Short Communication

Acute-phase proteins in pregnant Sudanese women with severe
Plasmodium falciparum malaria


A R T I C L E  I N F O

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A B S T R A C T

A case–control study was carried out in Kassala and Medani Maternity Hospitals in Sudan to investigate acute-phase proteins [haptoglobin, C-reactive protein (CRP), ferritin and albumin] in three groups of pregnant women (32 in each arm) comprising those with severe Plasmodium falciparum malaria or uncomplicated P. falciparum malaria and healthy controls. Whilst there was no significant difference in the levels of albumin and haptoglobin, ferritin and CRP levels were significantly higher in pregnant women with severe P. falciparum malaria. There were significant positive correlations between parasite count and haptoglobin, and medium positive correlations between parasite count and CRP.

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1. Introduction

Pregnant women are more susceptible to severe malaria, which has a high mortality rate. In Sudan, pregnant women are susceptible to Plasmodium falciparum malaria irrespective of age or parity, and malaria is a leading cause of maternal and perinatal morbidity and mortality.

Acute-phase proteins (APP) have been implicated in the pathogenesis of severe P. falciparum malaria in children.1,2,3 Whilst there are few published data on the role of APPs in the pathogenesis of malaria during pregnancy,4,5 no published data exist on APPs and severe malaria during pregnancy. The current study was conducted to investigate the APPs haptoglobin, C-reactive protein (CRP), ferritin and albumin in severe malaria during pregnancy at Kassala and Medani Maternity Hospitals in Sudan.

2. Materials and methods

This study was conducted during the period of August–December 2010. Kassala and Medani are in eastern and central Sudan, respectively, in areas characterised by unstable malaria transmission.

Cases were pregnant women who presented with one of WHO manifestations of severe P. falciparum malaria.1 Two groups were considered as controls: pregnant women with uncomplicated P. falciparum malaria (without a severe form1); and healthy pregnant women volunteers who were matched for age, parity, gestational age and weight. After receiving signed informed consent from the patients or guardian, structured questionnaires were administered to collect medical and obstetric history. Blood films were prepared, the slides were Giemsa-stained and the number of
asexual *P. falciparum* parasites per 200 white blood cells was counted.

2.1. Determination of human acute-phase proteins

Blood was withdrawn in a plain tube, centrifuged and kept at −20 °C. Samples were transferred in dry-ice and analysed in Khartoum. An ELISA haptoglobin test kit (Immunology Consultants Laboratory, Newberg, OR, USA) was used to measure haptoglobin. All samples were run in duplicate and the mean value was used.

A sandwich-format immunometric NycoCard CRP Single Test (NycoMed Pharma, Brussels, Belgium) was used for determination of CRP. Colour intensity was measured quantitatively with a NycoCard Reader II. Ferritin was measured by immunofluorescent assay using an IMMULITE Immunoassay System (Siemens, Los Angeles, CA, USA). Serum albumin was measured using commercially available kits.

2.2. Statistics

Data were entered using SPSS for Windows V.16.0 (SPSS Inc., Chicago, IL, USA). Student’s t-test and ANOVA were used to compare the mean (SD) for normally distributed data. Mann-Whitney U-test was used to compare the significance of differences between the groups (severe vs uncomplicated malaria, uncomplicated malaria vs healthy controls, and severe malaria vs healthy controls) because the data for haptoglobin, CRP, ferritin and albumin were not normally distributed. Correlations between continuous variables were assessed by Spearman’s rank test. A p-value of <0.05 was regarded as significant.

3. Results

Whilst the three groups (32 women in each arm) were well matched with regard to age, parity, gestational age and weight, their temperature was significantly higher and haemoglobin was significantly lower in patients with severe *P. falciparum* malaria (Table 1). Different manifestations of severe *P. falciparum* malaria were observed, including: severe anaemia, 12 (37.5%); hypoglycaemia, 11 (34.4%); jaundice, 8 (25.0%); hypotension, 8 (25.0%); repeated convulsions, 5 (15.6%); and cerebral malaria, 4 (12.5%); there was more than one manifestation in 7 cases (21.9%).

Whilst there was no significant difference in the levels of albumin and haptoglobin, the levels of both ferritin and CRP were significantly higher in patients with severe *P. falciparum* malaria than in patients with uncomplicated malaria and healthy controls and were significantly higher in uncomplicated malaria compared with the healthy controls (Table 1).

Medium positive correlations were observed between parasite count and CRP ($r = 0.370$, $p = 0.01$). There were significant positive correlations between parasite count and haptoglobin ($r = 0.515$, $p = 0.001$) and non-significant correlations between albumin and haptoglobin ($r = 0.257$, $p = 0.03$). The same findings (data not shown) were obtained even if the groups of severe and uncomplicated malaria patients were analysed separately.

4. Discussion

In the current study, whilst there was no significant difference in the levels of haptoglobin and albumin, the levels of ferritin and CRP were significantly higher in patients with severe *P. falciparum* malaria compared with the levels in patients with uncomplicated *P. falciparum* malaria. Recently, O’Donnell et al. observed that ferritin concentration increased progressively with severity of malaria, whilst CRP concentration was markedly increased in children with uncomplicated malaria and was only moderately increased in children with severe malaria. Pregnant women with microscopic *P. falciparum* infections had high CRP levels compared with those carrying submicroscopic

### Table 1
Clinical and biochemical characteristics of the three groups of pregnant women with severe malaria (SM) or uncomplicated malaria (UM) and healthy controls (HC)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severe malaria (n = 32)</th>
<th>Uncomplicated malaria (n = 32)</th>
<th>Healthy controls (n = 32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravidity [median (range)]</td>
<td>3.0 (1.0–9.0)</td>
<td>2.0 (1.0–10.0)</td>
<td>3.0 (1.0–10.0)</td>
<td>0.8</td>
</tr>
<tr>
<td>Parity [median (range)]</td>
<td>2.0 (0.0–8.0)</td>
<td>1.0 (0.0–9.0)</td>
<td>2.0 (0.0–9.0)</td>
<td>0.9</td>
</tr>
<tr>
<td>Age (years) [mean (SD)]</td>
<td>28.5 (6.0)</td>
<td>27.0 (6.4)</td>
<td>26.3 (2.8)</td>
<td>0.1</td>
</tr>
<tr>
<td>Gestational age (weeks) [mean (SD)]</td>
<td>29.0 (7.8)</td>
<td>27.2 (8.2)</td>
<td>28.9 (2.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Weight (kg) [mean (SD)]</td>
<td>59.1 (6.7)</td>
<td>61.5 (6.6)</td>
<td>60.4 (4.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>Temperature (°C) [mean (SD)]</td>
<td>38.3 (0.6)</td>
<td>38.1 (0.6)</td>
<td>37.1 (0.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Haemoglobin (g/dl) [mean (SD)]</td>
<td>8.3 (1.7)</td>
<td>10.0 (1.1)</td>
<td>10.1 (1.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parasite count [mean (SD)]</td>
<td>30800 (3146)</td>
<td>14344 (17,499)</td>
<td>–</td>
<td>0.07</td>
</tr>
<tr>
<td>Blood glucose (mg/dl) [mean (SD)]</td>
<td>83.7 (59.5)</td>
<td>93.7 (29.0)</td>
<td>–</td>
<td>0.6</td>
</tr>
<tr>
<td>Duration of illness (days) [mean (SD)]</td>
<td>2.4 (1.0)</td>
<td>2.1 (1.1)</td>
<td>–</td>
<td>0.2</td>
</tr>
<tr>
<td>Ferritin (μg/l) [median (IQR)]</td>
<td>78.6 (44.1–148.9)</td>
<td>63.3 (30.5–113.2)</td>
<td>34.4 (7.9–60.3)</td>
<td>SM vs UM, p = 0.006; SM vs HC, p = 0.002; UM vs HC, p = 0.041</td>
</tr>
<tr>
<td>C-reactive protein (μg/ml) [median (IQR)]</td>
<td>79.0 (36.2–110.5)</td>
<td>63.0 (22.5–81.7)</td>
<td>8.5 (5.0–28.2)</td>
<td>SM vs UM, p = 0.001; SM vs HC, p = 0.001; UM vs HC, p = 0.021</td>
</tr>
<tr>
<td>Haptoglobin (mg/dl) [median (IQR)]</td>
<td>7.7 (0.1–38.6)</td>
<td>20.1 (5.9–40.9)</td>
<td>9.4 (18.2–23.5)</td>
<td>SM vs UM, p = 0.331; SM vs HC, p = 0.671; UM vs HC, p = 0.346</td>
</tr>
<tr>
<td>Albumin (g/dl) [median (IQR)]</td>
<td>3.2 (2.8–3.5)</td>
<td>3.2 (2.9–3.5)</td>
<td>3.1 (2.8–3.4)</td>
<td>SM vs UM, p = 1.000; SM vs HC, p = 0.416; UM vs HC, p = 0.346</td>
</tr>
</tbody>
</table>
P. falciparum infection and those free of detectable infection.\(^6\)

Ferritin levels were higher in women with severe P. falciparum malaria in the current study. This is in accordance with a previous study where ferritin levels were higher in children with severe malaria.\(^4\) In non-pregnant individuals, ferritin levels increase during both asymptomatic and symptomatic malaria, and the highest levels have been recorded in individuals with severe disease.\(^8\) In Tanzania, the levels of ferritin were increased in women with placental malaria.\(^5\) In Malawi, increased cord blood ferritin levels were associated with lower birth weight during placental malaria.\(^9\) However, in addition to inflammatory conditions, APPs are also released/elevated in normal physiological conditions such as pregnancy.\(^10\)

In the current study, haptoglobin levels did not differ between patients with severe malaria and controls. Previously, haptoglobin levels were significantly lower in children with severe malaria.\(^4,11\) The low haptoglobin levels were attributed to the contribution of haptoglobin to clear free haemoglobin from recent haemolysis.\(^10\)

This study had a small sample size and did not control for sickle cell anaemia, glucose-6-phosphate dehydrogenase deficiency, haptoglobin genotypes and other infections that might accompany severe malaria.

**Authors’ contributions:** AAS, AMB, NIA and MIE conceived the study; AAA, IA and OEM designed the study; AAS and IA analysed and interpreted the data; AMB interpreted the data. All authors drafted and critically revised the manuscript for intellectual content, and approved the final version of the paper. AAA and IA are guarantors of the paper.

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**Competing interests:** None declared.

**Ethical approval:** This study received ethical clearance from the Research Board at the Faculty of Medicine, University of Khartoum (Khartoum, Sudan).

**References**