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Placental Malaria in Colombia: Histopathologic Findings in *Plasmodium vivax* and *P. falciparum* Infections

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Abstract. Studies on gestational malaria and placental malaria have been scarce in malaria-endemic areas of the Western Hemisphere. To describe the histopathology of placental malaria in Colombia, a longitudinal descriptive study was conducted. In this study, 179 placentas were studied by histologic analysis (112 with gestational malaria and 67 negative for malaria). Placental malaria was confirmed in 22.35%, 50.0% had previous infections, and 47.5% had acute infections. Typical malaria-associated changes were observed in 37%. The most common changes were villitis, intervillitis, deciduitis, increased fibrin deposition, increased syncytial knots, mononuclear (monocytes/macrophages and lymphocytes), polymorphonuclear cell infiltration, and trophozoites in fetal erythrocytes. No association was found between type of placental changes observed and histopathologic classification of placental malaria. The findings are consistent with those reported for placental malaria in other regions. *Plasmodium vivax* was the main parasite responsible for placental and gestational malaria, but its role in the pathogenesis of placental malaria was not conclusive.

INTRODUCTION

Gestational malaria is an important cause of low birth weight, miscarriage, stillbirth, congenital malaria and other complications.[1–3] Most knowledge about gestational malaria comes from countries in Africa where *Plasmodium falciparum* is predominant.[4–8] However, *P. vivax* infection might also cause severe consequences in the mother and the neonate, although at lower rates than *P. falciparum*.[3,9–12]

Several factors are crucial in the epidemiology and the effects of gestational malaria, and most data firmly confirms that previous immune response against *P. falciparum* plays a central role. *Plasmodium falciparum*–infected erythrocytes accumulate within the placenta through interactions between the VAR2CSA protein on the infected erythrocyte surface and placental chondroitin sulfate proteoglycans.[13] Anti-adherence antibodies preventing this union were associated with protection against placental malaria, but such antibodies develop along successive pregnancies, explaining the particular susceptibility of women on their first pregnancy, in a process in which placental malaria and up-regulation of the *var2csa* gene might be crucial.[6,7,13,14] In the placenta, *P. falciparum* can also attach to glycosaminoglycans-hyaluronic acid[4] and other receptors.[15,16]

Whereas *P. falciparum* parasites are known to sequester in the placenta, *P. vivax* has not been confirmed to sequester. *Plasmodium vivax*–infected placentas showed no specific pathologic changes, suggesting that the presence of low birth weight might be secondary to systemic rather than local effects of the infection.[8] In 2010, it was reported that *P. vivax*-infected erythrocytes were able to cytoadhere, under static and flow conditions, to cells expressing endothelial receptors known to mediate *P. falciparum* cytoadhesion.[17] Although this finding challenges previous concepts on the inability of *P. vivax* to induce severe pathologic changes, its clinical significance remains to be elucidated.

The predominant pathologic change secondary to maternal *P. falciparum* infection is placental malaria. The placental intervillous space is the area where most placental malaria–associated injuries occur. In the case of *P. falciparum,* specific changes in the placenta are evident,[11,18–26] including hemozoin deposition, which might have an interesting role in the pathogenesis of gestational malaria or placental malaria.

Incidence and effects of gestational malaria in prospective follow-up studies (cohort studies) during pregnancy has been addressed by few researchers;[27,28] most authors have reported on the prevalence of gestational malaria at delivery. Moreover, prospective studies on either placental histopathologic changes in areas of low transmission or the pathologic changes of *P. vivax* in pregnancy, are scarce. Gestational malaria or placental malaria reports for the Americas are similarly scarce, regardless of the infecting species (Colombia,[29,30] Brazil,[31–33] Venezuela,[34,35] Perú,[36,37] Ecuador,[38] and French Guiana[39]).

The aim of this report is to describe the pathologic findings in the placentas of a group of pregnant women from two highly endemic malaria regions in Colombia. Samples were obtained from mothers with gestational malaria at any time point during their pregnancy. Healthy pregnant women were included as controls.

METHODS

Study sites and design. The study was conducted in the Uraba and Alto San Jorge regions in northwestern Colombia. These regions have similar ecoepidemiologic conditions for transmission of malaria. A description of the epidemiologic and social characteristics of the Uraba region has been reported elsewhere.[40,41] The mothers and their families have deprived social conditions and family incomes < US$337 per month. In addition, 80% of the women are housewives and the rest are sub-used. Housing, cultural behavior, and antimalarial self-medication practices strongly favor the presence of malaria;[42] > 50% population fail to fulfill their daily vitamin A requirement, 80% of the children had intestinal parasites, and ≥ 50% have malnutrition at rates that are 3–4-fold higher than those observed in the rest of the country.[43]

Mothers were recruited at malaria and antenatal clinics and local hospitals during July 2005–April 2011. Pregnant women fulfilling the inclusion criteria and who provided voluntary consent were included in the study. Persons were recruited in a consecutive manner.

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In several studies with published results[29,30] or in progress, 2,550 pregnant women were recruited. These women came from two types of studies: 1) a descriptive study of a cohort, and 2) cross-sectional descriptive studies. The cohort was composed of approximately 2,200 women who had at least two thick blood smear examinations during pregnancy. The cross-sectional studies evaluated approximately 350 women who had one thick blood smear examination at delivery.

Persons diagnosed with gestational malaria received antimalarial treatment according to the Colombian Ministry of health guidelines: a three-day course of chloroquine for *P. vivax* infection, a seven-day course of quinine plus clindamycin for *P. falciparum* (first trimester pregnancy), or after the first trimester, a three-day course of artesunate plus amodiaquine (in the Uraba region) or artemether plus lumefantrine (in the San Jorge region).

Among 2,200 women (cohort), 250 cases of gestational malaria were detected. Clinical and epidemiologic surveys were used to obtain information for these women. Data from medical records were also obtained. Therefore, a sample size from women with gestational malaria was calculated based on the population of placentas of women expected to have gestational malaria, i.e., 250; a 50% probability of detecting placental tissue changes; and a 95% confidence interval and 7% sampling error. This procedure resulted in a sample size of at least 110 placentas from women with gestational malaria. In the control group, one placenta from a normal-term pregnancy not affected by gestational malaria was recruited for each two placentas from women with gestational malaria (at least 55 placentas). The ratio of placentas from women with and without gestational malaria was defined by convenience. In all groups, a simple random sampling approach was applied. For sampling, we use lists of screened women.

The inclusion criteria for the study were to be pregnant and a permanent resident of the region, general good health condition, commitment to attend the antenatal clinic visits and hospital delivery. Persons were excluded if they withdrew their consent.

Sample collection, diagnosis of infection, and histologic study. Tissue samples were collected from the placenta immediately after delivery. Five fragments distributed throughout the whole area of insertion were obtained by sectioning the placental tissue (2 cm^2 surface area through whole placental thickness). Fragments were fixed in 10% neutral buffered formalin at room temperature and embedded in paraffin within 48 hours at the histopathology laboratory in Medellin. Placental biopsy specimens were processed at the Instituto de Patología of Universidad de Antioquia (Medellin, Colombia). All placental biopsy specimens were examined by a pathologist without prior knowledge of the maternal characteristics, pregnancy outcome, or malaria episodes in pregnancy.

Paraffin-embedded placental specimens were cut to produce at least 3 sections at a thickness of 5 μm, stained with hematoxylin and eosin, and examined by microscopy under 40× (magnification = 400) and 100× (magnification = 1,000; high- power field). Parasites and immune cells were counted in 10 fields at 100× and are presented as mean number per field. Numbers of syncytial knots were counted in three fields at 40× and are expressed as a mean. Histologic features were subjectively scored ranging from none/minimal to most severe on a scale of 0 to 3. The amount of hemozoin was assigned an arbitrary semi-quantitative score (scale: 0 = absent, 1 = scarce, 2 = moderate, 3 = abundant). Fibrinoid foci described as the accumulation of fibrin in the intervillous space was classified according to the amount observed in the whole slide at 40× (scale: 0 = absent, 1 ≤ 2 foci, 2 = 3–5 foci, and 3 > 5 foci).

Diagnosis of malaria was made by light microscopy of a thick blood smear, immune-chromatographic test (NOW ICT Malaria Pf/Pv®; Binax, Portland, ME), polymerase chain reaction (PCR), and histopathologic analysis using methods described elsewhere.[29,30]

Several histopathologic criteria to describe placental malaria have been reported; some addressed initial baseline aspects[18–22,44] and others addressed modified aspects.[11,23,45] In addition, criteria based on the immunopathologic characterization of damage have been introduced.[25,46,47] However, all classifications are similar and specific issues are explored to obtain advanced knowledge of the problem. The current study primarily used the criteria of Bulmer and others[21,22] and Galbraith and others[18,44] to identify placental malaria.

Definitions. Low birth weight was defined as < 2,500 grams. A premature neonate was defined as birth at < 37 weeks. Intra-uterine growth restriction was defined as ≥ 37 weeks of gestation and low birth weight. Abortion was defined as delivery before 22 weeks of pregnancy. Stillbirth was defined as a dead neonate at > 22 weeks. Anemia was defined as a hemoglobin level < 11 g/dL. Massive chronic intervillositis was defined as prominent inflammatory cell infiltrate of intervillous space, mainly by mononuclear cells (monocytes/macrophages) and lymphocytes. Gestational or pregnancy malaria was defined as plasmodial infection diagnosed in a pregnant woman by thick blood smear of maternal peripheral blood. Placental malaria at delivery was defined as plasmodial infection diagnosed in placenta by thick blood smear of placental blood, or by observation of parasites and/or hemozoin during histopathologic analysis of placental tissue.

Statistical analysis. Data were analyzed using SPSS version 10.0 (SPSS IBM, Armonk, NY) and Epi-Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA). Normally distributed continuous data were compared between two groups by using the Student's *t*-test, and non-normally distributed continuous data were compared by using the Mann-Whitney U test or Kruskal-Wallis test. Categorical data were analyzed by using the chi-square test or Fisher exact test.

Ethics The study protocol was reviewed and approved by the bioethics committees of the Sede de Investigaciones Universitarias SIU (Act 07-32-126) and the Instituto de Investigaciones Medicas (Act 012 of 18-07-09), Universidad de Antioquia, Medellin (act 07-32-126). Each participant signed an informed consent form.

RESULTS

A total of 179 placentas (one/person) were studied: 112 from women with gestational malaria and 67 from uninfected controls. Women had a mean ± SD age of 23 ± 6 years and a mean SD pregnancy length of 39 ± 3 weeks (range = 19–43 weeks). Gravidity ranged from 0 to 10, and 25% were primigravidae; 46% had 1–2 previous pregnancies, 22% had 3–5 previous pregnancies, and 7% had 6–10 previous pregnancies. The mean ± SD gestation at the time of the primary malaria episode was 28.9 ± 9.2 weeks. The duration of the malaria attack was 1–4 days in 62%, 5–8 days in 28%,

TABLE 1

Observed frequencies of species distribution (*Plasmodium vivax*, *P. falciparum*, and mixed) and time of diagnosis (antenatal care or at delivery) in 112 women with gestational malaria confirmed by thick blood smear examination of peripheral blood, Colombia

Species/time of diagnosis	No.	%	Total frequency by species (%)
P. vivax			74.11
Antenatal care	73	65.18	
Delivery	10	8.93	
P. falciparum			22.33
Antenatal care	16	14.29	
Delivery	9	8.04	
Mixed			3.56
Antenatal care	0	0	
Delivery	4	3.56	
Total	112	100.0	100.00

and > 9 days in 10%. Mean ± SD hemoglobin level at week 20 of pregnancy was 11.6 ± 1.5 g/dL. The mean SD placental weight was 564 ± 125 grams and the mean ± SD newborn weight was 3,283 ± 533 grams. Premature rupture of membranes was not reported.

According to thick blood smear, maternal peripheral blood at delivery was positive for parasites in 63% of cases, of which 74% were *P. vivax*, 22% *P. falciparum*, and 4% mixed malaria (Table 1). Mean ± SD maternal peripheral parasitemia was 6,124 ± 9,769 parasites/μL, and mean ± SD placental parasitemia was 1,923 ± 5,316 parasites/μL.

Characteristics of women with and without placental malaria by histologic analysis. Characteristics of all study participants are shown in Table 2. No significant differences among the infected and non-infected women were observed, except for three variables: hemoglobin levels in the second half of pregnancy, presence of anemia, and Apgar score at 5 minutes. Positive correlations were detected between gestational age and newborn weight (r = 0.513, $P = 0.0001$); placental weight and weeks of gestation (r = 0.313, $P = 0.006$); and newborn weight and placental weight (r = 0.358, $P = 0.001$).

TABLE 2

Characteristics of women, placentas, and newborns in cases of placental malaria (n = 139) and negative (n = 40) for placental malaria based on histopathologic (according to the presence of parasites and/or hemozoin), Colombia*

Characteristic	Placental malaria	
	No	Yes
Weeks of follow up (mean)†	16.0 (8.5)	11.2 (7.2)
Age (years)	23.61 (6.13)	22.48 (6.42)
Years of residence in endemic zone (years)	14.92 (8.52)	14.61 (9.58)
No. previous pregnancies	1.95 (2.18)	1.78 (1.58)
First pregnancy	23.7%	22.5%
Pregnancy malaria at any time	51.8%	100%
Pregnancy malaria at delivery	7.9%	23.1%
Weeks of gestation at primary episode of malaria	27.7 (9.9)	31.2 (7.8)
Hb level in first half of pregnancy (g/dL)	11.66 (1.38)	11.42 (2.03)
Anemia (Hb level < 11 g/dL): first half of pregnancy	27.4%	29.2%
Hb level in second half of pregnancy (g/dL)†	10.90 (1.62)	9.98 (1.72)
Anemia (Hb level < 11 g/dL): second half of pregnancy†	50.5%	69.7%
Preeclampsia	3.8%	3.8%
Gestational age (weeks)	38.4 (2.7)	38.0 (3.1)
Prematurity (< 37 weeks)	8.3%	2.9%
Placental weight (grams)	563.69 (132.37)	537.27 (121.35)
Birth weight (grams)	3,165.7 (542.8)	3,202.4 (675.0)
Low birth weight (< 2,500 grams)	7.8%	2.6%
Cephalic perimeter (cm)	33.70 (2.67)	33.84 (2.79)
Length (cm)	48.84 (3.02)	48.45 (4.18)
Apgar score at 1 minute	7.98 (0.97)	8.15 (0.61)
Apgar score at 5 minutes†	9.72 (0.67)	9.94 (0.24)
Ratio birth weight:placental weight	5.62	5.96

*Values are mean (SD) or proportion (%).
†$P < 0.05$.

Histologic placental changes. Fifty-one percent (91 of 179) of the placentas showed no change or alteration, and 49% (88 of 179) showed at least one type of change or alteration. The 88 affected placentas showed at least 1 of 39 type of changes (Table 3), and the number of changes ranged between 1 and 4 per placenta (mean ± SD = 1.63 ± 0.84). The most common alterations were infarction (in 36% of placentas with changes); followed by chorioamnionitis, funisitis, placentitis, and microabscess (22%); villitis, intervillitis, and deciduitis (20%); and intervillous thrombi (14%). Increased fibrinoid deposition and syncytial knots were detected in 10% and 9%, respectively, and lymphocyte or neutrophil infiltrate (polymorphonuclear neutrophils [PMNs]) was detected in 3%.

Histopathologic examination according to the classifications of Galbraith and others[18,44] and Bulmer and others[21,22] showed that 22.3% (40 of 179) of placentas had placental malaria: 47.5% (19 of 40) acute malaria, 2.5% (1 of 40) chronic malaria, and 50.0% (20/40) past malaria.

A strong significant association was detected between diagnosis of gestational malaria by thick blood smear and diagnosis of placental malaria by histopathologic analysis and defined by presence of parasites and/or hemozoin among 112 placentas of women with a diagnosis of gestational malaria. A total of 36% of their placentas had placental malaria compared with none in 67 cases without gestational malaria (Table 4).

Agreement between *Plasmodium* species detected in placenta by histopathologic analysis and species detected in placental blood by thick blood smear was significantly high and non-absolute (89.7%) ($P = 0.0000001$, by Yates' test). In 119 placentas examined by histopathologic analysis, five had at least one parasite per high-power field and were negative by thick blood smear. Of nine cases of *P. vivax* diagnosed by thick blood smear, none could be confirmed by histopathologic analysis (Table 5).

TABLE 3

Detailed description of histologic changes detected in 88 cases of placental malaria in 144 characterized placentas, Colombia

Change or alteration	Absolute frequency (n = 144)	Overall proportion in 144 (%)	Relative proportion in 88 (%)
Infarction (basal, suprabasal, marginal, subchorionic)	32	22	36
Chorioamnionitis, funisitis, placentitis, microabscess	19	13	22
Villitis, intervillitis, deciduitis	18	13	20
Intervillous thrombi	12	8	14
Increased fibrinoid deposition	9	6	10
Increased syncytial knots	8	6	9
Sickle cell trait	6	4	7
Lymphocyte or neutrophil infiltrate	3	2	3
Vasculitis	3	2	3
Trophozoites in fetal erythrocytes	1	1	1
Other (various low frequency changes)	33	23	38
Total	144	100	100

TABLE 4

Association between the diagnosis of placental malaria by histopathology (defined as presence of parasites and/or hemozoin) and the diagnosis of gestational malaria by thick smear in mother's peripheral blood, Colombia

	Placental malaria		
Gestational malaria	No	Yes	Total
No	67	0 (0% in 67)	67
Yes	72	40 (36% in 112)	112
Total	139	40*	179

*Prevalence of placental malaria by histopathologic analysis = 40 (22.35%) of 179 (P = 0.0001, by Fisher exact test.

Placental changes were slightly more frequent, but not significantly more frequent ($\chi^2 = 0.434$, by Fisher one-sided test) because infection occurred closer to delivery. The frequency of placental changes was statistically similar in placentas regardless of the presence of placental malaria detected by histologic analysis ($\chi^2 = 0.561$, by Fisher one-sided test).

Placental malaria detected by microscopy was significantly associated with patent peripheral parasitemia in the mother, i.e., a placental malaria-positive smear was detected only in 2% (2 of 117) when the mother was negative for malaria compared with 79% (15 of 19) when the mother was positive for malaria ($P = 0.0000001$, by χ^2 test). A total of 47% (9 of 19) of cases of placental malaria detected by histologic analysis were associated with patent peripheral parasitemia compared with 20% (29 of 146) of cases with negative peripheral blood smears ($P = 0.017$, by χ^2 test).

In 11% (19 of 179) of the placentas, parasites were observed by histopathologic analysis, and the number ranged between 1 and 131 parasites/high-power field (mean ± SD = 2 ± 12 parasites/high-power field). Seventeen placentas were positive by microscopy of a placental thick blood smear (*P. vivax* =15 and *P. falciparum* = 2), and mean ± SD parasite counts were 1.1 ± 1.6 for *P. vivax* and 67.0 ± 90.5 for *P. falciparum* ($P = 0.052$, by Mann-Whitney U test).

Placental malaria was detected in 57% of cases of gestational malaria diagnosed at delivery versus 30% of cases of gestational malaria diagnosed at the antenatal clinic before delivery ($P = 0.019478$). In addition, a significant association was observed between type of placental malaria detected by histologic analysis and time of diagnosis of gestational malaria. Acute placental infection was observed in 30% (8 of 27) of cases of gestational malaria diagnosed before delivery compared with 77% (10 of 13) of cases diagnosed at birth. Chronic placental infection was absent in cases of gestational malaria diagnosed before delivery and present in 8% (1 of 13) in cases diagnosed at birth. Past malaria infection was detected in 70% (19 of 27) of cases of gestational malaria diagnosed before delivery and 15% (2 of 13) of cases diagnosed at birth ($P = 0.003$).

TABLE 5

Concordance between diagnosis of *Plasmodium* species by histology and placental blood thick smear, Colombia*

	Thick blood smear			
Histologic analysis	Negative	*Plasmodium vivax*	*P. falciparum*	Total
Negative	114	9	0	123
Positive	5	6	2	13
Total	119	15	2	136

*P = 0.0000001, by Yates' test; raw concordance (efficiency) = 122 (89.7%) of 136; Kappa (Cohen) = 0.4766 (95% confidence interval = 0.2433–0.7100); and Youden index = 0.43 (95% confidence interval = 0.19–0.67).

TABLE 6

Relationship between presence of placental malaria and presence of immune cells and foci of fibrinoid according to PM (yes, no), Colombia*

	Placental malaria by histologic analysis			
Characteristic	No (n = 139)	Yes (n = 40)	P(t)	P(M-W)
Mononuclear cells	10.26 ± 11.32	12.50 ± 9.11	0.200	0.188
PMNs	16.50 ± 9.60	13.90 ± 8.63	0.107	0.170
Fibrinoid deposits	11.42 ± 6.17	16.35 ± 9.92	0.005	0.000

*Values are mean ± SD scores. PMNs = polymorphonuclear neutrophils.

When placental malaria was confirmed by histopathologic analysis (according to presence of hemozoin and/or parasites), this finding was significantly associated with increased fibrinoid deposits (Table 6). However, intensity of PMNs or mononuclear cell infiltrates was similar regardless of the presence of placental malaria, and parasite count, hemozoin score, and PMN infiltrate significantly varied according to the species observed (Table 7). Mean parasite count, hemozoin score, mononuclear cell infiltrate, PMN infiltrate, and fibrinoid foci were statistically different in groups according to the presence of parasites. All factors, except PMNs, increased as the number of parasites increased ($P = 0.0001$ for parasites, $P = 0.002$ for hemozoin, and $P = 0.029$ for fibrinoid foci, by Mann-Whitney U test). In contrast, the mean number of knots was statistically similar in relation to the number of placental parasites ($P = 0.320$, by Mann-Whitney U test).

Analysis of some variables obtained after histopathologic examination confirmed existence of a bivariate correlation (Table 8). The number of parasites was significantly associated with hemozoin, mononuclear cell count, PMN cell count, and fibrinoid foci. Conversely, syncytial knots did not show a correlation with the amount of parasites or any of the other four variables. It is noteworthy that coefficient correlations are strengthened, but the probability of significance is weakened when the analysis is limited to cases with placental malaria detected by histopathologic analysis (n = 40). Also, bivariate correlation analysis of the 19 cases detected with parasites in placental blood (microscopy) showed no significant correlation between presence of parasites and the four other variables. Therefore, no additional statistical analysis was performed.

General and histologic features of placental malaria. The association between placental malaria detected by histologic

TABLE 7

Relationship between presence of placental and placental histopathology changes depending on the species of *Plasmodium* Colombia*

	Species by microscopy		
Characteristic	*Plasmodium vivax* (n = 15)	*P. falciparum* (n = 2)	P(M-W)
Mean no. parasites	1.1 ± 1.6	67 ± 90.5	0.052
Total hemozoin score (median)	0.40 ± 0.51	2.0 ± 1.41	0.043
Mean no. mononuclear cells	12.47 ± 10.64	21.00 ± 9.90	0.133
Mean no. PMNs	13.20 ± 5.65	3.50 ± 2.12	0.044
Mean no. fibrinoid deposits	15.20 ± 6.60	39.50 ± 34.65	0.295

*Values are mean ± SD scores. PMNs = polymorphonuclear neutrophils.

TABLE 8
Bivariate analysis of the relationship between histopathologic findings in placentas, Colombia*

Variable (n = 179)	ρ coefficient (*P*)			
	Mononuclear cells	PMNs	Fibrinoid foci	Hemozoin
Mean no. parasites	0.240 (0.001)	–0.161 (0.031)	0.334 (0.0001)	0.537 (0.0001)
Mononuclear cells		–0.199 (0.008)	0.262 (0.0001)	0.079 (0.290)
PMNs			0.103 (0.171)	–0.125 (0.096)
Fibrinoid deposits				0.238 (0.001)

*PMNs = polymorphonuclear neutrophils. Bivariate correlation analysis of the 19 cases with parasites in placental blood did not detect a significant correlation between number of parasites and the four variables listed.

analysis and a positive species diagnosis test result (microscopy, ICT, or PCR) was 36% for *P. vivax* and 46% for *P. falciparum*. Hemozoin was found in 92.5% (37 of 40) of cases of placental malaria detected by histologic analysis ($\chi^2 = 0.0001$, by Fisher one-sided test), but in most (34 of 37) cases, it was scarce. The mean ± SD number of fibrinoid foci was significantly higher in placentas with malaria than in placentas without malaria (16.3 ± 9.9 versus 11.4 ± 6.2, respectively; $P = 0.0001$). A total of 2–18 (mean = 13) fibrinoid foci were detected in 5% (9 of 179) of the placentas, only one placenta was diagnosed with past placental malaria, and the remaining placentas were negative for malaria by histopathologic analysis.

Neutrophils, mononuclear cells, or fibrinoid foci were found in 91% (177 of 179) of the placentas. Numbers of PMNs ranged from 2 to 54 (mean ± SD = 16 ± 9), numbers of mononuclear cells ranged from 1 to 131 (mean ± SD = 11 ± 11), numbers of fibrinoid foci ranged from 2 to 64 (mean ± SD = 12 ± 7). These values were statistically different when a diagnosis of placental malaria was made by histologic analysis (Tables 6 and 7). Mean ± SD PMN numbers were similar ($P = 0.317$) according to the presence and type of placental malaria (16.27 ± 9.73 for no placental malaria, 12.72 ± 9.30 for acute malaria, 5.00 ± 0.00 for chronic malaria, and 15.33 ± 8.03 for past malaria). A similar finding was confirmed for mean ± SD numbers of mononuclear cells ($P = 0.056$) (16.27 ± 9.73 for no placental malaria, 15.83 ± 10.47 for acute malaria, 28.0 ± 0.00 for chronic malaria, and 8.90 ± 5.75 for past malaria).

The mean score for hemozoin was statistically higher in infections with *P. falciparum* detected by microscopy than in infections with *P. vivax* detected by microscopy ($P = 0.043$) and independent of parasitemia ($P = 0.052$) (Table 6). Similarly, mean numbers of PMNs varied among the two species ($P = 0.044$, by Mann-Whitney U test), and mean mononuclear cell number and fibrinoid foci were similar ($P = 0.133$ and $P = 0.295$, respectively, by Mann-Whitney U test). Mean number of PMNs was similar in any type of placental infection (past or acute) ($P = 0.304$, by Kruskal-Wallis test). However, significant differences were observed in mean number of mononuclear cells ($P = 0.027$) and fibrinoid foci ($P = 0.0007$).

Cases of *P. vivax* infection with positive histopathologic results were observed in 87% (7 of 8) of placentas with acute malaria and 13% (1 of 8) of placentas with past malaria by the criteria of Bulmer and others.[21,22] Based on the criteria of Rogerson and others,[26] these cases were classified as initial acute infections (25%, 2 of 8), advanced acute infections (50%, 4 of 8), chronic infection (12.5%, 1 of 8), and past infection (12.5%, 1 of 8). According to this classification, the five cases of *P. falciparum* placental malaria were advanced acute infections (40%), chronic infection (20%), and past infections (40%). The single case of mixed placental malaria infection (*P. vivax* and *P. falciparum*) was negative for infection by histopathologic analysis.

The proposed histopathologic classification presented in this study was based on those of Galbraith and others[18,44] and Bulmer and others,[21,22] which are similar. Another classification, which differs from the one we used because of the inclusion of location of hemozoin as a definitive criteria, was proposed by Rogerson and others.[26] Therefore, our results regarding histopathologic assessment of placental malaria incorporated variables from three types of criteria. With exception of two cases, the two classification systems showed good agreement (Table 9).

Placental infection by *P. vivax*. Placental malaria caused by *P. vivax* was confirmed in 26 placentas by thick blood smear, PCR, or ICT. However, parasites detected by histologic analysis were observed only in eight of these placentas. Descriptions of the two histological classifications used are shown in Table 10, and general characteristics of the women examined are shown in Table 11. Histologic analysis of these cases of *P. vivax* placental malaria confirmed the presence of parasites in 27% and hemozoin in 23% (mononuclear or PMNs and/or in fibrinoid in 4 of 6 and in fibrinoid in 2 of 6). All placentas had

TABLE 9
Comparison of placental malaria classification by different criteria*[21,24,25,29]

Rogerson and others[26] (negative = 139, positive = 40)		Galbraith and others [18,44] and Bulmer and others[21,22] (negative = 139, positive = 40)				
		No infection, P^-H^-	Past, P^-H^+	Chronic, P^+H^+	Acute, P^+ and/or H^+	Total
No infection	P^-H^-	139	0	0	0	139
Past infection	P^-H^+ in fibrinoid deposits	0	19	0	0	19
Initial acute infection	P^+H^-	0	0	0	3	3
Advanced acute infection	P^+H^+ in MN and/or in fibrinoid deposits	0	2†	0	11	13
Chronic infection	H^+ in fibrinoid deposits	0	0	1	4	5
Total		139	21	1	18	179

*Classifications were based of presence of hemozoin (H) and *Plasmodium* (P) parasites. MN = mononuclear cell. Two cases were classified according to some of the authors, but correspondence with others could not be made.
†Cases failing to meet criteria for a specific classification according to Rogerson and others;[26] they had hemozoin in monocytes and absence of parasites.

TABLE 10

Histopathologic classification of 26 cases of *Plasmodium vivax* placental malaria, Colombia*

Rogerson and others[26]	Galbraith and others[18,44] and Bulmer and others[21,22]			
	No infection	Past	Acute	Total
No infection	18	0	0	18
Past infection	0	1	0	1
Initial acute infection	0	0	2	2
Advanced acute infection	0	0	4	4
Chronic infection	0	0	1	1
Total	18	1	7	26

*Identification of species was performed by microscopy of thick blood smears, immunochromatographic test, or polymerase chain reaction ($P = 0.0001$).

placental intervillous space monocytes, PMN infiltrates, and fibrinoid foci (high in one case); and one placenta had increased syncytial knots. Statistical analysis of variables shown in Table 11 and results of histologic analysis (according to the two classifications applied) confirmed a significant relationship between mean number of parasites and hemozoin ($P = 0.0001$) and foci of fibrinoid ($P = 0.061$).

DISCUSSION

A relatively young population and long-time residents in a malaria-endemic area was studied. In addition, the recorded parity (< 1, 26%; range = 1–2, 46%) in the groups was low. Placental malaria was observed in 22.3% by histologic analysis and in 2.5% by microscopy of placental thick blood smears. The pre-dominance of *P. vivax* over *P. falciparum* might result from the high frequency of *P. vivax* in the study regions. However, the ratio of *P. vivax* to *P. falciparum* infections was higher than expected; 3.45 in persons with gestational malaria and 7.5 in persons with placental malaria, versus 2.0 in non-pregnant women.[48] Similar results for gestational malaria have been reported for in Manaus (western Amazon region of Brazil).[33] The authors attributed these results to a higher frequency of *P. vivax* in pregnant women and contraindication of primaquine administration. In addition, the authors proposed that mechanisms of resistance/susceptibility to malaria infection and/or pathogenesis in pregnant women may differ depending on the plasmodial species.[33]

TABLE 11

Characteristics of 26 cases of *Plasmodium vivax* placental malaria, Colombia*

Variable	No.	Mean ± SD	Range
Mothers			
Age (years)	26	25.08 ± 7.92	13–39
Years in malaria zone	24	16.06 ± 10.51	0–33
Gravidity (no.)	26	2.00 ± 2.38	0–9
Hemoglobin level in first half of pregnancy (g/dL)	13	11.80 ± 1.84	9.30–16.00
Hemoglobin level in second half of pregnancy (g/dL)	22	10.72 ± 1.01	9.00–12.50
Gestational age (weeks)	23	40.0 ± 1.7	36.3–43.7
Placental weight (grams)	14	547.1 ± 110.8	400–720
Birth weight (grams)	25	3,253.6 ± 358.57	2,700–3,940
Neonates			
Cephalic perimeter (cm)	25	34.60 ± 3.27	30–48
Length (cm)	25	49.32 ± 1.28	48–53
Apgar score at 1 minute	25	8.24 ± 0.78	7–9
Apgar score at 5 minutes	25	9.72 ± 0.68	8–10
Placentas			
Parasites/10 fields (100×)†	26	0.65 ± 1.32	0–4
Hemozoin (arbitrary unit)†	26	0.23 ± 0.43	0–1
PMNs/10 fields (100×)†	26	13.04 ± 5.50	3–26
Mononuclear cells/10 fields (100×)†	26	11.69 ± 8.62	3–40
Fibrinoid deposits‡	26	14.19 ± 6.17	4–23

*PMNs = polymorphonuclear neutrophils. Diagnosis of species was performed by microscopy of thick blood smears, immunochromatographic test, or polymerase chain reaction.
†$P = 0.0001$ vs. no placental malaria.
‡$P = 0.061$ vs. no placental malaria.

The presence of placental malaria was significantly associated with gestational malaria, a finding that has also been reported in Thailand.[24] Nevertheless, in the current study, similar frequencies of the association between placental malaria and gestational malaria were observed in *P. vivax* or *P. falciparum* infections (19.4% and 17.7%, respectively). However, this association was stronger for *P. falciparum* in Thailand (33% and 65%, respectively).[24] The frequency of placental malaria was higher in women with gestational malaria at delivery than in women given a diagnosis during the antenatal period (56% versus 30%, respectively), suggesting that malaria treatment was effective and prevented placental damage, as reported.[24] Placental infection, as diagnosed by thick blood smear or histologic analysis, was associated with patent peripheral parasitemia in the mother, and this finding was consistent with previous findings.[24] A high frequency of placental malaria detected by histologic analysis in women with circulating parasites seems logical because of the higher chance of parasites reaching deep organs, including the placenta.

The observed mean birth weight was normal regardless of the presence of placental malaria. Similarly, the mean placental weight was unaffected by the presence of placental malaria, but it was lower than that observed in other populations in regions of Colombia to which malaria is not endemic.[49] Women of the Karen ethnic group from Thailand with a lower mean placental weight than that reported in our study had a birth weight:placental weight ratio of 6.0,[24] which is similar to 5.62 (placental malaria negative) and 5.96 (placental malaria positive) detected in this study. Presence of placental malaria had no statistically significant effect on frequencies of prematurity and low birth weight. This finding is in contrast to findings from Thailand, in which high prematurity and low birth weight were reported, mainly in *P. falciparum* infections.[24]

Histologic alterations were similar in both groups of placentas whenever a diagnosis of placental malaria was made by histologic analysis or microscopy. Most of these lesions were seen during normal placental development[50] and may explain the lack of a difference between infected and uninfected placentas.

The type of placental malaria detected by histologic analysis varied according to the classification criteria applied. This finding was apparent for cases of active infection, in which substantial differences were observed. The classification of Rogerson and others is based on the inflammatory immune process triggered by parasite hemozoin, and the immune response shown by placental migration of maternal mononuclear cells into the intervillous space.[26] The importance of these cells has also been highlighted by other authors.[46,47,51,52] Ismail and others proposed a semi-quantitative assessment of inflammation and hemozoin within the placenta, and considered the number of parasitized erythrocytes as another element for measuring placental damage.[11] Muehlenbachs

and others[25] proposed a different semi-quantitative scale to measure inflammation and hemozoin, regardless of the presence of infected erythrocytes; and used similar, but not identical, scales as proposed by Ismail and others.[11] However, all approaches have been controversial.[53] Therefore, a standardized system of placental damage score is needed for regions to which malaria is endemic.

The type of placental malaria detected by histopathologic analysis was also associated with the time when gestational malaria was diagnosed and showed a predominance of acute placental malaria in cases diagnosed at birth. Cases of past infection were more common when the diagnosis was made throughout pregnancy. In *P. vivax* infections, acute and past infections were found. However, the number of parasites was low and less than that reported in *P. falciparum* infections. This finding is consistent with those of previous reports on the level of parasitemia in non-pregnant residents in Uraba.[54,55]

A correlation between the number of placental parasites and the number of mononuclear cells, fibrinoid foci, or hemozoin indicates that the intensity of inflammation and injury are related to parasite load. This association might explain the higher severity of placental malaria in primigravidae than in multigravidae, a process well known in *P. falciparum* malaria and largely uncharacterized in *P. vivax* malaria.[20] In the current study, a high correlation between parasitemia and number of mononuclear cells and PMNs infiltrating the intervillous space was observed. The mean number of PMNs was higher and statistically different in women infected with *P. vivax*. Because these results were observed in small groups, significant linear correlations between these variables must be validated in larger studies.

Pregnant women with *P. vivax* infections had lower levels of placental hemozoin, with respect to *P. falciparum*, thus confirming previous theories of reduced accumulation of *P. vivax*-infected erythrocytes in the placenta.[56] In the current study, hemozoin significantly and positively correlated with the number of parasites and fibrinoid foci, but not with mononuclear or PMNs. The significance of this association in *P. vivax* infections, along with the effect on anemia and pregnancy outcome, should be further explored in specifically designed studies, particularly in view of the recommendation of antimalarial prophylaxis against both species made by other authors after confirmation of the deleterious effect of *P. vivax* infection in primigravidae.[3]

Studies conducted in populations in Africa confirmed the association between *P. falciparum* submicroscopic infection during pregnancy with maternal anemia and low birth weight[57] and with a pro-coagulant/anti-fibrinolytic effect, but not with inflammation.[58] Submicroscopic infection in gestational malaria and placental malaria is a common finding in northwestern Colombia.[29] Preliminary results of ongoing studies in the region indicate an association between increased apoptosis and some pro-inflammatory cytokines during placental infection by any species (Agudelo O and others, unpublished data). Also of major interest is the problem of hidden placental malaria, which represented approximately 10% (by microscopy)[29] of malaria cases in Colombia. In women in Africa, hidden malaria has been reported as a concern, and significant low levels of soluble biomarkers have been detected.[59]

In conclusion, the frequency of gestational malaria (10.4% by thick blood smear examination) and placental malaria (22.3% by histologic analysis and 12.5% by thick blood smear examination) were high in Uraba and Alto San Jorge, Colombia, and there was a frequency of association of 64% between gestational malaria and placental malaria. The risk of placental malaria in cases of gestational malaria increased as the diagnosis was made closer to birth. Therefore, to reduce such risk, diagnosis of gestational malaria should be performed as early as possible to provide adequate antimalarial treatment. The histopathologic characteristics of placental malaria are generally consistent with those reported elsewhere, particularly in Thailand, where *P. vivax* predominates. Finally, *P. vivax* was the most common species responsible of gestational malaria and placental malaria, but its role as an agent capable of producing histopathologic and placental lesions was inconclusive because of the small number of cases.

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