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Haematological Aspects of Malaria during Pregnancy at Brazzaville University Hospital

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ABSTRACT

Introduction: *Malaria is a major public health problem. Pregnant women are among those at risk of developing the infection. This infection is responsible for hematological changes of varying severity.*

Objective: *To study the haematological manifestations of malaria in pregnant women at Brazzaville University Hospital.*

Patients Materials and Method: *This was a retrospective case-control analytical study carried out in the Gynaecology-Obstetrics Department of the CHUB from January 2014 to December 2019, a period of five years. The study included all pregnant women hospitalized for malaria with proof of positivity of the thick drop for hematzoa and the results of the blood count. Data were analyzed using R software, with test significance at $p<0.05$.*

Results: *Malaria frequency was variable from 2014 to 2019. Malaria-infected pregnant women were aged between [20-25 years] with a mean age of 26, uneducated, single, in the third trimester of pregnancy, with low socioeconomic status ($p<0.05$). Hemoglobin level was less than 10.9 g/dl ($p<0.05$), thrombocytopenia was present ($p>0.05$) and leukocyte count was less than 10000 elements/ml ($p<0.05$). The hemoglobin<10.9 g/dl level was found more in multigestates than in primigates, more in the 2nd and 3rd trimesters of pregnancy than in the first trimester ($p<0.05$).*

Conclusion: *The haematological changes caused by malaria during pregnancy call for vigilance in the management of malaria in pregnant women, in order to reduce morbidity. The need for awareness campaigns to reduce the scourge must remain a major concern.*

Keywords

Pregnant women, Hemoglobin, Haematology, Brazzaville.

Introduction

Malaria is a major public health problem in sub-Saharan Africa. It is caused by the development in erythrocytes of Plasmodium, transmitted to humans by the bite of the female Anopheles mosquito. The most dreaded species is Plasmodium falci-parum [1,2], whose development in the body causes hematological changes of varying severity. These include anemia, thrombocytopenia and changes in leukocyte count [3]. These changes occur in all parasitized individuals, including pregnant women, who physiologically present various hematological changes such as folate-deficiency anemia [4,5]. However, the severity of malaria affects not only the

mother, but also the fetus she is carrying and even the newborn to be born [3]. Malaria, which affected more than 11 million pregnant women in 2018 [2], varies in severity depending on whether they are in their first, second or third trimester, and whether they are primigravida primiparas or multigesta multiparas [3].

Several authors have demonstrated that malaria has an impact on the hematological parameters of pregnant women. Vallesesha NC in India showed that anemia was present in 87.3% of pregnant women in their study [6], and San OT et al. in Thailand showed that thrombocytopenia also affected pregnant women [7]. In Africa, Anigo KM et al. in Nigeria have also noted changes in certain haematological parameters such as leukopenia and anaemia [8]. Similarly, Bouyou-Akotet et al. in Libreville [9-11] demonstrated anemia, thrombocytopenia and leukocyte modi-fication. These different changes in pregnant animals can be aggravated by malaria or provoked by plasmodial infection. We set out to study the haematological manifestations of malaria in pregnant women admitted to the Centre Hospitalier Universitaire de Brazzaville, in order to identify the different haematological manifestations of malaria during pregnancy.

Patients Materials and Methods

This was a retrospective analytic case-control study involving pregnant women hospitalized in the Gynecology-Obstetrics Department of the Centre Hospitalier Universitaire de Brazzaville from January 02, 2014 to December 30, 2019, a period of five (05) years.

The study population consisted of pregnant women hospitalized in the gynecology and obstetrics units divided into two groups:

- Case group: pregnant women hospitalized for malaria at any stage of pregnancy
- Control group: pregnant women hospitalized for any other non-febrile pathology.

Matching: a case was matched with a control.

We included in the study all hospitalized pregnant women for whom malaria had been diagnosed, and who provided information from prenatal consultation forms, positive results of the thick blood drop for haematozoa and blood count reports.

We took into account all records of non-impaluted pregnant women hospitalized for a non-febrile pathology and which contained information from prenatal consultation forms, biological examinations and blood counts.

For cases, we excluded all records of malaria-infected pregnant women without biological examinations such as thick drop, haematozoa and blood count; for controls, we excluded all records of non-malaria-infected pregnant women without biological examinations and all records of pregnant women with fever.

We took an exhaustive sample of the records of pregnant women hospitalized for malaria.

The sample size was calculated using SCHLESSELMAN's formula with P0: proportion exposed in the control group; P1: proportion exposed in the case group; $Z\alpha = 1.65$ and $Z\beta = 1.2$.

We used a study of Plasmodium falciparum malaria in pregnant women published in Toamasina, Madagascar in 2014. In this study, the prevalence of malaria in pregnant women was 26.5%.

The minimum sample size was 299 pregnant women. We retained 354 files.

The variables studied were socio-demographic variables (age; occupation; level of education; socio-economic level; marital status); reproductive variables (gestational age, parity, gestational age) and haematological variables (blood count).

An elaborate survey sheet was used for data collection. Thick drop slides were stained for haematozoa using the 10% Giemsa technique for 15 minutes. The smear was taken from 20µl of whole blood collected in an EDTA tube and stained using the May Grunwald Giemsa technique for 20 minutes. The stained thick drop and blood smear were read by an experienced microscopist.

The complete blood count (CBC) was obtained from 5 ml of blood in an EDTA tube, using an automated haematology system to determine haematological constants. Anemia was defined as a hemoglobin level below 11 g/dl. Thrombocytopenia was considered when platelet count was below 150000 /ml. Leukocytosis was considered when the leukocyte count was greater than 10000/ml.

Data were entered using Microsft Excel version 2013, and statistical analyses were performed using R (Core Team) version 4.0.3. Quantitative variables were expressed as mean ± standard deviation, and these means were compared using Student's t-test or the Mann Whitney test. For qualitative variables, frequencies (n) and proportions (%) were calculated. These frequencies were compared using Pearson's Chi-square test of independence, or Fisher's exact test when the expected number of participants was less than 5.

Univariate logistic regression analyses were performed to identify risk factors for infection at the alpha =5% threshold. Subsequently, all variables showing a significant association with parasitic infection were used to perform a multivariate analysis (logistic regression). Receiver operating characteristic (ROC) analysis was performed to assess the area under the curve (AUC) of the multivariate model. All tests were significant at the $p < 0.05$ level.

Results

Sociodemographic and reproductive characteristics of cases and controls

The median ages of cases 26 years [22.0;32.0] were significantly different from controls 28 years [23.2;34.0] (p=0.001). The extremes of age were 15 and 45 in the cases, and 15 and 44 in the controls.

Haematological changes in malaria-infected pregnant women and controls

Anemia was more significantly observed in malaria-affected than in non-affected pregnant women (p=0.018). When present, it was 18 times more severe in cases than in controls. Thrombocytopenia, on the other hand, was present in cases and controls with no statistically significant difference (p>0.05), as shown in Table 3.

Haematological changes by age group in cases

Table 4 shows that the leukocyte count was significantly below 10,000/ml in all age groups (p=0.036). However, hyperleukocytosis was observed in almost half the cases of malaria-affected pregnant women aged [15-20 years].

Haematological changes according to gestational age in cases

Pregnancy age was statistically (p=0.001) associated with moderate anemia (hemoglobin between 7-10.9 g/dl). 55.9% of pregnant women affected by malaria in the 2nd trimester had moderate and severe anemia. Table 5 also shows the results of other haematological constants.

Haematological changes according to gender in cases

Haematological manifestations were not associated with gestational status in cases (p>0.05) as shown in Table 6. However, it was noted that moderate anemias (hemoglobin: 7-10.9 g/dl) were more observed in multigestates, whereas severe anemias (hemoglobin < 7 g/dl) when it came to primigravidas. Similarly,

Table 1: Socio-demographic characteristics of malaria-infected and non-malaria-infected pregnant women during the study period.

	Case		Control		OR[IC 95%]	p-value+	p-value*
	N=354	(%)	*N=354*	(%)			
Age ranges:							0.013
[15-20 years]	70	19.8	44	12.4	1.54 [0.95;2.48]	0.078	
[20-25 years]	92	26.0	83	23.4	1.07 [0.71;1.63]	0.746	
[25-30 years]	91	25.7	88	24.9	Ref.	Ref.	
[30-35 years]	56	15.8	1	22.9	0.67 [0.43;1.05]	0.080	
[35-45 years]	45	12.7	58	16.4	0.75 [0.46;1.22]	0.25	
Profession:							0.229
Gainful activity	84	23.7	99	28	Ref.	Ref.	
Non-gainful activity	270	76.3	255	72.0	1.25 [0.89; 1.75]	0,199	
Educational level:							0.001
Primary	33	9.32	20	5.65	1.04 [0.55;1.97]	0.916	
Secondary	97	27.4	61	17.2	Ref.	Ref.	
Higher	77	21.8	96	27.1	0.50 [0.33;0.78]	0.002	
Out-of-school	147	41.5	177	50.0	0.52 [0.35;0.77]	0.001	
Marital status:							<0.001
In couple	59	16.7	103	29.1	0.49 [0.34; 0.70]	<0.001	
Single	295	83.3	251	70.9	Ref.	Ref.	
Socio-economic level:							0.074
Medium	59	16.7	80	22.6	Ref.	Ref.	
High	7	1.98	11	3.11	0.86 [0.32;2.36]	0.788	
Low	288	81.4	263	74.3	1.48 [1.02;2.16]	0.039	

*Test Khi² d'indépendance; +P-value de régression logistique.

Table 2: Reproductive characteristics of malaria-infected and non-malaria-infected pregnant women during the study period.

	Case		Control		OR[IC 95%]	p-value+	p-value*
	N=354	(%)	*N=354*	(%)			
Gestity:							0.181
Primigeste	83	23.4	69	19.5	1.41 [0.95;2.09]	0.089	
Paucigeste	137	38.7	128	36.2	1.25 [0.90;1.75]	0.185	
Multigeste	134	37.9	157	44.4	Ref.	Ref.	
Parity:							0.042
Nullipare	116	32.8	107	30.2	0.89 [0.61;1.30]	0.558	
Primipare	93	26.3	100	28.2	0.77 [0.52;1.13]	0.185	
Paucipare	113	31.9	93	26.3	Ref.	Ref.	
Multipare	32	9.04	54	15.3	0.49 [0.29;0.82]	0.006	
Gestational age:							<0.001
1st trimester	40	11.3	7	1.9	9.76 [4.28;22.2]	<0.001	
2nd trimester	125	35.3	16	4.52	13.3 [7.69;23.2]	0.000	
3rd trimester	185	52.3	316	89.3	Ref.	Ref.	
≥ 42 SA	4	1.13	15	4.24	0.46 [0.15;1.39]	0.163	

Table 3: Lineage-specific haematological changes in malaria-infected pregnant women and controls during the study period.

	Case		Control		OR[IC 95%]	p-value+	p-value*
	N=278	(%)	*N=352*	(%)			
Hemoglobin:							<0.001
11-14,6	81	29.1	125	35.5	Ref.	Ref.	
7-10,9	149	53.6	223	63.4	1.03 [0.73;1.46]	0.865	
<7	48	17.3	4	1.14	18.5 [6.43;53.3]	<0.001	
Mean corpuscular concentration of hemoglobin:							0.417
[30-37,5]	152	67.3	216	72.2	Ref.	Ref.	
[27-30[	42	18.6	44	14.7	1.36 [0.85;2.17]	0.207	
<27	32	14.2	39	13.0	1.17 [0.70;1.94]	0.557	
Platelets:							0.080
Normal	87	32.8	90	26.0	Ref.	Ref.	
Thrombocytopenia	178	67.2	256	74.0	0.72 [0.51;1.02]	0.067	
Thrombocytopenia classes							0.144
Slight	16	8.99	16	6.25	Ref.	Ref.	
Moderate	14	7.87	11	4.30	1.27 [0.45;3.64]	0.665	
Severe	148	83.1	229	89.5	0.65 [0.31;1.33]	0.243	
White blood cells:							<0.001
≤10000	171	64.3	150	43.7	Ref.	Ref.	
>10000	95	35.7	193	56.3	0.43 [0.31;0.60]	<0.001	

Table 4: Hematological changes by age group in cases during the study period.

	[15-20 ans]		]20-25 ans]		]25-30 ans]		]30-35 ans]		]35-45 ans]		p-value
	N=57	(%)	*N=77*	(%)	*N=66*	(%)	*N=42*	(%)	*N=36*	(%)	
Hemoglobin:											0.258
11-14,6	11	19.3	25	32.5	24	36.4	12	28.6	9	25.0	
7-10,9	31	54.4	37	48.1	33	50.0	25	59.5	23	63.9	
< 7	15	26.3	15	19.5	9	13.6	5	11.9	4	11.1	
Mean corpuscular concentration of hemoglobin:											0.970
[30-37,5]	29	67.4	45	68.2	35	67.3	24	68.6	19	63.3	
[27-30[	10	23.3	11	16.7	10	19.2	6	17.1	5	16.7	
< 27	4	9.30	10	15.2	7	13.5	5	14.3	6	20.0	
Platelets:											0.397
Normal	12	22.6	24	32.0	23	36.5	14	35.0	14	41.2	
Thrombocytopenia	41	77.4	51	68.0	40	63.5	26	65.0	20	58.8	
Thrombocytopenia classes											
Slight	4	9.76	3	5.88	1	2.50	4	15.4	4	20.0	
Moderate	4	9.76	1	1.96	3	7.50	4	15.4	2	10.0	
Severe	33	80.5	47	92.2	36	90.0	18	69.2	14	70.0	
White blood cells:											**0.036**
≤ 10000	28	52.8	54	73.0	36	57.1	26	63.4	27	79.4	
> 10000	25	47.2	20	27.0	27	42.9	15	36.6	7	20.6	

severe thrombocytopenia was more common in impalarial primigravida than in paucigravida and multigesta (p=0.084).

Discussion

We conducted a 5-year retrospective study based on data from archived files in the CHUB obstetrics and gynecology department. As with most retrospective studies, we observed a lack of certain data, reflected by a lack of completeness of certain haematological parameters. As a result, we were unable to assess certain haematological aspects rigorously. The loss of data prevented us from accurately and consistently matching cases and controls in the study. Hence the variability in the matching factor.

These different findings, in terms of data loss, were also noted by Fomba S in Bamako [11].

The chosen study population is characterized by physiological disturbances that can be biasing factors in the assessment of certain results, such as anemia. Pregnant animals are often subject to physiological anemia [12-14]. However, the absence of fever in the controls could be an element that would allow a better comparison of the two groups.

Socio-Demographic and reproductive characteristics

The median age of pregnant women affected by malaria was 26 years, with extremes of 15 and 45 years. This age of the

gestating women corresponds roughly to the average pregnancy age of women of childbearing age according to the 2018 general population and housing census of Congo [15]. The same average age of 26 was found in Libreville by Bouyou-Akotet MK et al. [10]. These results are also similar to those of Fomba S in Bamako [11], Ouedraogo CMR in Ouagadougou [16] and Fenomanana MS in Madagascar [17]. However, it should be noted that Ouédraogo A in Boussé, north of Ouagadougou, found an average age of 23 years among pregnant women with malaria [18]. The notable difference between our results and those of Ouédraogo A can be explained by the fact that his study was carried out in a rural area where the influence of tradition is still strong, implying that women start childbearing early. In urban areas, on the other hand, women start childbearing later and later.

In Nigeria, in the province of Osogbo, Akinboro RA [9] showed that the average age of pregnant women affected by malaria was 31 years. This is much higher than our results. Cultural and religious realities could explain this difference.

In our study, the majority of pregnant women affected by malaria had a poor level of education (no schooling and primary education). This may be due either to a lack of adequate care, or to a failure to understand the malaria prevention instructions given to pregnant women by health workers during pregnancy follow-up.
This trend has been found in several African studies [15,19].

Table 5: Hematological lineage changes according to gestational age in cases during the study period.

	1er trimestre		**2è trimestre**		**3è trimestre**		**42 SA**		**p-value**
	N=34	(%)	***N=102***	(%)	***N=139***	(%)	***N=3***	(%)	
Hemoglobin:									**0.001**
11-14,6	12	35.3	18	17.6	51	36.7	0	0.00	
7-10,9	15	44.1	57	55.9	74	53.2	3	100	
< 7	7	20.6	27	26.5	14	10.1	0	0.00	
Mean corpuscular concentration of hemoglobin:									0.549
[30-37,5]	18	58.1	55	67.9	77	69.4	2	66.7	
[27-30[	7	22.6	12	14.8	22	19.8	1	33.3	
< 27	6	19.4	14	17.3	12	10.8	0	0.00	
Platelets:									0.411
Normal	14	41.2	27	27.6	45	34.6	1	33.3	
Thrombocytopenia	20	58.8	71	72.4	85	65.4	2	66.7	
Thrombocytopenia classes									**0.004**
Slight	4	20.0	4	5.63	8	9.41	0	0.00	
Moderate	1	5.00	2	2.82	9	10.6	2	100	
Severe	15	75.0	65	91.5	68	80.0	0	0.00	
White blood cells:									0.377
≤ 10000	23	71.9	68	68.7	78	59.1	2	66.7	
> 10000	9	28.1	31	31.3	54	40.9	1	33.3	

Table 6: Hematological changes in lineages according to gestational age in cases during the study period.

	Primigeste		**Paucigeste**		**Multigeste**		**p-value**
	N=70	**(%)**	***N=103***	**(%)**	***N=105***	**(%)**	
Hemoglobin:							0.380
11-14,6	17	24.3	36	35.0	28	26.7	
7-10,9	37	52.9	52	50.5	60	57.1	
<7	16	22.9	15	14.6	17	16.2	
Mean corpuscular concentration of hemoglobin:							0.139
[30-37,5]	39	65.0	55	67.1	58	69.0	
[27-30[	12	20.0	20	24.4	10	11.9	
<27	9	15.0	7	8.54	16	19.0	
Platelets:							0.690
Normal	21	31.3	35	36.1	31	30.7	
Thrombocytopenia	46	68.7	62	63.9	70	69.3	
Thrombocytopenia classes:							0.277
Slight	5	10.9	3	4.84	8	11.4	
Moderate	1	2.17	7	11.3	6	8.57	
Severe	40	87.0	52	83.9	56	80.0	
White blood cells:							0.084
≤10000	37	56.1	71	72.4	63	62.4	
>10000	29	43.9	27	27.6	38	37.6	

Our results are in line with the literature, which shows that primigravida, paucigravida and primiparous women are the most prone to malaria among pregnant women.

Niang MM at Dakar University Hospital showed that nulliparous and pauciparous women were more likely to suffer from malaria than multiparous women [20]. Similarly, Fenomanana MS et al. [17] showed a predominance of pauciparous women.

This predominance of nulliparous and pauciparous women could be explained, on the one hand, by their lack of experience of pregnancy and, on the other, by immunological aspects that would make pregnant women more resistant to malaria as pregnancies progress [21]. However, our study shows that, although not significant, the primigravida had less malaria. This could be an indication of the success of malaria prevention campaigns in this category of pregnant women.

The reversal of trends in gestational age revealed by our study, i.e. a preponderance of malaria in the third trimester of pregnancy, could be further proof that the first trimesters of pregnancy are the focus of attention on the part of pregnant women. But, on the other hand, our results also show that the third trimester of pregnancy could become more dangerous for pregnant women than the first two.

Balogun ST et al. in Oyo State, Akinboro RA et al. in Ile-Ife, Nigeria; Ouédraogo CMR obtained results similar to ours [21-23]. On the other hand, Fenomanana MS et al. in Toamasina found a predominance of pregnant women in the second trimester of pregnancy [17]. Such an increase in malaria cases in the third trimester of pregnancy should raise fears of a resurgence of congenital malaria cases, as it is during the second and third trimesters of pregnancy that placental infestation occurs.

Hematological Manifestations

Hematological manifestations in malaria have been described by several authors [21]. The most common manifestations concern the red, platelet and white blood lines. Involvement of these different lineages is most often manifested by anemia of variable severity, inconstant thrombocytopenia and either leukocytosis or leukopenia [24].

These haematological manifestations of malaria are also present in pregnant women, but may be more severe due to the immune depression caused by pregnancy [25,26].

Our results showed the existence of anaemia in pregnant women, as shown by several authors in Africa and elsewhere [2,12,15,19,27-29]. This almost constant presence of anemia in our study could either raise the question of inadequate iron and folic acid supplementation, although malaria alone could justify the presence of anemia [24,30,31], or prove the significant morbidity of malaria in pregnant women.

Anemia, most often moderate, was significantly associated with gestational age in our study. This has been found by Bouyou-Akotet in Libreville, Fenomanana in Madagascar, Singh N in India [10,17,22]. These results support data in the literature, which make malaria one of the leading causes of anemia in pregnant women [32].

Thrombocytopenia is a frequent change observed during pregnancy. It is often multifactorial, and is only associated with pregnancy once all other etiologies have been ruled out [26]. In addition to thrombocytopenia in pregnancy, thrombocytopenia is also observed in malaria [16,21]. These two associated physiological and pathological phenomena would explain the presence of thrombocytopenia in pregnant women in our study, as noted in the literature.

The association of thrombocytopenia with malaria could be explained by the increased splenic sequestration and role of platelets in malaria in pregnant women.

Our findings are in line with descriptions of thrombocytopenia in malaria in general, and in pregnancy in particular. Indeed, thrombocytopenia is the haematological complication most frequently observed in malaria, making it almost an element of orientation in areas of high malaria endemicity [8].

This may explain the thrombocytopenia found in our study. Thrombocytopenia, when present, although not significant overall, was moderate in paucigravida and severe in pregnant women in the second trimester of pregnancy. Although several studies have been carried out on the subject, it is accepted that the malaria transmission zone in which the pregnant women are located plays an important role in the impact on red blood cells or haemoglobin from the first to the third trimester of pregnancy, from primigravida to multigesta [33].

Leukocyte Count

The leukocyte count in our study was below 10,000 cells/ml, with no significant difference. Ango Kola Matthew et al. also found leukocytes below 10,000 cells/ml [8]. The leukocyte count is usually normal during pregnancy. In other studies, the leukocyte count has been found to exceed 10,000 cells/ml, attesting to a leukocytosis in cases of malaria. This was the case in the study by Saw Oo Tan, who found a leukocytosis of almost 14,000 elements/ml [7]. Other authors found leukopenia.

This variability in leukocyte count depends on several parameters apart from pregnancy, severity of infection, parasitemia and host immune status. In most cases, the leukocyte count is normal [5].

Conclusion

The haematological manifestations of malaria in pregnant women at Brazzaville University Hospital are varied. The present study reported that malaria in pregnant women was much more common in nulliparous, uneducated pregnant women in the 2nd and 3rd trimesters of pregnancy. Hematological manifestations associated with malaria were anemia and thrombocytopenia. These results confirm the need for anti-malarial and anti-anemic chemoprophylaxis in pregnant women.

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