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Malaria Parasite Density and Anaemia in Pregnant Women Attending Antenatal Consultation in the Regional Hospital Bamenda, Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CBE and FNEE designed the study and carried out the field work. Author CBE did the statistical analysis and wrote the manuscript, Authors CY, FNN, ONN and HKK read and edited the manuscript. Author HKK did the overall supervision. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aims were to investigate the variation of malaria parasite density (GMPD) with sociodemographic and pregnancy related characteristics; the prevalence of anaemia and anamia levels; assess the variation of malaria parasite density (GMPD) with level of anaemia; and to assess the variation of anaemia levels with parity among malaria parasite infected pregnant women attending ANC.
Study Design: It was a cross-sectional hospital-based study.
Place and Duration of Study: This study was carried out at the ANC unit of the Bamenda Regional Hospital.
Methodology: Pregnant women with malaria parasitaemia from a preliminary study were involved in this study. A questionnaire was used to collect data on sociodemographic characteristics and pregnancy related parameters. Capillary blood samples were used for the preparation of thick and thin blood smears for malaria parasite microscopy; determination of haemoglobin concentration and WBC count. Data was analyzed using SPSS version 20. Kruskal-Wallis' test was used to assess differences in GMPD. The student t-test was used to determine the variation of GMPD with trimester, parity and level of anaemia while the χ^2 and OR were used to compare the prevalence of anaemia. *P*-values < .05 were considered significant.
Results: GMPD was higher in second trimester (475±510) than first trimester (261±190) (*P=.00*), higher at third trimester (300±206) than first trimester (261±190) (t=12.7, *P=.01*) as well as in second trimester than in third (t=2.3, *P=.03*). Participants with malaria parasitaemia were at 7 times more odds to have anaemia (OR = 6.8; *P=.001*). The prevalence of mild anaemia was higher in women who were positive for malaria parasitaemia (17; 37.8%), (N=45) than those who were negative (19; 9.3%). Moderate anaemia was higher in women with positive malaria parasitaemia (2; 4.4%) than those who were negative (1; 0.5%) (χ2= 30.2; *P=.01*). GMPD was higher in mild anaemic (650±523/µL) than in non-anaemic (236±469/µL) participants (t= 2.5*; P=.03*).
Conclusion: GMPDs vary with trimester while mild and moderate anaemias were present. Higher GMPDs were observed in mild anaemia than others. Anaemia levels were most recorded among the primigravidae. Frequent checks on GMPD and haemoglobin level on primigravidae could reduce severe malaria anaemia in pregnancy.

Keywords: Malaria parasite density; anaemia; pregnant women; Regional Hospital Bamenda; Cameroon.

1. INTRODUCTION

Malaria remains the most prevalent parasitic disease of man and an important cause of anaemia in the tropics. It is life-threatening and is caused by coccidian parasites of the genus *Plasmodium* that are transmitted by the bites of infected female *Anopheles* mosquitoes. Five species of *Plasmodium* cause disease in man namely: *Plasmodium falciparum, P. vivax, P. malaria, P. ovale* and *P. knowlesi* which was recently discovered in North East Asia [1]. *P.falciparum* is the most prevalent in tropical Africa including Cameroon [2]. Unfortunately, it causes the most severe form of the disease [3] and is an indirect cause of maternal mortality due to its contribution to anaemia in high malaria transmission settings [4] such as Cameroon. The WHO estimates that there were 241 million cases of malaria in 2020 up from 227 million cases in 2019. The estimated number of malaria deaths stood at 627 000 in 2020, an increase of 69 000 deaths over the previous year. Those that are more vulnerable to the disease are children less than five years of age and pregnant women [5, 6]. Malaria is a febrile disease with an incubation period of about seven days or more and can occur as simple or severe malaria. The most common manifestations of simple malaria are fever, joint pains, headache, chills, abdominal disorder and vomiting [7]. Severe malaria usually occurs when simple malaria cases are left untreated and they deteriorate. Progression to severe malaria is most common with *Plasmodium falciparum* due to very high parasitaemia levels [8]. Associated manifestations include respiratory distress, hypoglycaemia, metabolic acidosis, cerebral malaria, anaemia and death [9, 10]. Although this is common with children, adults with suppressed immune status may also suffer severe malaria.

In pregnancy, malaria is considered a severe disease with anaemia standing out as a frequent

manifestation [3, 11]. Although malaria anaemia is reported to decrease with age due to a progressive build up in protective immunity, anaemia in pregnancy continues to be quite nefarious with significant complications on the foetus and the pregnant woman. These may include poor birth outcomes in the new born and increased risk of maternal mortality in some settings [12-14]. With reduced levels of haemoglobin [15], there is poor development of the foetus which if associated with high parasitaemia, may result in spontaneous abortions and death *in utero*. These occur as a result of infection, multiplication and destruction of red blood cells by malaria parasite asexual stages. Also, placental malaria characterized by a transformation of the maternal parasites and infected of red blood cells within the placenta reduces placenta permeability to blood flow [16, 17]. The number of red cells involved is usually very high in *P.falciparum* infections. However, in the tropics, anaemia can also be due to other factors including hookworm infection, malnutrition, HIV and AIDS, bacterial infections, and deficiencies in vitamins B2, B3 and B12. Malaria anaemia has also been associated with haemoglobinopathies and other inherited red cell abnormalities notably glucose 6 phosphate dehydrogenase (G6PD) deficiency. Albeit other factors, anaemia is reported to be strongly associated with malaria and especially in pregnancy [4, 12, 13].

In areas of stable malaria transmission, the prevalence of moderate-to-severe anaemia (haemoglobin<7 g/dL) has been reported to be a more sensitive correlation factor of a reduction in malaria exposure than parasite prevalence. It is reported that anaemia may reduce more quickly than mortality as coverage of malaria interventions, such as insecticide-treated bed nets (ITNs), malaria chemoprophylaxis and indoor residual spraying are scaled up [18, 19].

Pregnant women attending antenatal consultation at the Bamenda Regional Hospital receive sulphadoxine pyrimethamine oral treatment, long-lasting insecticide-treated nets (LLINs) and health education on malaria prevention. These measures are intended to reduce the prevalence of malaria and its burden on pregnant women as well as the complications of malaria on the foetus and the mother including anaemia. Our literature search did not find studies that assessed the impact of these measures among pregnant women attending ANC in the Bamenda Regional Hospital. Against this background, the objectives of this study were to investigate the variation of malaria parasite density (GMPD) with sociodemographic and pregnancy related characteristics; determine the prevalence of anaemia and anamia levels; assess the variation of malaria parasite density (GMPD) with level of anaemia; as well as assess the variation of anaemia levels with parity among malaria parasite infected pregnant women attending antenatal clinic (ANC) consultations at the Bamenda Regional Hospital (BRH). Data from this study could help to guide policy on the prevention and control of malaria related anaemia in pregnancy.

2. MATERIALS AND METHODS

2.1 Study Site

This study was carried out at the RHB during the period April to May 2020. Participants were recruited from the antenatal clinic during their routine consultations. The RHB is a category three (second referral level) health facility within the Cameroon health system. Details of the study site and the prevalence of malaria have been earlier described [20].

2.2 Study Design

This was a hospital based cross sectional study. A questionnaire was used to collect data from pregnant women attending antenatal consultation at the BRH. Capillary blood samples were collected by finger prick. The blood was used for the preparation of thick and thin blood smears for malaria parasite detection, quantification and speciation; and for determination of haemoglobin concentration and white blood cell count.

2.3 Study Population, Sample Size and Sampling Methods

Participants were pregnant women attending ANC at the BRH. Pregnant women who were suffering from other severe diseases such as AIDS, sickle cell anaemia, cancers, tuberculosis as well as women on malaria treatment and blood transfusion were excluded from the study. A convenient sampling technique was used to select 250 pregnant women for the baseline survey. This sample size calculation has been reported earlier in another publication [20]. In this paper, participants who were positive for malaria parasitatemia (45, 18.0%) were included in most of the inferential analysis. The initial sample size (N=250) was only considered when the proportions of anaemia and anaemia levels were

compared among women who were positive for malaria parasitaemia and those who were negative.

2.4 Data Collection

2.4.1 Administration of questionnaires

A pretested structured questionnaire was used to collect sociodemographic data from the participants. This included age, profession, level of education, parity, age and trimester of pregnancy. The questionnaire was administered in English and exceptionally in Pidgin English and French depending on the participant.

2.4.2 Laboratory procedures

2.4.2.1 Haemoglobin measurement and white blood cell count

Capillary blood was collected by pricking the finger using sterile lancets after disinfecting the site to be punctured with 70% alcohol. A drop of blood was placed on the test trip attached to a semi-automated haemoglobin photometer (Haemocuehaemoglobin analyzer) for the detection of haemoglobin concentration following manufacturer's prescriptions. In the procedure, a single-use micro-cuvette of about 10µL in volume was filled with capillary blood. The cuvette is coated with sodium nitrite and sodium azide. The nitrite converts the haemoglobin to haemiglobin which is converted to haemiglobinazide by the azide. The haemocue photometer then measures the absorbance of the haemoglobinazide and displays the concentration of haemoglobin on the digital screen after 15-45 seconds. Normal values of haemoglobin were considered to be ≥11g/dl of blood; mild anaemia: 9-10.9g/dL; moderate anaemia: 7-8.9g/dL; severe: <7 g/dL [21]. For the white blood cell count (WBCC), 1 in 20 dilution of blood was done in a solution of glacial acetic acid tinged with 1% v/v gentian violet and loaded on improved Neubauer counting chamber. The number of WBCs per µl of blood was determined following standard procedures [22].

2.4.2.2 Blood specimen collection, preparation of blood smears and diagnosis of malaria parasite

A drop of the capillary blood was used to prepare thick and thin blood films for malaria diagnosis. The blood films were air-dried and the thin films fixed with absolute methanol. Both films were stained with 10% giemsa solution for ten minutes, rinsed and air-dried [22]. Malaria parasite density per µl of blood was done by counting the number of parasites per 200 white blood cells and multiplying by the white blood cell count per µl of each participant.

2.5 Statistical Analysis

Data was entered into an excel sheet and exported to SPSS version 20 for analysis. Kruskal-Wallis' test was used to assess differences in geometric mean parasite density (GMPD) per µL of blood in the various categories of particpants based on demographic characteristics. The student t-test was used to determine the variation of GMPD with trimester of pregnancy, parity and level of anaemia while the chi square test and odds ratio were used to compare the prevalence of anaemia among participants. P-values < .05 were applied as a measure to get an indication of statistical strength.

3. RESULTS

3.1 GMPDs with Respect to Sociodemographic Characteristics of the Study Population

The description of the study population and the prevalence of malaria parasitaemia for this study had been reported in a publication earlier [20]. The overall GMPD for the participants who were positive for malaria parasitaemia was 362±364 parasites per µL of blood with a range from 100-2000. Using the Kruskal-Wallis's test to compare GMPDs, no significant statistical difference was observed with respect to the sociodemographic characteristic as seen in Table 1.

3.2 Variation of Malaria Parasite Density with Trimester of Pregnancy

Using the paired sample t-test to compare GMPDs of the study participants at the various trimesters of pregnancy revealed that, from pair 1, the GMPD was found to be higher in women at second trimester (475±510) than their counterparts of the first trimester (261±190) and the difference was statistically significant (*P=.00*). Similarly, from the second pair it was observed that the GMPD was higher (t=12.7, *P=.01*) in women at third trimester (300±206) than those at first trimester (261±190). The third pair also revealed a significant statistical difference in GMPD (t=2.3, *P=.03*) with a higher value for the women in the second trimester than those of the third. This is shown in Table 2.

3.3 Variation of Malaria Parasite Density with Parity

Using the paired sample t-test to compare the GMPDs of the participants with respect to the different parities showed no significant statistical difference. However, the highest GMPD was observed in women at 3rd parity (581±482) while the least was observed in the those at >3 parities (142±268) as shown in Table 3.

3.4 Prevalence of Anaemia amongst the Study Participants in the Baseline Study

The mean±SDhaemoglobin concentration for the participants was 11.96 ±1.2 varying from 8.2-15.5 g/dL of blood. Majority of the pregnant women (211; 84.4%) had haemoglobin values within normal range. Thirty-nine (15.6%) of the participants were anaemic (Hb values less than 11g/dL of blood). Mild anaemia was the most prevalent (36; 14.4%)) with respect to other levels of anaemia. No case of severe anaemia was recorded as shown in Table 4.

3.5 Anaemia and Malaria Parasitaemia among the Baseline Study Participants

Comparing the prevalence of anaemia amongst participants who tested positive for malaria parasitaemia (19; 42.2%) and their malaria negative counterparts (20; 9.8%), it was observed that those with malaria parasitaemia were at 7 times more odds to have anaemia (OR = 6.8; *P=.001*). With respect to level of anaemia, it was observed that the prevalence of mild anaemia was higher in women with positive malaria parasitaemia (17; 37.8%) than their counterparts with negative malaria parasitaemia (19; 9.3%). Similarly, moderate anaemia was higher in women with positive malaria parasitaemia (2; 4.4%) than those who were negative (1; 0.5%) and the difference was statistically significant ($\chi 2$= 30.2; *P=.01*) as shown in Table 5.

Table 1. Comparing GMPDs/µL of blood with respect to socio-demographic characteristics

Characteristic	Category (n)	GMPD(SD)/µL of blood	Range	Kruskal-Wallis (*P*-value)
Age (years)	≤30 (33)	394 (415)	100-2000	0.02 (0.89)
	>30 (12)	275 (314)	100-500	
Occupation	Business (27)	405.6 (415)	100-2000	1.74 *(.63)*
	Civil servant (6)	183.3 (40.8)	150 - 250	
	Student (5)	390 (454.7)	150 - 1200	
	Others (7)	328.6 (211.9)	100 - 750	
Level of education	Primary (9)	611.1 (587.8)	100 - 2000	2.92 *(.23)*
	Secondary (16)	290.6 (230.4)	100 - 1000	
	Tertiary (20)	307.5 (287.6)	100 - 1200	
Parity	1 (14)	342.9 (324.6)	100 - 1200	0.40 *(.94)*
	2 (11)	481.82 (581.06)	100 - 2000	
	3 (9)	361.11 (279.26)	100 - 900	
	>3 (11)	268.18 (141.90)	100 - 500	
Overall		362 (364)	100-2000	

Table 2. Comparing GMPD/µL of blood at the different pregnancy trimesters of participants

Pair of trimesters	Trimester	GMPD (±SD)/ µL of blood	t-test	p-value
Pair 1	1st trimester	261 (190)	-3.9	.00
	2nd trimester	475 (510)		
Pair 2	1st trimester	261(190)	12.7	.01
	3rd trimester	300 (206)		
Pair 3	2nd trimester	475 (510)	2.3	.03
	3rd trimester	300 (206)		

3.6 Variation of Malaria Parasite Density with Levels of Anaemia

Comparing the GMPDs of participants with different levels of anaemia using the paired sample t-test depicted a statistically significant difference (t= 2.5; *P=.03*) in GMPD with higher values in mild anaemic participants (650±523/µL) than those who were non-anaemic (236±469/µL). However, GMPD values were similar between other pairs as shown in Table 6.

Table 3. Comparing GMPD/µL of blood of the different parities of the participants

Pair	Parities	GMPD(±SD)/µL of blood	t-test	*P-value*
Pair 1	Parity 1	353 (391)	0.5	*.60*
	Parity 2	581 (482)		
Pair 2	Parity 1	353 (391)	0.3	*.81*
	Parity 3	276 (367)		
Pair 3	Parity 1	353(391)	1.0	*.42*
	Parity >3	142 (268)		
Pair 4	Parity 2	581(482)	0.5	*.60*
	Parity 3	276 (367)		
Pair 5	Parity 2	581 (482)	1.2	*.32*
	Parity >3	142 (268)		
Pair 6	Parity 3	276 (367)	1.1	*.33*
	Parity >3	142 (268)		

Table 4. Prevalence of anaemia among the study participants

Characteristic	Category	Frequency (%)
Anaemia	No (Hb≥ 11g/dL)	211 (84.4)
	Yes (Hb< 11g/dL)	39 (15.6)
Anaemia level	Mild	36 (14.4)
	Moderate	3 (1.2)
	Non-anaemic	211 (84.4)

Table 5. Anaemia status/level with respect to malaria parasite status

Anaemia status	Category	Malaria status (%)		Level of significance
		Negative (N=205)	Positive (N=45)	
Anaemia	No	185 (90.2)	26 (57.8)	OR = 6.8; *P*=.001
	Yes	20 (9.8)	19 (42.2)	
Anaemia level	Mild	19 (9.3)	17 (37.8)	χ^2= 30.2; *P=.01*
	Moderate	1 (0.5)	2 (4.4)	
	Normal	185 (90.2)	26 (57.8)	

Table 6. Variation of GMPD/µL of blood with anaemia level

Pair	Anaemia levels	GMPD(±SD)/µL of blood	t-test	*P*-value
Pair 1	Non anaemic	236 (193)	2.5	*.03*
	Mild anaemia	650 (523)		
Pair 2	Non anaemic	236 (469)	0.43	*.74*
	Moderate anaemia	425 (247)		
Pair 3	Mild anaemia	650 (469)	0.7	*.61*
	Moderate anaemia	425 47)		

3.7 Variation of Anaemia Levels with Parity of Participants

The difference in the prevalence of anaemia levels with respect to parity of the pregnant women was statistically significant (χ^2=295.2, *P=.000*). It was observed that mild anaemia (57.7%) and moderate anaemia (37.2%) were the two levels detected in this study. A minority (4.4%) of the malaria infected participants were non-anaemic as shown in Table 7.

4. DISCUSSION

Anaemia stands out as one of the most common complications in malaria. This is usually exacerbated when it occurs during pregnancy due to physiological changes in the pregnant woman, but also as a result of the immunological changes that occur on the parasite when they infect the placenta [16, 17]. These changes seem to vary with individual's sociodemographic characteristics, parasite density as well pregnancy related factors such as parity and trimester [23-26].

Findings from this study, showed no difference in GMPD in pregnant women with respect to age. This is strange, given that malaria parasite density is reported to be associated with the level of immunity which is generally acquired through exposure. Exposure generally comes with time and consequently elderly individuals in a malaria endemic area will more likely present with greater immunity. This phenomenon which is contrary to the findings in this study has been reported in other studies in endemic areas by Nnaji et al in Nenwi, Nigeria [23] and Steketee et al in Malawi [24]. In spite of the above, it is also possible that the women may have attained an age where they have acquired maximum immunity to malaria parasite.

Based on trimester of pregnancy, it was observed that GMPDs were highest in the second and lowest in the first. It is possible that during the first trimester, the immunity against malaria parasite is gradually shared between the mother and the foetus as it develops. At the peak of the second trimester, it is likely that the maternal immunity would have reduced significantly giving way for an increase in parasitaemia during an infection and especially infection of the placenta [27]. The mother could possibly start adapting and producing specific immunity by the third trimester and hence reducing the parasite density. The absence of a significant difference in GMPDs among women of different parities was surprising given that reports have shown that malaria parasite density decreases with parity as women acquire more immunity as they increase parity [23, 24]. Probably the strategies put in place in the antenatal care package which include malaria prevention measures, nutrition education help to place the immunity of the women at a similar level and may present similar resistance in the face of an infection with malaria parasite.

The prevalence of anaemia in this study 15.6% is lower than values reported in similar studies in the middle belt of Ghana [25], sub-Saharan Africa [28,29] and the Mount Cameroon area [30]. This could be as a result of the uptake of malaria prevention measures such as sulphadoxine pyrimethamine given during ANC visits from second trimester, use of LLINS among others put in place by the Government and implemented by the Bamenda Regional Hospital ANC personnel. Similarly, the prevalence was lower than that reported in another study carried out during same season on apparently health pregnant women in Burkina Faso [15]. There were more cases of mild anaemia than moderate. No case of severe anaemia was recorded. A similar study in the Fako Division of Cameroon reported similar findings although with a very low prevalence of severe anaemia [12] Individuals who were apparently very sick alongside others with underlying diseases were excluded from the study. This might have reduced the chances of reporting severe anaemia cases even in those who may have been very sick due to malaria infection. A significantly higher value of GMPD was recorded amongst pregnant women with mild anaemia compared to those who were non-anaemic. This is a confirmation that the anaemia cases in this study population were most likely due to malaria parasite infection which is usually associated with destruction of red blood cells exacerbated in *Plasmodium falciparum* infections. Although pathogenesis of malaria anaemia is multifactorial [28 -30], *Plasmodium parasite* is an intraerythrocytic parasite so there is inevitable destruction of red blood cells containing parasites as schizonts rupture. However, a more important contributor is the increased destruction of non-parasitized red cells that parallels disease severity [31]. It has been estimated that loss of unparasitized erythrocytes accounts for approximately 90% of the acute anaemia resulting from a single infection. Parasitaemia in *P. falciparum* malaria commonly

Table 7. Comparing levels of anaemia in pregnant women of different parities

Parity	Anaemia level (%)			χ^2 (p-value)
	Mild	Moderate	Non-anaemic	
1	8 (30.8)	6 (35.3)	0 (0.0)	
2	7 (26.9)	4 (23.5)	0 (0.0)	295.2 (.000)
3	6 (23.1)	1 (5.9)	0 (0.0)	
>3	5 (19.2)	6 (35.3)	2 (100)	
Total	26 (57.7)	17 (37.7)	2 (4.4)	

exceeds 1% of red cells parasitized, and in severe disease may exceed 10%. This haemolytic anaemia in malaria may be compounded by bone marrow dyserythropoiesis during and immediately after the acute illness [32].

This detection of mild and moderate anaemia amongst pregnant women in this study is an indication of reduced risk of anaemia associated with malaria in the study population. The implementation of malaria prevention measures by personnel of the Regional Hospital Bamenda as recommended by WHO [33], the fight against other factors related to anaemia such as the distribution of anti-helminthic drugs by the Ministry of Public Health are possible explanations for these results, given that helminth infections especially that due to hookworms contribute to anaemia. Similar findings were reported by Achidi and collaborators in another study in Fako Division of Southwest Cameroon [12]. However, this study did not assess the consumption of blood tonics which is a routine prescription for pregnant women during their ANC consultations. This might have affected the categorization of participants in the levels of anaemia.

The highest proportion of both mild and moderate anaemic cases were reported among the primigravida. Primigravida are at a greater risk of malaria due to lower adaptive immunity which is acquired with time. Consequently, women with lower parity may not exhibit tolerance to malaria parasitaemia and so more red blood cells are destroyed in these individuals resulting to anaemia. Similar results have been reported by Beeson et al [29] and Rogerson *et al* [30]. A study by Rouamba *et al* [15] in Burkina Faso also reported a difference in anaemia level with respect to parity.

5. CONCLUSION

Findings from this study show that GMPDs vary with trimester of pregnancy. Mild and moderate anaemias are quite present although the study population was apparently healthy. Higher levels of GMPDs were found in individuals with mild anaemia than others; and also, anaemia levels were most recorded among the primigravidae. More frequent checks on malaria parasite density and haemoglobin level on primigravidae could reduce the chances of developing severe malaria anaemia in pregnancy.

CONSENT

Informed consent was obtained from all participants. Participation in the study was totally free and voluntary. Participants had the liberty to quit the study at any point in time. Pregnant women who were diagnosed with malaria and/or anaemia were referred for management.

ETHICAL APPROVAL

An ethical clearance to carry out this study was obtained from the Institutional Review Board of the Faculty of Health Sciences of the University of Bamenda. Administrative authorizations were obtained from the Regional Delegation of Public Health for the North West Region, the Director of the BRH as well as permission from the heads of the laboratory and antenatal care units of the Hospital.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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