Decreased susceptibility to placental malaria in anaemic women in an area with unstable malaria transmission in central Sudan

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The interaction between iron level, iron supplementation, and susceptibility to infection, including malaria, remains a concern. A cross-sectional study was conducted at Medani hospital in central Sudan to investigate the relationship between anaemia and placental malaria. Obstetrical history was obtained; haemoglobin levels were determined. Placental tissue was obtained and malaria histology classified as active, chronic, past or no malaria infection. Among 324 women investigated, 7 (2.2%), 4 (1.2%), and 44 (13.6%) of the placentae showed active, chronic and past infection on histology examination respectively, while 269 (83.0%) of them showed no infection. Anaemia (haemoglobin <11 g/dl) was less frequent in women with placental *Plasmodium falciparum* infection, 27/55 (49.1%) vs 174/269 (64.7%), $P=0.02$. Anaemia was associated with a decreased risk for placental malaria, and the odds ratio for placental malaria (in both primiparae and multiparae group) was 0.2, 95% CI: 0.1–0.6, $P=0.002$ and it was 0.2, 95% CI: 0.03–0.7; $P=0.02$ for primiparae group. Thus, there is a strong relationship between anaemia and the absence of placental malaria.

Keywords: Anaemia, Malaria, Pregnancy, Placenta, Sudan

Introduction

Malaria and anaemia during pregnancy are major contributors to the maternal and perinatal morbidity and mortality.^{1,2} Therefore, programs that provide iron supplements to pregnant women have been implemented worldwide. Interaction between iron level, iron supplementation, and susceptibility to infection, including malaria, remains a concern.³ Studies involving children suggest that iron supplementation may increase the risk for malaria⁴ and the Pemba trial⁵ showed that giving iron supplements to iron-replete children does increase malaria risk.

Anaemia in pregnancy is associated with adverse outcomes.^{1,2} The control of anaemia in women of reproductive age is one of the Safe Motherhood Initiatives,⁶ and the World Health Organization recommended iron and folic acid supplementation for all women of child-bearing age.⁷ However, this dream of freedom from anaemia has been stalled somewhat to date. This failure may be mainly one of implementation; one often-mentioned contribution to

the problem is the unresolved concern as to the interaction among anaemia status, iron supplementation and susceptibility to infection. There is a controversy over the results of the clinical trials, e.g. in Papua New Guinea, the frequency of malaria increased among primiparae who received intravenous iron.⁸ However, use of oral iron supplementation by Gambian women did not increase the prevalence or the severity of peripheral or placental parasitaemia.⁹ Recently, two cross-sectional studies in Tanzania and Malawi showed that, women with placental malaria were less likely to have iron deficiency than women without placental malaria infection and iron deficiency was independently associated with a significantly decreased risk of placental malaria.^{10,11} The current study was conducted at Medani Maternity Hospital in an area characterized by unstable malaria transmission with the peak following the rainy and post rainy season during July–January¹² to investigate if anaemic women were at decreased risk of placental malaria. According to the Sudanese National Malaria Control Programme policy, artesunate sulfadoxine-pyrimethamine is the first line treatment for malaria during the second and third trimesters and quinine in the first

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trimester of pregnancy and Chemoprophylaxis is not yet recommended in the area.

Patients and Methods

Patients

The study was conducted between October 2010 and January 2011 at the labour ward of Medani Maternity Hospital, Central Sudan. Medani Maternity Hospital provides tertiary care for women who receive antenatal care at the hospital as well as for referrals from the other clinics and hospitals, and women who are close to the hospital facility. All women with risk factors or obstetric complications are referred to the hospital. The referral criteria are not strictly adhered to and many patients without any significant complications deliver at the hospital. After signing an informed consent, women with a singleton baby were approached to participate in the study. Those with antepartum haemorrhage and diabetes mellitus were excluded. A structured questionnaire was administered to collect information about socio-demographic and obstetric characteristics. Women were questioned about antenatal care attendance and iron and folic acid usage in the index pregnancy.

Sample size was calculated based on a two-sided hypothesis tests using Epiinfo with 80% power and confidence interval of 95%.

Haematology

Maternal, placental and cord blood films were prepared, the slides were Giemsa-stained and the number of asexual *Plasmodium falciparum* parasites per 200 white blood cells was counted and double-checked blindly by an expert microscopist. Maternal haemoglobin concentrations were estimated by HemoCue haemoglobinometer (HemoCue AB, Angelholm, Sweden). Maternal blood groups were determined by the agglutination method.

Placental histology

Full thickness placental blocks of around 2–3 cm were taken from the placenta, kept in neutral buffered formalin for histopathology examinations. Placental malaria infections were characterized based on the classification of:¹³ uninfected (no parasites or pigment), active (parasites in intervillous spaces), chronic (parasites in maternal erythrocytes and pigment in fibrin or cells within fibrin and/or chorionic villous syncytiotrophoblast or stroma), past (no parasites and pigment confined to fibrin or cells within fibrin). The placental samples were fixed in buffered formalin to avoid formalin pigment formation, which has similar optical characteristics and polarized light activity to malaria pigment.¹⁴ Then the placental samples were transferred to Khartoum, where they were processed and the slides were read by an expert pathologist in our group (A.A.M.).

Statistics

Data were entered in computer using SPSS for windows version 16.0. Means and proportions were compared by Student's *t*-test, Chi-square test and Fisher's exact test as appropriate. Univariate and multivariate analyses were performed where placental malaria was the dependent variable and maternal socio-demographic characteristics (age, parity, and maternal blood group, past history and anaemia (haemoglobin <11 g/dl) were possible influencing factors. $P \leq 0.05$ was regarded as significant.

Ethics

The study received ethical clearance from the Research Board at the Faculty of Medicine, University of Khartoum.

Results

General characteristics of the women

Placental histopathology and maternal haemoglobin data were available for 324 women; these women were included in the final analyses. Among these 324 women, 99 (30.5%) were primiparae. The mean (SD) age was 25.6 (6.4) years and 63 (19.4%) of the women were teenagers (age ≤ 19 years). Of the 324 women, 71 (22.0%), 55 (17.0%), 16 (5.0%), and 182 (56.0%) had blood group A, B, AB, and O, respectively. The mean (SD) haemoglobin was 11.4 (2.1) g/dl, 201 (62.0%) women were anaemic and 4 (1.2%) had severe anaemia (haemoglobin <7 g/dl). Only 47 (14.5%) women used bed nets and none of them received intermittent preventative treatment in the index pregnancy. Sixty (18.5%), 179 (55.2%), and 85 (26.2%) women did not attend antenatal care at all, attended once or twice, and attended more than two visits. Sixty (18.5%) women did not use iron in the index pregnancy. Three cases had positive placental blood films; in two of these, maternal blood films were also positive.

Placental malaria

Seven (2.2%), 4 (1.2%), and 44 (13.6%) of the placentae showed active, chronic and past infection on histologic examination respectively, while 269 (83.0%) of them showed no infection. However, there were no significant differences between women with and without placental malaria infection when the age, parity, educational levels, use of iron and folic acid, antenatal care attendance and blood groups were considered (Table 1).

Placental malaria and anaemia

Women with placental malaria infections were less likely to have anaemia than women with no placental malaria infections, 27/55 (49.1%) vs 174/269 (64.7%), $P=0.02$.

As shown in the Tables 2 and 3, anaemia was associated with a decreased risk for placental malaria, and the odds ratio for placental malaria (in both

primiparae and multiparae group) was 0.2, 95% CI: 0.1–0.6, $P=0.002$ and it was 0.2, 95% CI: 0.03–0.7; $P=0.02$ for primiparae group.

Discussion

The main findings of the current study were: anaemia was less frequent in women with placental *P. falciparum* infection and women with placental malaria infections were less likely to have anaemia. This is in agreement with a previous study, where it was found that placental malaria was less frequent and less severe among pregnant Tanzanian women with iron deficiency than among those with normal iron status and that this effect was greatest during the first pregnancy.¹⁰ Recently, it has been shown that Malawian women with placental malaria were less likely to have iron deficiency than women without placental malaria infection (OR=0.4, 95% CI 0.2–0.8, $P=0.01$), iron deficiency was independently associated with a significantly decreased risk of placental malaria and the association was greater in multigravidae.¹¹ Interestingly, in Gadarif maternity hospital in eastern

Sudan, haemoglobin level was not a predictor for placental malaria infection.¹⁵

Oppenheimer *et al.*^{4,8} reported that among women treated with intravenous iron in Papua New Guinea, the frequency of malaria increased among primiparous women but not among multiparous women. However, use of oral iron supplementation by Gambian multigravidae did not increase the prevalence or the severity of peripheral or placental parasitemia during a randomized placebo-controlled study.⁹ Likewise, in an area characterized by unstable malaria transmission, the risk of *P. falciparum* malaria did not increase (although the risk of *P. vivax* malaria did increase) during prospective follow-up among pregnant Thai women who received haematinic treatment.¹⁶ For many reasons, the current study should be compared cautiously with previous studies in term of parity-related susceptibility to malaria infections. First, the differences between gravidity groups may be related to acquired immunity. In areas of stable malaria transmission, women acquire antibodies against placental parasites

Table 1 Socio-demographic, clinical characteristics and placental malaria infections among pregnant women at Medani Maternity Hospital, Sudan*

Variables	Active infection (N=7)	Chronic infection (N=4)	Past infection (N=44)	Non-infection (N=269)	P
Primigravidae	2 (28.6)	0 (0)	15 (34.1)	82 (30.1)	0.7
Blood group O	6 (85.7)	3 (75.0)	27 (61.4)	146 (54.3)	0.8
Anaemia	5 (71.4)	1 (25.0)	21 (47.7)	174 (64.5)	0.02
Lack of antenatal care	0 (0.0)	0 (0.0)	8 (18.2)	52 (19.3)	0.5
History of malaria	2 (28.6)	0 (0.0)	9 (20.1)	34 (12.6)	0.1
Educational<secondary level	0 (0.0)	2 (50)	8 (18.2)	58 (21.6)	0.1

Note: *Data are shown as N (%).

Table 2 Factors associated with placental malaria in Medani Maternity hospital, Central Sudan using univariate or multivariate analysis

The variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Age	1.0	0.9–1.0	0.2	1.0	0.9–1.1	0.1
Primigravidae	1.1	0.5–2.4	0.6	1.6	0.6–4.6	0.3
History of malaria	1.8	0.8–3.7	0.1	3.1	1.2–8.0	0.01
Educational<secondary level	0.9	0.5–1.5	0.7	0.6	0.2–1.4	0.3
Lack of antenatal care	0.5	0.2–2.4	0.2	0.5	0.1–1.8	0.3
Did not use iron supplement	1.1	0.5–1.9	0.8	0.4	0.4–1.7	0.8
Blood group O vs non-group O	1.3	0.6–2.5	0.4	1.5	0.7–3.1	0.2
Anaemia	0.5	0.2–1.0	0.05	0.2	0.1–0.6	0.002

Table 3 Factors associated with placental malaria in primigravidae in Medani Maternity hospital, Central Sudan using univariate or multivariate analysis

The variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Age	2.5	1.5–4.4	0.001	1.0	0.9–1.1	0.1
History of malaria	3.7	1.8–7.7	0.001	3.1	1.2–8.0	0.01
Educational level<secondary	0.9	0.9–1.0	0.3	0.6	0.2–1.4	0.3
Lack of antenatal care	2.0	1.0–3.6	0.02	0.5	0.1–1.8	0.3
Blood group O vs non-group O	1.4	0.9–2.3	0.08	1.5	0.7–3.1	0.2
Anaemia	0.3	0.1–1.1	0.09	0.2	0.03–0.7	0.02

over successive pregnancies.¹⁷ Second, the low prevalence of placental malaria in these women in the current study and the relatively small sample size makes it hard to compare this study to other ones of placental malaria subtypes, e.g. acute, chronic and past. Third, the aetiology of anaemia was not investigated in the current study and it was not clear whether it was iron deficiency like the previous studies or it was due to other deficiencies. However, recently we have observed that iron deficiency (S-ferritin <15 µg/l), iron deficiency anaemia (<11 g/dl and S-ferritin <15 µg/l) were prevalent in 25 (12.5%) and 13 (6.5%) of 200 pregnant women in the same hospital.¹⁸ Because all data in the current study were collected at the time of delivery, it is difficult to draw conclusions about cause and effect of anaemia and placental malaria. Haemoglobin levels can change during the course of pregnancy; in the case of past infection, we do not know if the woman was anaemic at the time she was infected with malaria or not. In addition, other possible causes of anaemia such as haemoglobinopathies were not examined in this study and the prevalence of sickle cell and thalassaemia in this population are unknown. Although HIV serostatus was not investigated among these women, HIV infection has to be considered as a possible aetiological factor for anaemia among pregnant women in sub-Saharan Africa.¹⁹ Yet we have shown that pregnant women in central Sudan had a low HIV prevalence (1.0%).^{20,21}

Different mechanisms have been proposed to explain how the risk of malaria might increase with iron supplementation and decrease with iron deficiency. Supplemented non-anaemic pregnant women may be less attractive to the mosquitoes than those who are anaemic.²² Iron is an important nutrient for host requirements and for the metabolism of invading pathogens. Iron inhibits the expression of inducible nitric oxide synthase, which subsequently down-regulates the formation of nitric oxide in macrophages and appears to be critical to macrophage defense against *P. falciparum*.²³ Summary, anaemia was associated with a decreased risk for placental malaria, in both primiparae and multiparae group in this setting. Further research is needed.

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