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Effects of *Plasmodium falciparum* infection on umbilical artery resistance and intrafetal blood flow distribution: a Doppler ultrasound study from Papua New Guinea

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Abstract

Background: Doppler velocimetry studies of umbilical artery (UA) and middle cerebral artery (MCA) flow help to determine the presence and severity of fetal growth restriction. Increased UA resistance and reduced MCA pulsatility may indicate increased placental resistance and intrafetal blood flow redistribution. Malaria causes low birth weight and fetal growth restriction, but few studies have assessed its effects on uteroplacental and fetoplacental blood flow.

Methods: Colour-pulsed Doppler ultrasound was used to assess UA and MCA flow in 396 Papua New Guinean singleton fetuses. Abnormal flow was defined as an UA resistance index above the 90th centile, and/or a MCA pulsatility index and cerebroplacental ratio (ratio of MCA and UA pulsatility index) below the 10th centile of population-specific models fitted to the data. Associations between malaria (peripheral infection prior to and at ultrasound examination, and any gestational infection, i.e., 'exposure') and abnormal flow, and between abnormal flow and birth outcomes, were estimated.

Results: Of 78 malaria infection episodes detected before or at the ultrasound visit, 62 (79.5%) were *Plasmodium falciparum* (34 sub-microscopic infections), and 16 were *Plasmodium vivax*. *Plasmodium falciparum* infection before or at Doppler measurement was associated with increased UA resistance (adjusted odds ratio (aOR) 2.3 95% CI 1.0–5.2, $P = 0.047$). When assessed by 'exposure', *P. falciparum* infection was significantly associated with increased UA resistance (all infections: 2.4, 1.1–4.9, $P = 0.024$; sub-microscopic infections 2.6, 1.0–6.6, $P = 0.051$) and a reduced MCA pulsatility index (all infections: 2.6, 1.2–5.3, $P = 0.012$; sub-microscopic infections: 2.8, 1.1–7.5, $P = 0.035$). Sub-microscopic *P. falciparum* infections were additionally associated with a reduced cerebroplacental ratio (3.64, 1.22–10.88, $P = 0.021$). There were too few *P. vivax* infections to draw robust conclusions. An increased UA resistance index was associated with histological evidence of placental malaria (5.1, 2.3–10.9, $P < 0.001$; sensitivity 0.26, specificity 0.93). A low cerebroplacental Doppler ratio was associated with concurrently measuring small-for-gestational-age, and with low birth weight.

Discussion/conclusion: Both microscopic and sub-microscopic *P. falciparum* infections impair fetoplacental and intrafetal flow, at least temporarily. Increased UA resistance has high specificity but low sensitivity for the detection of placental infection. These findings suggest that interventions to protect the fetus should clear and prevent both microscopic and sub-microscopic malarial infections.

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Background

The identification and management of risk factors for fetal growth restriction (FGR) is key to optimizing pregnancy outcomes. The incidence of low birth weight [(LBW) <2500 g], FGR and infant death are substantially higher in low- and middle-income countries (LMICs) [1, 2].

Plasmodium falciparum and *Plasmodium vivax* infections are common during pregnancy in many LMICs, and are associated with LBW [3]; infection at any stage of pregnancy may affect birth weight and fetal growth [4–6]. Mechanisms by which malarial infection may cause FGR include deleterious effects on trophoblast migration and invasion capacity [5], placental vascular insufficiency through suppression of pro-angiogenic factors and reduced placental vessel development [7], impaired nutrient transport, and through causing imbalances in growth hormone levels [6, 8, 9].

Doppler ultrasound allows for non-invasive real-time assessment of uteroplacental and fetoplacental blood flow characteristics and intrafetal blood flow distribution. An increased umbilical artery resistance index (UARI) or absent/reversed UA end-diastolic flow in the second half of pregnancy indicates increased placental vascular resistance and uteroplacental insufficiency, due to poor villous angiogenesis or acute pathologies such as placental abruption [10]. A decrease in middle cerebral artery pulsatility index (MCAPPI) in third trimester indicates blood flow redistribution to the fetal brain as a physiological adaptation to placental insufficiency [11]. This is an adaptive response to maintain brain growth at the expense of other body parts when oxygen and nutrient supply is limited, resulting in asymmetrical growth restriction [12]. UA and MCA interrogations predict adverse outcomes best amongst pregnancies measuring small-for-gestational-age [(SGA), <10th centile of a growth standard] or with decreasing growth centiles, both fetal size indicators of FGR [13].

A small number of ultrasound-based studies in LMICs have examined factors associated with abnormal placental development and fetal growth, and have evaluated the potential effects of malaria parasitaemia on uteroplacental and fetoplacental blood flow [14–19]. Symptomatic falciparum malaria in early third trimester was associated with acutely increased UARI and reduced MCA resistance in French Guiana [14, 15]. In Kenyan women in third trimester, concurrent malaria parasitaemia was associated with increased uterine artery resistance indices, but

effects on UARI were not measured [16]. In another Kenyan cohort, increased UARI was seen amongst women with concurrent infection before 26 gestational weeks [17]. Infection in early pregnancy (<20 gestational weeks) was associated with increased uterine artery resistance in undernourished Congolese women, and with reduced UARI in later pregnancy amongst primigravidae, the latter perhaps being due to adaptive villous angiogenesis following a treated infection [18]. In a case–control study of *P. vivax* in pregnancy in Brazil, parasitaemia early in pregnancy was associated with reduced fetal growth later in pregnancy, but UARI did not differ between infected/uninfected groups [19].

In rural coastal Papua New Guinea (PNG), both *P. falciparum* and *P. vivax* infection are endemic [20], and malaria is an important risk factor for LBW [21], together with maternal betel (areca) nut consumption [22], macronutrient undernutrition [23], and tobacco smoking [24]. Here, macronutrient undernutrition and anaemia were observed to be key risk factors for measuring SGA and for poor fetal weight gain [25]. In the same study, malaria parasitaemia was associated with SGA in univariable but not in adjusted analyses. To date, the relationship between malaria infection and fetoplacental and intrafetal blood flow has not been evaluated in PNG.

This study evaluated the impact of *P. falciparum* and *P. vivax* infection as well as other potential preventable and treatable risk factors for LBW and FGR, such as undernutrition and anaemia, on fetoplacental (UA) and MCA Doppler flow indices and fetal size in a cohort of pregnant women co-enrolled in a large malaria prevention study in PNG.

Methods

Study setting and population

The study cohort consisted of women who participated in both a randomized controlled trial investigating the impact of intermittent preventive treatment (IPTp) with azithromycin (AZ) plus sulfadoxine–pyrimethamine (SP) on birth weight [23] and a nested ultrasound study evaluating factors associated with reduced fetal size and fetal weight gain in PNG [25]. The studies were conducted between November 2009 and February 2013 at nine health centres in Madang and Sumkar districts situated on the north coast of PNG.

All participants provided written informed consent for participation in the original trial and the ultrasound

study. The research was approved by the PNG Institute of Medical Research, the PNG Medical Research Advisory Council and the Melbourne Health Human Research Ethics Committee, as previously described [23, 26].

The study area is characterized by perennial transmission of *P. falciparum* and *P. vivax*, and infant and adult macronutrient undernutrition is common; malarial infection, anaemia and undernutrition are important risk factors for FGR and LBW [21, 25, 27]. Up to 20% of newborns are LBW, which is an important contributor to PNG's high infant mortality rate of 47.4 per 1000 live births in 2013 [28].

Women aged 16–49 years with no co-morbidities, a fundal height of 26 cm or less above the symphysis pubis, and who resided near to participating antenatal clinics, were invited to join the parent trial following screening at their first antenatal clinic visit [23]. Dating ultrasound scans were performed at or shortly after enrolment when possible. Trial participants with a singleton pregnancy and a dating ultrasound before 25 completed gestational weeks were eligible for inclusion in the present study [29].

At enrolment sociodemographic and clinical characteristics were recorded, anthropometric indicators of nutritional status were evaluated, and haemoglobin (Hb) was measured (HemoCue Ltd., Angelholm, Sweden). As per national guidelines, moderate-to-severe anaemia (Hb < 90 g/L) was treated with iron/folate supplements and albendazole, malaria with quinine (first trimester) or artemether–lumefantrine, and insecticide-treated bed nets were provided [30].

Women were subsequently invited to attend further ultrasound scan sessions during which fetal size parameters were measured and UA and MCA flow was interrogated. Women with no Doppler data, or who had a stillborn or congenitally abnormal baby, were not included in the study cohort [31].

Clinical, sonographic and laboratory evaluations

At enrolment, information on educational and socio-economic status as well as risk behaviours such as tobacco smoking and betel (areca) nut chewing was obtained. Maternal anthropometrics such as height, weight and mid-upper arm circumference (MUAC) were recorded, and pulse, temperature and blood pressure were measured.

Maternal blood was collected for diagnostic purposes during subsequent scheduled and unscheduled visits, and birth weight (BW) was determined using a digital scale (Charder Medical, Taiwan, accuracy: 10 g).

Malaria infection was diagnosed by light microscopy (LM) and quantitative real-time polymerase chain

reaction (qPCR), using established methodologies [32, 33]. Placental malaria on histology was classified as active (presence of parasitized cells) and past (haemozoin only) [34]. Presence of inflammation at enrolment was assessed by using a high-sensitive enzyme-linked immunosorbent assay for C-reactive protein (hs-CRP) (RD Systems, Minneapolis, USA).

Transabdominal ultrasound scans were performed using a portable ultrasound scanner (Logiqbook XP, General Electric Medical Systems, UK). The crown-rump length (<13 gestational weeks) or head circumference (HC) (femur length (FL) if unavailable) were used to estimate GA (gestational age), in accordance with published guidelines [30]. The study did not use other estimators of GA, such as last menstrual period, given their poor performance in this and similar cohorts [35, 36].

A colour-pulsed Doppler ultrasound, using a 2–5 MHz convex abdominal probe, was performed to assess UA and MCA flow. Measurements were performed in the absence of fetal breathing and body movement, and were recorded over a minimum of three uniform heart cycles. UA Doppler flow was assessed on a free loop of cord. Absent or reverse end-diastolic flow was not observed in the cohort. MCA flow was interrogated from near field MCAs only using a transtemporal view: flow velocity waveforms were obtained from the area where the MCA joins the Circle of Willis. Fetal abdominal circumference (AC), HC and FL were measured as previously described [26]. Fetal weights were estimated using a formula developed by Hadlock et al. [37] using a combination of all three measurements (or AC plus FL when HC could not be measured adequately due to fetal position, $n = 9$). A random sample of image stills were sent for external quality control. Of 90 randomly selected UA Doppler stills, all were deemed of good quality (adequate cord position, adequate and good quality wave obtained, adequate Doppler beam placement). Quality control criteria and results of fetal size parameters for this cohort have previously been reported [26].

Exposures and outcome measures

Exposure variables to be examined a priori were selected based on the literature and findings from other secondary analyses of the parent trial. These included malaria infection, anthropometric indicators of women's nutritional status, risk behaviour (tobacco smoking, betel nut chewing, alcohol consumption), socio-economic markers, reported educational attainment, and malaria prevention regimen received.

Protein-energy undernutrition was defined as low body mass index (BMI < 18.5 kg/m²) or low MUAC (<22 cm) at enrolment, and short stature as height <150 cm [38].

Moderate-to-severe anaemia was defined as Hb <90 g/L at enrolment, and inflammation as a hs-CRP level ≥ 5 mg/L. These exposures were evaluated at study enrolment only.

Malaria infection was defined as positivity by light microscopy and/or qPCR. Speciation into *P. falciparum* or *P. vivax* was based on qPCR results [33]. Malaria infections were categorized into microscopic and sub-microscopic *P. falciparum* infections and *P. vivax* infections. Due to the low number of *P. vivax* infections, not all statistical analyses could be performed for this species.

Analyses were conducted for infections preceding or coinciding with Doppler ultrasounds in order to assess the effect of malaria infection on the subsequent/contemporaneous measurement. Most infections were detected at study enrolment. In addition, the analysis considered all infections detected (including those at delivery or after a Doppler scan) as a measure of exposure to malaria in general (similar to a 'force of infection' estimate). This is reasonable, as it can be expected that a considerable proportion of asymptomatic and low-level infections are only intermittently detected by PCR. Previous analyses of longitudinal cohort infection data has shown that PCR detection of infection is imperfect [39]. In pregnancy, this may be more pronounced as a result of placental sequestration. In addition, malaria transmission intensity on the north coast of PNG can vary considerably between villages [40]: it is likely a proportion of women is frequently exposed to infection, while the majority of women are not exposed to infection at all. As such, the detection of malaria infection during pregnancy is likely to be associated with undetected infection instances in the same woman at other study contacts, including those at or before Doppler scan. Analyses were also conducted considering only infections that were detected in the first 20 weeks of pregnancy.

Outcome variables were UARI [calculated as (peak systolic velocity – end diastolic velocity)/peak systolic velocity], MCAPI (calculated as (peak systolic velocity – end diastolic velocity)/time averaged velocity [41]), and cerebroplacental Doppler ratio (CPR), which is the ratio of the MCAPI and the UA pulsatility index.

For each, z-scores were calculated, to adjust for variations in GA at the time of the study, because fetoplacental and cerebral blood flow vary with GA [10, 11]. Previous research indicates that ethnic differences in fetoplacental flow may be limited [42], but sex-specific differences in umbilical and cerebral blood flow may exist [43].

Lastly, associations of measures indicating aberrant fetal growth with the Doppler measurements were evaluated. Specifically associations of Doppler indices with suspected SGA episodes at the time of scan, LBW, and placental malaria were investigated.

Statistical analysis

Statistical analyses were performed using Stata 12.0 (StataCorp, USA) and Mathematica 9.0 (Wolfram Inc., USA). The majority of MCAPI and CPR measurements fell outside the previously published reference ranges, (see Additional file 1). As such, an abnormal MCAPI and CPR was defined as below the 10th centile, and abnormal UARI as above the 90th centile, of ranges determined in this population. These ranges were derived by fitting linear mixed effects models using the fractional polynomial approach described by Royston et al. [44], which accounts for repeat measurements per woman. Response variables (i.e., UARI, MCAPI and CPR) were transformed if required as indicated by Box-Cox regression of the left-hand side of the model. Measurements outside of these 10th/90th centile bands were selected using z-scores (10th/90th centiles are equivalent to z-score of ± 1.28). In addition data analysis was performed using a categorical cut <1.0 to define an abnormal CPR.

To account for repeat measurements, associations were estimated using generalized estimating equations (GEE) with an exchangeable working correlation structure (binary outcomes) [45]. Analyses were adjusted a priori for fetal sex [18], gravidity and GA. Interactions (effect measure modification) were tested for nutritional indicators (BMI, MUAC, short stature), anaemia, inflammation, gravidity, and fetal sex as well as potential risk behaviour (tobacco smoking, betel nut chewing and alcohol) using multiplicative terms (equivalent to factorial interactions). $P < 0.15$ for the interaction term was used to define presence of effect measure modification.

Results

Baseline characteristics

The study cohort consisted of 396 singleton pregnancies that resulted in a congenitally normal live birth. The mean GA at enrolment was 19.6 weeks (Table 1). More than half of the women were primigravidae (53.3%), most lived rurally and 89 (22.47%) had a Hb reading below 90 g/L at enrolment (Table 1).

The 396 women were tested a total of 1565 times for malaria with either PCR or LM, 72 women tested positive for malaria infection in peripheral blood once, 13 twice and two women tested positive three times. Eighteen *P. falciparum* and seven *P. vivax* infections were detected prior to 20 completed weeks of gestation. Three infections episodes (all *P. falciparum*) were clinical.

A total of 1179 peripheral malaria infection screening episodes preceded or coincided with Doppler scans. Overall, 59 were positive for *P. falciparum* by LM or qPCR, and 27 infections were sub-microscopic. A total of 16 *P. vivax* infections were detected by either LM or qPCR (one LM and qPCR, 15 by qPCR only).

Table 1 Characteristics of study participants (N = 396)

Characteristic	N/total (%) or mean [SD]
Age (years)	24.3 (5.3)
Rural residence	239/381 (62.7)
Literate	375/396 (93.7)
Smoker	82/396 (20.7)
Areca nut user	320/395 (81.0)
Number of previous pregnancies	
0	211/396 (53.3)
1	87/396 (22.0)
≥2	98/396 (24.8)
Gestational age at recruitment (weeks)	19.6 (3.8)
Peripheral parasitaemia at recruitment by microscopy	19/396 (4.6)
Low mid-upper arm circumference	53/396 (13.4)
Low body mass index	17/396 (4.3)
Moderate-to-severe anaemia	89/396 (22.5)
Delivery outcomes	
Female newborn sex	212/395 (53.7)
Low birthweight (<2500 g)	67/396 (16.9)
Placental malaria on histology ^a	46/308 (14.9)

^a Active and past placental infection

Doppler velocimetry measurements

Of 396 women, 361 had UA Doppler studies: 282 had one, 67 had two and 12 had three studies (total: 452). MCA studies were performed on 298 women who had 370 MCA Doppler scans: 235 women were studied once, 55 women twice, seven women three times, and one woman four times. CPR data was available for 252 women, who had 303 paired UA and MCA studies. Of these, 206 women were studied once, 41 women twice and five women three times.

Regression of Doppler velocimetry indices versus gestational age

The best fit equations (mean and standard deviations) for the relationship between UARI (Eqs. 1a, 1b), MCAPI (Eqs. 2a, 2b) and CPR (Eqs. 3a, 3b) with GA (weeks) are given below, where μ (a) are means and σ (b) are standard deviations. The equations were used to determine potentially abnormal values based on a 10th/90th centile cut-off (z = 1.28).

$$\mu_{UARI}[X] = 0.12220 + 7.01831X^{-1}; X = GA^2 \tag{1a}$$

$$\sigma_{UARI}[X]^2 = 0.01905 + 3.89066 X^{-2} - 0.41972 X^{-1}; \tag{1b}$$

$$\mu_{MCAPI}[X] = -0.4569 + 0.298861 \text{ Sqrt}[X]; X = GA^{0.6835705} \tag{2a}$$

$$\sigma_{MCAPI}[X]^2 = 1.3561 - 0.488423 \text{ Sqrt}[X] + 0.0455214 X \tag{2b}$$

$$\mu_{CPR}[X] = 0.287651 + 0.0291729 X; X = GA^{0.4537937} \tag{3a}$$

$$\sigma_{CPR}[X]^2 = 0.0288508 - 0.00101161 X + 0.0000351 X^2 \tag{3b}$$

The Doppler velocimetry data and the respective regression curves and confidence intervals are shown in Fig. 1.

Impact of malaria infection on Doppler velocimetry measurements

Tables 2, 3, 4 show the associations of malaria infections with the Doppler velocimetry measurements. Associations with other characteristics such as nutritional and socio-economic indicators, IPTp consumption, betel nut chewing, alcohol drinking and tobacco smoking, were not significant and are given in Additional files 2, 3 and 4.

There was a significant association between the presence of *P. falciparum* parasitaemia before or at time of Doppler interrogation and a raised UARI measurement [adjusted odds ratio (aOR) 2.3, 95% CI 1.0–5.2; P = 0.047] (Table 2 and Additional file 5). When all peripheral infections were evaluated (before, during and after the Doppler measurements), similar, if not stronger effects were noted. *Plasmodium falciparum* infection during study participation was significantly correlated with having an elevated UARI measurement (>90th centile) [2.4 (95% CI 1.1–4.9); P = 0.024] (Table 2). In addition, there was a tendency towards an increased UARI amongst women who had only sub-microscopic *P. falciparum* infection (2.6, 1.00–6.6; P = 0.053). Malaria infections in the first 20 weeks of pregnancy were not associated with abnormal Doppler measurements.

There were no statistically significant associations between peripheral infections detected prior to or at the scan episode and reduced MCAPI. When all peripheral infections detected during pregnancy were considered, *P. falciparum* parasitaemia was associated with reduced MCAPI (2.7, 1.2–5.3; P = 0.012); a similar association was observed for sub-microscopic *P. falciparum* infections (Table 3), and the odds of abnormal MCAPI measurements increased with the number of infections detected (6.0, 1.7–20.8; P = 0.005).

There were no statistically significant associations of malaria infection with a reduced CPR when only peripheral infections detected prior to or at scan were analysed, although the association with previous *P. falciparum* infection approached significance (P = 0.11) (Table 4). However, there was a tendency towards an association

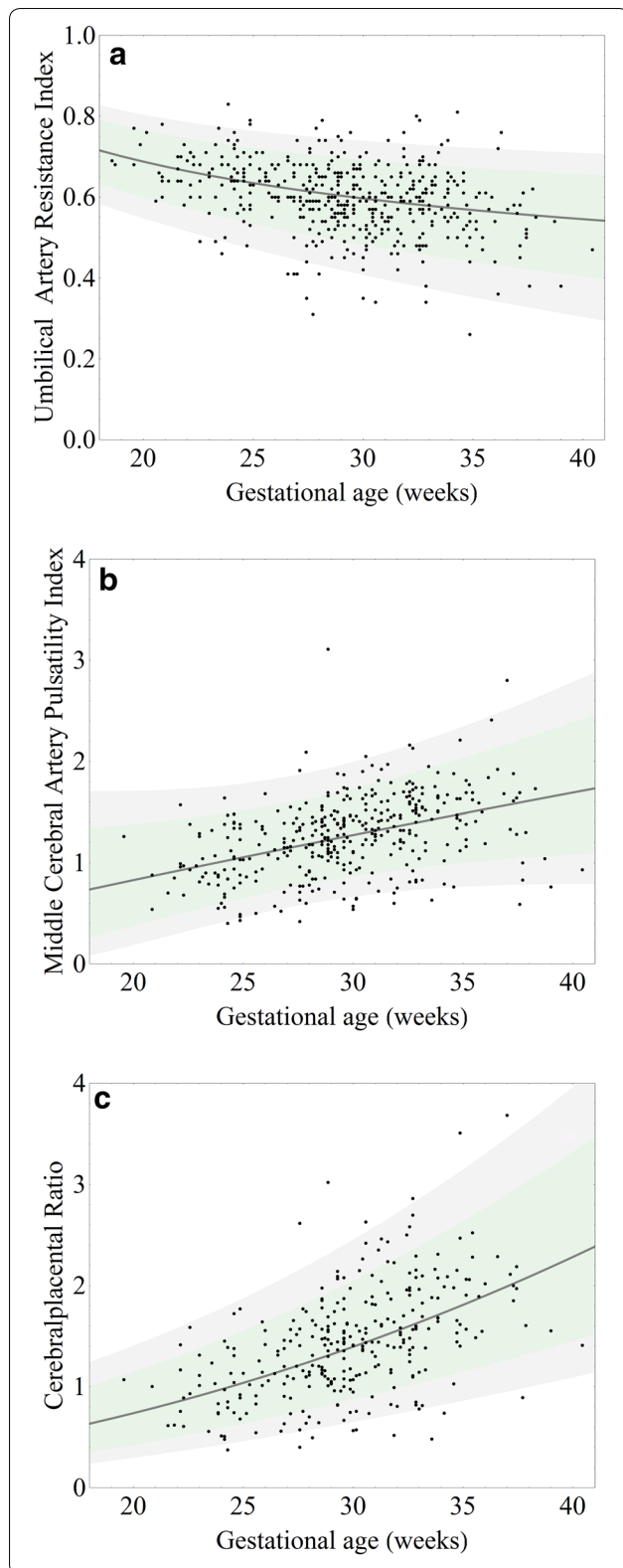


Fig. 1 Umbilical artery resistance index (a), middle cerebral artery pulsatility index (b) and cerebral/placental Doppler ratio (c) change with gestational age and regression curves. Black lines are the means (μ) of the mixed effects model regression (given in Eqs. 1a, 2a, 3a). The shaded areas are the intervals between which 95% (grey) and 80% (light green) of the data are located, equivalent to the 2.5th/97.7th and 10th/90th centiles and corresponding to Eqs. (1b, 2b, 3b) calculated as per Royston [18]

between exposure to *P. falciparum* and a reduced CPR ($P = 0.09$, Table 4). In particular, sub-microscopic infections (3.6, 1.2–10.9, $P = 0.021$) and cumulative parasite detection (6.2, 1.5–25.5; $P = 0.012$) were associated with increased risk of reduced CPR. When a cut-off of 1.0 was used to define an abnormal CPR only cumulative parasite detection remained associated with an increased risk of an abnormal CPR ($P = 0.024$) (Additional file 6).

Association of abnormal Doppler velocimetry with SGA, LBW and placental malaria

There were 50 episodes of SGA (estimated fetal weight <10th centile of the Hadlock reference standard) at the time of Doppler ultrasound in the cohort, 67 newborns (16.9%) had LBW and 46 out of the 308 histological analyses (14.9%) indicated placental infection (past and active infection).

Table 5 shows the associations between Doppler velocimetry measurements and measuring SGA at the time of scan, LBW (<2500 g) or placental infection.

A reduced CPR was associated with SGA (2.8, 95% CI 1.0–7.7, $P = 0.053$) and LBW (2.5, 1.01–5.7; $P = 0.033$), and similar results were observed when low CPR was defined as $CPR < 1.0$ (Table 5). An increased UARI was associated with placental infection (5.1, 2.3–10.9, $P < 0.001$). However, an UARI <10th centile had comparatively low sensitivity for the detection of (occult) placental malaria (sensitivity: 0.26; positive predictive value: 0.41) but may be a useful tool to exclude women with placental infection (specificity: 0.93; negative predictive value: 0.87).

There was no effect measure modification of the association between malaria infection and UARI, MCAPI or CPR by characteristics such as MUAC <22 cm, BMI <18.5 kg/m² or height <150 cm, risk behaviours such as smoking, drinking alcohol or betel nut chewing habits, or gravidity or fetal sex.

Discussion

In this first Doppler ultrasound study from PNG to determine factors related to abnormal fetoplacental and intrafetal blood flow, peripheral *P. falciparum*

Table 2 Association of peripheral blood malaria infection with elevated umbilical artery resistance index (>90th centile)

	% (N)	OR (95% CI)	P	aOR (95% CI) ^a	P
Peripheral malaria infection prior to Doppler scan ^b					
Any species	13.9 (63/452)	1.8 (0.8–3.9)	0.17	1.7 (0.8–3.9)	0.20
<i>P.f.</i>	11.5 (52/452)	2.3 (1.0–5.1)	0.048	2.3 (1.0–5.2)	0.047
<i>P.v.</i>	3.1 (14/452)	–	–	–	–
<i>P.f.</i> (sub)	4.8 (21/442)	2.3 (0.7–7.3)	0.15	2.1 (0.7–6.5)	0.20
Peripheral malaria infection at any stage in pregnancy (as a measure of exposure) ^c					
Any species	21.1 (76/361)	2.0 (1.0–4.0)	0.05	1.9 (0.9–4.0)	0.07
<i>P.f.</i>	16.6 (60/361)	2.4 (1.1–4.9)	0.021	2.4 (1.1–4.9)	0.024
<i>P.v.</i>	6.7 (24/361)	1.6 (0.6–4.9)	0.37	1.6 (0.5–5.0)	0.42
<i>P.f.</i> (sub)	7.4 (26/350)	2.7 (1.1–7.0)	0.04	2.6 (1.0–6.6)	0.05
Cumulative malaria detections with any species (as measure of exposure) ^c					
1	17.5 (63/361)	1.8 (0.9–4.0)	0.12	1.8 (0.8–3.9)	0.15
≥2	3.6 (13/361)	2.6 (0.7–9.2)	0.14	2.6 (0.7–9.8)	0.15

P.f., *P. falciparum*; *P.v.*, *P. vivax*; sub, sub-microscopic

^a Adjusted for fetal sex, gravidity and gestational age

^b N (%) represents the number of scans

^c N (%) represents the number of women

Table 3 Association of peripheral malaria infection with reduced fetal middle cerebral artery pulsatility index (<10th centile)

	% (N)	OR (95% CI)	P	aOR (95% CI) ^a	P
Peripheral malaria infection prior to Doppler scan ^b					
Any species	14.8 (55/370)	1.5 (0.6–3.5)	0.35	1.4 (0.6–3.2)	0.42
<i>P.f.</i>	12.7 (47/370)	1.9 (0.8–4.5)	0.15	1.8 (0.8–4.2)	0.17
<i>P.v.</i>	3.2 (12/370)	–	–	–	–
<i>P.f.</i> (sub)	6.6 (24/362)	1.5 (0.5–4.5)	0.52	1.4 (0.5–4.2)	0.59
Peripheral malaria infection at any stage in pregnancy (as a measure of exposure) ^c					
Any species	22.8 (68/298)	1.9 (0.9–3.9)	0.09	1.8 (0.9–3.79)	0.10
<i>P.f.</i>	18.1 (54/298)	2.6 (1.3–5.5)	0.010	2.6 (1.2–5.3)	0.012
<i>P.v.</i>	7.1 (21/298)	0.5 (0.1–4.0)	0.53	0.5 (0.1–3.9)	0.51
<i>P.f.</i> (sub)	9.4 (27/287)	2.9 (1.1–7.5)	0.027	2.8 (1.1–7.5)	0.035
Cumulative malaria detections with any species (as a measure of exposure) ^c					
1	18.8 (56/298)	1.2 (0.5–2.8)	0.63	1.2 (0.5–2.7)	0.69
≥2	4.0 (12/298)	6.2 (1.8–21.5)	0.004	6.0 (1.7–20.8)	0.005

P.f., *P. falciparum*; *P.v.*, *P. vivax*; sub, sub-microscopic

^a Adjusted for fetal gender, gravidity and gestational age

^b N (%) represents the number of scans

^c N (%) represents the number of women

parasitaemia was associated with an increased UARI and a decreased MCAP. Associations were strongest when the impact of overall malaria exposure (peripheral infection during any stage of pregnancy) on flow was evaluated. Here, increased UARI and intrafetal flow redistribution were observed amongst women with microscopic or sub-microscopic *P. falciparum* infections. No such effects were observed for *P. vivax* infections or infections detected in early pregnancy (<20 gestational

weeks) but their numbers were low. Documented episodes of increased UARI were associated with histological evidence of placental malaria at delivery.

The present study contributes further evidence that *P. falciparum* infection can have deleterious effects on fetoplacental flow and intrafetal blood flow redistribution. Concurrent *P. falciparum* infection in early pregnancy was associated with increased UARI in two studies from Kenya [16, 17], and women with symptomatic falciparum malaria

Table 4 Association of peripheral malaria infection with reduced cerebroplacental Doppler ratio (<10th centile)

	% (N)	OR (95% CI)	P	aOR (95% CI) ^a	P
Any malaria infection prior to Doppler scan ^b					
Any species	14.8 (45/303)	1.7 (0.7–4.37)	0.25	1.7 (0.7–4.3)	0.25
<i>P.f.</i>	12.5 (38/303)	2.2 (0.9–5.7)	0.09	2.2 (0.8–5.5)	0.11
<i>P.v.</i>	3.3 (10/303)	–	–	–	–
<i>P.f.</i> (sub)	5.7 (17/296)	2.5 (0.7–8.2)	0.14	2.4 (0.7–8.2)	0.16
Peripheral malaria infection at any stage in pregnancy (as a measure of exposure) ^c					
Any species	21.4 (54/252)	1.6 (0.7–3.6)	0.29	1.6 (0.7–3.7)	0.29
<i>P.f.</i>	17.1 (43/252)	2.2 (0.9–5.0)	0.08	2.1 (0.9–5.0)	0.09
<i>P.v.</i>	7.1 (18/252)	1.1 (0.2–5.2)	0.91	1.2 (0.3–5.5)	0.84
<i>P.f.</i> (sub)	8.2 (20/244)	3.7 (1.3–10.7)	0.014	3.6 (1.2–10.9)	0.021
Cumulative malaria detections with any species (as a measure of exposure) ^c					
1	17.9 (45/252)	0.9 (0.3–2.5)	0.85	0.9 (0.3–2.5)	0.86
≥2	3.6 (9/252)	6.7 (1.6–27.5)	0.008	6.17 (1.5–25.5)	0.012

P.f., *P. falciparum*; *P.v.*, *P. vivax*; sub, sub-microscopic

^a Adjusted for fetal gender, gravidity and gestational age

^b N (%) represents the number of scans

^c N (%) represents the number of women

Table 5 Doppler velocimetry measurements and associations with measuring small for gestational age at delivery, low birth weight and placental infection on histology

	% (N)	OR (95% CI)	P	aOR (95% CI)*	P*
Small for gestational age (n = 50) ^b					
Umbilical artery resistance index >90th centile	12.0 (44/368)	1.3 (0.4–4.0)	0.65	1.2 (0.4–4.1)*	0.73
Middle cerebral artery pulsatility index <10th centile	11.6 (34/293)	2.0 (0.7–5.4)	0.16	2.3 (0.8–6.1)	0.10
Cerebroplacental Doppler ratio <10th centile	11.5 (28/243)	2.1 (0.8–6.0)	0.15	2.7 (1.0–7.7)	0.053
Cerebroplacental Doppler ratio <1.0	11.5 (28/243)	1.4 (0.6–3.4)	0.48	3.3 (1.2–9.2)	0.024
Low birth weight (n = 67) ^c					
Umbilical artery resistance index >90th centile	16.6 (60/361)	1.3 (0.6–2.7)	0.52	1.3 (0.6–2.8)	0.56
Middle cerebral artery pulsatility index <10th centile	17.8 (53/298)	1.9 (0.9–4.1)	0.08	2.0 (1.0–4.3)	0.07
Cerebroplacental Doppler ratio <10th centile	17.8 (45/252)	2.8 (1.3–6.3)	0.011	2.5 (1.1–5.7)	0.033
Cerebroplacental Doppler ratio <1.0	17.8 (45/252)	2.6 (1.3–4.9)	0.005	2.8 (1.3–6.0)	0.007
Placental malaria (n = 46) ^c					
Umbilical artery resistance index >90th centile	15.6 (44/281)	4.9 (2.3–10.7)	<0.001	5.1 (2.3–10.9)	<0.001
Middle cerebral artery pulsatility index <10th centile	14.5 (34/234)	1.8 (0.7–5.1)	0.24	1.8 (0.6–5.0)	0.23
Cerebroplacental Doppler ratio <10th centile	14.7 (29/197)	1.5 (0.5–5.0)	0.47	1.3 (0.4–4.6)	0.65
Cerebroplacental Doppler ratio <1.0	14.7 (29/197)	1.8 (0.8–4.3)	0.16	1.8 (0.7–4.8)	0.26

* Adjusted for fetal sex, gravidity and gestational age

^a N (%) represents the number of scans

^b N (%) represents the number of women

had increased UARI in research from French Guiana [14]. An increased UARI may be indicative of some level of placental insufficiency, either temporary or permanent. Previously proposed mechanisms include negative effects of *P. falciparum* infection on placental vascular neogenesis, or potentially on uterine spiral artery invasion and conversion by reducing trophoblast motility and invasion capacity [5, 7]. Alternatively, *P. falciparum* infection may lead to more acute processes affecting placental function and perfusion,

e.g., inflammation and placental thromboxane production. For the first time, the present research shows that falciparum malaria is associated with intrafetal blood flow redistribution, the diversion of blood flow from peripheral organs to more 'essential' organs, such as the brain. This can be a sonographic manifestation of growth restriction, and in clinical practice MCAP is used to assess severity of suspected FGR in fetuses measuring SGA and who have normal umbilical artery Doppler studies [10]. Although malaria

infection was not associated with SGA, malarial infection alters Doppler flow indices in a manner suggesting at least temporary placental insufficiency: anti-malarial treatment provided as part of the parent trial and unscheduled visits for ill health may have limited the establishment of the potentially more deleterious chronic placental infections.

Interestingly, malaria infection was not associated with measuring SGA in pregnancy or at birth in this cohort [25]. Most malaria infections were detected at enrolment, when all women received presumptive anti-malarial treatment. Clearance of infections at study enrolment may have limited their chronic effects on placental development and function [23]. Consistent with this, there were no differences in UARI or MCAPI by treatment arm (single treatment with SP and chloroquine or three doses of SP and AZ (see Additional files 2, 4, 5) or SGA [25]. Nevertheless, in the trial analysis active placental infection was reduced amongst women receiving SPAZ, and placental infection was a risk for LBW [23].

The present study observed stronger associations between malaria 'exposure' (peripheral infections detected at any stage during pregnancy) and Doppler indices than for malaria infections detected in peripheral blood at or before the Doppler study time point. The rationale for assessing associations of infections detected after ultrasound scan is that there is increasing evidence that in areas with declining malaria transmission a large number of infections remain undetected even by qPCR, in particular in pregnancy [46–48]. In addition, infection risk is clustered, i.e., the same individuals tend to get re-infected, and this is particularly common with *P. falciparum* [48]: women with malaria detection after scan are more likely to have been infected prior or at scan, than those who were not.

Plasmodium vivax infections have been previously associated with LBW, and research indicates that such infections also affect umbilical artery flow [18, 49]. The present study was unable to demonstrate an effect of vivax malaria on UA or MCA flow, possibly because there were few detectable *P. vivax* infections and the study lacked power to demonstrate such relationships. There was no observable effect measure modification of the *P. falciparum* parasitaemia-blood flow relationship by maternal nutritional status or gravidity, in contrast to what has been demonstrated in other similar studies [17, 50].

Plasmodium falciparum infection was associated with increased UA resistance in all analyses (infection before or at scan, any peripheral infection), yet the 'exposure' analysis revealed important additional associations, such as the impact of sub-microscopic *P. falciparum* infections on flow, with particularly strong impacts on the MCAPI and the CPR. Of note, a low CPR was the Doppler ultrasound measurement most strongly associated with LBW in this

cohort. There is evidence to suggest that sub-microscopic *P. falciparum* infections can cause LBW, anaemia and possibly preterm birth [45, 46]. This is of great concern, as these infections are common and are frequently undetectable by rapid diagnostic tests. As such, strategies such as intermittent screening and treatment for malaria are likely to miss infections that are directly involved in causing fetal pathology and adverse pregnancy outcome.

In contrast to other cohorts, gravidity, fetal sex [18] and MUAC, height and anaemia [51] were not associated with UARI or MCAPI. Risk behaviour such as smoking tobacco, chewing betelnut and drinking alcohol did not statistically significantly increase the proportion of abnormal Doppler episodes, although smoking tobacco, as a risk factor for high UARI, approached significance ($P = 0.085$, Additional file 2) [52].

Women with increased UARI measurement during pregnancy were more likely to have evidence of placental infection on histology. Although UARI had low sensitivity (0.26) for the detection of placental malaria, it had a good negative predictive value (0.87). Where ultrasound is available, further evaluation of its ability to predict placental malaria infection is indicated. The sensitivity of UARI could be improved by combining this measurement with other simple investigations that may help identify women with occult placental malaria [53].

This study has some limitations. Firstly, the study was insufficiently powered to evaluate the effect of early pregnancy malaria and vivax malaria on fetoplacental and intrafetal flow. Secondly, the number of Doppler velocimetry measurements and malaria screening episodes was comparatively small, and thus episodes of abnormal flow or infection may have been missed. Only ~60 women had Doppler indices measured at two or more timepoints during pregnancy. In addition, only one measurement per assessment (UA, MCA) was taken during each scanning episode, instead of the recommended three measurements [10]. Furthermore, there was no external quality control for the MCA measurements, although all image stills for UA measurements randomly selected for review passed quality control. Thirdly, the study may not have measured and adjusted for all confounding factors, such as indoor pollution. Lastly, previously published reference ranges for MCAPI and CPR could not be used for analysis as distribution of these variables in the study population differed significantly from published reference standards [10, 11]. As such, a within-population analysis was performed deriving 10th/90th centiles using an established approach [44] and defining likely 'abnormal' Doppler indices as those outside of the 10th/90th centile boundaries. In addition, analyses were repeated using a categorical cut off of <1.0 to define an abnormal CPR. It is possible that the observed difference in population averages is due to the known lower

blood pressures in rural PNG populations than those of Westernized populations [54, 55].

Conclusion

In PNG, both microscopic and sub-microscopic *P. falciparum* infections in pregnancy are associated with increased UARI and reduced MCAPI, which are indicators of placental insufficiency and fetal blood flow redistribution, respectively, and are both associated with FGR. Malaria prevention strategies for pregnant women must be able to clear all infections, including sub-microscopic ones. In malaria-endemic areas an abnormal UARI could indicate presence of placental infection, and women may benefit from presumptive treatment with a highly effective anti-malarial.

Additional files

Additional file 1. Umbilical artery resistance index (UARI, Panel A), middle cerebral artery pulsatility index (MCAPI, Panel B) and cerebroplacental Doppler ratio (CPR, Panel C) data from this study and reference ranges for change with gestational age from previous studies [10, 11]. While the UARI reference range overlaps significantly with the data collected in the present study, the MCAPI and CPR reference ranges are markedly different from the present data. This necessitated the derivation of population-specific definitions of abnormal Doppler velocimetry measurements, as described in "Methods" section.

Additional file 2. Malaria infection, nutritional factors, smoking, and other potential associations with elevated umbilical artery resistance index (UARI, >90th centile) during pregnancy in PNG women. In contrast to other cohorts, nutritional factors such as MUAC, height and anaemia were not associated with increased UARI. Smoking tobacco, a known risk factor for elevated UARI approached significance ($P = 0.085$) while other risk behaviours, such as chewing betelnut and drinking alcohol, showed no association.

Additional file 3. Malaria infection, nutritional factors, smoking, and other potential associations with decreased middle cerebral artery pulsatility index (MCAPI, <10th centile) during pregnancy in PNG women. No significant differences were observed when various known nutritional and behavioural risk factors were assessed.

Additional file 4. Malaria infection, nutritional factors, smoking, and other potential associations with decreased cerebroplacental Doppler ratio (CPR, <10th centile) during pregnancy in PNG women. Cerebroplacental ratio was not significantly influenced by various nutritional, behavioural and other factors.

Additional file 5. Associations of recent malaria infection with Doppler indices. A total of 533 malaria tests preceded the Doppler scan by no more than 6 weeks and were thus considered 'recent'. In this category, 17 were positive for *P. falciparum* with either method (13 by qPCR; 11 by LM) and two were positive for *P. vivax*, by qPCR. Of these, seven infections were sub-microscopic. These data are not shown in the main text as the numbers of recent malaria episodes were too low to confidently perform statistical analyses.

Additional file 6. Association of peripheral malaria infection with reduced cerebroplacental Doppler ratio (<1.0).

Abbreviations

UA: umbilical artery; MCA: middle cerebral artery; CPR: cerebroplacental ratio; GEE: generalized estimating equations; FGR: fetal growth restriction; LBW: low birth weight; LMICs: low-and middle-income countries; UARI: umbilical artery

resistance index; MCAPI: middle cerebral artery pulsatility index; SGA: small-for-gestational age; PNG: Papua New Guinea; IPTp: intermittent preventive treatment in pregnancy; SP: sulfadoxine pyrimethamine; AZ: azithromycin; Hb: haemoglobin; MUAC: mid-upper arm circumference; BW: birth weight; qPCR: quantitative polymerase chain reaction; hs-CRP: high sensitive C-reactive protein; HC: head circumference; FL: femur length; GA: gestational age; BMI: body mass index; LM: light microscopy; aOR: adjusted odds ratio; EFW: estimated fetal weight; AC: abdominal circumference; LM: light microscopy.

Authors' contributions

SJR, MO and HWU conceived and designed the study; MO, HWU and RAW supervised enrolment and follow-up of participants; MO and HWU performed scans; MO, SK and HWU verified, analysed and interpreted the data; MO, SK and HWU wrote the first draft of the manuscript. All authors critically revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

As per PNG Institute of Medical Research policy, the data are available upon request addressed to the corresponding author.

Ethics approval and consent to participate

All participants provided written informed consent for participation in the original trial and the ultrasound study. The research was approved by the PNG Institute of Medical Research, the PNG Medical Research Advisory Council and the Melbourne Health Human Research Ethics Committee, as previously described [23, 26].

Consent for publication

All participants provided written consent for publication.

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