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Evaluation of the OptiMAL Rapid Antigen Test and Species-Specific PCR To Detect Placental *Plasmodium falciparum* Infection at Delivery

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During pregnancy, *Plasmodium falciparum* infection of the placenta frequently occurs in the absence of parasites in peripheral blood. We investigated the abilities of the OptiMAL rapid immunochromatographic strip test for *P. falciparum* lactate dehydrogenase and species-specific PCR performed on peripheral blood to detect placental infection or malaria-associated low birth weight. Of 509 Malawian women screened by microscopy, 76 had malaria infection. Among these 509 women, the frequency of peripheral blood parasitemia was low. The OptiMAL test gave positive results in 37 of 171 women tested (one of whom had placental but not peripheral blood parasitemia) and had sensitivities of 71% for peripheral parasitemia and 38% for placental parasitemia compared to the microscopy values. The specificity for peripheral parasitemia was 94%. In 135 women, PCR had sensitivities of 94% for peripheral blood malaria detected by microscopy and 72% for placental infection. In samples examined by PCR, the prevalence of malaria in peripheral blood increased from 26.7% by microscopy to 51.9%. Women with placental malaria and women with malaria in peripheral blood samples by microscopy or OptiMAL testing, but not women with malaria detected only by PCR, had lower-birth-weight babies than did women without malaria by these criteria. Positive results by PCR in the absence of microscopic parasitemia were not associated with low birth weight. Neither OptiMAL nor PCR testing of peripheral blood is adequately sensitive to detect all placental malaria infection, but a positive result by OptiMAL testing identifies women with a high proportion of low-birth-weight babies.

There are more than 20 million pregnancies annually in African countries where malaria is endemic, and malaria infection is more common in pregnant women than their nonpregnant counterparts (8). Malaria in pregnancy is associated with adverse outcomes for mother and fetus, notably maternal anemia (which predisposes to maternal mortality) and low birth weight (which predisposes to infant mortality) (3, 5). Antimalarial drug interventions may decrease the incidence of these complications of malaria in pregnancy but do not abolish them (12, 14). In many studies, the strongest associations with poor outcomes are with placental parasite density, but placental parasitemia is often not associated with peripheral parasitemia and can be diagnosed only at delivery (7, 12).

Species-specific PCR for *Plasmodium falciparum* is a highly sensitive tool for detection of peripheral blood parasitemia. In adults, submicroscopic *P. falciparum* infection is frequent (2). More pregnant women are found to be malaria infected by PCR than by peripheral blood film examination (9), but PCR has not been evaluated for its ability to detect placental malaria infection in the presence or absence of malaria parasites in peripheral blood.

Rapid antigen tests make use of the secretion of soluble parasite-derived proteins into the circulation, which may be detected immunochromatographically using strips impregnated with antibody to the protein. Recently, tests based on two such proteins, *P. falciparum* histidine-rich protein 2 (HRP-2) and *P. falciparum* lactate dehydrogenase (LDH) have been developed and marketed, primarily as alternatives to microscopy for diagnosis of malaria infection (11). The sensitivity of the OptiMAL rapid test, an immunochromatographic strip test, has proved similar to the sensitivity of microscopy in both developing and developed countries (4, 10). An enzyme-linked immunosorbent assay (ELISA) and a strip test for HRP-2 were both sensitive for *P. falciparum* infection in pregnant women in Cameroon (7).

The ability to detect clinically significant malaria infection of the placenta prior to delivery, at prenatal clinics, could lead to more-effective testing and management of malaria in pregnancy. Clearance of infection with antimalarial therapy could then permit catch up growth of the fetus. Using peripheral blood samples, we evaluated the OptiMAL test and a species-specific PCR and compared these tests to microscopy for their ability to detect placental malaria infection at delivery in pregnant Malawian women. We wished to determine whether these tests had advantages over microscopy for diagnosis of placental malaria and how well detection of parasite DNA or protein correlated with malaria's principal negative outcome in pregnancy, decreased infant birth weight.

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TABLE 1. Sensitivity and specificity of peripheral blood microscopy, OptiMAL test, and PCR for detection of *P. falciparum* infection

Method	Peripheral blood malaria		Placental blood malaria		All malaria infection	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Peripheral blood microscopy			38/73 (52.1)	38/41 (92.7)	41/76 (53.9)	
OptiMAL test	29/41 (70.7)	122/130 (93.8)	28/73 (38.4)	89/98 (90.8)	30/76 (39.5)	88/95 (92.6)
Species-specific PCR	34/36 (94.4)	63/99 (63.6)	47/65 (72.3)	47/70 (67.1)	50/68 (73.5)	47/67 (70.1)

MATERIALS AND METHODS

The study was performed between October and December 2000 at Queen Elizabeth Central Hospital, Blantyre, Malawi, a tertiary hospital serving the southern region of the country. Women delivering singleton live-born infants were eligible for the study, if they delivered while study staff were in attendance on the Labour Ward. Written consent was obtained for the collection of a 2-ml venous blood sample (for blood film preparation and antigen test) and a placental smear was made by incision of the maternal surface of the placenta. A single smear from each site was examined by two observers. Blood films were air dried and stained with Field's stain, and the number of parasites in 200 leukocytes was counted. Parasitemia was determined using an assumed leukocyte count of 8,000/μl. Whole-blood samples were kept at 4°C until microscopy results were available. After the first 19 patients, a case control design was introduced, such that each woman with parasites detected in peripheral and/or placental blood (case) was matched to a woman of the same gravidity, ± 1 year of age, and receiving the same number of doses of antimalarials during pregnancy. Blood was tested for the presence of *P. falciparum* LDH, using the OptiMAL test (Flow, Inc., Portland, Oreg.) performed according to the manufacturer's instructions.

For species-specific PCR, DNA was extracted from the cell pellet of 2-ml blood samples using the Nucleon BACC 3 kit (Technel Life Science PLC, Manchester, United Kingdom) and resuspended in 100 μl of Tris-EDTA (TE). Five microliters of DNA solution was used in a total reaction mixture volume of 20 μl for the first round of a nested PCR, and 1 μl of this reaction mixture was used for the second round of PCR. Primers were derived from the small-subunit rRNA gene of *P. falciparum*, and conditions were as previously described (15).

Data were entered into Epiinfo version 6 (Centers for Disease Control and Prevention, Atlanta, Ga.) and analyzed with Epiinfo and with Stata version 6 (Stata Corporation, College Park, Tex.). For normally distributed data (age and birth weight), Student's *t* test was used to compare groups.

Ethical considerations. The study was approved by the College of Medicine Research Committee, University of Malawi.

RESULTS

A total of 509 women entered the study, of whom 202 (39.7%) were primigravidae, 132 (25.9%) were in their second pregnancy, and 175 (34.4%) were multigravidae (gravida of 3 or more). Mean age was 23.2 ± 5.2 years. Malaria infection was detected in 76 of 509 women (cases; 14.9%), of whom 35 had placental infection alone, 38 had placental and peripheral infection, and 3 had peripheral infection alone. For the 38 women with peripheral and placental parasitemia, parasite densities in the two sites were not significantly correlated with one another ($P = 0.42$, not statistically significant). Antigen tests were performed on 171 women (76 cases, 76 matched controls, and 19 unmatched uninfected women). OptiMAL testing was performed on women who were younger (mean age, 22.1 ± 4.6 years) than untested women (23.7 ± 5.5 years; $P = 0.0008$) and less likely to be multigravidae (24.0% compared to 39.6%; $P < 0.001$), reflecting the higher incidence of malaria in first and second pregnancies (18.9%) than later pregnancies (7.4%; $P = 0.001$). The 76 women with malaria had significantly lower-birth-weight babies (mean ± standard deviation [SD], 2,667 ± 514 g) than 433 women without infection (3,040 ± 521 g; $P < 0.0001$) and than age- and parity-matched controls (3,092 ± 541 g; $P < 0.0001$).

OptiMAL test results of peripheral blood samples were positive in 30 (39.5%) of 76 women with *P. falciparum* detected in peripheral and/or placental blood and 7 (7.4%) of women with negative peripheral and placental blood microscopy results (Table 1). In all cases, results consistent with *P. falciparum* infection were obtained. Among women with peripheral blood infection, the sensitivity of OptiMAL was 70.7% (29 of 41), and for placental malaria, it was 38.4% (28 of 73). The OptiMAL sensitivity for peripheral blood infection, and to a lesser extent placental infection, was significantly higher with increasing parasitemia (Table 2).

OptiMAL test results were significantly associated with birth weight, and low birth weight (<2,500 g) was significantly more common in women who tested positive by OptiMAL (Table 3). Among women with placental infection, those with positive OptiMAL test results had significantly lower-birth-weight babies and higher prevalence of low-birth-weight babies than did women with placental malaria who were OptiMAL negative. For 338 women not tested by OptiMAL, the mean birth weight ± SD was 3,017 ± 520 g, and low birth weight was seen for 40 of their babies (11.8%; $P = 0.25$ [not significantly different from the results for women with negative OptiMAL results]).

PCR was performed on peripheral blood samples from 135 women, of whom 120 also had OptiMAL tests performed. Malaria infection was detected by PCR in 70 of 135 women (51.8%), including 50 of 68 women (73.5%) with malaria in peripheral blood samples and/or placental film, and 20 of 67 women (29.8%) with negative results for malaria by microscopy. PCR was highly sensitive for peripheral blood infection

TABLE 2. Relationship of antigen test and PCR results to peripheral and placental parasitemia

Sample and parasite density[a]	Antigen positive[b]	PCR positive[b]
Peripheral blood		
0	8/130 (6.2)	36/99 (36.4)
1–499	3/11 (27.3)	9/9 (100)
500–1,499	5/9 (55.6)	8/8 (100)
1,500–5,000	10/10 (100)	7/9 (77.8)
>5,000	11/11 (100)	10/10 (100)
Total	37/171 (21.6)	70/135 (51.9)
Placental blood		
0	9/98 (10.1)	23/70 (32.9)
1–499	1/19 (5.3)	10/19 (52.6)
500–1,499	4/18 (22.2)	10/14 (71.4)
1,500–5,000	10/21 (47.6)	17/19 (89.5)
>5,000	13/15 (86.7)	10/13 (76.9)
Total	37/171 (21.6)	70/135 (51.9)

[a] Parasite density given in the number of parasites per milliliter of blood.
[b] Number of women with positive test result/total number of women tested (percentage positive).

TABLE 3. Comparison of birth weights and proportions of low birth weight in women positive for malaria by microscopy, OptiMAL, or PCR

Test and/or malaria (*n*)	Birth wt[a]		OR (95% CI), probability[b]	Probability (positive against negative)[c]
	Positive test result	Negative test result		
Microscopy, maternal malaria (509)	2,550 ± 515 (41) [39.0]	3,022 ± 522 (468) [10.7]	5.4 (2.7–10.7), <0.0001	<0.0001
Microscopy, placental malaria (509)	2,639 ± 503 (73) [27.4]	3,043 ± 520 (436) [10.6]	3.2 (1.8–5.8), <0.0001	<0.0001
OptiMAL (171)	2,608 ± 594 (37) [40.5]	3,007 ± 525 (134) [8.2]	7.6 (3.1–18.8), <0.0001	0.0001
OptiMAL, placental malaria (73)	2,428 ± 425 (28) [53.6]	2,770 ± 447 (45) [11.1]	9.2 (2.8–30.3), <0.0001	0.004
PCR (135)	2,812 ± 509 (70) [24.9]	2,964 ± 602 (65) [9.2]	3.2 (1.2–8.6), 0.025	0.11
PCR, placental malaria (65)	2,628 ± 430 (47) [34.0]	2,640 ± 711 (18) [16.7]	2.6 (0.65–10.2) 0.18	0.93

[a] Mean birth weight (in grams) ± SD with the number of individuals shown in parentheses and the percentage of low-birth-weight babies (<2,500 g) shown in brackets.
[b] Odds ratio (OR), 95% confidence interval (95% CI) shown in parentheses, followed by the probability of low birth weight.
[c] Probability of low birth weight (positive test result against negative test result).

and detected almost twice as many infections as did microscopy. Thirty-six women had positive results by PCR but negative results by microscopy of peripheral blood samples; of these women, 16 had placental infection by microscopy. The other 20 had negative results by microscopy and may have had either subpatent peripheral parasitemia or parasites sequestered in other sites than the placenta. Of seven women with positive OptiMAL test results with negative results by microscopy, PCR gave positive results for two, suggesting that these were false-negative results for microscopy and that the other five women had no malaria infection.

PCR-positive mothers had babies with lower birth weights than PCR-negative mothers (Table 3), although not significantly so. Maternal or placental parasitemia found by microscopy was more closely associated with birth weight and risk of low birth weight. Birth weight of babies of 20 women with submicroscopic malaria was 3,164 ± 465 g compared to 3,088 ± 511 g for 47 babies of PCR-negative women and 3,020 ± 524 g for babies whose mothers did not have PCR performed (not significant).

DISCUSSION

The OptiMAL test has been evaluated in both developing and developed countries for its ability to diagnose infection caused by *Plasmodium* species, usually in individuals with symptoms suggestive of malaria infection (4, 10, 11). In these circumstances, it has yielded high sensitivities and specificities when parasitemia is moderate to high (>500/μl).

We evaluated the OptiMAL test in pregnant Malawian women (test given at delivery) to determine whether it could detect placental parasitemia in women who had negative results by microscopy of peripheral blood samples. Such a tool would be useful in detection of occult infection, which may otherwise have negative consequences for fetal growth. However, we found the OptiMAL test to have a low sensitivity for the detection of placental *P. falciparum* infection in Malawian women and to be no more sensitive than conventional microscopy of peripheral blood for detection of placental parasites. In this study, only 1 of 171 women tested had placental infection and a positive OptiMAL test without peripheral infection. No infections with other *Plasmodium* spp. were recognized.

The sensitivity of the OptiMAL test agreed quite closely with peripheral parasitemia and somewhat less closely with placental parasitemia (Table 1). No woman with a peripheral parasitemia of >1,500/μl (or approximately 0.03%) had a negative result by OptiMAL, whereas 12 of 20 with lower-grade parasitemia had negative OptiMAL test results. There were, however, moderate to dense placental infections which were associated with negative OptiMAL test results; this suggests that either placental parasites may have been nonviable or that *P. falciparum* LDH levels in peripheral blood samples resulting from placental infection are not high enough to be detected by OptiMAL. We have previously shown that placental parasite isolates can adhere to host receptors and can be agglutinated by human serum (1), and we have adapted isolates from placenta to ongoing culture (J. G. Beeson and S. J. Rogerson, unpublished data), which suggests that placental parasites are usually healthy. Leke et al. evaluated the ICT strip test for *P. falciparum* HRP-2 in pregnant Cameroonian women and found it to have a sensitivity of 89% and specificity of 94% for malaria infection (7). No breakdown of parasitemias was provided for this study, although in a related study of an HRP-2 ELISA, the sensitivity was less than 80% in women with placental parasitemia below 10% (7). The densities of placental and peripheral parasitemia were generally low in our study, which may have contributed to the low sensitivity of the OptiMAL test. Our study was performed at a time of low malaria transmission and prevalence (13). In a separate study comparing the OptiMAL test and a *P. falciparum*-specific immunocapture enzyme activity assay performed in the same population, the sensitivities of both tests were below 50% for peripheral parasitemia and 40% for placental infection, respectively (S. Wurster et al., submitted for publication).

Of eight women with positive OptiMAL test results and negative peripheral blood films, one had placental malaria infection and seven had no malaria infection by microscopy. Two of these women were PCR positive; the other five were PCR negative and their false-positive OptiMAL test results are unexplained. It is possible that these women had peripheral blood malaria infection, which was not detected by microscopy (which had a threshold of 40 parasites/μl), or that infecting parasites were sequestered in sites other than the placenta. Recognized causes of false-positive OptiMAL test results include rheumatoid factor positivity (although rates are lower than for *P. falciparum* HRP-2-based tests) (6). We are not aware of other evaluations of OptiMAL in pregnant women, which might have identified other, pregnancy-specific causes of false-positive test results.

Importantly, those women who had positive OptiMAL test

results had much higher rates of low-birth-weight babies and babies with lower mean birth weights than women with negative OptiMAL test results (Table 3). We have previously found that women with placental malaria have approximately twice as many low-birth-weight babies as women without infection at delivery (12). Placental malaria infection was associated with decreased birth weight, and of the women who had placental malaria, women in whom *P. falciparum* LDH was detected had babies with lower birth weights than did women with placental malaria and negative OptiMAL test results. Women with negative OptiMAL test results had babies with similar birth weights and similar prevalence of low-birth-weight babies compared to the babies of those women screened but not recruited to the case-control study. This suggests the OptiMAL test may be detecting parasite burdens which are associated with poor infant outcome. Ours was a relatively small study, and while our findings are quite striking, a larger evaluation of the OptiMAL test as a potential predictor of low birth weight is indicated.

The low specificity of PCR is a reflection of the insensitivity of microscopy. Microscopy remains the "gold standard" for diagnosis of malaria infection. Although it is less sensitive than PCR in detecting infection, it has not been shown that infections in the submicroscopic range are associated with specific pathology. While PCR was more sensitive than OptiMAL in detecting malaria infection in the placenta, it still missed 16 of 32 (50%) placental infections associated with negative peripheral blood smears and detected malaria infection in 20 women without peripheral or placental parasitemia. These additional cases of malaria infection were without adverse associations with birth weight. PCR, therefore, does not appear to offer any advantage in detection of women at risk of low-birth-weight babies associated with malaria infection. We were not able to measure maternal hemoglobin in this study, but Mockenhaupt et al. reported that pregnant women with submicroscopic malaria infection had slightly lower mean hemoglobin concentration, but not a higher prevalence of anemia, than did PCR-negative women (9). The importance of submicroscopic malaria for mother or baby seems to be low.

Although the OptiMAL test proved disappointing in its ability to detect placental malaria infection, it did appear to identify a group of women at high risk of having low-birth-weight babies. This observation, if supported by further studies, would suggest that OptiMAL screening in late pregnancy could be useful to identify women at high risk of delivering babies with low birth weight due to malaria infection, who might benefit from targeted antimalarial treatment.

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