

SHORT REPORT: SOUTHEAST ASIAN OVALOCYTOSIS AND PREGNANCY IN A MALARIA-ENDEMIC REGION OF PAPUA NEW GUINEA

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Abstract. The band 3 deletion for southeast Asian ovalocytosis (SAO) occurs commonly in southeast Asia and the western Pacific. Southeast Asian ovalocytosis is associated with protection against cerebral malaria in children and therefore could reduce sequestration of erythrocytes parasitized by *Plasmodium falciparum* in the brain microvasculature. Sequestration of parasitized erythrocytes in the placenta accounts for much of the pathology of malaria during pregnancy. Therefore, we investigated the effect of SAO on malaria during pregnancy in the malaria-hyperendemic north coastal region of Papua New Guinea. The frequency of SAO in 927 women attending hospital for delivery was 8.7% (95% confidence interval = 6.9–10.5). Markers of fertility, the frequency of miscarriages and stillbirths, maternal anemia, placental and peripheral malaria at delivery, and birth weight were similar in women with and without SAO. In summary, although we can not exclude an interaction between SAO and malaria during pregnancy, we found no evidence that it provided a clinical benefit in this population.

Southeast Asian ovalocytosis is common in the malarious regions of southeast Asia and the western Pacific, and occurs in up to 35% of people in the north coastal region of Papua New Guinea.¹ It is a hereditary condition that is characterized by rigid red blood cells with altered morphology and reduced anion transport.² The molecular basis for SAO is a 27-basepair deletion in the gene encoding band 3, the major erythrocyte transmembrane protein on chromosome 17.³ Although asymptomatic in the heterozygous state, the homozygous state is thought to be lethal *in utero*.⁴

Malaria during pregnancy is associated with miscarriage, maternal anemia, and reduced birth weight, especially in primigravidae.⁵ Much of the pathology associated with malaria during pregnancy is caused by sequestration of parasitized erythrocytes in the intervillous space of the placenta.⁶ Cerebral malaria is believed to result from the sequestration of parasitized erythrocytes in the vascular endothelium in the brain.⁷ Southeast Asian ovalocytosis was shown to protect against cerebral malaria in children in Papua New Guinea.^{8,9} Because the brain and the placenta are sites of sequestration of parasitized erythrocytes, we investigated the effect of SAO on reproductive fitness and malaria infection during pregnancy.

As described previously, between July 1994 and January 1996, 987 women from the north coastal region of Papua New Guinea who attended Madang Hospital for delivery were recruited in a prospective study to assess the effect of hemoglobin and red blood cell genetic variants against malaria during pregnancy.^{10,11} We recorded markers of reproductive fitness (age in primigravidae, gravidity, pregnancy interval, and the number of miscarriages and stillbirths). The effect of malaria on the outcome of pregnancy was assessed by the rate of preterm delivery and low birth weight. Hemoglobin concentration during pregnancy was recorded from ante-natal clinic cards, when available. Venous blood was collected into tubes containing EDTA from each mother as soon after delivery as possible. A full blood count was measured (Coulter Electronics, Luton, United Kingdom) and blood films were made,

stained with Giemsa, and examined by microscopy for malaria parasites. Blood samples were centrifuged and DNA was extracted from the cell pellet by phenol-chloroform extraction. The SAO genotype was determined in DNA samples by the polymerase chain reaction.¹ Blood films were also prepared from the maternal surface of each placenta, stained, and examined for malaria parasites as described above. Statistical analysis was conducted with SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL). A *P* value < 0.05 was considered statistically significant. Ethical permission was obtained from the Medical Research Advisory Committee of Papua New Guinea and informed consent was obtained from all mothers participating in the study.

Southeast Asian ovalocytosis was present in 8.7% (95% confidence interval [CI] = 6.9–10.5%) of the pregnant women and in women from the rural area (51 of 499, 10.2%) or those living in Madang town or peri-urban settlements (25 of 367, 6.8%; *P* = 0.10). Residence could not be determined reliably for 61 women. Markers of reproductive fitness were similar in women with and without SAO (Table 1). The frequency of placental *Plasmodium falciparum* infection and systemic malaria infection after delivery did not vary significantly according to SAO genotype (Table 1). Placental parasitemia was present in 24.5% (95% CI = 21.4–27.6%) of women with a normal genotype compared with 21.1% (95% CI = 11.9–30.3%) with SAO. Malaria parasitemia after delivery was present in 19.0% (95% CI = 16.3–21.7%) of normal women and in 16.1% (95% CI = 8.1–24.1%) of women with SAO. Malaria infection was more common in primigravidae than in multigravidae, but was similar according to SAO genotype (Table 1). Overall, 854 (92.2%) of 926 women reported that they had taken chloroquine and 830 (89.6%) of 926 women had taken iron and folate during pregnancy. Use of these drugs was similar in women with and without SAO (*P* = 1.00; no information was available for one woman).

Median hemoglobin concentrations during pregnancy and after delivery were similar in women with and without SAO (Table 1). The median hemoglobin concentration during pregnancy was 9.6 g/dL (95% CI = 9.4–10.0 g/dL) for normal women and 10.2 g/dL (95% CI = 9.1–10.8 g/dL) for women with SAO. The median hemoglobin concentration after delivery was 10.5 g/dL (95% CI = 10.3–10.6 g/dL) for normal women and 10.7 g/dL (95% CI = 10.1–11.1 g/dL) for women

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TABLE 1
Reproductive fitness, malaria, and outcome of pregnancy according to maternal SAO genotype*

	Normal	SAO	P
Gravidity†			
1	310	32	
2	202	20	
3-6	304	26	
7-12	30	3	
Total	846	81	0.88
Age in primigravidae (years)	20 (18-21) [288]	20 (18-21) [26]	0.82
Multigravidae			
Pregnancy interval,‡ weeks	85 (54-137) [346]	79 (47-118) [37]	0.44
Stillbirths§			
0	508	46	
1	25	3	0.92
≥ 2	3	0	
Miscarriages¶			
0	468	43	
1	58	5	0.99
≥ 2	10	1	
Hemoglobin during pregnancy (g/dL)	9.6 (8.1-10.8) [341]	10.2 (8.8-11.3) [28]	0.39
Hemoglobin after delivery (g/dL)	10.5 (9.2-11.6) [791]	10.7 (9.5-11.8) [77]	0.29
Mother positive for <i>Plasmodium falciparum</i> after delivery, no. (%)			
All women	159/838 (19.0)	13/81 (16.1)	0.65
Primigravidae only	76/308 (24.7)	8/32 (25.0)	1.00
Density of <i>P. falciparum</i> (parasites/μL blood)	3.4 (3.0-3.95)	4.04 (3.15-4.29)	0.12
Placenta positive for <i>P. falciparum</i> , no. (%)			
All women	180/736 (24.5)	16/76 (21.2)	0.58
Primigravidae only	89/265 (33.7)	9/30 (30.0)	0.84
Infant			
Preterm, no. (%)	55/846 (6.5)	4/81 (4.9)	0.40
Birth weight < 2.50 kg, no. (%)	160/846 (18.9)	13/81 (16.0)	0.32
Low birth weight risk in primigravidae (95% CI)#	2.22 (1.21-4.69)	2.45 (0.86-6.65)	-
Birth weight in term infants (kg)	2.94 (2.66-3.24) 791	2.86 (2.67-3.25) 77	0.59

* Values are the median (interquartile range) [no.] unless otherwise indicated. SAO = Southeast Asia ovalocytosis; CI = confidence interval.

† For gravidity, all previous pregnancies including miscarriages and stillbirths were included.

‡ No. of weeks since last delivery of live infant or stillbirth or miscarriage.

§ Delivery occurring at gestation > 20 weeks.

¶ Delivery occurring at gestation ≤ 20 weeks.

Risk of low birth weight (< 2.50 kg) in primigravidae compared with all multigravidae.

with SAO. Values for median hemoglobin concentration during pregnancy and after delivery in primigravidae were similar to those in multigravidae. Severe anemia (hemoglobin < 7.0 g/dL) was uncommon in all women and occurred in 26 (7.1%) of 369 women during pregnancy and in 23 (2.7%) of 868 women after delivery. The frequency of severe anemia was similar in women with and without SAO ($P > 0.24$).

Median birth weight among term infants was 2.94 kg (95% CI = 2.91-2.98 kg) in women without SAO and 2.86 kg (95% CI = 2.73-3.08 kg) in women with SAO. The frequency of preterm delivery and low birth weight did not vary significantly according to maternal genotype for SAO (Table 1).

Reproductive fitness as assessed by maternal age at first delivery, gravidity, and the interval since the previous preg-

nancy in multigravidae were similar in women with and without SAO. Also, the frequency of SAO in women in this cohort was greater than that in girls recruited as community controls for severe malaria cases in the same geographic area (6 [4.29%] of 140, 95% CI = 0.93-7.65%).⁹ Therefore, in this hospital-based study of women attending for delivery, SAO did not appear to impair fertility. Previous fetal loss (the number of miscarriages and stillbirths in multigravidae) did not vary according to SAO genotype, which suggests that SAO does not impair early fetal survival and development.

Southeast Asian ovalocytosis was more common in women of Madang ethnicity (60 of 559, 10.7%) than in women of other ethnicity (21 of 368, 5.7%; $P = 0.011$). However, markers of reproductive fitness, malaria infection, hemoglobin status, and birth weight according to SAO genotype in women of Madang ethnicity were similar in all women.

Our findings that SAO was not associated with maternal hemoglobin concentration, birth weight, and frequency of low birth weight are similar to those of Benet and others in a recently reported community based, case-control study of 685 pregnant women and 245 non-pregnant women also recruited from Madang Province in Papua New Guinea.¹² We have reported previously that SAO was associated with more severe anemia in children with severe malaria.⁹ However, severe anemia during pregnancy was relatively uncommon in this series and did not differ according to genotype for SAO.

Despite the association between SAO and protection against cerebral malaria in children,^{8,9} maternal SAO did not significantly reduce the frequency of placental malaria, including in primigravidae, as assessed by microscopy of placental smears in our study. This is in contrast to Benet and others, who reported that placental malaria (either active or chronic infection) was less common in SAO than control women.¹² Although we did not examine histologic sections of placenta to assess chronic infection, there was good agreement between microscopy of placental smears and histologic examination of placental tissue in women with active or active-chronic malaria infection in the study by Benet and others.¹² Therefore, this difference in methodology is unlikely to account for the discrepancy between the studies. The findings in both studies that birth weight was similar in women with SAO and in normal women suggests that any reduction of placental parasitemia in SAO does not result in a significant clinical benefit.

Sequestration of parasitized erythrocytes contributes directly to *P. falciparum* pathogenesis in both cerebral and placental malaria.⁷ However, the dominant host receptors for parasite-derived antigens differ between the brain and placenta, with a predominance of binding to chondroitin sulfate A and hyaluronic acid in the latter. Adherence of parasitized erythrocytes to receptors in the placenta would not appear to be impaired by SAO. This is consistent with findings *in vitro*, where adhesion to chondroitin sulfate A was not impaired in parasitized erythrocytes in persons with SAO.¹³

Systemic parasitemia also contributes to the adverse effects of malaria during pregnancy.¹⁴ Although we were not able to assess malaria infection during pregnancy in this study, maternal parasitemia after delivery did not vary significantly according to SAO genotype.

Benet and others also reported that the frequency of SAO in pregnant women was lower than that in non-pregnant women and proposed that SAO in combination with another

as yet undefined genetic trait produces sterility.¹² Although we can not exclude this possibility, the fact that the frequency of SAO in pregnant women delivering in hospital was not lower than that observed in girls recruited as community controls for cases of severe malaria from the same geographic area suggest that this is unlikely.⁹ In conclusion, within the limits of a hospital-based cohort of women attending for delivery, we found no evidence that SAO either impaired fertility or protected against malaria during pregnancy.

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