SHORT REPORT: INCREASED SUSCEPTIBILITY TO *PLASMODIUM MALARIAE* IN PREGNANT \(\alpha^+\)-THALASSEMIC WOMEN

FRANK P. MOCKENHAUPT, BIRGIT RONG, HOLGER TILL, WILLIAM N. A. THOMPSON, AND ULRICH BIENZLE

Institute of Tropical Medicine Berlin, Medical Faculty Charité, Humboldt-University Berlin, Germany; Presbyterian Mission Hospital, Agogo, Ghana

Abstract. The influence of \(\alpha^+\)-thalassemia on malaria in pregnancy was assessed in a cross-sectional study of 530 women in Ghana. Plasmodial infections, \(\alpha^+\)-thalassemia, serum levels of C-reactive protein, and antimalarial drugs in urine were determined. The \(\alpha\)-globin genotypes did not correlate with the prevalence of *Plasmodium falciparum*-infection and parasite densities. However, *Plasmodium malariae* tended to be more frequent in \(\alpha^+\)-thalassemic women (\(P = 0.05\)). Excluding women with residual antimalarials, a significant excess of *P. malariae* was observed in \(\alpha^+\)-thalassemic individuals. Febrile responses (\(P = 0.05\)) and inflammation (CRP > 0.6 mg/dl, \(P = 0.06\)) appeared to be less common in infected \(\alpha^+\)-thalassemic women and were also comparatively rare in parasitemic individuals who harbored double species infections with *P. falciparum* and *P. malariae*. *Plasmodium malariae* may influence the pathogenesis of falciparum malaria leading to a low prevalence of inflammation and febrile responses in \(\alpha^+\)-thalassemic women.

\(\alpha^+\)-thalassemia is considered to ameliorate the clinical course of malaria. We have performed a cross-sectional study on the impact of \(\alpha^+\)-thalassemia on malaria in 530 pregnant women attending the Agogo District Hospital, Ghana. Women seeking antenatal care were enrolled after informed consent had been obtained. The study was approved by the Ghanaian Ministry of Health. Malaria parasite densities were counted per 500 white blood cells (WBC) on Giemsa-stained thick films and, based on the individual WBC count, calculated as parasites per microliter. After extraction of DNA from blood, parasite species and submicroscopic infections were assessed by polymerase chain reaction (PCR) assays. The \(\alpha^+\)-type of \(\alpha^+\)-thalassemia was also identified by PCR assays. The hemoglobin (Hb) AS trait was screened for by cellulose acetate electrophoresis and confirmed by fast protein liquid chromatography. Chloroquine and pyrimethamine in urine were detected by ELISA dipsticks. C-reactive protein (CRP) in serum from 528 individuals was measured by immunoturbidimetry (Biokit, Kirchheim, Germany).

*Plasmodium falciparum*, *P. malariae*, and *P. ovale* were found in 63%, 3.4%, and 2.6% of the women, respectively (Table 1). Forty-nine percent (163/335) of the *P. falciparum*-infections were identified by PCR only but no parasites were visible by microscopy. These proportions were 39% (7/18) for *P. malariae* and 36% (5/14) for *P. ovale*. *Plasmodium malariae* occurred only in combination with *P. falciparum*. One triple-species infection and one exclusive *P. ovale* infection were seen. \(\alpha^+\)-thalassemia was identified in 33% of the women (29% heterozygous, 4% homozygous). HbAS was observed in 13% (46/357) of women with a normal \(\alpha\)-globin genotype, in 17% (26/153) of individuals with heterozygous and in 5% (1/20) of those with homozygous \(\alpha^+\)-thalassemia. The prevalence of *P. falciparum* infection and parasite densities did not correlate with the \(\alpha\)-globin genotypes. However, *P. malariae* infections appeared to be more frequent in \(\alpha^+\)-thalassemic individuals as this parasite was observed in 2.5% of normal, 4.6% of heterozygous and 10% of homozygous women (\(P \chi^2 \text{ for trend} = 0.05\)). Only 0.9% of women (3/343) with residual antimalarials but 8% (15/187) of those without detectable antimalarial drugs harbored *P. malariae*. Antimalarial drugs were detected in 64% (n = 228), 68% (n = 104), and 55% (n = 11) of normal, heterozygous, and homozygous \(\alpha^+\)-thalassemic women, respectively. Excluding women with antimalarials, a significant excess of *P. malariae* infections was observed in \(\alpha^+\)-thalassemic individuals (Table 1). Among *P. falciparum*-infected women without drugs, concurrent infection with *P. malariae* was seen in 6% (6/100) of normal, 19.4% (7/36) of heterozygous, and 28.6% (2/7) of individuals with homozygous \(\alpha^+\)-thalassemia (\(P \chi^2 \text{ for trend} = 0.006\)). In contrast, the prevalence of *P. malariae* did not differ significantly between individuals with HbAA (3.7%, 14/374) and HbAS (0/73) even if individuals with antimalarials were excluded (11/138 versus 0/20).

Evidence of inflammation (CRP > 0.6 mg/dl) and febrile responses (\(\geq 37.5^\circ\text{C oral}\)) tended to be less frequent in infected \(\alpha^+\)-thalassemic women (Table 1). We also observed a low prevalence of inflammation in women having double-species infections with *P. falciparum* and *P. malariae*. In these, inflammation was less frequent (41%, 7/17) than in other types of plasmodial infection (66%, 211/318; \(P \text{ Fisher exact} = 0.04\)). Moreover, febrile responses occurred in only 6% (1/17) of the *P. malariae*/*P. falciparum* double infections but in 19% (60/319) of the remaining infections. Median parasite densities were similar in women with (139/\mu l, range [17–2,069], \(n = 10\) ) and without *P. falciparum*/*P. malariae* double infections (291/\mu l [11–31,988], \(n = 162\) ).

The manifestation of malaria appears to be milder in \(\alpha^+\)-thalassemic than in non-thalassemic individuals. Increased levels of CRP and fever have poor specificity in the estimation of disease severity. In the present study, no attempt was made to establish a pyrogenic level of parasite density. However, it is noteworthy that at almost identical parasite levels of CRP and fever have poor specificity in the estimation of disease severity. In the present study, no attempt was made to establish a pyrogenic level of parasite density. However, it is noteworthy that at almost identical parasite densities in women with the three \(\alpha\)-globin genotypes, inflammation and febrile responses were relatively rare in malariaous \(\alpha^+\)-thalassemic individuals. In Melanesia, an increased susceptibility to *P. vivax* in \(\alpha^+\)-thalassemic infants has been suggested to afford protective immunity against severe falciparum malaria later in childhood. In West Africa, *P. vivax* is virtually absent. We propose that in \(\alpha^+\)-thalassemic pregnant women increased susceptibility to *P.
malariae may partly be responsible for mild courses of *P. falciparum* malaria. Other studies have also demonstrated reduced rates of symptomatic falciparum malaria in patients with previous or simultaneous *P. malariae* infections. 8,9 It is suggested that persisting *P. malariae* parasitemia down-regulates inflammatory responses and thereby induces tolerance to malarial infections. 8 *Plasmodium malariae* is believed to preferentially parasitize small and aged erythrocytes. 10 Invasion by *P. malariae* depends on erythrocyte receptors yet to be identified. These could be increased on α-thalassemic membranes. 11

The HbAS trait could have biased the distribution of *P. malariae* among women with and without α-thalassaemia. However, HbAS was not associated with the α-globin genotype and did not influence the frequency of *P. malariae*. This is in agreement with recent findings from Gabon. 12

In highly endemic areas, premunition, i.e., the persistence of asymptomatic plasmodial infections, confers protection against severe malarial disease. 13 We propose that one mechanism involved in the milder manifestations of *P. falciparum* malaria in α-thalassaemia is increased susceptibility to concurrent infection with a less virulent parasite.

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Authors addresses: Frank P. Mockenhaupt, Birgit Rong, and Ulrich Bienzle, Institut für Tropenmedizin, Spandauer Damm 130, 14050 Berlin, Germany; Tel: +49-30-30116-750, Fax: +49-30-30116-888. Holger Till and William N. A. Thompson, Presbyterian Mission Hospital Agogo, P. O. Box 2, Agogo, Ashanti-Akim, Ghana; Tel: +233-51-20201.

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<tr>
<th>No.</th>
<th>α-globin genotype</th>
<th>(\Delta n)</th>
<th>(\Delta\alpha)</th>
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<td>357</td>
<td>153</td>
<td>20</td>
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| Plasmodium falciparum | 63 (224/357) | 64 (98/153) | 65 (13/20) | NS |
| Plasmodium malariae   | 2.5 (9/357)  | 4.6 (7/153) | 10 (2/20)  | 0.05‡ |
| Plasmodium ovale      | 2.5 (9/357)  | 2.6 (4/153) | 5.0 (1/20) | NS |

Median parasite density [range]†

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<tr>
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<th>n = 117</th>
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<th>n = 9</th>
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<tr>
<td>P. falciparum</td>
<td>78 (100/129)</td>
<td>73 (36/49)</td>
<td>78 (7/9)</td>
<td>NS</td>
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<tr>
<td>P. malariae</td>
<td>4.7 (6/129)</td>
<td>14.3 (7/49)</td>
<td>22.2 (2/9)</td>
<td>0.008‡</td>
</tr>
<tr>
<td>P. ovale</td>
<td>4.7 (6/129)</td>
<td>2.0 (1/49)</td>
<td>11.1 (1/9)</td>
<td>NS</td>
</tr>
</tbody>
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% febrile among infected women (n = 336)

|            | 21 (46/224) | 15 (15/99) | 0 (0/13) | 0.05‡ |

% inflammation (CRP > 0.6 mg/dl) among infected women (n = 335)

|            | 68 (151/223) | 63 (62/99) | 38 (5/13) | 0.06‡ |

a Parasite/µl, microscopically positive samples only.
‡Only value missing in a woman with a normal α-globin genotype.

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