensity of malaria transmission

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There are few published studies on the burden of malaria during pregnancy from areas of sub-Saharan Africa where the intensity of malarial transmission is low, and few on submicroscopic malarial infections in pregnant women. The present study was conducted in New Halfa, an area of low-intensity transmission in eastern Sudan, between August 2003 and July 2004. The main aims were to assess the prevalences of submicroscopic and multiple *Plasmodium falciparum* infections in pregnant women (using the *P. falciparum* merozoite surface protein-2 as a polymorphic marker in PCR-based assays) and to determine the effects of such infections on anaemia during pregnancy. Of the 142 pregnant women who were recruited, only 17 (11.9%) were found smear-positive for *P. falciparum* by microscopy. The results of the PCR-based assays revealed, however, that 40 (32%) of the 125 smear-negative women had submicroscopic *P. falciparum* infections. Blood samples from 32 (80%) of those with submicroscopic infections showed only the FC 27 allele (of merozoite surface protein-2), six (15%) showed only the ICI allele, and two (5%) showed both of these alleles. Although the age, parity, gestational age and haemoglobin concentrations of the women with submicroscopic *P. falciparum* infections were not significantly different from those of the women who were smear- and PCR-negative, such infections may have a significant impact on materno–foetal health.

Of the 50 million pregnancies that occur in malaria-endemic areas every year, approximately half are to be found in sub-Saharan Africa (Steketee et al., 2001). The problem posed by, and epidemiology of, malaria during pregnancy depends on the local level of transmission. In areas where the intensity of transmission is low, malaria can cause severe materno–foetal syndromes, such as cerebral malaria, abortion, stillbirth and low birthweight. In contrast, those women who live in areas of stable and intense transmission enjoy considerable immunity and generally experience few symptoms during episodes of malarial infection, although some may develop severe anaemia (McGregor, 1984; Brabin, 1993; Newman et al., 2003; Okoko et al., 2003).

Eastern Sudan is an area characterized by low-intensity malaria transmission and a predominance of *P. falciparum* infection among cases of human malaria (Al Gadai, 1986). Malaria is the main cause of anaemia, low birthweights and maternal mortality in this region, where many pregnant women who are infected with *P. falciparum* develop the cerebral form of the disease (Taha et al., 1993; Dafallah et al., 2003; Adam et al., 2004a, b).

In endemic areas, submicroscopic *P. falciparum* infections appear to be common among children and could have an important role in the acquisition of natural immunity against malaria (Wagner et al., 1998; Farnert et al., 1999). So far, little is
known about such infections, or multiple *P. falciparum* infections, during pregnancy, and all the research on this topic that has been published was based in areas with intense transmission (Mockenhaupt *et al.*, 2000; Beck *et al.*, 2001; Saute *et al.*, 2002; Mayengue *et al.*, 2004). The main aims of the present study were to assess the prevalence of submicroscopic *P. falciparum* infection among pregnant women in eastern Sudan, and to explore the possible association of such infection with anaemia.

**SUBJECTS AND METHODS**

**Patients**

The study took place at the Alhara Aloua health centre in New Halfa, eastern Sudan, between August 2003 and July 2004. The aim was to enroll all the pregnant women visiting the centre’s antenatal clinic for the first time. After verbal consent was obtained, each woman’s obstetric history was recorded in detail, including the date of her last menstrual period, gravidity, and parity. Each woman was then asked specifically if she had any symptoms indicative of malaria: fever, headache, sweating, joint pain and/or vomiting. Body temperature was recorded, obstetric and physical examinations were conducted, and blood pressure, pallor and fundal level were recorded. Pregnancy was confirmed by ultrasound and its duration was calculated from the date of the last menstrual period.

**Parasitological and Haematological Parameters**

Thick and thin smears of capillary blood were prepared, stained with 10% Giemsa in phosphate-buffered saline (pH 7.0) for 10 min, and then examined under a light microscope. A thick smear was only considered negative if no malarial parasites were seen in 100 oil-immersion fields. For each positive thick smear, the level of parasitaemia was estimated by counting asexual stages against 200 leucocytes and assuming each subject had 6000 leucocytes/μl. All the thick smears were re-checked by a second microscopist, who was unaware of the results of the earlier microscopy; the opinion of a third microscopist was taken if the first and second microscopists recorded discrepant results.

The blood concentration of haemoglobin was estimated using a colorimetric method and a CO700D colorimeter (Walden Precision Apparatus, Cambridge, U.K.).

**Parasite DNA Extraction and PCR**

Two drops of blood were collected, on to a piece of filter paper, from each subject. The spots were allowed to dry and then each piece of filter paper was stored separately, in a plastic bag, until the malarial parasites in the blood spots could be genotyped.

Genomic DNA was extracted from the blood spots and checked, in an assay based on a nested PCR, for DNA from *P. falciparum* (Snounou *et al.*, 1993). The primers used allowed sequences coding for the highly polymorphic merozoite surface protein-2 (MSP-2) of *P. falciparum*, as well as oligonucleotides specific for the IC1 and FC27 alleles, to be amplified. Size variations within the alleles were used to discriminate between different strains of *P. falciparum*, by analysis of the PCR-fragment-length polymorphism (Plowe *et al.*, 1995; Robert *et al.*, 1996).

**Data Analysis**

The data were entered in a computer database and double-checked before being analysed in the SPSS. software package (SPSS Inc., Chicago, IL). Comparisons were made using Student’s *t*-tests, Mann–Whitney *U*-tests (for means of variables that were not normally distributed), and *χ*² and Fisher’s exact tests, as appropriate. A *P*-value of ≤0.05 was considered indicative of a statistically significant difference.
Ethics
The study received ethical clearance from the Faculty Research Board of the University of Khartoum’s Faculty of Medicine.

RESULTS

Overall, 142 pregnant women were enrolled as they attended antenatal clinic for the first time. Only 17 (11.9%) were found smear-positive for malarial infection (all *P. falciparum*) but 40 (32%) of the 125 smear-negative women were found PCR-positive and therefore had submicroscopic *P. falciparum* infections. Among the smear-negatives, 14 (38.8%) of the 36 primigravidae, five (22.7%) of the 22 secundigravidae and 21 (31.4%) of the 67 multigravidae (i.e. those who had been pregnant more than once before) were found PCR-positive ($P<0.05$). Twenty-one (37.5%) of the 56 smear-negative women investigated during the wet season and 19 (27.5%) of the 69 women smear-negative women investigated during the dry season had submicroscopic *P. falciparum* infections ($P<0.05$).

In terms of age, parity, gestational age, haemoglobin concentration, and the frequencies of anaemia ($<11$ g haemoglobin/dl) and severe anaemia ($<8$ g haemoglobin/dl), the smear-negative pregnant women with submicroscopic *P. falciparum* infection were similar, when enrolled, to those without such infection (see Table).

Blood samples from 32 (80%) of those with submicroscopic infections showed only the FC 27 allele (of merozoite surface protein-2), six (15%) showed only the ICI allele, and two (5%) showed both of these alleles. Each submicroscopic infection appeared to consist of just one or two clones of *P. falciparum* ($mean=1.016$; median=1).

DISCUSSION

This appears to be the first research on submicroscopic *P. falciparum* infections during pregnancy in an area of unstable, low-intensity malaria transmission (eastern Sudan). Approximately one in every three of the pregnant Sudanese women who had been found smear-negative for malaria had PCR-based evidence of submicroscopic *P. falciparum* infection. This prevalence is comparable with those, of 18%–46%, previously reported from areas of other African countries with high-intensity transmission (Mockenhaupt et al., 2000; Mayengue et al., 2004). Among both the pregnant (present study) and non-pregnant women of eastern Sudan (Roper et al., 1996), the prevalences of submicroscopic *P. falciparum* infections in the wet season appear similar to those in the dry season. The reasons for this surprising lack of seasonality have yet to be elucidated.

<table>
<thead>
<tr>
<th>Variable</th>
<th>With submicroscopic infection</th>
<th>Without submicroscopic infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigated</td>
<td>40 (35.0)</td>
<td>85 (25.8)</td>
</tr>
<tr>
<td>Primigravidae</td>
<td>14 (35.0)</td>
<td>22 (25.8)</td>
</tr>
<tr>
<td>Secundigravidae</td>
<td>5 (12.5)</td>
<td>17 (20.0)</td>
</tr>
<tr>
<td>Multigravidae</td>
<td>21 (52.5)</td>
<td>46 (54.1)</td>
</tr>
<tr>
<td>With anaemia</td>
<td>29 (72.5)</td>
<td>66 (77.6)</td>
</tr>
<tr>
<td>With severe anaemia</td>
<td>2 (5.0)</td>
<td>7 (8.2)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.02 (6.0)</td>
<td>26.31 (5.1)</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2.01 (2.1)</td>
<td>2.50 (2.4)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>27.2 (6.8)</td>
<td>25.4 (7.7)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>9.97 (1.3)</td>
<td>9.90 (1.2)</td>
</tr>
</tbody>
</table>
In the present study, the age, gravidity and gestational age of the pregnant women with submicroscopic *P. falciparum* infection were similar to those of the other smear-negative pregnant women. Similar observations were recently made in an area of Gabon with intense malaria transmission (Mayengue et al., 2004). In Ghana, however, Mockenhaupt et al. (2000) reported that the prevalence of submicroscopic *P. falciparum* parasitaemia among pregnant women increased with increasing gravidity and gestational age — each pregnancy in a woman’s life enhancing her immune protection and reducing the likelihood that she would develop a parasitaemia that was intense enough to be detected by routine microscopy.

The most prevalent MSP-2 allelic family in the parasite populations characterized in the present study was of the FC27 type, as also recently seen in Gabon (Mayengue et al., 2004). The mean number of clones/submicroscopic infection seen in the pregnant women of New Halfa was only 1.016. In areas with intense transmission, the multiplicity of *P. falciparum* infections in pregnant women tends to be higher than this, with means of one to four clones/infection (Beck et al., 2001; Saute et al., 2002; Mayengue et al., 2004). The extent of allelic diversity in the MSP-2 gene, as estimated by the total number of different alleles found in a given parasite population and the mean multiplicity of infections, appears to be positively correlated with the local level of endemicity (Hoffmann et al., 2001).

In the present study, as in Mozambique (Saute et al., 2002) and Gabon (Mayengue...
et al., 2004), there was no evidence that submicroscopic P. falciparum infections led to increased risk of anaemia in pregnant women. In endemic areas of Ghana, however, such infections may be associated with anaemia among pregnant women (Mockenhaupt et al., 2000), and 20%–25% of children with anaemia do not have parasitaemias that are detectable by routine microscopy, although many such children have other evidence of malarial infection (Kurtzhals et al., 2003).

Although the present study failed to demonstrate a significant difference in age, parity, gestational age or haemoglobin concentration between the pregnant women with and without submicroscopic P. falciparum infections, the prevalence of malarial infection during pregnancy in Sudan is clearly much higher than indicated by the results of routine microscopy. Many women found smear-negative at the delivery of their babies probably have the placental malarial infections that are associated with low birthweights and other adverse outcomes. Submicroscopic P. falciparum infections could represent a significant problem in terms of materno–foetal health.

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REFERENCES


