Research

Prevalence of *Plasmodium falciparum* infection in pregnant women in Gabon

Marielle K Bouyou-Akotet*[1,2,3], Denisa E Ionete-Collard[4], Modeste Mabika-Manfoumbi[1], Eric Kendjo[1], Pierre-Blaise Matsiegui[2], Elie Mavoungou[2,3] and Maryvonne Kombila[1]

Address: [1]Département de Parasitologie, Mycologie, Médecine Tropicale, Université des Sciences de la Santé, Libreville, Gabon, [2]Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon, [3]Department of Human Parasitology, Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany and [4]UNICEF, Niamey, Niger

Email: Marielle K Bouyou-Akotet* - mar_bouyou@yahoo.fr; Denisa E Ionete-Collard - dionet@unicef.org; Modeste Mabika-Manfoumbi - mabikmamfoumbi@yahoo.fr; Eric Kendjo - eriked@yahoo.fr; Pierre-Blaise Matsiegui - chercheurs@inet.ga; Elie Mavoungou - elie.mavoungou@uni-tuebingen.de; Maryvonne Kombila - m.kombila@internetgabon.com

* Corresponding author

Abstract

Background: In areas where malaria is endemic, pregnancy is associated with increased susceptibility to malaria. It is generally agreed that this risk ends with delivery and decreases with the number of pregnancies. Our study aimed to demonstrate relationships between malarial parasitaemia and age, gravidity and anaemia in pregnant women in Libreville, the capital city of Gabon.

Methods: Peripheral blood was collected from 311 primigravidae and women in their second pregnancy. Thick blood smears were checked, as were the results of haemoglobin electrophoresis. We also looked for the presence of anaemia, fever, and checked whether the volunteers had had chemoprophylaxis. The study was performed in Gabon where malaria transmission is intense and perennial.

Results: A total of 177 women (57%) had microscopic parasitaemia; 139 (64%)of them were primigravidae, 38 (40%) in their second pregnancy and 180 (64%) were teenagers. The parasites densities were also higher in primigravidae and teenagers. The prevalence of anaemia was 71% and was associated with microscopic *Plasmodium falciparum* parasitaemia: women with moderate or severe anaemia had higher parasite prevalences and densities. However, the sickle cell trait, fever and the use of chemoprophylaxis did not have a significant association with the presence of *P. falciparum*.

Conclusions: These results suggest that the prevalence of malaria and the prevalence of anaemia, whether associated with malaria or not, are higher in pregnant women in Gabon. Primigravidae and young pregnant women are the most susceptible to infection. It is, therefore, urgent to design an effective regimen of malaria prophylaxis for this high risk population.

Background

In areas where malaria is highly endemic, a protective semi-immunity against *Plasmodium falciparum* is acquired during the first 10–15 years of life, and the majority of malaria-related morbidity and mortality happens in young children [1]. However, in contrast with low malaria prevalence in adults, pregnant women in endemic areas are highly susceptible to malaria, and both the frequency and the severity of disease are higher in pregnant than non-pregnant women [2]. In pregnancy, there is a transient depression of cell-mediated immunity that allows foetal allograft retention but also interferes with resistance to various infectious diseases [3]. Cellular immune responses to *P. falciparum* antigens are depressed in pregnant women in comparison with non-pregnant control women [4,5]. Anti-adhesion antibodies against chondroitin sulphate A-binding parasites are associated with protection from maternal malaria, but these antibodies develop only over successives pregnancies, accounting for the susceptibility of primigravidae to infection [6]. Indeed, women in first and second pregnancies are the most affected, with both gravidity and premunition influencing susceptibility to malaria infection [7–10]. Numerous epidemiological studies have reported a broad range of conditions during pregnancy which are a result of malaria [11–15]. As with peripheral parasitaemia, placental infection is also most frequent and heaviest in primigravidae [16]. Furthermore, malaria reduces birth-weight most in this group [16]. Although this data has been reported in many African countries, hardly anything is known about malaria in pregnant women from Gabon, a central African country where *P. falciparum* malaria is endemic. Thus, we conducted a community-based cross-sectional study to describe the prevalence of malaria parasitaemia and anaemia in pregnant women living in Libreville. As the prevalence of infection is lower in individuals with sickle cell trait compared with their normal haemoglobin counterparts [17], we also studied the frequency of the sickle cell trait.

Patients and methods

Study site and population

The study area is in Libreville, Gabon, where the climate is equatorial. Two rainy seasons occur in Gabon, from February to May and September to December, and two dry seasons. The annual temperature ranges from 24°C to 31°C (average 25.9°C), and the average humidity is greater than 80%. In this area, malaria is hyperendemic, predominantly caused by *P. falciparum*. The entomological inoculation rate (EIR), measured in an adjoining urban area, is 50 infected bites per person per year [18] and the main vectors are *Anopheles gambiae* and *Anopheles funestus*. This study was conducted during both the high rainy and dry seasons, from April 1995 to September 1996. Pregnant women (first and second pregnancy)

attending the SMI de la Peyrie (a maternal and child health centre located in an urban area) were enrolled in the study.

Ethical considerations

All work was performed according to the guidelines for human experimentation in clinical research stated by the Ministry of Public Health and Population of Gabon. All women gave oral informed consent.

Data collection

Women in their first and second pregnancies were included in the study when they came for their first monthly antenatal visit. A number was attributed to each one, which was recorded on a prenatal consultation form. After a complete medical examination by nurse midwives and two physicians, the volunteer's age, weight, length of pregnancy, parity, exposure to antimalarial drug and axillary temperature were noted. Women were considered as having a fever when their temperature was ≥ 38°C. Gestational age was assessed using a gestational calendar, trimester was defined as first (<14 weeks), second (14–27 weeks) and third (>27 weeks). The biological check-up included measuring the haemoglobin rate (Hb), using haemoglobin electrophoresis to detect sickle cell trait and detecting parasitaemia.

Laboratory methods

Thick and thin blood films were stained with Giemsa and read for malaria parasites by two trained microscopists following standard, quality-controlled procedure [19]. Parasitaemia was expressed as the number of asexual forms of *P. falciparum* per microlitre: a result was considered negative after a reading of 1,000 leucocytes in the microscope (× 1,000). Haematological measurements were verified using a Coulter Hycel HC plus HD 21, an automated analyser. Parasitaemia was graded as low (1–999/μl), moderate (1000–9999/μl) and high (>10000/μl), haemoglobin levels as normal (>11 g/dl), low anaemia (11–9 g/dl), moderate anaemia (8.9–7 g/dl) and severe anaemia (<7 g/dl), and age as teenagers (<20 years old), young women (20–24 years old) and older women (>24 years old). *P. falciparum*-positive women were treated with quinine at 25 mg/kg/day during five days. The alkaline pH electrophoresis of haemoglobin was performed for sickle cell trait determination.

Statistical analysis

Data was analyzed using EpiInfo 6.04b and the Statview software programme. For paired and unpaired comparisons of continuous variables, the non-parametric Wilcoxon sign rank, the Kruskal-Wallis, and Mann-Withney U-tests were used. Chi square analysis was used to compare proportions within and among groups. Adjusted tests were performed with the presence of malaria

Table 1: Characteristics of the study groups

	Number of women (n= 311)	Primigravidae (n= 217)	Secundigravidae (n= 94)	P^a
Age mean years, (SD)[b]	19 (3.33)	19 (3.31)	21 (2.93)	< 0.001
Age of pregnancy mean, months (SD)	4 (1.3)	4 (1.2)	4 (1.4)	
Thick blood smear				
Negative No[c], (%)	134 (43)	78 (36)	56 (60)	
Positive No, (%)	177 (57)	139 (64)	38 (40)	< 0.001
Low[d] No, (%)	92 (52)	66 (47)	26 (68)	
Moderate No, (%)	75 (42)	64 (46)	11 (29)	
High No, (%)	10 (6)	9 (6)	1 (3)	
Parasitaemia[e]				
Median [IQR][f]	930 [348–2808]	1050 [510–3060]	434 [149–2008]	0.002
Haemoglobin				
Mean g/dl, (SD)	10.2 (1.4)	10.1 (1.4)	10.3 (1.3)	NS[g]
Non anaemic No, (%)	90 (29)	61 (28)	29 (31)	
Anaemic No, (%)	221 (71)	156 (72)	65 (69)	NS
Low[h] No, (%)	157 (71)	108 (69)	49 (75)	
Moderate No, (%)	59 (27)	45 (29)	14 (22)	
Severe No, (%)	5 (2)	3 (2)	2 (3)	
EpHb[i]				
AA No, (%)	248 (80)			
AS No, (%)	56 (18)			
AC No, (%)	7 (2)			
Fever				
yes No, (%)	84 (27)			
no No, (%)	227 (73)			
Chemoprophylaxis				
yes No, (%)	17 (5)			
no No, (%)	294 (95)			

[a] *P* values for comparison between primigravidae and multigravidae. [b] Standard Deviation. [c] Number of pregnant women. [d] for definitions of parasitaemia see Patients and Methods. [e] parasitaemia in parasitaemic women. [f] interquartile range. [g] Not significant. [h] for definitions of anaemia see Patients and Methods. [i] Electrophoresis of Hb.

parasitaemia as a dependent variable using age and gravidity as possibly variables influencing malaria. $p < 0.05$ was considered significant.

Results

Patient characteristics

311 women were selected at their first ACC (antenatal clinic care) at SMI de la Peyrie, patients' characteristics are summarised in Table 1. 217 (70%) were primigravidae. The mean age was 19 years (13–37) with 93% less than 25 years. Primigravidae were significantly younger than secundigravidae (z = 3.20 p < 0.001). The mean length of pregnancy was four months (1–6). 17 (5%) women had started chemoprophylaxis with chloroquine (600 mg/week). 248 (80%) had a normal haemoglobin (AA), 56 (18%) had haemoglobin AS and seven (2%) had haemoglobin AC. There was no difference in distribution of these variables between primigravidae and women in their second pregnancy.

Malaria prevalence

Malaria prevalence was 57% (n = 177) (Table 1). All infections were diagnosed as *P. falciparum*. The median of parasitaemia in all infected women was 930 parasites/microlitre (p/µl). Malaria prevalence was significantly higher in primigravidae women (64%) than in women in their second pregnancy (40%) (Odds ratio [OR] = 2.41, 95% confidence interval [CI] = 1.43 - 4.07, *p* < 0.001). Parasitaemic primigravidae women had higher median parasites density than parasitaemic secundigravidae (1050 versus 434 p/µL, z = 3.11, *p* = 0.002). (Table 1). The prevalence of parasitaemia did not vary with season (dry or rainy). Overall, the parasite prevalence was 52% in the dry season and 59% in the rainy season (OR = 1.3, 95% IC = 0.77 - 2.30, *p* = 0.48)(data not shown).

Relation with age

Although pregnant women were young, and only in their first or second pregnancy, we compared the prevalence of *P. falciparum* infection between teenagers, young and older women. (Table 2). Parasitaemia was significantly

Table 2: Relation of age with parasitaemia and anaemia

Age	years, (No)[a]	≤ 19 (180)	20 – 24 (109)	> 24 (22)	P
Non parasitaemic	No, (%)	65 (36.1)	57 (52.3)	12 (54.5)	
Parasitaemic	No, (%)	115 (63.9)	52 (47.7)	10 (55.5)	0.01
Parasitaemia	median [IQR][b]	1200 [425–3260]	820 [224–1984]	784 [385–2668]	NS [c]
Non anaemic	No, (%)	47 (26.1)	35 (32.1)	8 (36.4)	
Anaemic	No, (%)	133 (73.9)	74 (67.9)	14 (67.6)	NS
Hb	mean g/dl, (SD)[d]	10 (1.3)	10.3 (1.4)	10.4 (1.5)	NS

[a]Number of pregnant women. [b] [IQR] = interquartile range. [c]Not significant. [d]Standard Deviation

more common in teenagers (63.9%) than young (47.7%) and older women (55.5%) (χ^2= 8.68, *p* = 0.013). No significant difference was found between younger and older women (OR = 1.09, 95% CI = 0.4 - 3.02, *p* = 0.85) (not shown). We separately examined teenagers (< 20 years old) and older (≥ 20 years old) women as well as primigravidae and secundigravidae for influences on malaria. Malaria prevalence was higher in teenagers than older women (64% versus 47%, OR = 1.99, 95% CI = 1.22 - 3.25, *p* = 0.002). After correcting for parity, this difference was also significant (OR = 1.77, 95% CI = 1.10 - 2.85, *p* < 0.01).

Relation with haemoglobin AS
Malaria prevalence was not influenced by the sickle cell trait. Although 64% of Hb AS women were infected compared to 55% Hb AA women, this difference was not statistically significant (OR = 1.4, 95% CI = 0.77 - 2.79), nor were the medians of parasitaemia.

Relation with fever
In the groups recruited, 84 (27%) women had a fever. Within this group, 39 women (46%) had a plasmodial infection. The prevalence of plasmodial infection was significantly higher in women without fever (61%) (OR = 0.55, 95% CI = 0.33 - 0.95, *p* = 0.023). There was no difference in the medians of parasitaemia between these groups.

Relation with haemoglobin levels
Anaemia prevalence was 71% (n = 221) (Table 1). In order to evaluate the effect of *P. falciparum* peripheral infections in women with and without anaemia, we compared the prevalence of parasitaemia. The presence of *P. falciparum* asexual form in peripheral blood was highly associated with anaemia. Indeed, anaemic women were significantly more infected (63%) than non-anaemic (41%) women (OR = 2.38, 95% CI = 1.4 - 4.05, *p* < 0.001). However, anaemia prevalence was similar between primigravidae and secundigravidae (Table 1). There was no significant difference between teenagers, yound and older women (Table 1). Mean haemoglobin

level was 10.2 g/dl in all the enrolled women, and similar between women of different gravidity (Table 1). Parasites grade and anaemia classes are cross-tabulated in Table 1. Moderately and severely anaemic women had higher parasite densities (medians 2818 and 1166 p/μl) than women with low anaemia (981 p/μl). The lowest densities were observed in non-anaemic women. Association between sickle cell trait carriage and haemoglobin levels was assessed. The mean haemoglobin levels did not differ between Hb AS women and Hb AA women (10 versus 10.2 g/dl, z = -1.12, *p* = 0.26), nor were the prevalence of anaemia (71% versus 76.8%, OR = 1.08, 95% CI = 0.66 - 2.85, *p* = 0.37).

Relation with previous chloroquine chemoprophylaxis
Only 17 women (9 primigravidae and 8 secundigravidae) had taken chemoprophylaxis from the beginning of pregnancy. When we compared the levels of parasitaemia in this group to the other 294 women, we found that previous chloroquine prophylaxis had no effect on susceptibility to *P. falciparum* infection. The prevalence of infected women was not different: 47% in group with chemoprophylaxis had malaria compared to 57% of the women without previous chemoprophylaxis (OR = 1.19, 95% CI = 0.4 - 3.46, *p* = 0.73). The medians of parasitaemia were also similar (not shown).

Discussion
The susceptibility of pregnant women to malaria is well-established [1,20]. However, no epidemiological data have been recorded in Libreville, the capital city of Gabon, where *P. falciparum* malaria is highly endemic. The aim of this study was to evaluate the prevalence of malaria infection in Gabonese women who were pregnant and to determine the relationship between this and gravidity status, age, anaemia, the sickle cell trait and chemoprophylaxis.

We found that malaria prevalence was 57%. As expected, this prevalence was higher in primigravidae than secundigravidae, and in women under 20 years old. Anaemia prevalence was also higher, with a significant association

Table 3: Relationship between anaemia and parasitaemia

Parasitaemia[a]	0	Low	Moderate	High	Median [IQR][b]
Haemoglobin, No[c] (%)					
<7 g/dl (n = 5)	1 (20)	1 (20)	2 (40)	1 (20)	2818 [1047–8376]
7 – 8.9 g/dl (n = 59)	16 (27.1)	21 (35.6)	19 (32.2)	3 (5.1)	1166 [527–3268]
9 – 11 g/dl (n = 157)	66 (41.4)	47 (29.9)	42 (26.8)	3 (1.9)	981 [321–2475]
>11 g/dl (n = 90)	53 (59)	21 (23.3)	12 (13.3)	4 (4.4)	728 [278–2242]

[a]For definition of parasitaemia, see Patients and Methods. [b] [IQR] = interquartile range. Median for those parasitemic. [c]Number of pregnant women in each parasitaemic group.

between *P. falciparum* infection and anaemia. We also found that the presence of the sickle cell trait had no influence on malaria prevalence, nor did previous chemoprophylaxis. Likewise, age and gravidity had no effect on anaemia. These results are in agreement with those reported elsewhere, which show that *P. falciparum* infection is higher during pregnancy, more so in primigravidae and is usually associated with anaemia or reduced haemoglobin levels [21–27].

Our rationale for including pregnant women attending the SMI de la Peyrie is that they are representative of the general population in terms of accessibility to the health centers, and they had low income. In Gabon, early pregnancies are frequent, and 60% of population is below 25 years of age, the age of most of the pregnant women included in the study (93%). Women in their first and second pregnancies were chosen as they constitute one of the risk groups in stable malaria areas [20,28–31].

Previous studies conducted in different areas in Gabon, have reported the prevalence of *P. falciparum* infection in pregnant women in the same range of our results. These studies were done at delivery [32,33]. Only the study conducted by Garin *et al*. showed an increase of plasmodial parasitaemia during pregnancy in primigravidae and secundigravidae compared to multigravidae [34]. Our findings are in general accordance with findings from other areas (i.e. Malawi, Tanzania, Ghana, India), four urban sites with similar malaria epidemiological characteristics [21–24,31]. In these vulnerable populations, primigravidae remain unquestionably the most susceptible: they are more often infected than multigravidae. In the same epidemiological context, these levels of prevalence are similar to those reported by other authors: 65% in Dielmo, Senegal [28], 62% in Tanzania [25], 65% in Malawi [26] and 41% in Kenya [35]. The levels of parasitaemia are also high in primigravidae. This could be explained by gravidity status, and as well as by the fact that the women were, in most cases, recruited at the fourth month of pregnancy. In the second trimester, parasitaemia is usually high compared to the first and the third tri-

mester [24,35]. Age and gravidity appeared to be the principal influences of malaria prevalence, mostly in teenagers.

Anaemia prevalence was also high in all women, i.e. 71%, in agreement with the mean percentage for Africa (61%) [36,37]. This prevalence is identical to that reported in a group of pregnant women in Malawi [27] and Tanzania [24]. A significant role in the determination of anaemia has been demonstrated in this study. Women with low anaemia had the highest prevalence of malaria (50%), and higher parasite load was most common in women with severe anaemia. Similar finding were reported from Blantyre, Malawi [31]. In our study, gravidity and age did not influence the prevalence of anaemia and the mean heamoglobin levels. Therefore, all anaemia aetiologies in pregnant women should be further investigated.

Fever has not been found to be a good indicator of clinical malaria. Despite the fact that in endemic areas asymptomatic *P. falciparum* infections are frequent in adults, our study showed that malaria was not the main aetiology of fever during pregnancy. Other causes like urinary and genital infection could be the cause and should be treated to avoid subsequent obstetrical problems.

The prevalence of sickle cell trait in Gabon is about 22,5% [38]. Furthermore, the prevalence of infection has been reported to be lower in individuals with sickle cell trait compared to the normal-haemoglobin counterparts [17]. In our study population, the prevalence of the sickle cell trait was similar to the general population, and it did not influence the prevalence of malarial infection. This is not surprising: studies conducted by Van Dongen in Zambia and Mockenhaupt in Ghana have also shown that they no have no influence on each other [22,39].

Previous chloroquine chemoprophylaxis has no effect on the prevalence of infection and parasitaemia. Chloroquine is still recommended as the first-line anti-malarial drug in preventive treatment for pregnant women in Gabon. It is widely used, affordable and available, thus, a

reasonable choice for the majority of the population. Considering that the drug has been taken in correct doses, and that pregnant women generally use insecticides, is chloroquine still efficient in chemoprophylaxis since it is known that its efficacy in curative treatment in children and adults is less than 20% [40,41]?

Unfortunately, we did not evaluate some confounding factors such as type of housing, stool hookworm burden or human immunodeficiency virus serostatus that seem to influence malaria and/or anaemia.

Conclusion

This study illustrates high prevalence of malaria and anaemia in pregnant women living in Libreville. Both are more common in first pregnancies, and primiparity and young age appear to be greater determinants of malaria risk. Chloroquine is still recommended by national health policy in Gabon, and chloroquine resistance in curative doses has been reported. Thus, an effective regimen of malaria prophylaxis of women during their first and second pregnancies is strongly recommended.

Authors'contributions

MK B-A participated in study and in the statistical analysis and the drafting of the manuscript. DE I-C participated in the design of the study and the statistical analysis. M M. carried out the study and participated in biological tests. E K participated in the statistical analysis. PB M participated in the medical and biological tests. E M participated in the design of the study and the drafting of the manuscript. M K coordinated and participated in the design of the study, statistical analysis, and the drafting of the manuscript. All authors read and approved the final version.

Acknowledgements

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