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Malaria Parasitaemia and Variations in Haematological Parameters among Pregnant Women in Buea

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Authors' contributions

This work was carried out in collaboration with all authors. Author JLNN designed the work, wrote the protocol, supervised the field and laboratory work, participated in the data analysis and wrote the first draft of the manuscript. Author NJ did the laboratory work, participated in the data analysis and edited the manuscript. Author NCN did the final editing and proof-reading of the manuscript. Author GCMW participated in the design of the work, the field and laboratory work and the drafting of the manuscript. Author NBY did the data analysis, read and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Malaria is a life threatening parasitic disease that is entirely preventable and curable. Pregnant women are among the most vulnerable groups to this deadly disease. Prompt diagnosis and treatment is needed to reduce morbidity and mortality. Haematological changes that

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occur during malaria infection have been suggested as a potential predictor that can aid in the diagnosis of malaria infection.

Methods: A cross sectional study involving 276 pregnant women with signs and symptoms of malaria was conducted at Regional Hospital Buea (RHB) from April to July 2018. Baselines characteristics of the study population were collected. Blood (4 ml) was collected from each pregnant woman and dispensed into an EDTA tube. Identification of malaria parasite was done using a rapid diagnostic test (Care Start^{TM}). Complete blood count was performed using an automated haematology analyzer (Mindray®, BC-5300).

Results: Out of the 276 pregnant women, 98 (35.51%) had malaria. A total of eighty eight (31.88%) of the pregnant women were anaemic, with forty eight (17.4%) being malaria infected and forty (14.5%) non-malaria. The mean (± SD) of total white blood cell count in the malaria infected group was 5.9 (±2.4) and was significantly different (p = 0.039) from that of non-malaria infected (6.7[±1.9]) population. Red blood cells significantly (p = 0.001) decreased in malaria infected patients (3.7 [± 0.6]) as compared to non-malaria infections (4.2 (±1.0). The mean (± SD) of platelets counts in malaria infected group was significantly different from that of non-malaria infected pregnant women.

Conclusion: Pregnant women infected with malaria exhibited changes in haematological parameters with low platelets (thrombocytopenia) and Haemoglobin concentration being the most significant predictors of malaria in our study area. These parameters could improve malaria diagnosis when used in combination with other clinical diagnosis.

Keywords: Pregnant women; haematological parameters; malaria; anaemia.

1. INTRODUCTION

Malaria is one of the most widespread parasitic infections in the world and still constitutes a major public health problem [1,2]. Malaria is caused by a protozoan parasite of the genus; *Plasmodium.* Five species of plasmodia are known to cause human malaria namely; *Plasmodium falciparum. Plasmodium ovale, Plasmodium vivax, Plasmodium malariae and Plasmodium knowleski* with *Plasmodium falciparum* being the most pathogenic [3,4,5]. All these species share a similar life cycle which involves two hosts; the human host and the female anopheles mosquito (vector). The life cycle is composed of mainly schizogony occurring in human and sporogony that takes places in the mosquito [6,7]. Sporozoites injected by the female anopheles mosquito into the human enter the circulation and are carried to the liver where they penetrate the liver cells and undergo exo-erythrocytic cycle eventually resulting to the formation and release of merozoites which invades erythrocytes to start an erythrocytic cycle [8].

The prevalence and severity of malaria infection have been reported to be higher among children and pregnant women than in any other age group [9] and it is the main cause of morbidity and mortality in endemic areas [10]. Globally, about 3.4 billion people are at risk of being infected by malaria and developing disease and about 1.1 billion are at high risk. According to WHO, an estimated 216 million cases of malaria occurred in 2016 and the disease led to 445,000 deaths [11] and about 25 million pregnant women were at risk for malaria. The burden was heaviest in W.H.O. African region, where 528,000 (92 %) of all deaths occurred in children aged under 5 years who accounted for more than two thirds of all deaths. Fifteen countries all in sub-saharan Africa carried 80% of the global malaria burden [11].

In Cameroon, malaria is endemic in the 10 regions of the country [12]. According to the 2011 demographic and health survey, this infection is responsible for 40 to 45% of medical consultations, 30% of hospitalizations, and 5 % pregnancy (24% in total) deaths in Cameroon. It is also the cause of 26% of absences in the workplace and 40% of the health expenditure of households. Malaria is responsible for 49 % of consultations and 59% of hospitalizations during pregnancy leading to abortions and premature labour and deliveries as well as low birth weight all exposing the babies and mothers to maternal mortality [13].

Microscopic detection and identification of *Plasmodium species* in Giemsa stained thick blood film and a thin blood film is the accepted worldwide “gold standard” used for routine diagnosis of malaria. However, different rapid

diagnostic tests have been developed, tested and reported to meet up with WHO standards [14,15,16]. Presently theses rapid diagnostic tests are used in many hospitals for diagnosis including the Buea Regional Hospital in which this study was carried out [17].

Haematological alterations that characterize malaria are related to the biochemical changes that occur during the asexual stage of the life cycle of the malaria parasite. Entry of the merozoite into the erythrocytes usually leads to a marked increase in the secretion of inflammatory cytokines (IL-1, IL-10), activation of coagulation cascade (due to platelet consumption and endothelial damage) and sequestration of parasitized red blood cells (due to over expression of cell adhesion molecules) [18]. These along with other mechanisms set in motion, events that result in morphological and numerical changes of the various blood cells [13].

Since pregnancy reduces a woman's immunity to malaria, making her more susceptible to malaria infection and increasing the risk of illness, severe anaemia and death, pregnant women are more vulnerable to this disease than their non-pregnant women counterparts and other adults**.** Maternal malaria tends to increase the risk of spontaneous abortion, stillbirth, premature delivery and low birth weight (a leading cause to child mortality) for the unborn child. Even though this problem has long been neglected, the creation of new approaches will offer hope for reducing the prevalence or burden of this disease in pregnant women thus, improving the health condition of both mothers and new-borns [8].

There is limited study on the haematological profile of pregnant women with malaria parasitemia in Sub-Saharan Africa looking into the prevalence of malaria in pregnancy and the effect on the haematological profile. Haemato-logical changes (for example a change in the number of red blood cells (RBC), white blood cells (WBC) and platelets (PLTS) are some of the most common complications in malaria and play a major role in malaria pathology. These haematological changes can help improve on the diagnosis of malaria by increasing suspicions and promoting a punctilious search of the parasite using a microscope. Thus this work was designed to determine the prevalence of malaria parasitaemia in relation to some haematological parameters of pregnant women attending the Regional Hospital Buea.

2. METHODS

2.1 Study Area

This study was conducted at the Buea Regional Hospital, found in Fako Division, South West Region of Cameroon. Buea is the capital of South West Region of Cameroon. The town is located on the eastern slope of Mount Cameroon. The population of Buea consist mainly of the indigenous Bakweri people but due to its position as a university town and regional capital, there are significant numbers of people from other ethnic groups. Buea has humid climatic conditions with two main seasons: the rainy season that runs from March to September and the dry season which runs from October to February. The vegetation type is that of tropical rain forest that has been cleared off in some areas to provide land for construction and Agriculture. The inhabitants of the town carry out activities such as farming, trading, and some are workers in government offices, para-statal and private institutions while others are students.

The Buea Regional Hospital in which this study was carried out is the main reference hospital in Fako Division and the South west Region. It is situated precisely between the delegation of Education and the army barracks, along the highway to the Bokwango neighbourhood. The hospital (with about 200 bed spaces) is made up of many units headed by specialist doctors and other hospital staffs such as nurses, laboratory scientist, midwives, and clerical workers of all categories.

2.2 Study Design, Study Population and Sample Size Estimation

A cross sectional hospital-based study was undertaken between the months of April 2018 to July 2018. This study consisted of 276 pregnant women who attended the Regional Hospital Buea during the research period. Pregnant women of all ages, social status, religion who read and willingly signed the consent forms took part in the study.

Using the formula for sample size calculation as described by Swinscow [19] the number of pregnant women to participate in the study was determined.

$$n = \frac{Z^2 xp(1-p)}{e^2},$$

where

n= Sample size
p= is the prevalence based on previous studies= 19.3% (Laurentine et al. 2015).
Z=95% confidence interval =1.96
e=error =0.05

$$n = \frac{1.96^2 x\ 0.193(1-0.193)}{0.05^2}$$

n= 239

However, consent forms were issued out to 300 pregnant women during the entire period of study. Two hundred and seventy six (276) women signed and returned the forms indicating their consent to participate in the study. Thus the study involved 276 pregnant women.

Inclusion Criteria: all pregnant women who willingly gave their consent (signed the consent form), where HIV negative, had no underlining haematological condition such as leukaemia or sickle cell were included in the study.

Exclusion Criteria: all pregnant women who refused to sign the consent form, where HIV positive or had report of any serious haematological conditions such as sickle cell anaemia or leukaemia were excluded from the study.

2.3 Sample Collection, Preparation, Malaria Parasite Detection and Complete Blood Count Test

Once participants gave their informed consent, their blood samples were collected. About 4 ml of blood was collected and dispensed into an EDTA anticoagulant test tube following aseptic techniques, to perform the complete blood count (CBC) and malaria rapid diagnostic test (RDT).

Malaria parasite was detected using the SD Bioline® Malaria Ag P.f/Pan (Standard diagnostic, inc.). SD Bioline® Malaria Ag P.f/Pan Test is a rapid qualitative and differential test for the detectioin of histidine-rich protein II (HRP-II) antigen of *Plasmodium falciparum* and common plasmodium lactate dehydrogenase (pLDH) of *Plasmodium species* in human whole blood.

The test preparation and interpretation was done following manufacturer's instruction. 5 µl of whole blood is drawn from the EDTA tube using the loop provided with the test kit and is dispense into the round specimen touching pad. The tab of the assay diluent was twisted and pulled to open and all the content was dispense into the square well of the test device. The results were read after a minimum time of 15 minutes and maximum of 30 minutes.

The CBC was performed using the Mindray® automatic haematology analyser (BC-5300, Shenzen Mindray Bio-Medical Electronics Co., Ltd, China).

2.4 Data Analysis

Data collected was entered into an Excel spreadsheet (Microsoft excel, 2010) and analyzed using SPSS (Statistical package for social science, version 20). The statistical tests performed were the Pearson's Chi-square for comparison of proportions, the Student's T-test and ANOVA for the comparison of group means, and correlation analysis to determine the haematological profile of pregnant women with malaria.

3. RESULTS

3.1 Baseline Characteristics of the Study Population

The study involved two hundred and seventy six (276) pregnant women who were suspected to be suffering from malaria due to presenting signs and symptoms after due consultation with the physician. The mean age was 27.55 years. Most of the women were above 25 years of age (58.0%), the majority were married (76.1%) and most had attended secondary education (54.3%) (Table 1).

3.2 Prevalence of Malaria

Of the 276 participants, 98 (35.51%) had malaria, while the rest 178 (64.49%), tested negative. The prevalence of malaria according to their trimester showed that pregnant women in the first trimester were the most infected with malaria parasite (52, 18.8%), followed by second trimester (32, 11.6%) (Table 2).

Malaria prevalence varied with variations in demographic characteristics. Although not

statistically significant, the prevalence was higher in pregnant women who were older, married, and had acquired university education (56, 20.3%;68, 24.6% and 50, 18.1% respectively) as shown in Table 3.

3.3 Haematological Parameter of Malaria Infected and Non-Infected Pregnant Women

A comparison of the haematological parameters revealed a significant difference in White blood cells, Red blood cell, Haemoglobin, Haematocrit and Platelets values between the malaria infected and non-malaria infected pregnant women. The mean values for all haematological parameters were lower in malaria positive pregnant women than in malaria negative pregnant women as shown in Table 4.

3.4 Anemia in Pregnancy

Anaemia was defined as haemoglobin level < 10 g/dl. The mean (± SD) of haemoglobin value of malaria infected women 9.7(± 1.4) was significantly lower than that of malaria non-infected pregnant women 10.7 (± 1.1). Eighty eight (31.88%) of the pregnant women were anaemic. For the anaemic cases only, forty eight

Table 1. Demographic characteristics of the study population

Variable	Total	Percentage (%)
Age Groups		
< = 25 yrs.	114	41.3
>= 26 yrs.	160	58.0
Marital Status		
Single	62	22.5
Married	210	76.1
Divorced	0	0
Level of Education		
University	106	38.4
Secondary	150	54.3
Primary	20	07.2
Total	**276**	**100**

Table 2. Prevalence of malaria according to trimester

Frequency (Percentage)	Malaria		P_value
Trimester	**Positive**	**Negative**	
First trimester	52 (18.8 %)	80 (29 %)	0.468
Second Trimester	32 (11.6 %)	58 (21.0 %)	
Third trimester	14 (5.1 %)	40 (14.5 %)	
Total	98 (35.5 %)	178 (64.5 %)	

Table 3. Malaria prevalence with respect to demographic characteristics of the study population

Variable	Malaria(Yes)	Malaria(Neg)	p-value
	N= 98	**N=178**	
Age (Mean +/- SD)	28.0 (±6.1)	27.1 (±5.5)	
Age Groups			
< 26 yrs.	42 (15.2 %)	74 (26.8 %)	0.883
>= 26 yrs.	56 (20.3 %)	104 (37.7 %)	
Total	98 (35.5 %)	178 (64.5 %)	
Marital Status			
Single	30 (10.9 %)	32 (11.6 %)	0.150
Married	68 (24.6 %)	146 (52.9 %)	
Divorced	0	0	
Total	98 (35.5 %)	178 (64.5 %)	
Level of Education			
University	42 (15.2 %)	64 (23.2 %)	0.150
Secondary	50 (18.1%)	100 (36.2 %)	
Primary	6 (2.2 %)	14 (5.1 %)	
Total	**98 (35.5 %)**	**178 (64.5 %)**	

Table 4. Mean values for haematological parameters in malaria infected and non-infected pregnant women

Variable	Malaria(Pos) Mean (± SD)	Malaria(Neg) Mean (± SD)	P_Value
Haemoglobin (HB)	9.7 (± 1.4)	10.7 (± 1.1)	**< 0.001**
Haematocrit (HCT)	29.8 (±4.4)	33.6 (± 4.6)	**< 0.001**
Red Blood Cells (RBC)	3.7 (±0.6)	4.2 (± 1.0)	**< 0.001**
Platelets (PLT)	148.5 (± 67.8)	213.0 (±58.8)	**< 0.001**
White blood cells (WBC)	5.9 (±2.4)	6.7 (±1.9)	**< 0.039**

Table 5. Prevalence of anaemia in pregnancy

Variable Anaemia	Frequency	Percentage	P_ value
Yes	88	31.88 %	P < 0.05
No	188	68.12 %	
Total	276	100.00 %	

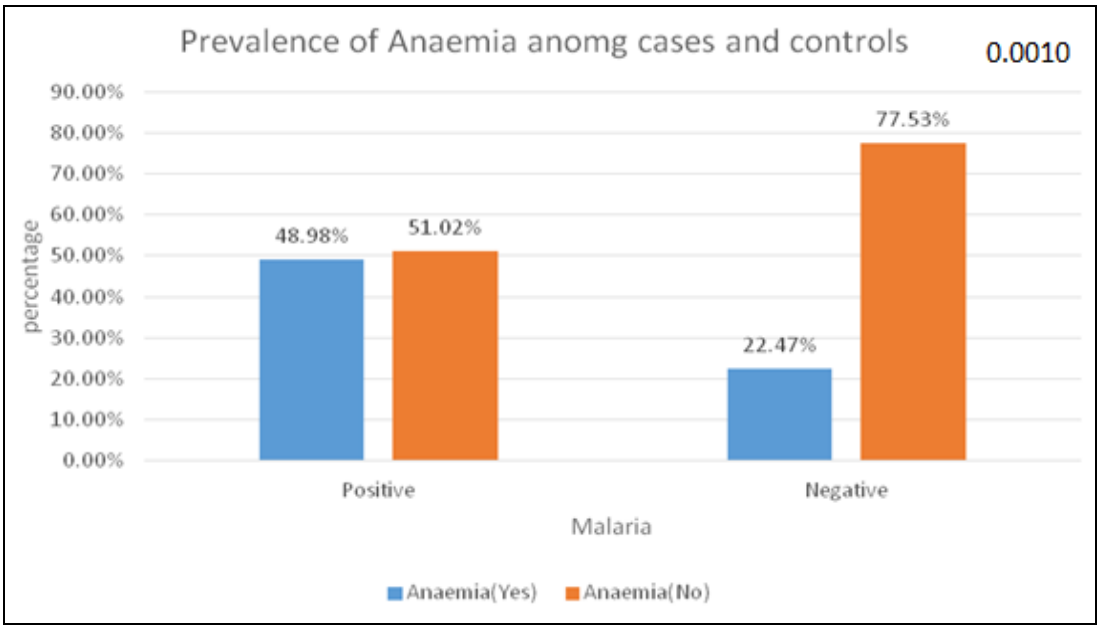

Fig.1. Anaemia in relation to malaria status of pregnant women

Table 6. Prevalence of thrombocytopenia in pregnancy

Variable	Frequency	Percentage
Thrombocytopenia		
Yes	76	27.54 %
No	200	72.46 %
Total	276	100.00 %

Table 7. Thrombocytopenia in relation to malaria status

Thrombocytopenia	Malaria		P_Value
	Positive	Negative	< 0.001
Yes (Positive)	58 (59.18 %)	18 (10.11 %)	
No (Negative)	40 (40.82 %)	160 (89.89 %)	

(48.98%) were malaria positive and forty were (51.02%) malaria negative (Table 5, Fig. 1). Anaemia was significantly higher among pregnant women with malaria ($p < 0.001$) than in those without malaria.

3.5 Thrombocytopenia in Pregnancy

Thrombocytopenia was defined as Platelets < 150000 /mm^3. The mean (SD) of platelet count for the malaria infected women was 148.5 (±

67.8) and it was significantly lower than that of malaria non-infected pregnant women (213.0 ± 58.8). Overall, the prevalence of thrombocytopenia in the study population was 27.54% (Table 6). In relation to malaria status, there was significant difference ($p < 0.001$) in the prevalence of thrombocytopenia among malaria positive and malaria negative pregnant women (Table 7).

4. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

The overall prevalence of malaria from our study was 35.51%. As compared to statistics of malaria prevalence reported by Ndamukong-Nyanga et al. in 2014 (35% prevalence) in Buea [14], it is observed that, the prevalence of malaria ha s not changed significantly from 2014 to 2018. This could be an indication that inhabitants of Buea do not adhere strictly to the control measures put in place by the government. In other parts of the country lower prevalence values have been recorded by other authors such as *Sumo* et al. (2015) in Ndop [20,21]. The lower prevalence recorded in other areas may be due to adherence to measures recently implemented by the Cameroon government through the Ministry of Public Health. These include the free distribution of insecticides treated bed nets (ITNs) to all pregnant women and public education on the use in prevention and control of malaria. First trimester pregnant women had the highest prevalence of malaria (53.06%), followed by the second trimester (32.65%) and lastly the third trimester (14.29%). This result was consistent with the study of Accrombessi et al. 2018 in Benin [22]. This can be explained by the fact that pregnant women generally go for antenatal care late. Most often, it is during the first ANC visit that most of them do a malaria test and then begin treatment if found to be positive. By the second and third trimesters most pregnant women would have been treated for malaria, so the prevalence drops. Also, intermittent preventive treatment (prohpylaxis) with sulfadoxine-pyrimethamine is not recommended before the second trimester, leaving women insufficiently or not protected in early pregnancy. Initiatives put in place by the Ministry of Public Health in Cameroon and other bodies such as 'Initiative of Cameroon coalition against malaria and Centre for Development and Best Practices in Health' demand that all pregnant women be given free insecticide treated mosquito nets. However, most pregnant women may not have nets because of late start of ANC consultation. Hence, they may experience mosquito bites in early pregnancy with a risk of malaria infection.

The study revealed significant decrease in haemogloblin (9.7 [± 1.4]), haematocrit (29.8 [±4.4]), platelets (148.5 [± 67.8]), RBC (3.7 [±0.6]) and WBC (5.9 [±2.4]) respectively of malaria infected pregnant women compared to non-infected pregnant women. The findings of this work are consisted with previous study reported by Alo Moses et al. In 2014 [23]. Decrease in haemoglobin and haematocrit were reportedly caused by increased plasma volume during pregnancy (23). Hence there is need of managing their blood profiles with dietary supplements and the need for proper and balanced diet during pregnancy.

Anaemia is one of the most common complications in malaria especially in younger children and pregnant women [9,24]. The current study reports a significant decrease in haemoglobin level in pregnant women infected with malaria as compared to those non-infected with malaria. This finding agrees with the previous work done by Sumbele et al. (2014) on anaemia status in pregnant women [24]. The mild anaemia reported in 22.74% of the non-malaria infected pregnant women may be due to poor nutritional status and no intake of iron and vitamin B12 drugs. Knowledge deficit may also be a factor contributing to anaemia and malaria prevalence among pregnant women. In line with Kimbi et al. [25] lack of knowledge and poor perception of malaria control measures can lead to non-applicability of the existing preventive measures. This will lead to increased malaria transmission and increased levels of anaemia especially among vulnerable populations (e.g. In pregnant women).

Thrombocytopenia, one of the complications of malaria in pregnancy and in younger children was found to have a significant decrease in pregnant women with malaria as compared with non-malaria pregnant women and it is consistent with the study performed by Alo Moses et al. [23]. These variations in blood parameters indicate clearly that malaria significantly affect the blood picture of pregnant women. Anaemia in pregnancy can cause severe side effects ranging from general body weakness, to intra-uterine death [23].

4.2 Conclusion

The overall malaria prevalence in the study was 35.51%. Malaria prevalence was insignificantly higher in the first trimester. The study revealed a high prevalence of malaria, anaemia and thrombocytopenia in pregnant women seeking for healthcare services in the study area. It also demonstrated a significant decrease in haematological parameters such as haemoglobin, RBC, PLTS, HCT and WBC. These parameters could improve malaria diagnosis when used in combination with other diagnosis and microscopy.

4.3 Recommendations

Based on the findings, the following recommendations can be made:

- Haematological parameters should be measured and evaluated in pregnant women with signs and symptoms of malaria as a routine test for ANC.
- Intensified teaching of the pregnant women during ANC on malaria prevention and control could also help to reduce the burden of the disease.

CONSENT

Pregnant women of all ages, social status, religion who read and willingly signed the consent forms took part in the study.

ETHICAL APPROVAL

Authorisation was obtained from the delegation of public health in Buea and Biaka University Institute of Buea giving the necessary approval to conduct this research. Clearance was received from the general supervisor of Regional Hospital Buea. All participating women were asked to sign a consent form after explaining the research nature and objectives to them.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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