Research Article

Correlation between Malaria and the Red Blood Cell Indices of Pregnant Women in Buea and Tiko Health Districts, South West Region of Cameroon

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ABSTRACT

This study was designed to determine the level of malaria endemicity and correlate between malaria parasitaemia and the red blood cell indices of the pregnant women in the Buea and Tiko health districts of the south west region of Cameroon. Samples were drawn from: The CDC Central Clinic Tiko, the Mutengene Baptist Hospital, the Buea Regional Hospital, the Government Health Centre Muea and the Buea Road Integrated Health Centre. The non-probabilistic sampling method, the volunteer sampling technique was used to recruit a total of 377 pregnant women. Questionnaires were used for the collection of primary data. A total of 41.4% (156/377) of the pregnant women were infected with malaria parasitaemia. Due to repeated infections in some of the research subjects, a total of 204 infected cases were recorded. Mild parasitaemia (<1000Troph/ µl of blood) was most frequent (106/204), and severe parasitaemia (<10000Troph/ µl of blood) was the least frequent (08/204). Amongst the infected cases, 15.4% (24/156) had decreased levels of red blood cell counts ($<3.5 \times 10^{12}$/L), 47.4% (74/156) had decreased haematocrit values (<33%), 34.6% (54/156) had decreased values of MCV (<82fl), 53.2% (83/156) had decreased values of MCH (< 27pg), 55.8% (87/156) had decreased values of MCHC (<320g/l) and 37.2% (58/156) had increased levels of RDW-CV (<14.5%). Significant correlation between malaria parasitaemia and haemoglobin measurements was reported only in the pregnant women of the CDC Central Clinic Tiko, during their second trimester of pregnancy. There were no significant correlation between any of the red blood cell indices and malaria parasitaemia, at P<0.05. Other etiologic factors were contributed to the anaemia reported in the pregnant women
of the study area.

RESUME

Cette étude a été conçue pour déterminer le niveau d'endémicité du paludisme et comparer sa parasitémie aux constantes globulaires chez les femmes enceintes dans les centres de santé des districts de Buea et Tiko de la région du Sud-ouest Cameroun. L'échantillon a été obtenu de la clinique centrale CDC de Tiko, de l'hôpital Baptiste de Mutengene, de l'hôpital régional de Buea, du centre de santé gouvernemental de Muea et du centre de santé intégré de Buea Road. La méthode d'échantillonnage non probabiliste et la technique du volontariat ont été utilisées pour recruter 377 femmes enceintes. Des questionnaires ont été utilisés pour recueillir les données préliminaires. Un total de 41,4%(156/377) de parasitémie au paludisme a été observé lors de cette étude chez les femmes enceintes. Du fait d'infections répétées chez certaines des sujets étudiés, un total de 204 sujets infectés ont été relevés. La parasitémie modérée (<1000 troph/µl de sang) a été la plus fréquente (106/204), et la sévère (<10000 troph/ µl de sang), la moins fréquente (08/204). Parmi les cas infectés, 15,4% (24/156) avaient une diminution de la numération des globules rouges ($<3,5 \times 10^{12}$ /L), 47,4% (74/156), une diminution de l'hématocrite (<33%), 34,6% (54/156) une diminution des valeurs du VGM (<82fl), 53,2% (83/156) une diminution des valeurs de la TCMH (< 27pg), 55,8% (87/156) une diminution des valeurs de la CCMH (<320 g/l) et 37,2% (58/156) une augmentation du niveau d' IDR (<14,5%). Une corrélation significative entre la parasitémie au paludisme et les concentrations en hémoglobine a été observée uniquement chez les femmes enceintes de la clinique centrale CDC de Tiko pendant le deuxième trimestre de la grossesse. Aucune corrélation significative n'a été relevée entre les constantes globulaires et la parasitémie au paludisme à p<0,05. D'autres facteurs étiologiques auraient contribué à l'anémie observée chez les femmes enceintes dans la région étudiée.

Keywords: Malaria, parasitaemia, red blood cell indices (MCV, MCH, MCHC and RDW), haematocrit, haemoglobin, anaemia.

INTRODUCTION

Malaria remains a devastating global health problem especially for those living in the tropics and equator. Worldwide, an estimated 300-500 million people contract malaria each year, resulting in approximately 1.5 -2.7 million deaths annually (WHO, 2012). During pregnancy, immunity to malaria is altered and infection is frequently asymptomatic, and so may go unsuspected and undetected, but may result in severe maternal anaemia (Warrell *et al.*, 2002)

In regions where malaria is endemic, it is commonly considered to be a principal cause of severe anaemia (Calis *et al.*, 2008). In areas of malaria endemicity, malaria may be responsible for about 44% of anaemia (Takem *et al.,* 2010). Anaemia in pregnancy has been described as one of such enormous medical challenges because it is a major public-health problem in Africa and it is an important factor associated with an increased risk of poor pregnancy outcomes. In 1993, the World Health Organization (WHO) instituted its safe motherhood initiative with a goal of reducing the number of maternal deaths by half, before the year 2000. A key component was to eradicate anaemia in pregnancy, focusing on the greater risk in younger women. But anaemia during pregnancy, particularly iron deficiency anaemia (IDA), continued to be of worldwide concern (Abel *et al.,* 2000).

Malaria anaemia is estimated to affect up to 16 million women and children by the year 2015. In September 2000, the largest gathering of world leaders in human history convened for the Millenium Summit, on the Millenium Development Goals, in which a plan was adopted to achieve the Millennium Development Goals by the year 2015. As one of the steps to achieving the goals, stakeholders committed 40 billion US dollars in resources to a global effort to save the lives of the 16 million women and children who are likely to be affected by malaria induced anaemia by the year 2015.

As one of the malaria endemic countries, Cameroon has made efforts in the fight against malaria. Between 2006 and 2010, the Ministry of Public Health in Cameroon distributed over eight million long-lasting treated mosquito nets. The Government of Cameroon in her efforts to fight against malaria, embarked on one of its biggest efforts in the protracted battle against malaria. The distribution of over 8 million treated mosquito nets (loaded with insecticides to keep mosquitoes at bay, for five years) was launched on the 20th August 2011. All Governors of Cameroon's 10 administrative regions headed the distribution committees, that comprised medical doctors and security officers in order to ensure maximum coverage of the national territory. This campaign was just after the President of Cameroon decided to offer free malaria treatment for all children under five, in January 2011.

Despite all these, cases of malaria anaemia are still identified among pregnant women in Cameroon and beyond (Cameroon, South West Region, Buea, Ministry of Public Health, 2011). For the year 2010, Buea, Limbe, Muyuka and Tiko health districts offered antenatal care services to 4314, 7191, 4568, and 6733 pregnant women respectively. This gives a total of 22,806 pregnant women who received antenatal care in 2010 in Fako division (Cameroon, South west Region, Buea, Ministry of Public Health, 2011). Statistics also show that, while in the South West Region as a whole, from January to June 2006, 2690 pregnant women consulted for different diseases, 1274 were diagnosed of malaria and 513 of them were hospitalized (Cameroon, South West Region, Buea, Ministry of Public Health, 2011).

The aim of the present investigation was to determine the factors that favour the development of anaemia in pregnant women in the semi-urban areas of South Western Cameroon. Accordingly therefore, the socioe-conomic status, haematological indices and nutritional factors were determined.

MATERIALS AND METHODS

Study Area.

The Buea and Tiko health districts are found in the Fako Division of the South West Region of Cameroon. Buea and Tiko are located between longitude 8.6-10 and latitude 4-5.2 degrees. The climate alternates from the hot humid coastline, the Tiko plain, to the montaine on the slopes of Mt Cameroon,where Buea is situated. Fako division is divided into four health districts; the Buea, Limbe, Muyuka and Tiko health districts. In these health districts, there are health units, district hospitals and a regional hospital which offer services of antenatal care to pregnant women.

Study Design

This intensive field research was out during the period of nine months, from the 3rd of August 2011 to the 30th of April 2012. Pregnant women were recruited from the Cameroon Development Corporation (CDC) Central Clinic Tiko, Mutengene Baptist Hospital, Buea Regional Hospital, Government Health Center Muea and the Buea Road Integrated Health Centre. Newly registered pregnant women for antenatal care visits were considered eligible for recruitment as research participants. The sampling method used was the non probabilistic sampling method, or the volunteer sampling technique. This study took a longitudinal form, from the time of recruitment into the study, to term.

Administration of questionaires

Newly registered pregnant women for antenatal care visits were contacted on one-to-one basis and after a brief explanation about the study, informed consent forms were handed to them. Those who accepted to participate in the study signed the form, and were assisted in filling them. Recruitment into the study was based upon the signing of the informed consent form.

Laboratory diagnostic methods

Parasitological examination and haemoglobin measurement

Collection of blood

A cotton wool soaked in 70%v/v alcohol was used to disinfect a finger lobe by swabbing the area. The disinfected area was pricked with a sterile lancet. The first drop of blood was swabbed with dry cotton and the finger slightly squeezed to get a larger drop of blood.

Preparation of thick and thin blood films for diagnosis of malaria

A small drop of blood from the pricked finger was placed at the centre of a clean grease free microscope slide and a larger drop to the right of it. Without delay, the smaller drop of blood was spread using a smooth edged slide spreader, with the spreader held at the steeper angle, to get a thin film. The larger drop of blood was also spread several times in a circular pattern of about 2cm in diameter, also using an edge of another slide, to get a thick film. Each slide was labelled and both films allowed to air-dry with the slide in a horizontal position and placed in a safe place.

Staining for malaria parasite

The thin blood film was fixed using absolute methanol, by placing the slide horizontally on a staining rack and applying a drop of the absolute methanol, making sure the alcohol did not touch the thick film. The thin film was fixed for one to two minutes. 3% Giemsa staining solution was prepared by mixing gently 1.5ml of Giemsa stain to 50ml of buffered water (PH 7.2). With the

slide placed horizontally on a staining rack and the smear facing upward, the diluted stain was covered on it for 30 minutes. Buffered water was used to flush the stain from the slide, to avoid the films being covered with fine deposit of the stain.

Determination of malaria parasite density

When the films were dried, a drop of immersion oil was placed on the thick film. It was mounted and examined using the high power 100x objective lens. Parasite numbers was determined by counting the number of parasites against at least 100 leukocytes, or 200 leukocytes for the definitive count. The number of the parasites/µl was calculated using the formula (WHO, 2000b):

Parasites/µl of blood =

Number of parasites counted x 8000

leukocytes Number of WBC Counted.

-For the positive cases, parasitaemia was classified as follows:

1-999 Parasites/µl of blood = Mild parasitaemia.

1,000-9,999 Parasites/µl of blood = Moderate parasitaemia.

>10,000 Parasites/µl of blood = Severe parasitaemia (WHO, 2000b).

3.4.5.1.5: Haemoglobin measurements

The photometric method – Reflectance Photometry was employed.

The Stanbio STAT-Site M^{Hgb} (Haemoglobinometer) was used (Campbell, 2005).

Test Principle:

The STAT-Site M^{Hgb} Test provides a direct reading of haemoglobin concentration in whole blood between 6 and 21g/dL. Values below or above this range was reported as **<Lo> or <Hi>** respectively.

The STAT-Site M^{Hgb} Test consists of a plastic card with reagent pad for determining the concentration of haemoglobin. When a drop of whole blood is applied to the top of the STAT-Site M^{Hgb} Test card, haemolysis occurs, with releases of haemoglobin. Sodium nitrite converts the haemoglobin to methaemoglobin. Sodium azide then reacts with methaemoglobin to form azide-methaemoglobin, which is brown in color and is detected at 565nm with a small portable reflectance analyzer. The amount of the colour produced due to azide-methemoglobin is proportional to the concentration of haemoglobin in the sample (Campbell, 2005).

Test proceedure:

Upon turning the meter on, the test card was slided under the guide tabs and firmly pushed to the back, to lock into place. A drop of blood from the pricked finger was carefully laid directly from the finger, to the centre of the test card. The meter then automatically counted down to the result. The result was recorded and the degree of anaemia classified as follows:

H b< 7g/dL = Severe Anaemia.

Hb7-8.9g/dL = Moderate Anaemia.

Hb 9-11g/dL = Mild Anaemia.

Hb > 11g/dL= Normal Haemoglobin level (WHO, 2000).

3.4.5.2: Full blood count and measurements of serum ferritin, transferrin and iron

3.4.5.2.1: Blood collection

Using a cotton wool soaked in 70% v/v alcohol as disinfectant, the venous area from where blood was collected was swabbed. Blood was collected from the vein using a 10mL syringe. Approximately 3 to 4 mL was dispatched into a K_3EDTA anti-coagulated tube and mixed gently. Approximately 4 mL of blood was also dispatched into a dry tube and centrifuged. Serum was separated and stored in an ependoff tube at -20°C.

3.4.5.2.2: Full blood count

The Mindray BC-2800 Auto-Haematology Analyzer was used for the full blood count, with reagents from the Mindray Company, China according to the instructions of the manufacturer.

Data analysis

Descrptive statistics (mean, median and mode), with the use of tables and charts were used to present the results on the status of malaria endemicity and intensity amongst the pregnant women of the study area.

The Pair-wise correlation analysis technique was employed to assess the relationship between malaria infection and haemoglobin measurements and the relationship between malaria infection and the red blood cell indices. The correlation coefficient(r) for each research centre and for the study area as a whole was obtained from the Pairwise Correlation Analysis Matrix, using E-views version 7. The correlation coefficients (r) were used to test for significant correlations, in the following formulae:

$Z = r\sqrt{N-1}$ and $t = r\sqrt{\frac{N-2}{1-r^2}}$ where;

Z stands for the Z-test and was used with sample sizes of 30 and above;

t stands for the t-test and was used with sample sizes of less than 30;

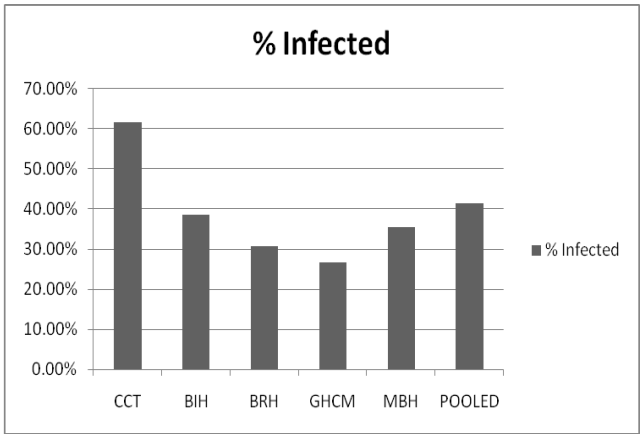
r was checked on the critical table directly;

RESULTS

A total of 482 pregnant women were recruited in this study, and 377 of them went through the study successfully, constituting about 78.2% of the recruited population. Table 1 shows the number of those recruited and those who participated in the study while FIG 1 shows malaria endermicity in the study area by study centres and FIG 2 shows the degree of malaria parasitaemia in the study area by trimesters.

TABLE 1: No (%) of those recruited and those who participated in the study

Centres	Nº Recruited	Nº(%) Participation
CDC Central Clinic Tiko (CDC Tiko)	115	109(95%)
Mutengene Baptist Hospital (MBH)	98	65(66%)
Government Health Centre Muea (GHCM)	84	60(71%)
Buea Regional Hospital (BRH)	75	65(87%)
Buea Road Integrated Health Centre (BIH)	110	78(71%)
Total	482	377(78%)



CCT = CDC Central Clinic Tiko, BIH = Buea Road Integrated Health Centre, BRH = Buea Regional Hospital, GHCM = Government Health Centre Muea, MBH = Mutengene Baptist Hospital

FIG 1: Malaria endermicity in the study area by centres

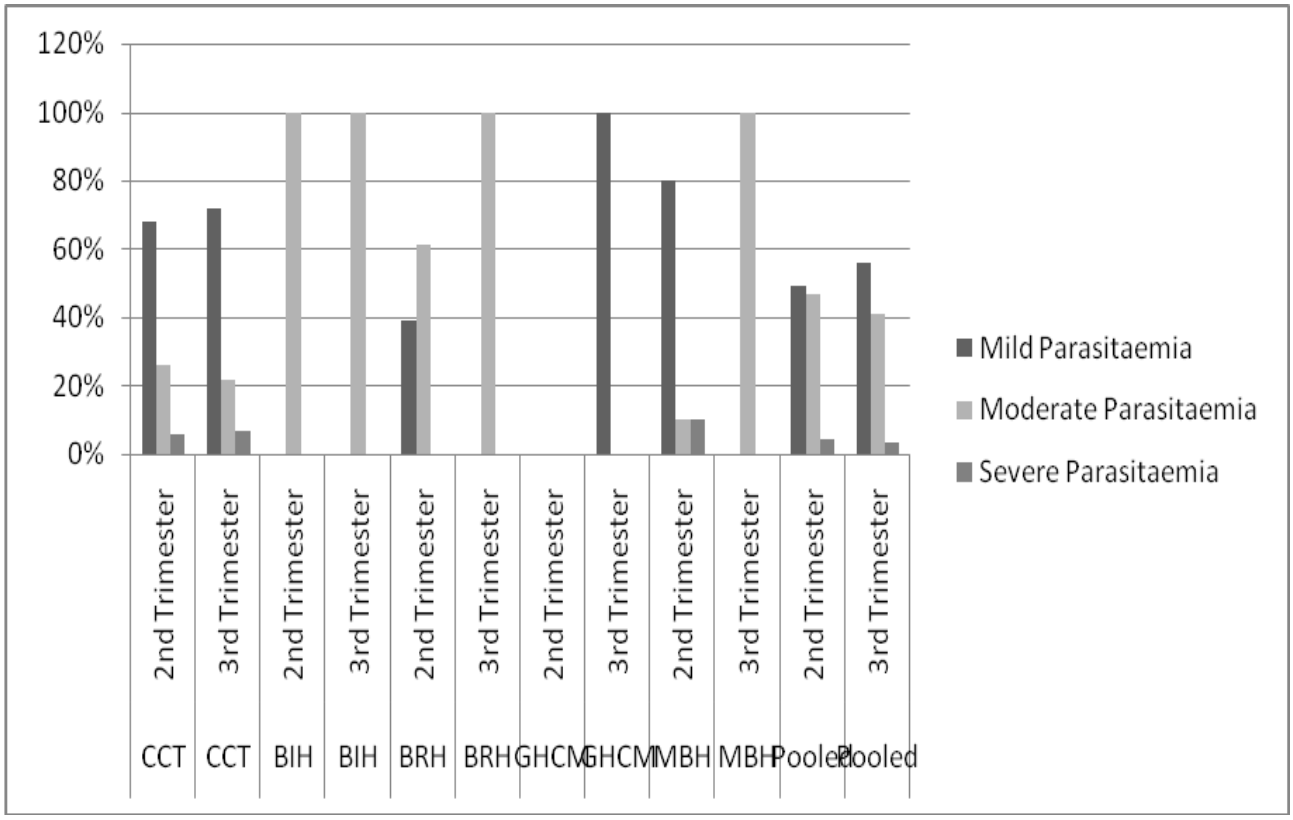


FIG 2: Degree of Parasitaemia in the study centres by trimesters

FIG 3 shows malaria endemicity in the study area by trimester and FIG 4 and 5 shows the classification of anaemia according to the presence/absence of malaria in the second and third trimesters respectively.

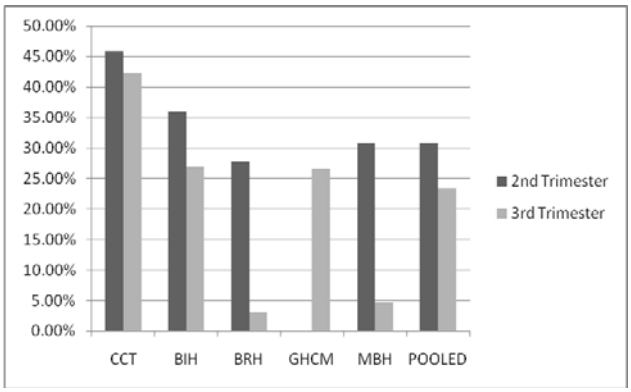


FIG 3: Malaria endemicity in the study area by trimester

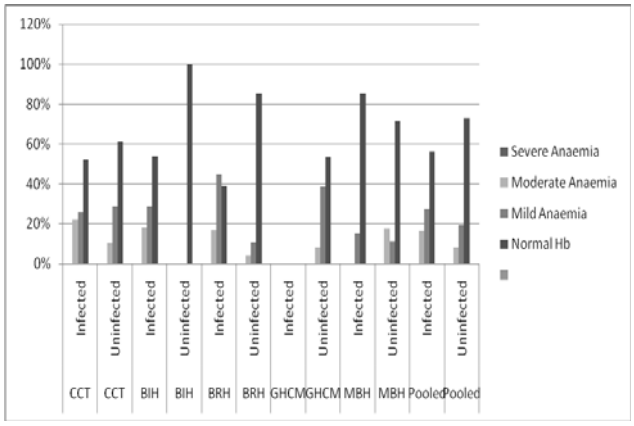


FIG 4: Classification of anaemia according to the presence/absence of infections in the second trimester of pregnancy

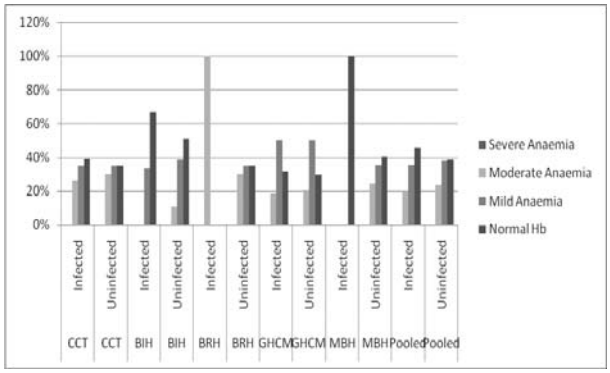


FIG 5: Classification of anaemia according to the presence/absence of infections in the third trimester of pregnancy

TABLE 2 shows the relationship between malaria parasitaemia and haemoglobin levels in the study area, by trimesters and TABLE 3 shows the results of the Pair wise correlation analysis between haemoglobin measurements and the presence of malaria in the study area.

TABLE 2: Relationship between malaria parasitaemia and haemoglobin levels in the study area, by trimesters

Status of infection /Trimester	**Moderate Anaemia (%)**	**Mild Anaemia (%)**	**Normal Hb (%)**	**Total (%)**
Mild Parasitaemia / 2nd Trimester	14(24.6)	5(08.8)	38(66.7)	57(49.1)
Moderate Parasitaemia / 2nd Trimester	5(09.3)	24(44.4)	24(44.4)	53(45.7)
Severe Parasitaemia / 2nd Trimester	00	3(60.0)	3(60.0)	6(05.2)
Total / 2nd Trimester	19 (16.4)	32 (27.6)	65 (56.0)	116
Mild Parasitaemia / 3rd Trimester	11(22.5)	19(38.8)	19(38.8)	49(55.7)
Moderate Parasitaemia / 3rd Trimester	6(16.7)	9(25.0)	21(58.3)	36(40.9)
Severe Parasitaemia / 3rd Trimester	00	3(100)	00	3(03.4)
Total/ 3rd Trimester	17(19.3)	31(35.2)	40(45.5)	88

Mild Parasitaemia = 1-999 trophozoites/µL of blood, Moderate Parasitaemia = 1,000-9,999 trophozoites/µL of blood, Severe Parasitaemia = e"10,000 trophozoites/ µL of blood (WHO, 2003).

Moderate anaemia = Hb7-9.9g/dl, Mild Anaemia= Hb 10.0-10.9g/dl, Normal haemoglobin = Hb > 11g/dl (WHO, 1992).

TABLE 3: Pair wise correlation analysis between haemoglobin measurements and the presence of malaria in the study area

Trimester		CCT MP	BIH MP	GHCM MP	MBH MP	BRH MP	Pooled MP
Second trimester	Hb	-0.667	0.016	NA	-0.337	-0.185	-0.023
Third trimester	Hb	0.234	0.234	0.118	0.328	0.053	0.024

MP=Malaria Parasitaemia, Hb =Haemoglobin, NA= not available (Pair wise correlation analysis matrix table Eview version 7).

Using the correlation coefficients from TABLE 3 above, the Z-test and the t-tests were used to test for significant correlation. The correlation between malaria infections during the second trimester, and haemoglobin measurements, in the pregnant women of the CDC Central Clinic Tiko was significant at P>0.05. The correlation between malaria parasitaemia and haemoglobin measurements of the pregnant women in the BIH, GHCM, MBH and the BRH were all insignificant.

FIG 6, 7 and 8 illustrates the classification of blood indices according to the infected and non-infected cases in study centers, while table 4 and 5shows the classification of these indices for the infected cases, by the study centres

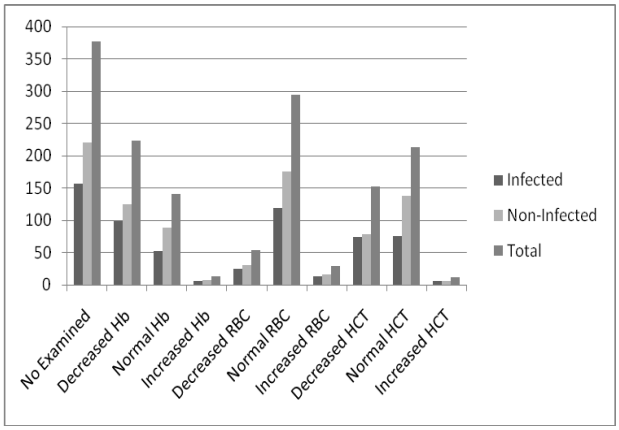


FIG 6: Classification of Hb, RBC, and HCT according to infected and non-infected cases in the study area (RBC= Red blood cell count, HCT= Haematocrit, MCV= Mean corpuscular volume, MCH = Mean corpuscular haemoglobin)

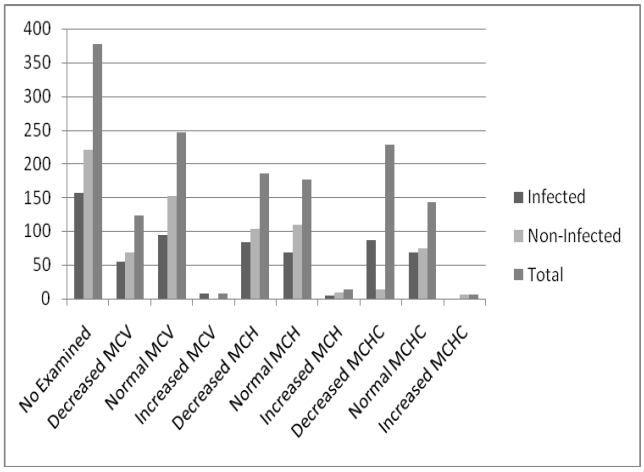


FIG 7: Classification of MCV, MCH, and MCHC according to the presence/absence of malaria
MCHC = Mean corpuscular haemoglobin concentration, RDW-CV = Red blood cell distribution width-Coefficient of Variation, RDW-SD= Red blood cell distribution width-Standard Deviation)

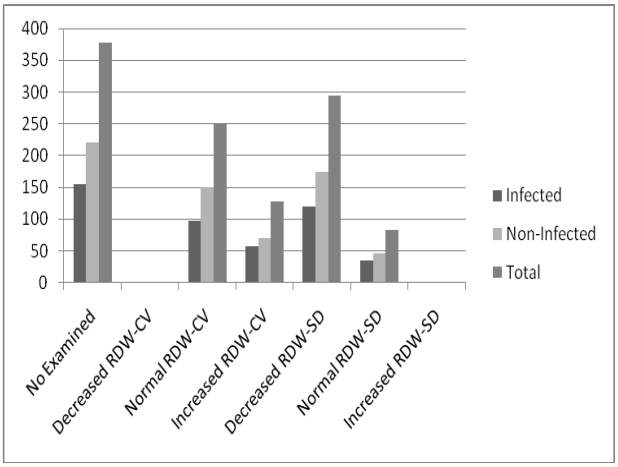


FIG 8: Classification of RDW-CV and RDW-SD according to the presence/absence of malaria

TABLE 4: Classification of Hb, RBC, HCT and MCV of the infected cases, by study centres

Study Centre	No infected (%)	Decreased Hb (%)	Normal Hb (%)	Increased Hb (%)	Decreased RBC (%)	Normal RBC (%)	Increased RBC (%)	Decreased HCT (%)	Normal HCT (%)	Increased HCT (%)	Decreased MCV (%)	Normal MCV (%)	Increased MCV (%)
CCT	67 (61.5)	42 (62.7)	21 (31.3)	04 (06.0)	11 (16.4)	48 (71.6)	08 (12.0)	33 (50.0)	30 (44.8)	04 (06.0)	25 (37.3)	37 (55.2)	05 (07.5)
BRH	20 (30.8)	20 (100)	00	00	08 (40.0)	12 (60.0)	00	18 (90.0)	02 (10.0)	00	08 (40.0)	10 (50.0)	02 (10.0)
GHCM	16 (26.7)	11 (68.6)	05 (31.3)	00	03 (18.8)	10 (62.5)	03 (18.8)	03 (18.8)	13 (81.3)	00	11 (68.8)	05 (31.3)	00
MBH	23 (35.4)	13 (56.5)	10 (43.5)	00	00	23 (100)	00	10 (43.5)	13 (56.5)	00	06 (26.1)	17 (73.9)	00
BIH	30(38.5)	12 (40.0)	16 (53.3)	02 (06.7)	02 (06.7)	26 (86.7)	02 (06.7)	10 (33.3)	18 (60.0)	02 (06.7)	04 (13.3)	26 (86.7)	00

Hb = Haemoglobin, RBC= Red blood cell count, HCT= Haematocrit, MCV= Mean corpuscular volume

TABLE 5: Classification of MCH, MCHC, RDW-CV and RDW-SD for the infected cases, by study centres

Study Centre	No Infected (%)	Decreased MCH (%)	Normal MCH (%)	Increased MCH (%)	Decreased MCHC (%)	Normal MCHC (%)	Increased MCHC (%)	Decreased RDW-CV (%)	Normal RDW-CV (%)	Increased RDW-CV (%)	Decreased RDW-SD (%)	Normal RDW-SD (%)	Increased RDW-SD (%)
CCT	67 (61.5)	36 (53.7)	26 (38.8)	05 (07.5)	27 (40.3)	40 (09.7)	00	00	39 (58.2)	28 (41.8)	49 (73.1)	18 (26.9)	00
BRH	20 (30.8)	13 (65.0)	07 (35.0)	00	10 (50.0)	10 (50.0)	00	00	13 (65.0)	07 (35.0)	13 (65.0)	07 (35.0)	00
GHCM	16 (26.7)	14 (87.5)	02 (12.5)	00	14 (87.5)	02 (12.5)	00	00	06 (37.5)	10 (62.5)	11 (68.8)	05 (31.3)	00
MBH	23 (35.4)	06 (26.1)	17 (73.9)	00	16 (69.6)	07 (30.4)	00	00	16 (69.6)	07 (30.4)	21 (91.3)	02 (08.7)	00
BIH	30 (38.5)	14 (46.7)	16 (53.3)	00	20 (66.7)	10 (33.3)	00	00	24 (80.0)	06 (20.0)	26 (86.7)	04 (13.3)	00

MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, RDW-CV = Red blood cell distribution width, RDW-SD= Red blood cell distribution width-Standard Deviation

Table 6 and 7 shows the correlation relationship between malaria parasitaemia during the second and third trimesters, and the red blood cell indices, by study centers.

TABLE 6: The correlation relationship between malaria parasitaemia during the second trimester and the red blood cell indices of the pregnant women, by study centers

RBC Indices	CDC Tiko	BIH	GHCM	MBH	BRH	Pooled
Hb	-0.0442	0.01302	NA	-0.10244	-0.1860	-0.0442
RBC	-0.0419	-0.0891	NA	-0.0712	-0.30720	-0.04186
HCT	-0.2607	0.00669	NA	-0.08569	-0.16197	-0.02607
MCV	0.04147	0.12277	NA	0.10467	0.35907	0.04147
MCH	0.01733	0.15863	NA	0.05880	0.222619	0.01733
MCHC	0.0830	0.08041	NA	-0.1188	-0.12506	0.0830
RDW-CV	0.1189	-0.2469	NA	0.17186	-0.2504	0.118099
RDW-SD	0.1362	0.31999	NA	0.047799	0.39346	0.13622

(Pair wise correlation analysis matrix table Eview version 7)

MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, RDW-CV = Red blood cell distribution width, RDW-SD= Red blood cell distribution width-Standard Deviation, NA = Not available

TABLE 7: The correlation relationship between malaria parasitaemia during the third trimester and the red blood cell indices of the pregnant women, by study centers

RBC Indices	CDC Tiko	BIH	GHCM	MBH	BRH	Pooled
Hb	-0.01302	0.23299	0.1118	0.2002	0.05177	-0.01302
RBC	0.01186	0.09444	0.0004	-0.0361	-0.0078	0.01186
HCT	0.01307	0.27049	0.1333	0.0702	0.02586	0.01306
MCV	-0.02072	0.20427	0.14148	0.14238	0.11345	-0.0207
MCH	-0.05105	0.13345	0.12175	0.10552	0.11847	-0.0511
MCHC	-0.00925	-0.0975	-0.0497	-0.11046	-0.00789	-0.00925
RDW-CV	-0.11029	0.2636	0.35458	-0.08448	-0.11774	-0.11029
RDW-SD	-0.12065	0.2935	0.04779	0.16019	0.32869	-0.12066

(Pair wise correlation analysis matrix table Eview version 7)

MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, RDW-CV = Red blood cell distribution width, RDW-SD= Red blood cell distribution width-Standard Deviation.

Using the correlation coefficients from TABLES 6 and 7 above, the Z-test was used to determine significant correlation. The calculated Z values were all less than $\pm$ 1.96, meaning that there were no significant correlation between any of the red blood cell indices and malaria parasitaemia, at P d" 0.05.

Discussion

The percentage of the pregnant women who were infected with malaria parasitaemia, in the study area was 41.4% (156/377). This means that close to 50% of the pregnant women were infected at least once. It can therefore be concluded that the study, which was carried out in an area described as hyper endemic for malaria (Wanji *et al.*, 2008) reported a high percentage of malaria (41.4%), as expected. This is in agreement with the findings reported by Meeusen *et al.*, (2001), that pregnant women in endemic areas are highly susceptible to malaria. This is also in agreement with the work of Achidi *et al.*, (2005), carried out in the Mutengene Maternity, in the South West Region of Cameroon, indicating 44.7% rate of infection with malaria parasite among pregnant women, at the point of antenatal enrolment. This study is also in line with another work carried out in Kassena-Nankana district of Ghana, in which, the overall prevalence of malaria parasitaemia during pregnancy was 47%, out of 3642 pregnant women of all gravidities and gestational age of 18-32 weeks (Clerk *et al.,* 2009).

The percentage of infected cases was highest (61.47%) in the CDC Central Clinic Tiko study center (FIG 1). The temperature at the hot humid coastline, the Tiko plain, where the CDC Central Clinic is located is favorable for the survival of the malaria parasite vectors. Another contributing factor for high infection percentage in this centre is the nature of the occupation of the pregnant women, who are mostly workers in the CDC plantations. This kind of jobs exposes them to mosquito bites, especially during the dusk's periods of the day. These women equally live in camps, with poor environmental sanitation and high levels of congestion amongst the inhabitants. This is in line with the findings of Danis-Lozano *et al.*, (2007) who reported that individual risk factors for *Plasmodium vivax* infection in the residual malaria transmission focus in Oaxaca, Mexico, which were significantly associated with

increased risk of malaria, were; sleeping on dirty floor or with two or more people in the same bed. The lowest percentage of infected cases was in the Government Health Centre, Muea. The GHCM study centre recorded zero percent of infected cases during the second trimester of pregnancy. This result is quite contrary to the results gotten from the other study centers. Notwithstanding, 26.7% of the pregnant women in this center were infected during the third trimester of pregnancy. Malaria infections were therefore not completely absent in this center.

Considering the study area as a whole, approximately 30.8% (116/377) of the pregnant women were infected during their second trimester of pregnancy and 23.3% (88/377) were infected during their third trimester of pregnancy. This indicates a slightly higher infection rate during the second trimester compared to that in the third trimester. This observation is in agreement with the work of Clerk *et al*., (2009), in which it was reported that third trimester of pregnancy was associated with a decreased risk of parasitaemia. A report by Falade *et al*., (2008) indicates that the prevalence of malaria by trimester at booking among antenatal clients in a secondary health care facility in Ibadan was 4% in the first trimester, 8.8% and 8.3% in the second and third trimesters respectively. In the present study, the percentage of infection in the third trimester was equally slightly less than the infection percentage in the second trimester. This shows that, second trimester of pregnancy is a more vulnerable stage at which pregnant women get infected with malaria parasites.

Up to 55.7% (210/377) of the women had not been on any form of malaria prophylaxis prior to registering for antenatal, this might have as well contributed to the high infection percentage in this trimester. Strict measures against malaria should therefore be practiced by pregnant women, at every stage of the pregnancy.

Mild parasitaemia was the most dominating type of parasitaemia in both trimesters. In all ,only 3.9% (08/204) of the parasitaemia were severe, 44.12% (90/204) were moderate, while 51.96% (106/204) were mild. The least frequent infection intensity was severe parasitaemia. This result disagrees with the report of Steketee *et al*., (2001), which states that pregnancies in women living in malaria endemic regions are associated with a high frequency and density of *Plasmodium falciparum* parasitaemia. Falade *et al.,* (2008) reported similar results to this, in which 4.1% of pregnant women at booking, in a secondary health care facility in Ibadan Nigeria, had moderate parasitaemia and only about 0.7% had high parasitaemia.

An important feature of malaria in pregnant women, as reported by Warrell and Gilles, (2002) is that peripheral blood films may be negative despite heavy placental infection. Given the fact that the uterine environment (in particular the placenta) appears to act as a privileged site for parasite replication, the absence of severe peripheral parasitaemia in this study, might not necessarily mean complete rolling out of heavy parasitisation at the level of the placenta.

There was a negative correlation relationship between malaria infection rate during the second trimester and haemoglobin concentrations in the peripheral blood of pregnant women in the CDC Tiko Central Clinic, at $P<0.05$ (TABLE 6). Malaria infections in the other centers had no significant correlation relationship with haemoglobin measurements. This significant negative correlation relationship is actually in line with scientific expectations because the pathophysiology of the anaemia caused by *P. falciparum* malaria is both complex and multifactorial. Among several factors, the major mechanisms are those of red cell destruction and decreased red cell production as potential causes of haemolysis include loss of infected cells by rupture or phagocytosis, removal of uninfected cells due to antibody sensitization or other

physicochemical membrane changes and increased recticuloendothelial activity particularly in organs such as the spleen. These results equally tie with the results of the work carried out by Achidi *et al.*, (2005) in which it was reported that, the mean haemoglobin levels of malaria parasite positive pregnant women were significantly lower than those who were malaria parasite free. This result is also in line with the results of the studies carried out by Erhabor *et al.,* (2010), in which mean values of haemoglobin concentrations were significantly lower among *Plasmodium sp*. parasitized pregnant women, compared to non-parasitized pregnant and non-pregnant women in the Niger Delta of Nigeria. The insignificant correlation relationship between malaria and haemoglobin measurements of the pregnant women in the other study centers at Pe" 0.05 is contrary to scientific expectations (WHO, 2000). This contradiction could be explained by the fact that the degree of parasitaemia in these research subjects was mainly mild (during the second trimesters 49.1% and the third trimester 55.72) and moderate (during the second trimester 45.7% and during the third trimester 40.9%). Severe parasitaemia in the study area as a whole was just 5.2% during the second trimester, and 3.4% during the third trimester. Because of this, anaemia (decreased haemoglobin levels) which is one of the complications of malaria was mild.

Most of the pregnant women in the Tiko centre are either workers or spouses of workers in the Cameroon Development Cooperation (CDC) and spend their working hours in the farms/ plantations, where they are further exposed to mosquito bites, which transmit the malaria parasite. Given the nature of their jobs and their areas of habitations the research participants of this centre have probably had repeated attacks of malaria which, according to Warrel and Gilles, (2002), eventually leads to profound anaemia. That notwithstanding, in these same pregnant women, an association was not found between malaria and anaemia during the third trimester. This could be so probably because of the emphasis on iron supplementation as the pregnancy progressed with time.

There were no significant correlation between any of the red blood cell indices and malaria parasitaemia, at 5% level of significance, using a two tailed test. This shows a lack of relationship between the presence of malaria and the decreased values of red blood cell indices in both infected and non infected pregnant women.
TABLES 8 and 9 report the correlation analysis between malaria parasitaemia during the second and third trimesters and the red blood cell counts, in the study area. There was an insignificant correlation relationship between the two variables. This means that, no significant association existed between malaria and red blood cell counts. This result is not in agreement with the report of WHO, (2000), which states that, iron sequestration, erythrophagocytosis and dyserythropoiesis were found in the acute phase of falciparum malaria, and that, survival of non-parasitized erythrophagocytosis was found to be reduced for several weeks, after clearance of parasitaemia in patients with falciparum and vivax malaria. Red blood cell counts are therefore expected to show a significant negative correlation with malaria parasitaemia. One of the reasons for this contradiction from our results could be caused by the fact that, malaria parasitaemia in the research subjects of this study was dominated by mild parasitaemia, whose effects on the red blood cell counts was equally mild, resulting to an insignificant correlation between these two variables.

The pooled results for the Pairwise correlation analysis between the haematocrit values and the malaria parasitaemia during the second and third trimesters show the same results as that with the

red blood cell counts. This is not surprising, as haematocrit is a measure of the percentage of the total blood volume that is made up by the red blood cells. This therefore means that, the conclusions drawn concerning the relationship between malaria parasitaemia and red blood cell counts is equally applicable to the relationship between malaria parasitaemia and haematocrit values, in the different research centres and in the study area as a whole. This result is in disagreement with that of Falade *et al*., (2008) in which the difference between the PCV values of malaria parasite infected cases and non infected cases was statistically significant at $P< 0.001$.

Recommendations

Due to the fact that some pregnant women recruited for this study were inconsistent in their antenatal care visits, it is likely that many women are still ignorant about the importance of regular antenatal care visits, and about the consequences of their failures in doing so. It is therefore recommended that, strategies be put in place by the authorities concerned with the welfare of pregnant women in the study area and in Cameroon in general to encourage consistent antenatal care visits by women who are pregnant. Special emphasis should be placed on early registration for the antenatal care visits (ANC) since in this study, all the pregnant women came for registration for ANC only during their second trimester of pregnancy.

Further studies to check on the effect of malaria parasitaemia on the iron storage forms of serum iron, ferritin, and transferrin should be carried out.

Conclusions

Malaria is still a problem amongst the pregnant women living in the tropics, as a significantly high level of prevalence was recorded in the study area. The levels of haemoglobin, haematocrit, red blood cell counts and red blood cell indices were affected mainly by severe parasitaemia as there were no significant correlation relationship between malaria parasitaemia and haemoglobin, haematocrit, red blood cell counts and the red blood cell indices.

Eventhough mild parasitaemia had insignificant effects on the different haematological parameters, it should still be treated seriously because of the profound effects it could possibly have on the iron storage forms of the body, especially in the pregnant women living in the tropics, as it is the case at hand.

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