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

M. COT, L. ABEL, A. ROISIN, D. BARRO, A. YADA, P. CARNEVALE, AND J. FEINGOLD

ORSTOM Center Muruz, Bobo-Dioulasso, Burkina Faso; Unit of Research in Genetic Epidemiology (INSERM U153), Paris, France; Department of Biometrics and Mathematics (INSERM U194), Pitié-Salpêtrière Hospital, Paris, France; USAID, Ouagadougou, Burkina Faso; Ministry of Health, Ouagadougou, Burkina Faso; ORSTOM s/c OCEAC, Yaoundé, Cameroon

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 number of febrile episodes. As a result, a randomized trial was carried out in the city of Banfora, which is located in the southern part of the country. 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 Ministry of Health.

It is known that pregnancy predisposes women to malaria infection, which is a cause of maternal morbidity and retarded intrauterine growth. The mechanisms of this increased susceptibility, which may involve an impairment of immunity during pregnancy, are not clearly understood.

The randomized trial was carried out in the city of Banfora, which is located in the southeastern 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 Ministry of Health.

The details of the trial have been described elsewhere 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 recent 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 microliter was calculated assuming an average of 8,000 leukocytes/μl. 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 Plasmodium 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) were conducted using a logarithmic transformation based on log (PD + 1) to allow for zero counts; the log-transformed parasite 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 significance of two different factors that could influence the MPD values: the gestational period (i.e., the month of gestation when the parasitemia was measured) and the season when the measurement took place. The MPD 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-Dioulasso, 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 = 287), AS (n = 138), and AC (n = 36), 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 cumulating method was used for 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. 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. 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 were carried out using the computer program SKUMIX (Population Genetics Laboratory, University of Hawaii, Honolulu, HI).

RESULTS

Description of the population

Of the 719 women in the control group, we excluded women with twins (n = 8) and women who were pregnant at the beginning of the study (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 between the two groups.

A large majority of the 570 remaining women were enrolled in the study between the fourth
but the LPD values were significantly higher during the rainy season than during the dry season. However, 57 women independently took short treatments (usually at infratherapeutic doses) because they believed they had malaria. We first checked that MPD values were not different between the 57 self-treated women and the rest of the subjects (t = 3.39, degrees of freedom [df] = 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.

Risk factors influencing the MPD

We found that the MPD values were not different between the 57 self-treated women and the rest of the subjects (t = 3.39, degrees of freedom [df] = 559, P < 0.001). The MPD 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.

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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 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, 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. Whereas the sickle cell and thalassemia traits are thought to afford a certain degree of protection against malaria infection, 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 homozygous (AA) individuals.

We next report the adjusted standardized mean parasite densities (MPDs) after adjustment for parity and area of residence, and predicted normal curves under the hypothesis of two distributions.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>MPD</th>
<th>AS</th>
<th>AC</th>
<th>CC</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>-0.025 ± 0.05</td>
<td>0.004 ± 0.14</td>
<td>-0.085 ± 0.09</td>
<td>0.516 ± 0.49</td>
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<tr>
<td></td>
<td>387</td>
<td>38</td>
<td>85</td>
<td>3</td>
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</tbody>
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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.

Table 4: Distribution of standardized mean ± SEM parasite density (MPD) values by hemoglobin genotype

As a result, we have conducted a retrospective controlled study from India. The results of this study supported the view that 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, and findings in mice that demonstrated the role of host genetic factors in the outcome of experimental malaria infection. 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; 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. The role of the HLA genes on the ability to respond to malaria infections has also been reported. We intend to conduct familial studies in populations that are different from the one in our previous study to confirm the role of a major gene controlling susceptibility/resistance to malaria infection and to determine the relationship 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.

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Authors' address: M. Cot, ORSTOM Center Muraz, Bobo-Dioulasso, Burkina Faso, and Unit of Research in Genetic Epidemiology (INSERM U155), Paris, France. L. Abel, Department of Biomathematics (INSERM U194), Pitié-Salpêtrière Hospital, Paris, France. A. Roisin, USAID, Ouagadougou, Burkina Faso. D. Barro and A. Yada, Ministry of Health, Ouagadougou, Burkina Faso. P. Carnevale, ORSTOM n°CCEAC, Younde, Cameroon. J. Feingold, Unit of Research in Genetic Epidemiology (INSERM U155), Paris, France.

Reprint requests: L. Abel, Department of Biomathematics (INSERM U194), Hopital de la Pitié-Salpetrière, 91 Bd de l'hopital, 75013 Paris, France.

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