ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2007, p. 332–334 Vol. 51, No. 1
0066-4804/07/$08.00+0 doi:10.1128/AAC.00856-06

NOTES

Markers of Sulfadoxine-Pyrimethamine-Resistant *Plasmodium falciparum* in Placenta and Circulation of Pregnant Women▽

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Received 13 July 2006/Returned for modification 19 September 2006/Accepted 23 October 2006

Placental sequestration of *Plasmodium falciparum* in pregnancy may impair the usefulness of molecular markers of sulfadoxine-pyrimethamine resistance. In 300 infected, delivering women, the concordance of PCR-restriction fragment length polymorphism-derived parasite resistance alleles in matched samples from placenta and circulation was 83 to 98%. Sulfadoxine-pyrimethamine resistance typing in peripheral blood is reasonably representative of *P. falciparum* infecting pregnant women.

Malaria in pregnancy is a serious public health problem in sub-Saharan Africa. Although commonly asymptomatic, its clinical consequences involve anemia, low birth weight, preterm delivery, and an estimated annual 75,000 to 200,000 attributable infant deaths (11).

Lately, intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP) has been used for malaria control in pregnancy (13). IPT involves the administration of SP treatment three times during the second and third trimesters, irrespective of parasitemia. Monitoring SP resistance is essential for estimating the effectiveness of this policy, and molecular markers are increasingly applied for this purpose. SP resistance is associated with specific mutations in the *Plasmodium falciparum* dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) genes (8). In Ghana, SP failure was recently observed in 28% of treated children; the *dhfr* triple mutation (Ile51+Arg59+Asn108) increased the risk of treatment failure 10-fold (6). Due to preexisting immunity, antimalarial treatment commonly is more effective during pregnancy than it is in children (3). Moreover, pregnancy may influence the value of resistance markers because, due to specific ligand expression, *P. falciparum* sequesters in the placental intervillous space. The absence of microscopically visible parasites in peripheral blood despite placental parasitemia is one common consequence. Although the sensitivity of PCR in detecting placental infection in peripheral blood approaches 100% (7), it is unclear whether circulating parasite genotypes in pregnant women represent the actual parasite population or only part of it. Extensive discordance between placental and peripheral polymorphic merozoite surface protein (*msp*) genotypes has been reported, but rather homogenous parasite populations have been reported as well (2, 4, 10). So far, it is unknown whether and to what extent parasite genotype discordance in pregnant women affects the value of SP resistance typing. Here, we compared peripheral blood and placental *dhfr* alleles in 300 delivering Ghanaian women with microscopically proven placental malaria.

Between January 2000 and January 2001, 889 delivering and consenting women were recruited at the district hospital in Agogo, southern Ghana, an area of holoendemicity. The study protocol was approved by the Ethics Committee, University of Science and Technology, Kumasi, Ghana. The diagnostic pro-

TABLE 1. Characteristics of 300 delivering women with placental malaria

Parameter	Value
Median age (yr) (range)	23 (15–45)
% (Proportion) of primiparae	48.3 (144/298)
% (Proportion) febrile (>37.4°C axillary temp)	5.1 (15/295)
% (Proportion) anemic (hemoglobin <11 g/dl)	48.3 (145/300)
% (Proportion) with single live delivery of low birth wt (<2,500 g)	19.6 (56/286)
% (Proportion) with preterm single live delivery (<37 wk of gestation)	20.9 (59/282)
% (Proportion) with peripheral parasitemia by microscopy	50.3 (151/300)
Geometric mean parasite density (95% CI[a]) of peripheral parasitemia	733/μl (525–1023)
Geometric mean parasite density (95% CI) of placental parasitemia	0.84/HPF[b] (0.62–1.13)
% (Proportion) with pyrimethamine in plasma	32.5 (95/292)

[a] CI, confidence interval.
[b] HPF, high-power field.

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▽ Published ahead of print on 6 November 2006.

TABLE 2. Comparison of peripheral blood and placental *Plasmodium falciparum* dihydrofolate reductase genotypes

Placental *dhfr* genotype of sample[a]	No. of samples with indicated peripheral blood *dhfr* genotype[a]								Total (%)
	51 59 108	51 59 **108**	51 **59** 108	**51** 59 108	**51** 59 **108**	51 **59 108**	**51 59 108**	Nontypeable	
51 59 108	12	2	2				1		17 (5.7)
51 59 **108**		5			2	2			9 (3.0)
51 **59** 108					1				1 (0.3)
51 59 108	2			1					3 (1.0)
51 59 **108**					7		3		10 (3.3)
51 **59 108**	1			1	1	81	14	2	100 (33.3)
51 59 108			1		5	6	141	1	154 (51.3)
Nontypeable						4	2		6 (2.0)
Total (%)	15 (5.0)	7 (2.3)	3 (1.0)	2 (0.7)	16 (5.3)	93 (31.0)	161 (53.7)	3 (1.0)	

[a] Mutated codons are displayed in bold. The concordance of alleles between matched placental and peripheral samples was high for codon 108 (97.6%; 284/291), lower for codon 59 (94.2%; 274/291), and lowest for codon 51 (90%; 262/291) ($P = 0.0007$). Of 20 discordant peripheral isolates with the *dhfr* triple mutation, 17 matching placental genotypes exhibited double mutations, and 1 was of the wild type. Contrariwise, of the 13 placental isolates with *dhfr* triple mutation not identified as such by peripheral genotyping, 11 showed double *dhfr* mutations.

cedures used for and the malariologic characteristics of the majority of the women have been described previously (7). For the present study, all 300 available, matched samples of placental and peripheral blood of women with microscopically confirmed placental parasitemia were examined. DNA was extracted (QIAmp; QIAGEN), and stored at −80°C until the *dhfr* mutations Ser108Asn, Asn51Ile, and Cys59Arg were determined by PCR-restriction fragment length polymorphism (RFLP) in 2006 (1). Mixed alleles (wild type and with mutation present) were considered mutations. Plasma concentrations of pyrimethamine, at the time of study conduct recommended for the chemoprophylaxis of malaria in pregnancy, were measured by enzyme-linked immunosorbent assay (limit of detection, 10 ng/ml) (12).

The characteristics of the 300 women are shown in Table 1. Successful *dhfr* typing of all three alleles was achieved in 294 (98%) placental and 297 (99%) peripheral samples. *dhfr* mutations were frequent: only 5% of circulating genotypes were of the wild type, while 4%, 37%, and 54% comprised one, two, and three *dhfr* mutations, respectively (Table 2). Comparing the 297 peripheral genotypes to placental alleles, complete concordance was observed in 83.2% (247/297) of matched samples. The corresponding figures were 80.0% (128/160), 85.2% (75/88), and 89.9% (44/49) for placental samples with less than 1, between 1 and 10, and 10 or more parasites/high-power field ($P = 0.2$), respectively, and it was 73.3% (11/15) in febrile and 84.4% (234/277) in afebrile women ($P = 0.3$). Setting placental alleles as the reference, peripheral genotyping correctly identified 46.2% (6/13), 80.0% (88/110), and 91.6% (141/154) of isolates with one, two, and three *dhfr* mutations, respectively ($P < 0.0001$).

Pyrimethamine in plasma appeared to select for *dhfr* mutations. In women with and without pyrimethamine levels, placental wild-type isolates were seen in 2.1% (2/94) and 7.8% (15/193, $P = 0.06$), and isolates with two or three mutations were seen in 95.7% (90/94) and 87.6% (169/193, $P = 0.03$), respectively. Irrespective of plasma pyrimethamine, no influence of *dhfr* alleles on the clinical manifestation of malaria was observed (data not shown). However, the suppressive effect of

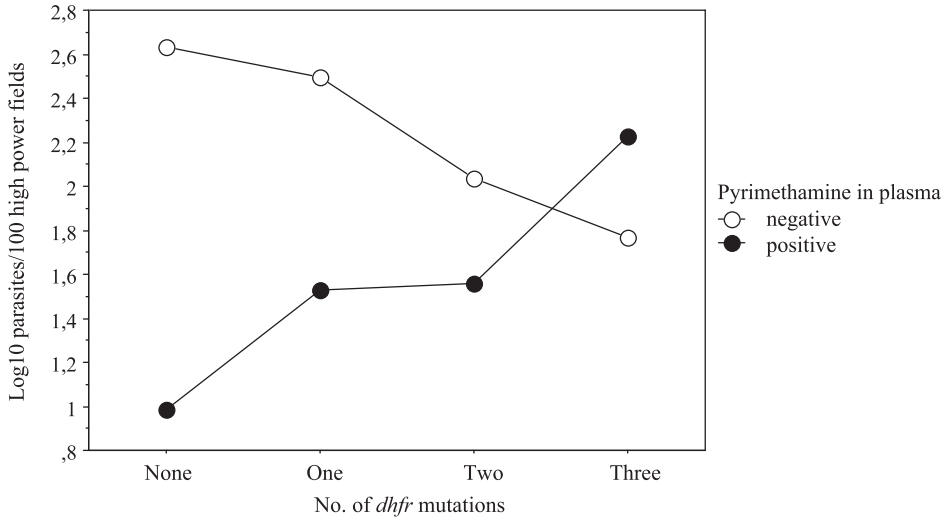

FIG. 1. Geometric mean placental parasite densities according to the presence of pyrimethamine in plasma and the dihydrofolate reductase genotype.

pyrimethamine on placental parasite density decreased with the number of *dhfr* mutations (Fig. 1) ($F = 5.1$; $P = 0.002$).

In Ghana, the *P. falciparum dhfr* triple mutation is highly predictive for SP treatment failure in children, whereas *dhps* alleles are not (and were thus not assessed here) (6). In the present study, the differential effect—depending on *dhfr* alleles—of plasma pyrimethamine on parasite density is suggestive of resistance. One major advantage of SP in IPT lies in its single-dose administration, but resistance is anticipated to spread and intensify. Here, we show that 93% and >50% of the women harbored parasites with the *dhfr* core mutation Asn108 and the high-resistance triple mutation, respectively. In 1998, these figures were 81% and 36% among infected antenatal care attendees (5), pointing to a rapid rise of SP resistance. Moreover, we report complete concordance between peripheral and placental *dhfr* genotypes obtained by PCR-RFLP in 83% of matched samples. Concordance was lower for the small group of wild-type and single-mutation parasites. Most discordant circulating genotypes differed from the matching placental ones in that double mutations were considered triple or vice versa. In both cases, SP resistance must be expected (6, 8). The concordance of placental and peripheral *dhfr* alleles was higher than was reported for *msp* genotypes (4, 10); less polymorphism in the former is one likely explanation. In practice, molecular markers need to be simple; we therefore grouped mixed *dhfr* alleles as mutations. When analyzed separately, concordance was still high (73% [data not shown]). PCR-RFLP is the most common technique for *dhfr* typing, and the assay used is highly specific. However, sensitivity might drop at very low parasitemia, particularly in polyclonal infections, and results occasionally differ from those obtained by other techniques (9). Consequently, our findings are only restrictedly transferable. Resistance markers are not routinely assessed in sub-Saharan Africa, and this is unlikely to change soon. Nevertheless, for epidemiological purposes we consider peripheral blood *dhfr* genotyping both useful and sufficiently precise to give an estimate of SP resistance in parasites infecting pregnant women. This should allow examination of the dynamics of SP resistance in infections occurring despite IPT. Once the predictive value of the *dhfr* mutations for IPT with SP has been established, monitoring these markers will provide valuable and rather easily accessible information, which is of basic importance to guide drug policy on the prevention and treatment of malaria in pregnancy.

We thank the mothers who participated in this study and the midwifes at Agogo Hospital.

This study was supported by Charité (grants 99-640, 2000-512, and 2001-613) and Merck Sharp & Dohme, Germany (grant "Infectious Diseases 1999") and forms part of the doctoral thesis of L.H. We do not have a commercial or other association that might pose a conflict of interest.

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