

Peripheral, Placental and Umbilical Plasmodic Incidence in a Stable Endemic Area at the Talangai Reference Hospital (R. du Congo)

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Abstract: ***Introduction:** malaria associated with pregnancy (MAP) remains a public health concern, taking into account the vulnerability of the populations involved (women and children). It is in this context that this study was to establish the plasmodic incidence of peripheral blood, placenta and cord during MAP. **Material and methods:** It was a cross-sectional and analytical study carried out at the maternity ward of the Talangai Reference Hospital (R. du Congo). 56 parturients were received at the hospital. The thick drop and appositions were made with peripheral blood, placenta and cord blood. **Results:** The average age was 25.29 with 19 years and 40 years as the extremes. Age, pregnancy and parity did not show any influence on the methods of prophylaxis. Peripheral blood, placenta and cord plasmodic incidence were 36.54%, 23% and 7.84%, respectively. There was no statistically significant difference in the distribution of peripheral blood, placental and cord parasitaemia and no associativity was observed between these parameters. **Conclusion:** The peripheral, placental and cord plasmodic index of malaria associated with pregnancy in our study remains of concern, especially since the study population was peri-urban, that is to say accessible to health services.*

Keywords: malaria, placenta, incidence, plasmodium, gestity, parity

1. Introduction

Pregnancy-associated malaria is a complex pathophysiological situation. Pregnancy which is an allograft which the maternal HLA system tolerates by a protective mechanism of immunosuppression; it is a state of vulnerability to possible infections.

The consequences of pregnancy-associated malaria which is characterized by an increased risk of death and impact on the health of the fetus, resulting in prematurity and low birth weight. In 2018, around 11 million pregnant women in sub-Saharan Africa developed a malaria infection, of which 16% of the children had a low birth weight [1]. It is within this framework that the WHO World Malaria Report 2019 includes a special section on the burden of malaria and its consequences for mothers and children.

Placental malaria infection is defined by the presence of trophozoites or schizonts in the placental apposition [2]. Congenital malaria is caused by the transplacental passage of parasitized red blood cells from mother to child and presents in two forms, one latent or congenital malaria infestation, the other patent or congenital malaria disease [3].

The Republic of Congo, a hyperendemic region for malaria; transmission is perennial there with an inoculation rate of 20

to 500 infectious mosquito bites per man per year. Malaria remains the leading cause of hospitalization with 55% of cases [4]. There are no recent data on the prevalence of congenital and especially placental malaria in the Republic of Congo. The rare studies of pregnancy-associated malaria have reported only observations on epidemiological data [5, 6, 7].

This work was an analytical study of the presence of plasmodium in peripheral blood, placenta and cord in the context of stable transmission contributing to the understanding of the pathophysiological mechanism of MAP. Thus, the objective of this study was to establish the plasmodic incidence of peripheral blood, placenta and cord during MAP.

2. Material and Methods

The study was carried out at the Talangai Base Reference Hospital in the Department of Brazzaville in the Republic of Congo. The biological analyzes took place in the Laboratory of Training, Research and Biomedical Analysis of the Faculty of Health Sciences of the University Marien Nguabi in the Republic of Congo.

It was an analytical cross-sectional study that took place over a period of 3 months. Simple random draw was the sample collection method used. The study population

consisted of all HIV-negative women coming to the center for childbirth. 56 pregnant women divided according to:

- The presence or absence of plasmodium in peripheral blood, placenta and cord;
- Taking prophylactic treatments: G1, no prophylaxis (intermittent preventive treatment with sulfadoxine-pyrimethamine or other treatments), G2, irregular prophylaxis, G3, regular prophylaxis.

The thick drop was performed routinely in all women giving birth. The thick drop of peripheral blood and cord was stained with 10% diluted Giemsa supplemented with a rapid diagnostic test (RDT). Parasite density was assessed by counting the number of parasitized in proportion to the number of leukocytes on a field microscope and a double-blind reading was taken by two experienced microscopists.

A volume of 2 to 5 ml of blood was drawn in an EDTA tube and in a dry tube during childbirth. Two appositions on the maternal side of the placenta were made, fixed in air and stained with MGG (May Grunwald Giemsa). The elements sought were parasitized red blood cells and intra-macrocyclic pigments. Placental malaria infection is diagnosed by the presence of trophozoites or schizonts. The Blood Formula Count was performed using an impedance variation technology hematology meter.

This work had received the approval of the Ethics Committee for Research in Health Sciences(CERSSA) and the statistical analysis was done on Excel and Graph pad.

3. Results

Sociodemographic characteristics of the study population

Distribution of pregnant women according to groups

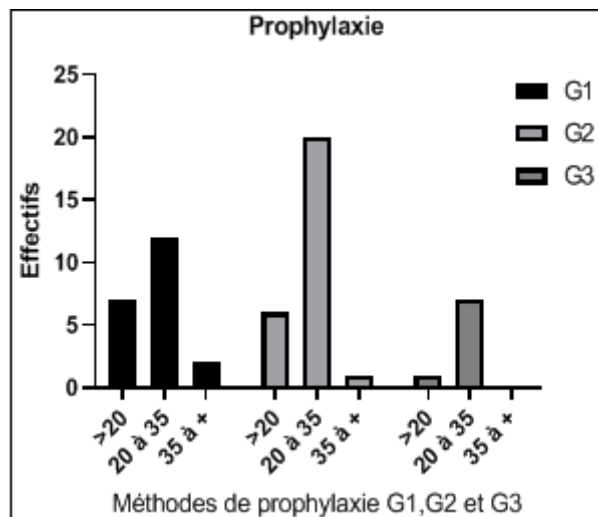
The sample size was 56 pregnant women. It was divided into three groups: no prophylaxis, G1 (IPT and others), irregular prophylaxis (G2), regular prophylaxis (G3). The G2 group of pregnant women with irregular prophylaxis methods was the most representative with 50.41%.

Maternal age

The distribution of pregnant women by age group showed that the age extremes ranged from 15 to 40 years with an average age of 25.29 ± 6.45 . The majority age group of pregnant women was 20 and 35, followed by the ≤ 19 age group with a statistically significant difference between age groups (ANOVA P value: 0.0003).

Antimalarial chemoprophylaxis

Graph 1 recapitulated the methods of prophylaxis for pregnant women according to age groups.



Graph 1: Breakdowns by age group according to prophylaxis methods,

The prophylaxis methods used did not show a statistically significant difference depending on the age groups of pregnant women (P value: 0.5046).

Comparison between different methods of antimalarial chemotherapy and socio-demographic characteristics

The comparison between different methods of antimalarial chemotherapy (G1, G2, G3) and socio-demographic characteristics was presented in Table I.

Table I: Comparison between different methods of antimalarial chemotherapy (G1, G2, G3) and socio-demographic characteristics.

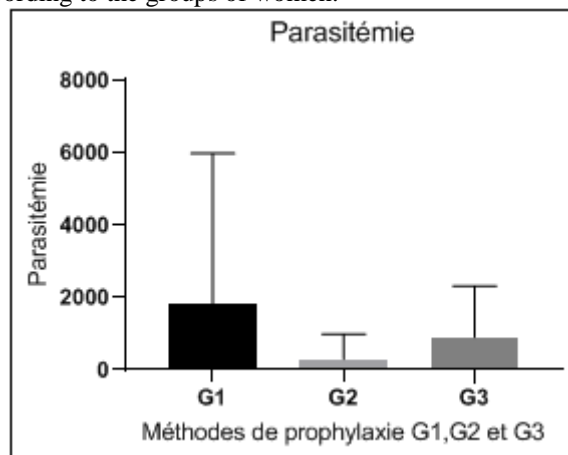
Parametres	P value	Observation
Niveau d'instruction vs G1, G2, G3	0,5859	NS
Age maternelvs G1, G2, G3	0,5046	NS
La gestité vs G1, G2, G3	0,3609	NS
La parité vs G1, G2, G3	0,8857	NS

NS: not Significant, vs; face

Biological parameters

The distribution of the parasitaemia according to the methods of prophylaxis

Graph 2 summarized the distribution of the parasitaemia according to the groups of women.

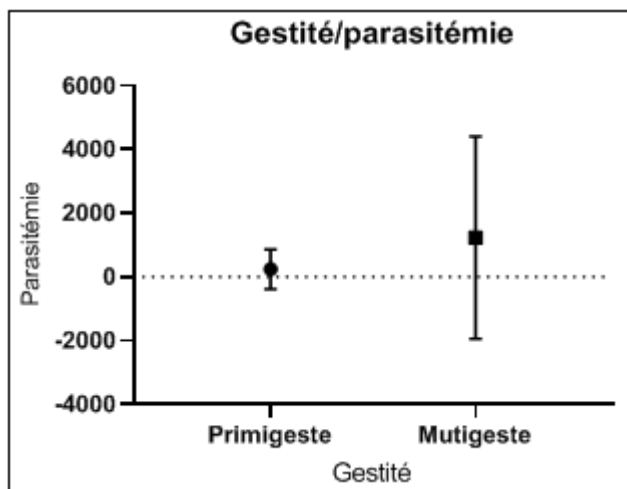


Graph 2: Distribution of parasitaemia according to prophylaxis methods

The distribution of parasitaemia between groups of women did not show a statistically significant difference (ANOVA, P value: 0.1793).

Parasitaemia and gestity

The distribution of parasitaemia according to pregnancy was shown in Graph 3.

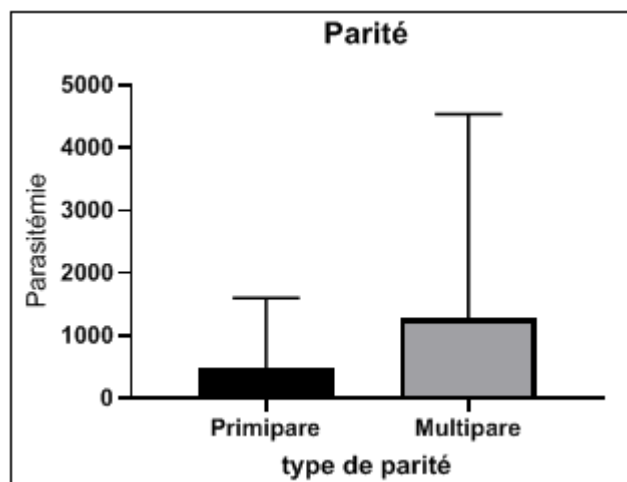


Graph 3: Distribution of parasitaemia according to gestity.

There was no statistically significant difference in parasitemia between the gestity types (P value: 0.2394).

Parasitaemia and parity

The distribution of parasitaemia as a function of parity was shown in Graph 4.

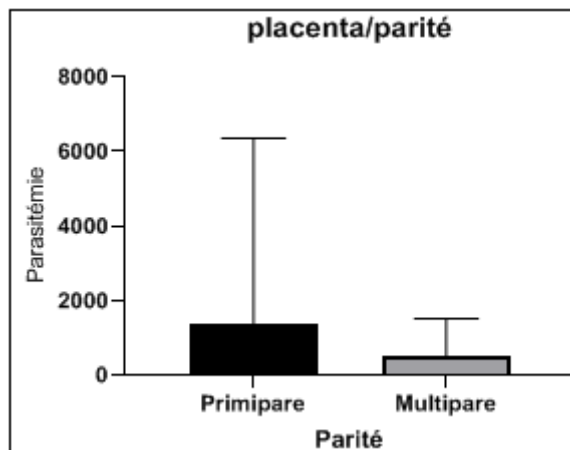


Graph 4: Distribution of parasitaemia as a function of parity.

Parity and parasitaemia did not show a correlation (P value: 0.8692). Likewise, the parasitaemia between the types of parity did not show a statistically significant difference (P value: 0.3536).

Placental parasitaemia and parity

The distribution of placental parasitaemia as a function of parity was shown in Graph 5.

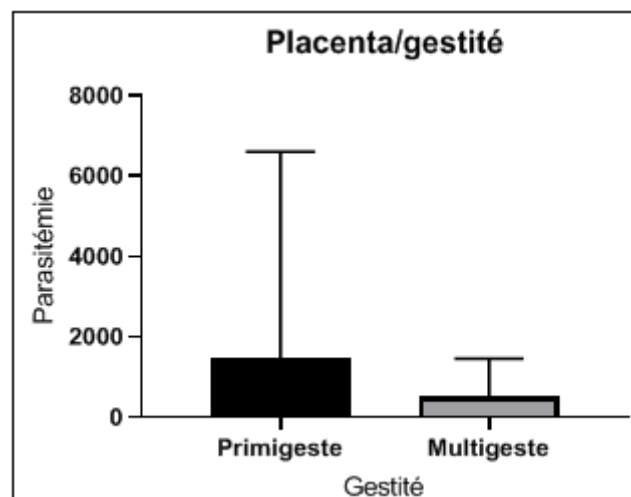


Graph 5: Distribution of placental parasitaemia according to parity.

The distribution of placental parasitaemia by parity did not show a statistically significant difference (Unpaired t test, P value: 0.3560).

Placental apposition and gestity

The distribution of placental parasitaemia according to pregnancy was shown in Graph 6.

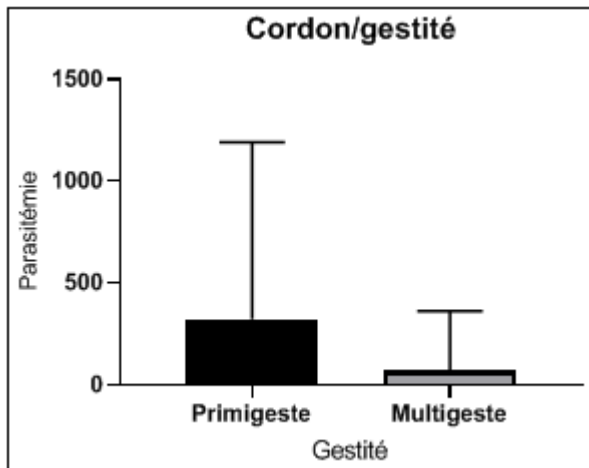


Graph 6: Distribution of placental parasitaemia according to gestity.

The distribution of placental parasitaemia according to pregnancy did not show a statistically significant difference (Unpaired t test, P value: 0.2841).

Cord blood and gestity

The distribution of cord blood parasitaemia according to pregnancy was shown in Graph 7.

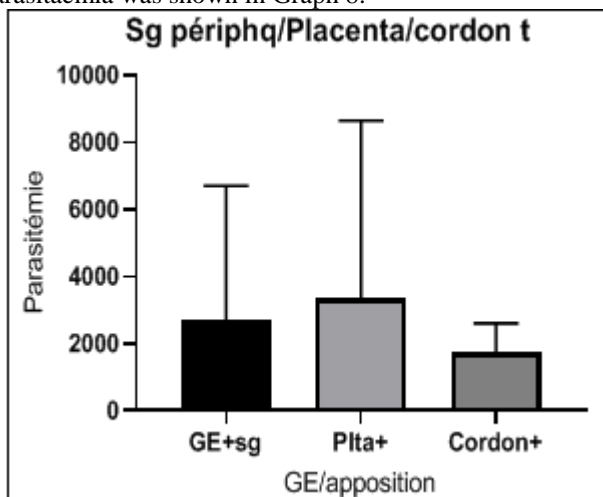


Graph 7: Distribution of cord blood parasitaemia according to gestity.

There was no statistically significant difference in the distribution of cord blood parasitaemia according to pregnancy (Unpaired t test, P value: 0.1211).

Analysis of peripheral blood, placenta and cord parasitaemia

The distribution of peripheral blood, placenta and cord parasitaemia was shown in Graph 8.



Graph 8: Distribution of parasitaemia of peripheral blood, placenta and cord

The distribution of peripheral blood, placenta and cord parasitaemia was not statistically significant (ANOVA, P: 0.7966).

4. Discussion

Constraints and limitations of the study

Childbirth is a sacred act in African culture so accessibility to the products resulting from this act is tedious. During this study, the consent of the parturients was a laborious exercise, taking into account the circumstances related to childbirth (pain, stress of miscarriages, etc.). The requirements related to the collection in particular the collection of placental blood were a step requiring technical skill characterized by speed and precision. Despite the above, the study objectives were met with exhaustive sampling.

Sociodemographic characteristics of the study population

The study of socio-demographic characteristics was based on the factors influencing the fight against malaria in pregnant women. Thus women giving birth were divided into three groups.

The G2 group of parturients using irregular prophylaxis methods was the most representative with 50.41%. These results were proof of the good knowledge by parturients of the risks of malaria during pregnancy. The irregularity in adherence to prophylaxis methods was evidence of the weaknesses of health programs in monitoring malaria control activities.

The size of the parturient population was 56 women. The average age was 25.29 ± 6.45 with extremes of 15 years and 40 years. These results were similar to those of Ndao et al. in Senegal, Famanta in Mali and Bakoua in Congo [8, 9, 10]. The majority age group was 20 to 35 years old. These data reflected the demographic characteristics of sub-Saharan African countries in comparison with those of Western countries which, through the accessibility of assisted reproduction methods, increased the chances reproduction of older women [11, 12].

The age factor had no impact on the use of antimalarial drugs for prevention. Famanta corroborated the same assertion in his study in Mali [8]. These results showed that the place of life of our population (periurban environment) was a positive factor in raising awareness in the fight against malaria. Ndao et al. concluded that an increase in the prevalence of placental malaria was even greater when the woman lived far from the city center [9].

Parity and gestity reflected the experience of parturients in managing a pregnancy. They did not have a significant influence on the use of antimalarial drugs for prevention (Table I). These results corroborate those of Bakoua et al. and Guindo et al. [10, 12].

The peripheral blood plasmodium index

The incidence of plasmodia in peripheral blood in our study was 36.54%. This result was similar to those of Samia Omer in Sudan (2017), Bouyou in Gabon (2010) and Achidi in Cameroon (2007) who had respectively 37.8%, 27.6% and 25.4% [13, 14, 15]. Diagne et al. obtained from thick peripheral blood drops a plasmodic index of 56% [16]. However, these results were superior to those of Bakoua in R. Congo (2010), of Guindo in Mali (2007) and of Elghazali in Sudan (2003) which had respectively 6.1%, 9.1% and 5.6% [10, 12, 17]. This difference could be explained by the following factors: stable or weak transmission zones, annual rainfall period, urban or peri-urban environment and the choice of the study population (on intermittent preventive treatment or not).

There was no statistically significant difference in the distribution of parasitaemia between groups of women. However, the mean G1 parasitaemia in parturients without prophylaxis was greater than in groups G2 and G3. These results demonstrated the impact of chemotherapy on the

parasite load corroborated by numerous previous studies. The results of the mean parasitaemia of group G3, with regular treatment, was higher than that of group G2, with irregular treatment, which were in contradiction with the literature. Responses from parturients during the survey may explain these observations.

Although some authors have reported an association between the Plasmodium Index and gestity, in our study the relationship between the two parameters was not established (P value: 0.6261). There was also no statistically significant difference between parasitaemia and parity (P value: 0.8692). Guindo and Elghazali corroborated this assertion [12, 17]. The random selection of largely asymptomatic parturients in our study could explain these results.

48.67% of parturients in our study presented with anemia with 4.44% severe anemia. The anemia rate in our study was characteristic of developing countries, i.e. from 9% to 60% (WHO, 2005) and remained superimposable to those observed by Sidibé in Mali with 58.4% and Diarra in Mali with 54, 5% [18, 19]. On the other hand, this rate was higher than those of Nosten in Thailand with 35.4% and of Luxemburger in Thailand with 40% [20, 21].

The peripheral blood plasmodic index correlated with hemoglobin level (P value: 0.0015). However, there was no correlation between the plasmodic index and anemia. This could be explained by the low parasitaemia of parturients. Unlike our results, many previous studies have established the relationship between the plasmodic Index and anemia. This would support the hypothesis that Plasmodium falciparum may be responsible for the massive destruction of red blood cells. However; it remains difficult to state as such the existence of such a link between anemia and malaria infection. Indeed, in addition to malaria infection; the etiology of maternal anemia remains multifactorial. It is subject to several influences, among others: diet poor in iron; absence of iron supplementation during pregnancy; geophagy; hemoglobinopathies; among other things, intestinal parasitosis remains additional factors found during pregnancy.

Plasmodium index of placental blood

We chose placental apportion as one of the indicators of malaria in childbirth because of its ease of realization and greater sensitivity compared to thick peripheral blood drop [11]. Compared to low birth weight or anemia, it is not multifactorial and depends only on the presence of the parasites.

The prevalence of placental malaria infection was 23% in our study. This result was significantly lower than that of Samia Omer in Sudan (58.9%) and higher than those obtained by Ndao in Senegal (8.1%), Guindo in Mali (10.3%) and Jyoti Singh in India (12%). [13, 9, 12, 22]. Taking preventive antimalarial treatments during pregnancy and the area of seasonal transmission could explain this difference. In fact, 72% of parturients in the Ndao study versus 8.13% in our study declared that they had followed their prevention on a regular basis [9].

The distribution of placental parasitaemia according to pregnancy did not show a statistically significant difference (Graph 5, 6). However, the mean placental parasitaemia in the primigravidae in our study was higher than the mean in the multigest. Likewise, the mean parasitaemia of primiparas was higher than that of multiparas. These results were consistent with the hypothesis that the acquisition of humoral immunity to placental malaria developed in proportion to the numbers of exposures. This assertion was not verified at the peripheral blood level in our study (Graph 3, 4). This lack of correspondence could be explained by the complexity of the immune response associated with the classification aspect (primi, pauci, multi ...) of gestity and parity.

Plasmodium index of cord blood

In our study the incidence of cord plasmodia was 7.84%. This result corroborated that of Diallo et al. with 7.8% [23]. Kisito et al. had a congenital malaria prevalence of 24.4% [24].

However, congenital malaria was considered until recently as a rare nosological entity, even in malaria endemic areas [24]. Numerous studies carried out in newborns have had low prevalence of congenital malaria: Diouf et al. and Mbongo et al. had a prevalence of 1.05% and 0.64%, respectively [7, 25]. These discrepancies could be explained by the hypothesis that 70% of the plasmodium transmitted by the mother to her child during pregnancy would be destroyed two to three days after the birth of the child [26, 27]. In addition, maternal-fetal transmission of anti-malaria antibodies, the presence of fetal hemoglobin in the baby and the enzymatic environment of the red blood cells of the newborn are unfavorable to the development of the parasite [26].

Our study showed no correlation between cord blood parasitaemia and pregnancy (P value: 0.1262). However, the mean value of the parasitaemia of the primigravidae was higher than that of the multigestia (Graph 7). In fact, primigravidae are much more likely than multi-gestures to contract malaria, to develop placental malaria and to transmit the disease to their fetus. Studies have shown that malaria is more common in primigravidae than in multigeste due to immunity acquired by the woman during pregnancy [28, 29].

Parasitaemia associativity of peripheral blood, placenta and cord

Plasmodium by a survival mechanism linked to a tropism of chondroitin sulfate A (CSA), adheres to the intervillous space of the placenta, thus causing placental malaria. These placental plasmodia, following metabolic disorders resulting from inflammation, cross the fetal-maternal placental barrier.

The distribution of peripheral blood, placental and cord parasitaemia did not show any statistically significant difference in our study (Graph 8). Likewise, no associativity was observed between parasitaemia of peripheral blood, placenta and cord. These results corroborated that of Guindo. This lack of associativity could be explained by the physiological particularity of these environments: the

peripheral blood, the placenta and the umbilical cord. In fact, in these environments, the plasmodium is exposed to factors which inhibit or activate the asexual reproduction of the parasite. However, the mean of placental parasitaemia in our study was higher than that of peripheral blood and cord. Indeed, the placenta by sequestration of the parasitized red blood cells thus constitutes a parasitic reservoir.

5. Conclusion

The peripheral, placental and cord plasmodium index of malaria associated with pregnancy in our study remains of concern, especially since the study population was peri-urban, that is to say accessible to health services. Thus, the reassessment of socio-demographic and therapeutic factors in the fight against malaria in pregnant women in this study area is highly desirable.

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