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ORIGINAL ARTICLE

Risk factors for malaria and adverse birth outcomes in a prospective cohort of pregnant women resident in a high malaria transmission area of Papua New Guinea

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Background: Low birth weight (LBW), anaemia and malaria are common in Papua New Guinean (PNG) women.

Methods: To identify risk factors for LBW, anaemia and preterm delivery (PTD), pregnant women recruited into a cohort study in Madang, PNG were followed to delivery.

Results: Of 470 women enrolled, delivery data were available for 328 (69.7%). By microscopy, 34.4% (113/328) of women had malaria parasitaemia at enrolment and 12.5% (41/328) at delivery; at each time point, PCR detected sub-microscopic parasitaemia in substantially more. Most infections were with *Plasmodium falciparum*; the remainder being predominantly *P. vivax*. Anaemia and smoking were associated with lower birth weight, and LBW (16.7%; 51/305) and PTD (21.8%; 63/290) were common. Histopathologically-diagnosed chronic placental malaria was associated with LBW (adjusted odds ratio [aOR] 3.3; p=0.048) and PTD (aOR 4.2; p=0.01). Lack of maternal education predisposed to PTD. Sub-microscopic parasitaemia at delivery appeared to increase risk of LBW. Of the genetic polymorphisms Southeast Asian ovalocytosis, α^{+}-thalassaemia and complement receptor 1 (CR1) deficiency, a CR1 heterozygous genotype was associated with decreased risk of anaemia, and substantial, but non-significant, effects were noted in other comparisons.

Conclusions: In coastal PNG, malaria and anaemia are important causes of adverse pregnancy outcomes.

Keywords: Genetics, Low birth weight, *Plasmodium*, Pregnancy, Preterm delivery

Introduction

Approximately 125 million women living in malaria-endemic areas become pregnant each year.[1] Pregnant women are more susceptible to malaria than non-pregnant adults partly due to the emergence of pregnancy-specific antigenic variants of *Plasmodium falciparum* that accumulate in the placenta.[2] Further, primigravid women are more susceptible than multigravid women, who typically develop pregnancy-specific immunity, including antibodies to pregnancy-specific parasite variants that confer protection against placental malaria in subsequent pregnancies.[3] The consequences of malaria during pregnancy include maternal deaths due to severe anaemia, and low birth weight babies (LBW; <2500 grams) who have increased perinatal and infant mortality.[4] LBW results from preterm birth (<37 weeks' gestation), or intrauterine growth retardation.

Coastal Papua New Guinea (PNG) experiences some of the highest levels of malaria transmission outside of Africa.[5] Malaria in pregnancy due to *P. falciparum* and *P. vivax* is common,[6] and its greatest impact on birth weight is in primigravid women.[6–8] *P. vivax* placental infection has been reported,[9] but *P. falciparum* is a more important cause of poor pregnancy outcomes.[1] Prospective studies of malaria in pregnancy from the area are rare; the most recent was performed in the 1980s, when malaria prevalence was significantly higher than at present.[7]

Several red cell genetic abnormalities are common in coastal PNG, including α^+-thalassaemia, Southeast Asian ovalocytosis (SAO) and complement receptor 1 (CR1) deficiency, and each has been associated with protection against severe malaria,[10–12] although they do not appear to protect against uncomplicated malaria, parasitaemia or parasite density.[13,14] Studies of SAO and α^+-thalassaemia in pregnant PNG women did not show clear evidence of protection against placental malaria, but suggested a decrease in intensity of placental infection in SAO heterozygotes, particularly in first-time mothers.[8,15,16]

To determine the prevalence, risk factors and consequences of malaria during pregnancy in Madang province, PNG, we performed a longitudinal study of women followed from first antenatal visit to delivery. The associations between malaria infections diagnosed by microscopy, PCR and placental histology and pregnancy outcomes were examined, and potential associations between locally prevalent red blood cell genetic polymorphisms and pregnancy outcomes were investigated.

Materials and methods

Study site

The study took place between September 2005 and October 2007 at the Antenatal Clinic, Alexishafen Health Centre, Madang province, PNG, where malaria transmission is high and perennial.

Study population

Pregnant women >16 years of age were enrolled at their first antenatal visit. Eligibility criteria included evidence of fetal movement, no history of multiple births or delivery complications and the intention to deliver at the Health Centre. Eligible women were read a statement describing the study and gave written informed consent. As staffing was limited, a convenience sample was enrolled, and data were not available on women not enrolled. Women with haemoglobin (Hb) <5 g/dl were excluded and referred for appropriate care.

Study design

Ethics approval was granted by the PNG Medical Research Advisory Council, the Melbourne Health Human Research Ethics Committee and Alfred Health Human Research Ethics Committee. Study participation occurred in parallel with clinic attendance at first antenatal visit, 30–34 weeks' gestation, delivery and 6–8 weeks post-delivery. Due to missing data, only enrolment and delivery visits are analysed here. At enrolment, women received chloroquine (9 or 12 tablets, 150 mg base) and (when available) sulphadoxine pyrimethamine (500/25 mg, three tablets), followed by weekly chloroquine prophylaxis (two 150 mg tablets weekly), and ferrous sulphate 270 mg and folic acid 0.3 mg daily, according to local policy. Prophylaxis was not monitored.

Sample and data collection

At each visit, clinical, malaria exposure (bed net use and residence) and demographic data were collected onto prepared case report forms. Participants were asked about history of fever, headache and chills within the previous 7 days. Gestational age was estimated from fundal height measurements. Weight was measured using sliding weight scales, and mid-upper arm circumference (MUAC) was measured using cloth tape measures. Temperature was recorded using a digital thermometer. At delivery, infants were weighed using SECA baby scales, and gestational age was estimated from Ballard scores.

At enrolment and delivery, 5 ml of venous blood was collected. At delivery, intervillous placental blood was collected from an incision in the maternal side of the placenta, and a 1 cm square full thickness placental biopsy was collected into 10% neutral buffered formalin.

At all time points, thick and thin blood films were prepared for microscopy to quantify parasitemia and blood was collected for molecular diagnosis of *Plasmodium spp.* infection (including from placental blood). Hb concentration was measured using a HemoCue haemoglobinometer (Hemocue, Ängelholm, Sweden). Residual blood was separated, with plasma and cellular fractions frozen separately.

Laboratory procedures

Blood films were stained with Giemsa and examined by two independent microscopists. Discordant results were referred for a third reading to adjudicate species and density of infection. DNA was extracted from erythrocyte pellets of 200 μl maternal blood (QIAmp DNA Blood Minikit, Qiagen, Valencia, CA, USA). Molecular diagnosis of *P. falciparum*, *P. vivax*, and *P. ovale* (*P. malariae* was not included in this assay) used a polymerase chain reaction/ligase detection reaction - fluorescent microsphere assay (LDR-FMA). Extracted DNA (2.5 μl) was used as a template for amplification of the multicopy Pf 18S small subunit ribosomal RNA gene.[17] Samples were considered positive only if both species-specific primers generated a positive signal. Participants were typed for red blood cell polymorphisms: SAO, CR1 and α^+-thalassaemia, using published methods.[13] Placental biopsies were embedded in paraffin and processed routinely, and Giemsa-stained sections were examined by light microscopy to identify changes consistent with acute, chronic or past infection.[18]

Exposures and outcomes

We determined predictors of maternal Hb concentration and peripheral or placental parasitaemia (by light microscopy) at delivery, and of birth weight, LBW and preterm birth. Exposures of interest included other malariometric variables e.g., peripheral parasitaemia at enrolment (by light microscopy or PCR); Hb at enrolment; participant characteristics including age, gravidity, parity, smoking, MUAC, education, residence and bed net use and host genetic polymorphisms (SAO, CR1, and α^+-thalassaemia). Smoking and MUAC were included as exposures for infant, but not maternal, outcomes. Malariometric exposures and outcomes include all species types, however the majority (>85%) of infections detected by microscopy were *P. falciparum*.

Statistical analysis

Logistic and linear regression analyses were performed to identify risk factors for adverse maternal and infant outcomes at delivery. Variables were compared using Pearson's χ^2 test for categorical

variables, and Student's t test for numerical variables. Changes in malariometrics within an individual woman between enrolment and delivery were quantified using McNemar's χ^2 test and Student's paired t test. Univariable associations were determined between malariometric, demographic and host genetic variables and Hb, birth weight, LBW and preterm delivery. Predictors of infant and maternal outcomes were identified from adjusted regression models. Adjusted models included all exposure variables, with some exceptions due to collinearity. Age and parity showed collinearity with gravidity, which was retained because of well-established differences in risk of malaria during pregnancy between primigravidae and multigravidae. Due to limited statistical power, both the magnitude of associations and the p-value were considered in the interpretation of potential risk factors.

Results

Study cohort

Table 1 shows the baseline characteristics of the 470 pregnant women recruited, and of the 328 women (69.7%) with delivery information. Of 377 women (80.2%; 377/470) who returned at delivery, we excluded six women with multiple births, and 43 who were not seen ≤3 days after delivery. There were no systematic differences between included women (n=328) and those who were excluded or lost to follow-up (n=142; all p>0.08), except that included women were slightly better educated (p=0.007; data not shown). Among women followed to delivery, the median age was 24 years (range 16–49), 127/328 (38.7%) were in their first pregnancy (primigravidae) and median (range) estimated gestational age was 25 (7–36) weeks. Seventy-five percent of women (234/321) used bed nets the night before enrolment.

At enrolment, 314/328 women (95.7%) were anaemic (Hb <11 g/dl) and 112/328 (34.1%) had severe anaemia (Hb <8 g/dl). Red cell polymorphisms associated with malaria in PNG were common: of 328 women, 47 (14.3%) had the SAO polymorphism; 297 (90.5%) had genotypes associated with intermediate or low CR1 expression on erythrocytes and 265 (80.7%) were heterozygous or homozygous for α^+-thalassaemia (Table 1).

Prevalence of malaria infection

At enrolment, 113/328 women (34.4%) had peripheral parasitaemia by microscopy, which decreased to 41/298 (12.5%) at delivery (p<0.001). In peripheral blood, *P. falciparum* was detected in 105/113 (92.9%) parasitaemic women at enrolment, and 35/41 (85%) at delivery. Additionally, 39/44 (89%) positive placental smears showed infection with *P. falciparum* (Table 2). By placental histology, 154/242 women (63.6%) had past or present malaria infection (Table 2).

More women were infected as determined by PCR than by microscopy (Table 2). Half the infections detected at enrolment, and two-thirds of infections at delivery, were sub-microscopic. Substantially more infections with *P. vivax* or *P. ovale* were detected by PCR than by microscopy, although *P. falciparum* still predominated.

Risk factors for malaria infection at delivery

We examined risk factors for blood slide parasitaemia at delivery (Table 3). On multivariate analysis, peripheral parasitaemia was more common in multigravid than primigravid women (OR 2.12 [0.95–4.73]; 0.07) and peripheral parasitaemia was less common in women who used a bed net (Table 3). No other factors, including host genetic polymorphisms, were significantly associated with risk of peripheral or placental parasitaemia (Table 3).

Risk factors for adverse infant outcomes–birth weight, low birth weight and preterm birth

Mean (SD) birth weight was 2870 (480) grams and the median (interquartile range) gestational age was 38 (37–40) weeks. The prevalence of LBW and preterm birth were high (51/305; 16.7% and 63/290; 21.7%, respectively). Malaria parasitaemia diagnosed by light microscopy was not associated with birth weight or LBW, but features of chronic placental infection on placental histology were associated with LBW and with preterm birth. In adjusted analyses, women with chronic placental malaria had significantly increased odds of LBW and preterm birth (3.3- and 4.2-fold, respectively) compared to uninfected women. Higher Hb at enrolment was associated with higher birth weight (61 g per 1 g/dl increase in Hb; p=0.005), but Hb concentration was not significantly associated with LBW or preterm delivery.

Other factors that were associated with birth weight in unadjusted, but not adjusted analyses included positive associations between MUAC and birth weight (p=0.022), and negative associations between birth weight and history of recent febrile symptoms (p=0.001) or parasitaemia at enrolment (p=0.034) (Table 4). In adjusted analyses, factors that were independently associated with birth weight included gravidity (p<0.001), smoking (p=0.005) and SAO genotype. Multigravid women had babies that were on average 325 g heavier than primigravidae (p<0.001), while smokers' babies were 197 g lighter than non-smokers' (Table 4).

Other participant characteristics associated with LBW and preterm birth were examined (Table 5). On multivariate analyses, multigravidae had significantly reduced odds of LBW (OR 0.28; p<0.001) and preterm birth (OR 0.38; p=0.0087) compared to primigravid women. Preterm birth was significantly less common in women who had had either primary (OR 0.22; p=0.0072) or secondary education (OR 0.13; p<0.001). Large magnitudes of effect with adverse infant outcomes were observed for CR1 and α^+-thalassaemia genotypes but these were not statistically significant; high-low (HL) and low-low (LL) CR1 genotypes reduced the odds of preterm birth by almost 50%, but had no effect on the odds of LBW, compared to the normal high-high (HH) genotype. Heterozygous and homozygous α^+-thalassaemia genotypes reduced odds of both preterm and LBW by up to 45%, compared to the wildtype (p>0.15). Women with SAO had increased odds of LBW (2.33-fold; p=0.07) compared to women of normal genotype (Table 5).

Risk factors for maternal haemoglobin and anaemia at delivery

In univariate analyses, both homo- and heterozygous α^+-thalassaemia genotypes were associated with decreases in mean Hb at delivery (0.79 g/dl and 0.74 g/dl) compared to women with normal genotype (Table 6). In adjusted analyses,

Table 1. Baseline characteristics of women enrolled in the cohort and of the sub-set who returned for a delivery visit

Variable		Enrolled (n=470)[a,b]	Delivered (n=328)[a,b]
Demographic			
Age (years)		24 (21–28), 16–49	24 (21–28), 16–49
Gestational age (weeks)		25.2 [4.28], 7–38	25.2 [4.17], 7–36
Gravidity	Primigravidae	179 (38)	127 (39)
	Multigravidae	292 (62)	201 (61)
MUAC (cm)		22.5 [1.9], 12–30	22.4 [1.8], 12–30
Weight (kg)		54.4 [6.1], 35–74	54.4 [5.9], 35–74
Mother's education	None	60 (13)	32 (10)
	Primary (1–7)	214 (46)	149 (46)
	Secondary (8+)	190 (41)	142 (44)
Mother's occupation	None	310 (66)	207 (63)
	Self-employed	114 (24)	83 (25)
	Earns wages	45 (10)	36 (11)
Smokes		88 (19)	63 (19)
Malaria exposure			
Owns bed net		382 (81)	268 (83)
Used bed net last night		335 (71)	234 (75)
Bed net last treated	Never	328 (70)	224 (75)
	<6 months	27 (6)	19 (6)
	>6 months	66 (14)	52 (17)
Residence	Town	16 (3)	9 (3)
	Village	452 (96)	316 (97)
Genetic			
SAO	Normal	410 (87)	281 (86)
	SAO	59 (13)	47 (14)
CR1	HH[c]	39 (8)	31 (9)
	HL[c]	197 (42)	136 (41)
	LL[c]	233 (49)	161 (49)
α-thalassaemia	Wildtype	87 (18)	63 (19)
	Heterozygous	185 (39)	128 (39)
	Homozygous	197 (42)	137 (42)
Clinical			
Clinical history[d]		122 (26)	82 (25)
Palpable spleen		86 (18)	63 (20)
Haemoglobin			
Haemoglobin (g/dl)		8.5 [1.4], 5.0–12.8	8.5 [1.4], 5.3–12.8
Severe anaemia (<8 g/dl)		163 (35)	112 (34)

Data are Mean [SD], range; or Median (25^{th}–75^{th} percentile), range; or n (%).
CR1: complement receptor-1; H: high; HH: high-high; HL: high-low; L: low; LL: low-low; MUAC: mid-upper arm circumference; SAO: Southeast Asian ovalocytosis.
[a] Enrolment: all women enrolled into study. Delivery: all women enrolled and included in final analyses. All p-values for the difference between study participants and excluded women >0.08, except for education (p=0.007).
[b] When totals do not add to sample size or percentages do not total 100%, missing values can be assumed.
[c] Abbreviations correspond to levels of expression on the cell surface.
[d] Clinical history of fever, headache or chills in the 7 days prior to enrolment visit.

the CR1 HL genotype was associated with protection against anaemia (OR 0.36; p=0.048), but not with Hb levels (p=0.50). Smoking was associated with a borderline increase in risk of anaemia in adjusted analyses (OR 2.0; p=0.075), while parasitaemia at enrolment was associated with a borderline increase in delivery Hb (0.42 g/dl; CI −0.04 to 0.88; p=0.073). There were non-significant but substantial associations between SAO and protection from anaemia (p=0.057), and between CR1 LL homozygosity and protection from anaemia (p=0.081).

Table 2. Malaria prevalence by microscopy and by PCR at enrolment and delivery

Variable	Enrolment (n=328) Mean [SD], range; or n (%)	Delivery (n=298) Mean [SD], range; or n (%)	Change (95% CI)[a]	p-value[b]
Peripheral blood microscopy				
All species	113 (34)	41 (14)	−22 (−29, −15)	<0.001
P. falciparum	105 (32)	35 (12)	−21 (−28, −15)	<0.001
P. vivax	12 (4)	7 (2)	−1.7 (−4.7, 1.4)	0.22
P. ovale	0 (0)	1 (0.3)	0.3 (−0.7, 1.3)	0.32
Placental blood microscopy				
All species	ND	44 (18)	NA	NS
P. falciparum	ND	39 (16)	NA	NS
P. vivax	ND	6 (2)	NA	NS
P. ovale	ND	0 (0)	NA	NS
Placental histology[c]				
No infection	ND	88 (36)	NA	NS
Acute infection	ND	93 (38)	NA	NS
Chronic infection	ND	41 (17)	NA	NS
Past infection	ND	20 (8)	NA	NS
Peripheral blood PCR				
All species	217 (66)	110 (47)	−18 (−28, 8.9)	<0.001
P. falciparum	160 (49)	86 (37)	−12 (−21, 2.7)	0.01
P. vivax	64 (20)	28 (12)	−8.1 (−15, −1.0)	0.02
P. ovale	38 (12)	11 (4)	−6.1 (−11, −0.9)	0.01
Haemoglobin				
Haemoglobin (g/dl)	8.5 [1.4], 5.3–12.8	9.2 [1.8], 4.2–14.8	0.69 (0.50, 0.89)	<0.001
Severe anaemia (<8 g/dl)	112 (34)	68 (23)	−11 (−17, −4.3)	0.001

Placental histology missing for 56 participants, PCR results at delivery missing for 65 participants.
NA: not applicable; ND: not done; NS: not significant.
[a] Between enrolment and delivery; discrepancies in percentage differences due to rounding.
[b] p-value derived from paired t tests for numeric variables, or McNemar's χ^2 test for binary variables.
[c] Placental histology of placental biopsies by light microscopy: no infection; acute infection (parasites without pigments); chronic infection (parasites with monocyte and/or fibrin pigments); past infection (pigment without parasites).

The contribution of sub-microscopic infections to adverse pregnancy outcomes

Sub-microscopic infections were seen in approximately one-third of women at enrolment and delivery. We compared our study endpoints of anaemia, birth weight and LBW in women with PCR-only infection and uninfected women at enrolment and delivery. Mean birth weight was not significantly different between women with sub-microscopic infection at delivery (2814±473 g) and uninfected women (2878±401 g; p=0.35), but there was a greater proportion of LBW deliveries among women with sub-microscopic infection (20/85; 24%) than in uninfected women (14/110, 12.7%; OR 2.41; CI 0.99–5.89; p=0.054; Table 7).

Discussion

We described clinical characteristics, malaria prevalence and prevalence of common red blood cell genetic polymorphisms in a cohort of pregnant women living in coastal PNG, and performed multivariable linear and logistic regressions to identify risk factors for malaria infection at delivery and for poor pregnancy outcomes including lower maternal Hb, decreased birth weight and increased risk of LBW and preterm delivery. This is the first reported study outside of Africa to show associations between placental infection and adverse birth outcomes.

Malaria is highly endemic in coastal PNG. At enrolment, one-third of women were parasitaemic by microscopy and two-thirds by PCR. Both *P. falciparum* and *P. vivax* infections are common in school-age PNG children,[19] but *P. falciparum* was responsible for >85% of microscopic infections, and three- quarters of sub-microscopic infections in pregnant women. Previous observations in PNG demonstrate the earlier acquisition of immunity to *P. vivax* than to *P. falciparum*, due to high force of infection.[20] Whether this extends to protection from asymptomatic parasitaemia is unknown. There also appears to be a greater increase in pregnancy-associated susceptibility to *P. falciparum* than to *P. vivax*.[21]

The great majority of women were anaemic (Hb <11 g/dl) and 35% had severe anaemia (defined here as Hb <8 g/dl). Apart from malaria, common causes of anaemia in pregnancy in PNG include

Table 3. Risk factors for peripheral and placental parasitaemia at delivery as determined by light microscopy

	Peripheral parasitaemia (n=298)				Placental parasitaemia (n=247)			
Variable	Normal (n=257)	Parasitaemic (n=41)	Univariable (OR [95% CI]; p)	Multivariable (OR [95% CI]; p)	Normal (n=203)	Parasitaemic (n=44)	Univariable	Multivariable
Age (years)	247 (86)	41 (14)	0.91 [0.85, 0.98]; 0.01	ND[a]	196 (82)	44 (18)	1.02 [0.96, 1.08]; 0.51	ND[a]
Gravidity								
Primigravidae	103 (40)	12 (29)	NA	NA	80 (39)	16 (36)	NA	NA
Multigravidae	154 (60)	29 (71)	1.62 [0.79–3.31]; 0.19	2.12 [0.95–4.73]; 0.07	123 (61)	28 (64)	1.14 [0.58–2.24]; 0.71	1.32 [0.61–2.84]; 0.47
Education								
None	24 (9)	2 (5)	NA	NA	19 (9)	3 (7)	NA	NA
Primary	120 (47)	15 (37)	1.5 [0.32–6.99]; 0.61	1.49 [0.29–7.71]; 0.63	95 (48)	18 (41)	1.2 [0.32–4.48]; 0.79	0.95 [0.23–3.93]; 0.95
Secondary	109 (43)	23 (57)	2.53 [0.56–11.47]; 0.23	2.95 [0.58–14.99]; 0.19	85 (43)	23 (52)	1.71 [0.47–6.30]; 0.42	1.62 [0.39–6.70]; 0.51
Used bed net								
No	56 (23)	14 (36)	NA	NA	43 (22)	13 (30)	NA	NA
Yes	188 (77)	25 (64)	0.53 [0.26–1.10]; 0.08	0.48 [0.22–1.04]; 0.06	148 (78)	30 (70)	0.67 [0.32–1.40]; 0.29	0.71 [0.33–1.55]; 0.40
Symptoms								
No	190 (75)	28 (68)	NA	NA	149 (74)	32 (73)	NA	NA
Yes	63 (25)	13 (32)	1.4 [0.68–2.87]; 0.36	1.42 [0.64–3.19]; 0.39	53 (26)	12 (27)	1.05 [0.51–2.20]; 0.89	1.09 [0.49–2.44]; 0.83
Enrolment Hb	257 (86)	41 (14)	0.95 [0.74, 1.21]; 0.70	1.03 [0.77–1.36]; 0.86	203 (82)	44 (18)	1.06 [0.83, 1.34]; 0.65	1.05 [0.80–1.37]; 0.73
Parasitaemia at enrolment								
No	166 (65)	25 (61)	NA	NA	128 (63)	32 (73)	NA	NA
Yes	91 (35)	16 (39)	1.17 [0.59–2.3]; 0.65	1.36 [0.62–2.97]; 0.44	75 (37)	12 (27)	0.64 [0.31–1.39]; 0.23	0.54 [0.24–1.22]; 0.14
SAO								
No	218 (85)	36 (88)	NA	NA	177 (87)	36 (82)	NA	NA
Yes	39 (15)	5 (12)	0.78 [0.29–2.10]; 0.62	0.52 [0.16–1.65]; 0.27	26 (13)	8 (18)	1.51 [0.63–3.61]; 0.35	1.27 [0.48–3.33]; 0.62
CR1								
HH	26 (10)	4 (10)	NA	NA	23 (11)	5 (11)	NA	NA
HL	107 (42)	16 (39)	0.97 [0.30–3.15]; 0.96	0.85 [0.25–2.9]; 0.80	85 (42)	12 (27)	0.65 [0.21–2.03]; 0.46	0.68 [0.21–2.20]; 0.51
LL	124 (48)	21 (51)	1.10 [0.35–3.48]; 0.87	1.01 [0.30–3.35]; 0.99	95 (47)	27 (61)	1.31 [0.45–3.76]; 0.62	1.36 [0.45–4.09]; 0.58
α-thalassaemia								
Wildtype	50 (19)	8 (19)	NA	NA	36 (18)	7 (16)	NA	NA
Heterozygote	101 (39)	18 (44)	1.11 [0.45–2.74]; 0.81	1.27 [0.45–3.46]; 0.64	76 (37)	19 (43)	1.30 [0.60–3.33]; 0.60	1.46 [0.53–4.01]; 0.46
Homozygote	106 (41)	15 (37)	0.88 [0.35–2.22]; 0.79	1.04 [0.37–2.89]; 0.94	91 (45)	18 (41)	1.02 [0.39–2.60]; 0.97	0.94 [0.34–2.62]; 0.91

Data are unadjusted and adjusted odds ratios [95% CI].
CR1: complement receptor-1; Hb: haemoglobin; HH: high-high cell surface expression; HL: high-low; LL: low-low; ND: not done; NA: not applicable (reference group); SAO: Southeast Asian ovalocytosis.
[a] Variables excluded due to collinearity.

Table 4. Factors associated with birth weight

Variable	Unadjusted	Adjusted[a]
Age (years)	8.4 [−1.5, 18]; 0.095	ND[b]
Gravidity		
Primigravidae	NA	NA
Multigravidae	304 [198, 410]; <0.001	325 [211, 439]; <0.001
MUAC (cm)	34 [4.9, 64]; 0.022	18 [−15, 50]; 0.288
Smokes		
No	NA	NA
Yes	−140 [−274, −6.4]; 0.04	−197 [−333, −61]; 0.005
Education		
None	NA	NA
Primary	54 [−146, 254]; 0.59	100 [−104, 305]; 0.34
Secondary	2.5 [−198, 203]; 0.98	79 [−127, 286]; 0.45
Used bed net		
No	NA	NA
Yes	−72 [−201, 56]; 0.27	−40 [−165, 84]; 0.52
Clinical history		
No	NA	NA
Yes	−221 [−345, −96]; 0.001	−104 [−230, 22]; 0.10
Enrolment Hb (g/dl)	61 [22, 100]; 0.002	61 [19, 102]; 0.005
Anaemia		
No	NA	ND[b]
Yes (Hb <8 g/dl)	−101 [−215, 13]; 0.083	
Parasitaemia (LM) at enrolment		
No	NA	NA
Yes	−121 [−234, −9.1]; 0.034	−30 [−149, 89]; 0.62
SAO		
Normal	NA	NA
SAO	−132 [−285, 22]; 0.093	−129 [−287, 29]; 0.11
CR1		
HH	NA	NA
HL	−179 [−370, 12]; 0.067	−168 [−350, 15]; 0.071
LL	−68 [−256, 120]; 0.48	−88 [−265, 90]; 0.33
α-thalassaemia		
Wildtype	NA	NA
Heterozygote	−61 [−212, 91]; 0.43	−12 [−159, 135]; 0.88
Homozygote	22 [−128, 171]; 0.78	47 [−100, 194]; 0.53

Data are mean differences in birth weight (grams) [95% CI]; p-value.
CR1: complement receptor-1; Hb: haemoglobin; HH: high-high cell surface expression; HL: high-low; LL: low-low; LM: light microscopy; MUAC: mid-upper arm circumference; ND: not done; NA: not applicable (reference group); SAO: Southeast Asian ovalocytosis.
[a] Adjusted model: n=271.
[b] Variables excluded due to collinearity. Missing data for age (11), smoking (1), MUAC (10), education (4), residence (3), bed net use (15), clinical history (4). Multivariable model includes all risk factors except age.

iron and/or folate deficiency.[7,22] Alpha thalassaemia was common, and may also contribute to anaemia. The increase in Hb levels between enrolment and delivery may reflect combined effects of malaria prophylaxis and iron and folate supplementation.

The only factor associated with a decreased risk of peripheral parasitaemia at delivery was increasing age, which may reflect the age dependent acquisition of immunity. Surprisingly, there was no significant gravidity-dependent decrease in risk of peripheral or placental parasitaemia at delivery,[4] which has been attributed largely to the acquisition of antibodies to pregnancy-associated *P. falciparum* parasite strains.[2] Previous studies from the area do show gravidity-dependent susceptibility to malaria,[23] and the gravidity-associated acquisition of antibodies to multiple different placental-binding *P. falciparum* isolates.[24]

Malaria prophylaxis was not highly effective, with parasitaemia detected in peripheral and/or placental blood at delivery in 24% of

Table 5. Risk factors for low birth weight and preterm delivery

Risk factors for low birth weight (LBW; <2500 grams), N=305					Risk factors for preterm birth (<37 weeks) gestation), N=290			
Variable	Normal (n=254)	LBW (n=51)	Univariable	Multivariable	Normal (n=227)	Preterm (n=63)	Univariable	Multivariable
Age (years)	245 (83)	29 (17)	0.96 [0.91, 1.02]; 0.23	ND[a]	218 (78)	61 (22)	0.97 [0.92, 1.03]; 0.31	ND[a]
Gravidity								
Primigravidae	87 (34)	30 (59)	NA	NA	79 (35)	32 (51)	NA	NA
Multigravidae	167 (66)	21 (41)	0.36 [0.20–0.67]; 0.0013	0.28 [0.13–0.57]; 0.001	148 (65)	31 (49)	0.52 [0.29–0.91]; 0.02	0.38 [0.19–0.79]; 0.0087
MUAC (cm)	246 (83)	50 (17)	0.88 [0.74, 1.0]; 0.12	0.91 [0.74–1.1]; 0.36	220 (78)	61 (22)	0.93 [0.80, 1.1]; 0.38	1.0 [0.83–1.2]; 0.84
Smokes								
No	202 (80)	40 (78)	NA	NA	183 (81)	48 (76)	NA	NA
Yes	51 (20)	11 (22)	1.1 [0.52–2.27]; 0.82	1.4 [0.62–3.35]; 0.40	43 (19)	15 (24)	1.3 [0.68–2.6]; 0.40	1.1 [0.50–2.62]; 0.75
Education								
None	22 (9)	5 (10)	NA	NA	15 (7)	11 (18)	NA	NA
Primary	119 (48)	22 (43)	0.81 [0.29–2.4]; 0.71	0.48 [0.13–1.7]; 0.26	102 (45)	31 (50)	0.41 [0.17–0.99]; 0.05	0.22 [0.07–0.66]; 0.0072
Secondary	109 (44)	24 (47)	0.97 [0.33–2.8]; 0.95	0.57 [0.16–2.0]; 0.38	107 (48)	20 (32)	0.25 [0.10–0.63]; 0.0033	0.13 [0.04–0.42]; <0.001
Used bed net								
No	62 (26)	12 (24)	NA	NA	52 (24)	15 (25)	NA	NA
Yes	179 (74)	37 (75)	1.1 [0.52–2.2); 0.86	1.2 [0.53–2.7]; 0.66	162 (76)	46 (75)	0.98 [0.50–1.9]; 0.96	0.78 [0.36–1.7]; 0.53
Clinical history								
No	191 (76)	NA	NA	NA	175 (77)	40 (66)	NA	–
Yes	59 (24)	16 (31)	1.5 [0.76–2.9]; 0.24	0.90 [0.42–1.9]; 0.79	51 (23)	21 (34)	1.8 [0.98–3.3]; 0.06	1.5 [0.70–3.1]; 0.30
Enrolment Hb (g/dl)	254 (83)	51 (17)	0.8 [0.68, 1.1]; 0.16	0.83 [0.63–1.08]; 0.16	227 (78)	63 (22)	0.86 [0.70, 1.1]; 0.16	0.85 [0.65–1.1]; 0.22
Anaemia								
No	168 (66)	34 (67)	NA	ND[a]	ND	ND	ND	ND
Yes (Hb <8) g/dl)	86 (34)	17 (33)	0.98 [0.52, 1.8] 0.94		ND	ND	ND	ND
Enrolment parasitaemia								
No	207 (86)	44 (90)	NA	NA	154 (68)	36 (57)	NA	NA
Yes	33 (14)	5 (10)	1.3 [0.71–2.4]; 0.37	1.0 [0.50–2.2]; 0.91	73 (32)	27 (43)	1.6 [0.89–2.8]; 0.12	1.3 [0.65–2.7]; 0.44
SAO								
Normal	221 (87)	40 (78)	NA	NA	194 (85)	55 (87)	NA	NA
SAO	33 (13)	11 (22)	1.8 [0.86–3.94]; 0.12	2.33 [0.94–5.75]; 0.07	33 (15)	8 (13)	0.85 [0.37–1.96]; 0.71	1.10 [0.40–2.99]; 0.85
CR1								
HH	25 (10)	5 (10)	NA	NA	20 (9)	9 (14)	NA	NA
HL	103 (41)	21 (41)	1.02 [0.35–2.97]; 0.97	1.08 [0.35–3.35]; 0.89	91 (40)	26 (41)	0.63 [0.26–1.56]; 0.32	0.56 [0.21–1.55]; 0.27
LL	126 (50)	25 (49)	0.99 [0.35–2.84]; 0.99	0.89 [0.29–2.72]; 0.84	116 (51)	28 (44)	0.54 [0.22–1.30]; 0.17	0.54 [0.20–1.43]; 0.21
α-thalassaemia								
Wildtype	46 (18)	12 (23)	NA	NA	43 (19)	11 (17)	NA	NA
Heterozygote	97 (38)	21 (41)	0.83 [0.38–1.83]; 0.64	0.66 [0.27–1.58]; 0.35	89 (39)	26 (41)	1.14 [0.52–2.52]; 0.74	0.93 [0.38–2.23]; 0.86
Homozygote	111 (44)	18 (35)	0.62 [0.28–1.40]; 0.25	0.55 [0.22–1.34]; 0.29	95 (42)	26 (41)	1.07 [0.48–2.36]; 0.87	0.63 [0.25–1.56]; 0.32

Placental histology	Normal (n=200)	LBW (n=37)	Univariable		Multivariable		Normal (n=178)	Preterm (n=49)	Univariable		Multivariable	
No infection	75 (37)	10 (27)	NA		NA		74 (42)	10 (20)	NA		NA	
Acute	82 (41)	9 (24)	0.82 [0.32, 2.1]; 0.69		0.66 [0.22, 2.0]; 0.47		65 (36)	20 (41)	2.3 [0.99, 5.2]; 0.052		2.08 [0.75, 5.8]; 0.16	
Chronic	28 (14)	13 (35)	3.5 [1.4, 8.8]; 0.01		3.3 [1.0, 10.6]; 0.048		24 (13)	15 (31)	4.6 [1.8, 11.6]; 0.001		4.2 [1.3, 13.4]; 0.01	
Past infection	15 (7)	5 (13)	2.5 [0.7, 8.4]; 0.14		1.5 [0.37, 6.1]; 0.58		15 (8)	4 (8)	2.0 [0.55, 7.1]; 0.30		1.34 [0.30, 6.1]; 0.70	

Data are unadjusted and adjusted odds ratios [95% CI].
CR1: complement receptor-1; Hb: haemoglobin; HH: high-high cell surface expression; HL: high-low; LBW: low birth weight; LL: low-low; LM: light microscopy; MUAC: mid-upper arm circumference; NA: not applicable (reference group); ND: not done; SAO: Southeast Asian ovalocytosis.
[a] Variables excluded due to collinearity.

women. Participants received curative doses of chloroquine and (usually) sulphadoxine-pyrimethamine and unsupervised weekly chloroquine prophylaxis. Chloroquine resistance in *P. falciparum* is common in PNG, with chloroquine resistant *P. vivax* also well described.[25] PNG recently introduced intermittent preventive treatment in pregnancy with sulphadoxine pyrimethamine, which should be more effective in controlling *P. falciparum* in pregnancy.

On placental histology, almost two-thirds of women had evidence of current or previous malaria, predominantly active infections (in which parasites were detected, frequently at low densities). Chronic infection was associated with LBW and preterm delivery. In African women, chronic placental infection (especially massive chronic intervillositis, the accumulation of large numbers of white cells in the maternal blood spaces of the placenta) has been particularly associated with growth restriction, while acute infection with high parasitaemia was associated with preterm delivery.[26] Our observations suggest that the pathogenesis of these complications may be similar in PNG and Africa.

Gravidity, smoking and low maternal Hb were associated with significant reductions in birth weight in adjusted analyses. Although mild anaemia may not decrease birth weight,[27] our cohort is notable for the severity of anaemia detected at enrolment, one-third of women had Hb <8 g/dl, as did 23% of women at delivery. Febrile symptoms were associated with lower birth weight in unadjusted analyses, but there was no association between febrile symptoms and malaria as determined by microscopy or PCR. The reductions in birth weight in first pregnancies translated into a high rate of LBW and preterm birth delivery in primigravid women, possibly due to their lack of pregnancy-specific immunity,[2] or the lower general risk of LBW associated with increased gravidity.

Most participants reported using bed nets that were not impregnated with insecticides, and although bed net users were less commonly parasitaemic at delivery, this did not translate into decreases in LBW or preterm delivery. The decrease in peripheral blood parasite prevalence at delivery associated with bed net use is consistent with insecticide treated bed net studies from Africa,[28] but bed net use and ownership might be a proxy for higher socio-economic status, better housing and decreased vector exposure, rather than directly mediating decreased malaria prevalence.

Previous studies performed in the region showed a similar prevalence of malaria infection, LBW and anaemia.[6,7] The prevalence of preterm delivery was 21.7%, whereas it was estimated at 6–10% in other studies,[6,7] probably due to differences in sensitivity of dating techniques used. We used Ballard scores, whereas fundal height supplemented by Dubowitz assessments, and Dubowitz assessments supplemented by cutaneous assessments were used in previous studies.[6,7] Although a comparison suggests similar performance of Dubowitz and Ballard scores in Malawi,[29] differences in staff training and interpretation may affect prevalence. Early ultrasound dating, the gold standard for estimating gestation was not available; in recent ultrasound studies in the same province (Unger et al., submitted), preterm delivery was observed in 10.6% of women receiving similar care.

Host genetics were associated with Hb levels and pregnancy outcomes, but not with malaria risk. Women with α^{+}-thalassaemia

Table 6. Risk factors for severe anaemia and associations with maternal haemoglobin at delivery

	Risk factors for severe anaemia at delivery				Associations with maternal haemoglobin at delivery	
Variable	Not anaemic (n=230)	Anaemic (n=68)	Unadjusted	Adjusted	Unadjusted	Adjusted
Age (years)	221 (100)	67 (100)	0.97 (0.92, 1.0); 0.27	ND[a]	0.01 [−0.03, 0.05]; 0.65	ND[a]
Gravidity						
Primigravidae	89 (39)	26 (38)	NA	NA	NA	NA
Multigravidae	141 (61)	42 (62)	1.0 (0.58, 1.8); 0.94	0.82 [0.41, 1.6]; 0.57	−0.17 [−0.58, 0.25]; 0.43	0.08 [−0.36, 0.52]; 0.72
MUAC (cm)	223 (100)	65 (100)	0.87 (0.74, 1.0); 0.077	0.91 [0.75, 1.1]; 0.36	0.06 [−0.05, 0.18]; 0.27	−0.05 [−0.17, 0.08]; 0.46
Smokes						
No	189 (82)	51 (75)	NA	NA	NA	NA
Yes	40 (18)	17 (25)	1.6 (0.83, 3.0); 0.17	2.0 [0.93, 4.4]; 0.075	−0.43 [−0.95, 0.08]; 0.1	−0.38 [−0.91, 0.15]; 0.162
Education						
None	21 (9)	7 (10)	NA	NA	NA	NA
Primary	107 (48)	28 (41)	0.79 (0.30, 2.0); 0.62	1.2 [0.38, 3.7]; 0.76	0.33 [−0.40, 1.06]; 0.37	−0.04 [−0.78, 0.71]; 0.93
Secondary	97 (43)	33 (49)	1.0 (0.40, 2.6); 0.97	1.5 [0.46, 4.7]; 0.52	0.18 [−0.56, 0.91]; 0.64	−0.23 [−0.99, 0.53]; 0.56
Used bed net						
No	53 (24)	17 (27)	NA	NA	NA	NA
Yes	167 (76)	47 (73)	0.9 (0.46, 1.7); 0.69	0.94 [0.43, 2.0]; 0.87	−0.12 [−0.60, 0.36]; 0.63	−0.09 [−0.57, 0.39]; 0.70
Clinical history						
No	169 (74)	50 (75)	NA	NA	NA	NA
Yes	58 (26)	17 (25)	0.99 (0.53, 1.8); 0.98	0.65 [0.30, 1.4]; 0.27	−0.09 [−0.56, 0.38]; 0.71	0.20 [−0.28, 0.68]; 0.40
Enrolment Hb (g/dl)	230 (100)	68 (100)	0.54 (0.42, 0.69); <0.001	0.55 [0.42, 0.73]; <0.001	0.54 [0.41, 0.68]; <0.001	0.56 [0.39, 0.72]; <0.001
Enrolment anaemia						
No	169 (73)	29 (43)	NA	ND[a]	NA	ND[a]
Yes (Hb <8 g/dl)	61 (27)	39 (57)	3.7 (2.1, 6.5); <0.001		−1.2 [−1.6, −0.77]; <0.001	
Enrolment parasitaemia						
No	148 (64)	44 (65)	NA	NA	NA	NA
Yes	82 (36)	24 (35)	0.98 (0.56, 1.7); 0.96	0.59 [0.29, 1.2]; 0.15	−0.05 [−0.47, 0.37]; 0.82	0.42 [−0.04, 0.88]; 0.073
SAO						
Normal	193 (84)	62 (91)	NA	NA	NA	NA
SAO	37 (16)	6 (9)	0.5 (0.20, 1.2); 0.14	0.28 [0.08, 1.0]; 0.057	0.24 [−0.33, 0.82]; 0.41	0.28 [−0.32, 0.89]; 0.35
CR1						
HH	20 (9)	11 (16)	NA	NA	NA	NA
HL	96 (42)	24 (35)	0.45 (0.19, 1.1); 0.073	0.36 [0.13, 0.99]; 0.048	0.34 [−0.36, 1.0]; 0.34	0.23 [−0.45, 0.91]; 0.50
LL	114 (50)	33 (49)	0.53 (0.23, 1.2); 0.13	0.44 [0.17, 1.1]; 0.081	0.49 [−0.20, 1.18]; 0.17	0.42 [−0.24, 1.07]; 0.21
α−thalassaemia						
Wildtype	50 (22)	7 (10)	NA	NA	NA	NA
Heterozygote	86 (37)	33 (48)	2.7 (1.1, 6.6); 0.026	2.1 [0.80, 5.6]; 0.13	−0.79 [−1.3, −0.23]; 0.006	−0.45 [−1.02, 0.11]; 0.11
Homozygote	94 (41)	28 (41)	2.1 (0.87, 5.2); 0.099	1.5 [0.56, 4.1]; 0.42	−0.74 [−1.3, −0.19]; 0.009	−0.39 [−0.96, 0.17]; 0.17

Data are number (%), unadjusted or adjusted odds ratio (95% CI), or adjusted or unadjusted coefficient [95% CI].
CR1: complement receptor-1; Hb: haemoglobin; HH: high-high cell surface expression; HL: high-low; LL: low-low; LM: light microscopy; MUAC: mid-upper arm circumference; NA: not applicable (reference group); ND: not done; SAO: Southeast Asian ovalocytosis.
[a] Variables excluded due to collinearity. n of adjusted model=263. Missing data for age (10; 1 anaemic), smoking (1; 0 anaemic), mid-upper arm circumference (10; 3 anaemic), education (5; 0 anaemic), residence (3; 0 anaemic), bed net use (14; 4 anaemic), clinical history (4; 1 anaemic). Thirty women missing haemoglobin at delivery.

Table 7. Association between submicroscopic *Plasmodium* infection, low birth weight and birth weight

Variable	Low birth weight (n,%)	Unadjusted	Adjusted	Mean [SD] birth weight	Unadjusted
Peripheral parasitaemia at delivery (PCR) (n=195)					
Negative	14/110; 12.7%	NA	NA	2878 [401]	NA
Positive	20/85; 23.5%	2.1 (0.99, 4.5); 0.052	2.4 (0.99, 5.9); 0.054	2814 [473]	−64 [−188, 59]; 0.31

Data are unadjusted or adjusted odds ratio (95% CI), or adjusted or unadjusted coefficient [95% CI]. Adjusted for gravidity, smoking, mid-upper arm circumference, education, residence, bed net use, clinical history, peripheral infection at enrolment (light microscopy), haemoglobin at enrolment, SAO, CR1 and alpha thalassaemia.
CR1: complement receptor-1; NA: not applicable (reference group); SAO: Southeast Asian ovalocytosis.

had decreased Hb at delivery, while CR1 and SAO polymorphisms were associated with increased maternal Hb at delivery. SAO was associated with a non-significant increase in risk of LBW, and decrease in mean birth weight (but not preterm birth). Previous studies in Madang have not shown a significant relationship between SAO and pregnancy outcome,[8,15,16] and in the absence of a clear mechanism by which it might affect fetal growth it seems unlikely to be a true effect. CR1 and α^+-thalassaemia polymorphisms were not significantly associated with birth weight, or with risk of LBW and preterm birth. The α^+-thalassaemia observations were consistent with an earlier study,[15] while this is the first study of the effect of CR1 genotype on pregnancy malaria and pregnancy outcomes.

In this cohort, one-third of women had sub-microscopic malaria infection at first presentation, and a similar number at delivery. Between half and two-thirds of all infections were sub-microscopic. Women with sub-microscopic infection at delivery had increased rates of LBW, which approached significance; there was no significant impact on birth weight. Further studies on larger sample sets are indicated. The importance of sub-microscopic infections for pregnancy outcome is not fully resolved,[30] and further studies are required to determine whether PCR can identify women at risk of adverse outcomes.

This study had some limitations. First, a convenience sample of women living close to the health centre was recruited, malaria or anaemia may be greater problems in more poorly served communities and we do not have information on non-enrolled women. Second, delivery data were only available on 70% of enrolled women, as many delivered at home. Third, the sample size was modest, which limited power to detect significant associations between maternal characteristics and malaria or pregnancy outcome. Few statistically significant associations were observed, and large magnitudes of effect for some non-significant associations may indicate that lack of statistical power could be concealing true associations. Nevertheless, we did demonstrate important associations between birth weight, risk of LBW or preterm delivery and factors such as smoking, Hb levels and education, and showed that histological diagnosis of chronic placental malaria is an important risk factor for LBW and preterm delivery, as well as confirming expected associations with gravidity.

Conclusions

Up to two-thirds of pregnant women living in an area of high malaria transmission of coastal PNG had malaria infection at the first antenatal visit and almost one-half at delivery. Of these, women with chronic placental infection had increased rates of LBW and preterm delivery; sub-microscopic infection may increase the risk of LBW delivery. Red blood cell polymorphisms that are common in PNG do not seem to explain the high rates of anaemia detected; malaria and possibly iron deficiency may instead be responsible. Strategies to better prevent malaria, and to improve maternal Hb, may decrease the burden of adverse pregnancy outcomes in settings like PNG.

Authors' contributions: SJR, IM, JGB, CLK and PMS conceived the study; SJR and DIS designed the study protocol; FB and DIS recruited patients; AU and CC carried out laboratory studies; KAM and SJR drafted the manuscript; FJF, IM, JGB and CLK critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. DIS and SJR are guarantors of the paper.

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Competing interests: None declared.

Ethical approval: Approval was granted by the PNG Medical Research Advisory Council, the Melbourne Health Human Research Ethics Committee and Alfred Health Human Research Ethics Committee. The procedures followed were in accordance with the ethical standards of the Helsinki Declaration (1964, amended most recently in 2008) of the World Medical Association.[31]

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