Am. J. Trop. Med. Hyg., 48(3), 1993, pp. 358–364

RISK FACTORS OF MALARIA INFECTION DURING PREGNANCY IN BURKINA FASO: SUGGESTION OF A GENETIC INFLUENCE

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Abstract. A cohort of 570 untreated pregnant women from Burkina Faso was studied to assess the influence of epidemiologic factors on malaria infection, which was quantified as the mean of serial, season-adjusted parasitemia measurements (mean parasite density [MPD]) carried out during the last five months of gestation. A significant effect of the area of maternal residence on the MPD was found ($P < 0.003$) and was probably due to geographic differences in mosquito transmission conditions. The strong relationship observed between parity and malaria infection ($P < 0.0001$), with MPD levels decreasing as the number of gestations increased, confirms that primigravidae are a high-risk group whose protection should be a priority. After adjustment for two relevant epidemiologic factors (i.e., area of residence and parity), the residual MPD values fitted a mixture of two distributions. This result supports the view that a major gene is involved in the determination of malaria infection intensities and is consistent with the results of a recent familial study in Cameroon.

It is known that pregnancy predisposes women to malaria infection, which is a cause of maternal morbidity and retarded intrauterine growth.[1–5] The mechanisms of this increased susceptibility, which may involve an impairment of immunity during pregnancy, are not clearly understood.[4, 6]. Cross-sectional epidemiologic studies have shown the influence of parity on malaria infection, with primigravidae being more frequently and more severely infected than multigravidae.[3–7] However, as far as we know, no longitudinal study with a followup of untreated pregnant women has been reported.

In Burkina Faso, chemoprophylaxis was not believed to significantly reduce morbidity and mortality, and the only recommendation of the Ministry of Health was chloroquine treatment of febrile episodes. As a result, a randomized trial of chloroquine prophylaxis in pregnant women was then conducted between 1987 and 1988 in the southwestern part of the country.[8] The control group in this trial consisted of a cohort of women who were not given any prophylaxis during pregnancy. The aim of this trial was to study the factors that influenced the levels of peripheral parasitemia observed in the women from this control group who were followed until delivery.

SUBJECTS AND METHODS

Subjects

The randomized trial was carried out in the city of Banfora, which is located in the southwestern part of Burkina Faso. The city is divided into seven administrative districts, with a total population of approximately 35,000. The study area is a semi-rural zone of Sudan savanna, where malaria transmission is seasonal and strongly influenced by rainfall. At the time of the study, there was a unique Mother and Child Health Centre in Banfora that was associated with the the Province Hospital.

The details of the trial have been described elsewhere[8] and will be briefly summarized. From February 1987 to February 1988, all pregnant women attending the Mother and Child Health Centre were included in the study and randomly divided into two groups. One of these groups (n = 745) received a weekly prophylaxis of 300 mg of chloroquine, while the control group (n = 719) did not receive any prophylaxis. All women were visited once a week by investigators, asked about febrile episodes or intake of drugs since the last visit, and bled by fingerprick every two weeks to measure parasite densities.

Laboratory methods

Thick blood films were stained with Giemsa. Asexual parasites and leukocytes were counted in 100 fields, and the parasite density per milliliter was calculated assuming an average of 8,000 leukocytes/ml.[3] Laboratory technicians had no information on the status of the individuals from whom the blood samples had been taken. *Plasmodium falciparum* accounted for 95% of the detected parasites and *P. malariae* for the remaining 5%. Since we were interested in the risk factors of global malaria infection, the parasite density considered for analysis was the total parasitemia due to both species of *Plasmodium*.

Statistical methods

Each of the women had several measurements of the parasite density (range 2–14, mean = 6) made during the followup period. Additional analyses of parasite density (PD) was conducted using a logarithmic transformation based on log (PD + 1) to allow for zero counts; the log-transformed parasites density will be designated as the LPD. To deal with a unique variable accounting for the intensity of malaria infection, a mean parasite density (MPD) was determined. Before calculating the MPD, we tested by analysis of variance the effect of two time-dependent factors that could influence the LPD values: the gestational period (i.e., the month of gestation when the parasitemia was measured) and the season when the measurement took place. The LPD values were then adjusted for the effect of the relevant factors as described in the Results, and for each woman, the MPD was then computed as the mean of her adjusted LPD values.

The five measured factors tested in this analysis as potentially influencing the MPD were 1) area of residence (seven areas), 2) parity, which was divided into four subgroups: a) first gestation (pregnancy) (n = 88); b) second gestation (n = 90); c) third gestation (n = 76); and d) more than three gestations (n = 316), 3) ethnic origin, classified as five major groups (Bobo-Dioula, Mossi, Peul, Senoufo, and others, including Dagari, Samo, and more than 20 additional minor groups), 4) hemoglobin genotype, determined for 511 women who agreed to venipuncture: AA (n = 387); AS (n = 38); AC (n = 83); and CC (n = 3), and 5) age in years, considered as a quantitative variable. Statistical analyses were performed using analysis of variance for categorical data and linear regression analysis for age by means of the statistical analysis system. The MPD values were then adjusted for the significant factors, as described in the Results.

To assess the possible role of genetic factors in the levels of parasite densities, a commingling analysis was performed on the MPD values adjusted for the relevant epidemiologic factors. Evidence for a mixture of normal distributions accounting for the adjusted data is consistent with a major Mendelian gene being involved in the determination of the MPD levels, but this can be confounded by skewness in the sample distribution.[9, 10] Therefore, the presence of a mixture of up to three normal distributions can be tested, while correcting for residual skewness by means of a classic power transformation.[9] The power transformation parameter p is estimated either on the assumption that the MPD values follow a normal distribution in the sample or on the assumption that the MPD values follow a mixture of normal distributions. Skewness in the distribution is tested by comparing a model in which p = 1 (corresponding to no transformation) against a model in which p is estimated. Maximum likelihood estimates of the relevant parameters and tests of various hypotheses using the likelihood-ratio criterion[11] were carried out using the computer program SKUMIX (Population Genetics Laboratory, University of Hawaii, Honolulu, HI).[9, 11]

RESULTS

Description of the population

Of the 719 women in the control group, we excluded women with twins (n = 8) and women whose pregnancy outcomes were unknown (n = 141), mostly because they had moved from the study area and/or because they gave birth at home. These 141 women differed significantly from the other women by their ethnic origin, since more women who originated from the northern part of the country (Peul group) left the study area before giving birth. However, the women excluded were comparable with the other women in the study for all other factors (area of residence, parity, and age), and the MPD values did not differ significantly in both groups.

A large majority of the 570 remaining women were enrolled in the study between the fourth

TABLE 1

Characteristics of the study population by intake of antimalarial drugs

Characteristics	Antimalarial treatment: No (%)	Antimalarial treatment: Yes (%)	P*
Age (years)			
<20	86 (16.3)	8 (14.0)	
20–24	161 (30.5)	17 (29.8)	
25–29	138 (26.1)	14 (24.6)	NS
30–34	87 (16.5)	9 (15.8)	
≥35	56 (10.6)	9 (15.8)	
Gestation rank			
1	82 (15.3)	7 (12.3)	
2	84 (15.6)	12 (21.0)	NS
3	71 (13.2)	5 (8.8)	
>3	300 (55.9)	33 (57.9)	
District			
Central†	271 (50.5)	48 (84.1)	0.0006
Peripheral‡	266 (49.5)	9 (15.9)	
Ethnic group			
Bobo-Dioula	90 (16.7)	15 (26.3)	
Mossi	106 (19.7)	11 (19.3)	
Peul	30 (5.6)	2 (3.5)	NS
Senoufo	232 (43.2)	20 (35.1)	
Other	79 (14.8)	9 (15.8)	

* By Pearson's chi-square test. NS = not significant.
† Districts 1 to 3.
‡ Districts 4 to 7.

and fifth months of pregnancy; therefore, only the measurements of parasitemia performed during the five last months of pregnancy were considered in the analysis. It was originally decided that every woman showing a parasitemia associated with clinical symptoms (body temperature > 37.5°C) would be treated with oral chloroquine (25 mg/kg for three days). None of the women demonstrated such an association during the followup. However, 57 women independently took a short treatment (usually at infratherapeutic doses) because they believed they had malaria. These women did not differ significantly from the remaining population, except for the area of residence (treatments were more frequent in the central districts [1–3] than in the peripheral districts [4–7]) (Table 1). Therefore, they were included in the analysis.

Influence of season and period of gestation

There was no effect of the gestational period, but the LPD values were significantly higher during the rainy season than during the dry season (t = 3.39, degrees of freedom [df] = 3,559, $P < 0.001$). The LPD values were consequently adjusted for the effect of the season by subtracting the mean LPD of the corresponding season from each individual LPD, and the MPD was computed for each woman.

TABLE 2

Results of statistical analysis of the effect of risk factors on the mean parasite density

Variable	P
Parity	<0.0001
Area of residence	<0.003
Parity × area	<0.008
Age*	<0.0001
Ethnic group	0.48
Hemoglobin genotype	0.69

* Effect of age disappeared after adjustment for parity.

Risk factors influencing the MPD

We first checked that MPD values were not different between the 57 self-treated women and the rest of the subjects (t = 0.26, df = 568, $P > 0.75$). The results observed when testing the effects of the five factors described above on the MPD are summarized in Table 2. No significant relationship between MPD and both the ethnic group and hemoglobin genotype was observed. The use of two-way analysis of variance showed that the effects of both the area of residence and the parity on MPD were significant ($P < 0.003$ and $P < 0.0001$, respectively), and the interaction between these two factors was also significant ($P < 0.008$). The distribution of MPD values by number of gestations and area of residence is shown in Table 3, and there is a clear trend of decreasing MPD with increasing parity. The MPD values decreased significantly with age and the correlation coefficient computed between age and MPD was −0.32 ($P < 0.0001$). However, after adjustment of the MPD values for parity, there was no effect of age.

The MPD values were then adjusted for the two remaining relevant factors, area of residence and parity. Since a significant interaction between these two factors was observed, the adjustment was performed by subtracting the mean effect observed for the combination of parity and area of residence to which she belonged from the MPD of each woman (28 possible combinations shown in Table 3).

Commingling analysis

Assuming a unimodal distribution, the adjusted MPD values were significantly skewed ($\chi^2 = 202.6$, df = 1, $P < 10^{-6}$). When we tested for a mixture of two skewed distributions against a single-skewed distribution, we found strong evidence for a bimodal distribution in the presence of residual skewness ($\chi^2 = 73.7$, df = 2, $P < 10^{-6}$). There was no evidence for a third component, regardless of whether skewness was accounted for or not. This result indicated that the high skewness observed in the adjusted MPD values comes from 1) a mixture of two distributions and 2) the fact that those two distributions are skewed and need a power transformation to restore normality. The distribution of the adjusted power-transformed MPD values and the predicted normal curves accounting for the presence of two distributions are shown in Figure 1. The proportions of subjects in these two distributions are 95.4% and 4.6%, respectively, for those who present the lower and the upper MPD levels.

DISCUSSION

The heaviest infection intensities were observed among primigravidae and decreased progressively with increasing number of gestations, confirming the major effect of parity on malaria infection during pregnancy reported in previous studies.[1–7] This finding can explain the high levels of placental malaria infection observed in primigravidae at delivery.[8] Because age and parity are strongly correlated in tropical Africa, it was important to test whether the observed effect of age on the MPD was actual or confounding. The disappearance of this effect after adjustment for parity and the results of previous studies demonstrating an increased prevalence of parasitemia in pregnant women compared with nonpregnant women of the same age[1, 2, 4, 12, 13] clearly show that parity is the relevant factor that influences susceptibility to malaria in pregnant women. The mechanisms underlying this increased susceptibility of primigravidae to malaria infection remain unclear and may involve nonspecific immunosuppression due to pregnancy-associated hormones such as cortisol,[14] and impairment of the immune response to certain malaria antigens[6, 15] An important practical implication of these results is that primigravidae are a high-

TABLE 3

*Distribution of standardized mean ± SEM parasite density (MPD) values by the number of gestations and area of maternal residence**

Area	No. of gestations: 1 n	1 MPD	2 n	2 MPD	3 n	3 MPD	≥4 n	≥4 MPD	Total n	Total MPD
1	15	0.19 ± 0.43	17	0.00 ± 0.29	11	0.31 ± 0.49	61	−0.33 ± 0.04	104	−0.13 ± 0.10
2	23	0.79 ± 0.30	24	0.76 ± 0.37	19	0.23 ± 0.18	67	−0.17 ± 0.07	133	0.22 ± 0.10
3	16	1.52 ± 0.43	14	−0.28 ± 0.10	9	−0.28 ± 0.13	40	−0.35 ± 0.05	79	0.05 ± 0.12
4	10	0.77 ± 0.40	6	0.71 ± 0.62	11	0.23 ± 0.21	37	−0.18 ± 0.10	64	0.12 ± 0.12
5	9	0.13 ± 0.39	8	−0.03 ± 0.21	7	−0.45 ± 0.02	37	−0.44 ± 0.03	61	−0.31 ± 0.07
6	7	1.31 ± 0.78	11	−0.05 ± 0.25	9	−0.44 ± 0.04	35	−0.38 ± 0.05	62	−0.14 ± 0.12
7	8	0.52 ± 0.67	10	0.68 ± 0.34	10	0.05 ± 0.19	39	−0.29 ± 0.07	67	0.00 ± 0.11
Total	88	0.77 ± 0.17	90	0.27 ± 0.14	76	0.01 ± 0.10	316	−0.30 ± 0.02	570	0.00 ± 0.04

* Since the MPD values are already adjusted for the effect of the season, there is no direct correspondence between the MPD values and the actual number of parasites.

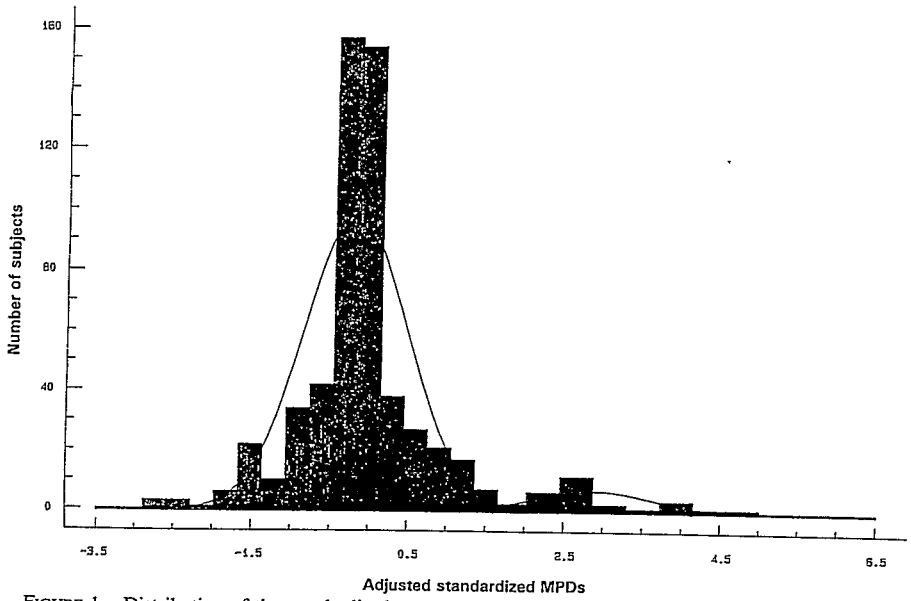

FIGURE 1. Distribution of the standardized power-transformed mean parasite densities (MPDs) after adjustment for parity and area of residence, and predicted normal curves under the hypothesis of two distributions.

risk group whose protection should be a priority to reduce the morbidity and mortality of both women and children.

The effect of the maternal residence is probably due to geographic differences in transmission conditions (with some districts being located close to larval breeding areas). The significant interaction observed between this factor and parity is more difficult to explain. The data seem to indicate a potentialization of the deleterious effect observed in primigravidae when they live in areas of high transmission. The mechanisms that account for this observation warrant further investigations.

As expected, the peripheral parasite densities were significantly higher during the rainy season when transmission is the highest. Whereas cross-sectional studies have shown a decrease in parasitemia prevalence with the period of gestation,[1–4] we did not observe any effect of this factor on the levels of parasite density. However, our data were collected during the second half of pregnancy, whereas parasitemia has been found to be particularly frequent during the first and second trimester of gestation.[1–4]

Whereas the sickle cell and thalassemia traits are thought to afford a certain degree of protection against malaria infection,[16] we did not find any effect of hemoglobin genotype on the MPD. In particular, the mean MPD values observed among individuals heterozygous for the sickle-cell trait (hemoglobin AS) were quite similar to those observed in homozygous (AA) individuals (Table 4), a finding that is consistent with previous studies that failed to demonstrate lower parasite rates in heterozygous (AS) individuals.[17, 18] However, AS heterozygous children have been shown to be less frequently and less severely infected than homozygous AA normal individuals.[19–21] These results support the view that the levels of immunity against *P. falciparum* observed in adults are such that the sickle-cell trait confers no advantage.[22]

TABLE 4

Distribution of standardized mean ± SEM parasite density (MPD) values by hemoglobin genotype

	Genotype			
	AA	AS	AC	CC
MPD	−0.025 ± 0.05	0.004 ± 0.14	−0.085 ± 0.09	0.516 ± 0.49
n	387	38	85	3

Ten percent of the women in the study took antimalarial drugs during their pregnancy, usually at infratherapeutic doses. They differed from the rest of the untreated women only in their area of residence, probably because access to medical care was easier in the central districts than in the peripheral ones. Their MPD values were similar to the MPD values of the other women of the study, and we do not think that this limited chemotherapy affected our results. On the other hand, some of these self-treated women might have selected themselves according to their susceptibility/resistance to malaria infection. In this case, their exclusion from the analysis could have introduced a bias that we wanted to avoid.

Finally, the MPD values adjusted for parity and area of residence fitted a mixture of two distributions. This result is consistent with the presence of a major gene being involved in the determination of the malaria infection intensities among pregnant women; however, this should be confirmed by familial studies in this population. This possible genetic involvement is strongly supported by both our recent segregation analysis performed on families from Cameroon, which showed evidence of a recessive gene controlling blood infection levels in malaria,[23] and findings in mice that demonstrated the role of host genetic factors in the outcome of experimental malaria infections.[24, 25] Certain genetic disorders of red blood cells, such as sickle-cell anemia, thalassemia, ovalocytosis, and glucose-6-phosphate dehydrogenase deficiency are known to increase resistance against malaria infection in humans;[16, 26] however, as mentioned above for abnormal hemoglobins, the importance of this protection remains unclear. Although genetic variants of the red blood cells have achieved polymorphic frequencies in many populations, the individual degree of protection afforded by them may only be quite small.[16]

The role of HLA genes on the ability to respond to malaria infections has also been reported.[27, 28] We intend to conduct familial studies in populations that are different from the one in our previous study[23] to confirm the role of a major gene controlling susceptibility/resistance to malaria infection and to determine the relationships of this gene with the genetic defects of red blood cells mentioned above and the HLA system. The identification of such a gene would have major implications in the control of malaria.

Financial support: This study was supported by the INSERM (Institut National de la Sante et de la Recherche Medicale) Reseau Nord-Sud no. 486 NS2.

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