ORIGINAL ARTICLE

RELATIONSHIPS BETWEEN BLOOD CELL COUNTS AND THE DENSITY OF MALARIA PARASITES AMONG PATIENTS AT THE REGIONAL HOSPITAL, LIMBE, CAMEROON.

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ABSTRACT

Malaria is one of the most important infectious disease in Cameroon and throughout the world [1]. Globally it results in an estimated 400 millions cases and about 3 millions deaths each year, most of these deaths in children aged 1 to 5 in Sub-Saharan Africa, making it the biggest single infections killer of children in the world [1]. It is a major public health problem in Cameroon, with its prevalence and incidence appearing to be on the increase owing to the lack of adequate control measures [6]. This study was designed to determine the correlation between blood cell counts and the density of malaria parasitemia amongst patients who presented for consultation at the Regional Hospital Limbe (RHL). A total of 100 consecutive patients suffering from malaria who consented to participating in this study were recruited and venous blood (3-5ml) was collected by venepuncture. Thick and thin blood films were prepared, stained and microscopically examined for the presence of malaria parasites. Total blood cells and differential white cell counts were performed using a coulter counter.
The findings depicted a negative correlation between parasite load and haemoglobin concentration [Hb], mean cell volume (MCV), and mean cell haemoglobin (MCH); a positive correlation of parasite density with white blood cell counts (WBC), red blood cell counts (RBC), and the differential white blood cell counts (lymphocyte, monocyte, and granulocytes); and no correlation was observed with the platelet counts.

INTRODUCTION

Malaria is a killer disease and despite efforts to eradicate malaria prevalence, the weight of malaria has not changed in countries where it is endemic. Although an ancient disease, environmental disturbances, malnutrition and the failure of drugs once used to control the disease, have conspired to make death by malaria more frequent now than at any point in history [3]. Malaria has been brought under control and even eliminated in many parts of Asia, Europe, and the Americas; yet in Africa, with various mosquito vectors, increasing drug resistance, and struggling health systems,

malaria infections have actually increased over the last 3 decades [1], [12].

From recent WHO statistic, about 350 to 500 million cases of malaria occur each year, resulting in 2 to 3 million deaths, 1,5 million of which are children (WHO, 2007; Schulman; 2007); thus, one person dies of malaria each minute. Over 90% of the global effect occurs in sub – Saharan Africa, where malaria dominates the chart of major public heath problems, just to compete with HIV/AIDS and tuberculosis today [2], [3]. Although in comparison, malaria Kills about as many person a year as AIDS has done in the last 15

years. In additions, malaria is an important cause of still birth, infant mortality, low birth weight and blood transfusions errors [4].

In Africa, at least 24 million pregnancies are threatened by malaria each year and malaria in Africa is estimated to cause up to 45 % of maternal anaemia and 35 % of preventable low birth weights [5], [6], [10], [11]. Experts foresee as much as a 20 % annual increase in Africa's rate of malaria related illnesses and death (Facer, 1997).

In Cameroon, malaria remains the first cause of morbidity and mortality. About 3 million clinical cases occur annually (about 95 cases in every 1 000 inhabitants) (PNTP, 1999) and it accounts for; 40 to 45 % of mortality in children below 5 year, 45 to 50 % of persons presenting complaints and 40 % of hospitalizations [6].

Generally, individuals living in endemic areas of malaria tend to acquire immunity, with age, such that susceptibility to the pathogen and pathology of malaria infections reduces with age [5], [7], [13], [14], [15]. Exceptions are seen in children below 5 years of age and in pregnant women [8] who are more vulnerable to malaria infection than non–pregnant women or adult men of the same age.

Malaria, which had been effectively suppressed in many parts of the world, is undergoing resurgence [8]. In developing countries, the burden of malaria has tremendous social and economic impact on a large scale. This caused the WHO to device a multi–component eradication program based on providing families with information necessary to prevent, recognise and treat malaria at home. Since the proper management of malaria is still a problem in Cameroon [8]. Even the use of anti–malaria prophylaxis during pregnancy and the development of a vaccine is still a problem. More centres for the study and control of malaria are being created and modern technique for the diagnosis being embraced [5], [15]. These programs also included vector control, the use of curative and prophylactic chemotherapy and the development of a vaccine.

The African summit on Roll Back malaria, which was held in Abuja, Nigeria on the 25th of march 2000, the Pan-African malaria conference on Roll Back malaria forum held in Yaounde Cameroon from the 13th to the 19th of November 2005 and the conference on Roll Back malaria, in the year 2007, reflects a real convergence of political momentum, institutional synergy and technical consensus on malaria mortality for Africa by the year 2010. This has caused the government of Cameroon to create a national program for the fight against malaria, HIV/AIDS and Tuberculosis under the ministry of public health and initiated by the Roll Back malaria, HIV/AIDS and Tuberculosis program.

Objective of Study

The overall objective of the investigation was to determine the correction between blood cell counts and the density of malaria infection amongst patients at the Regional Hospital Limbe (RHL).

MATERIALS AND METHODS

Study design and setting

This study was a cross sectional descriptive study. It was carried out at the Regional Hospital in Limbe (RHL), the administrative head quarters of Fako Division. informed consent of the experimental subjects and the approval of the regional ethical committee had been obtained.

The climate of Limbe is characterized by two main seasons; the rainy and the dry season with abundant rains ranging from $5500mm^3$ to $6500mm^3$ owing to the fact that Limbe is closer to Debundscha   known to be the wettest place in Africa recording rainfalls of about $10.000mm^3$. The rainy season starts in March to October while the dry season is between October and March. The temperatures are high and fairly constant ranging between 25°c and 30°c on the average that characterize this climate [9].

Study duration and population

This study was conducted from February to March 2008. Case recruitment covered the period from the 14th February to the 10th of March 2008.

The study population constituted patients who came from both the rural and urban settings of Limbe for consultation and diagnosis at the Limbe Regional Hospital. These were patients presenting with one or more of the following signs and symptoms: fever, headache, joint pains, abdominal upset, nausea, vomiting, diarrhoea, and digestive disorders; and whom malaria and full blood counts tests were requested. Venous blood was collected by veno-puncture of the antecubital vein into EDTA tubes.

The Giemsa staining method was used to detect malaria parasites in blood films on slides and the culter counter to determine full blood counts.

However, other processes could also be used to detect malaria parasites such as, the Diff-quick staining technique with the field stains which is not so oftenly used in many diagnostic laboratories.

The Giemsa staining technique involved the staining of two prepared blood films; the thick and the thin blood films.

- **Preparation of thin Blood Film**

The thin blood film was made as follows; a drop of blood was put at one end of a well cleaned and grease-free slide, and a spreader was used to produce a uniform spread of the blood over the slide such that a feathery tail end was achieved. The slide was then kept at a dry surface to air dry after which it was fixed with alcohol (95%) or with May Grunward fixative. The fixed slides were then stained with Giemsa stain made from a concentrated stock solution as follow; 3 drops of the stain to 2 ml of distilled water and allowed for a staining duration of 8 to 10 minutes after which it was washed, air dried and observed using the x100 oil immersion objective [5]. When the thin blood films were examined, the following ware seen: RBCs and WBCs .

- **Preparation of thick Blood Film**

Unlike the thin blood films, the thick blood films were made just by spreading a drop of blood at the centre of a clean grease-free slide in order to defibrinize the blood. It was

allowed to air dry after which it was stained with the Giemsa stain for 8 to 10 minutes without fixing with alcohol or may gunwale fixative [5]. When the thick blood film was examined, using the x100 objective and the x7 ocular, the following were seen: remains of red blood cells; white blood cells (Leukocytes) and platelets (thrombocytes).

The thick blood film consisted of many dehaemoglobinized red blood cells packed together in a thick mass since after staining with the Giemsa stain , the water in the stain acted on unpreserved red blood cells that caused the contents of the cells to dissolute in the water; hence dehaemoglobinization .

Examination of Blood Films for Malaria Parasite Recognition

Malaria parasites were found to take up Giemsa stain in a special way in both the thick and thin blood films.

Since the malaria parasites are known to pass through a number of developmental stages; in all the stages however, the same parts of the parasite were stained with the same colour: chromatin (part of the parasite nucleus) that is usually round in shape was stain deep red. The cytoplasm was presented in a number of forms; from a ring shape to a totally irregular shape. It was noticed to stain blue, although the shade of the blue could vary among the malaria species.

Stages of the Malaria Parasite

➢ The Trophozoite Stage

This stage was the most commonly seen and is often called the ring stage, although it sometimes took the form of an incomplete ring

The ring stage of malaria parasite in red blood cells

Owing to the fact that, the trophozoite is the growing stage; the parasite within the red blood cell varied in size from small to quite large. The malaria pigment appeared as the parasite grew. The pigment could be a by-product of the growth or metabolism of the parasite. It did not stain but had a colour of its own, which ranged from pale yellow to dark brown or black.

The Schizont Stage

At the schizont stage the malaria parasite is known to reproduce; a reproduction referred to as asexual because the parasite is known to be neither male nor female but reproduces itself by simple division. There were obvious phases in this stage, ranging from parasites with two chromatin pieces to parasites with a number of chromatin dots and definite cytoplasm [16]. It should be noted that, the parasite could be diagnosed using non-microscopic method as well [17].

Examination of the thick blood film

Routinely, thick blood films were examined. Provided that they had been well-made and stained before autofixation could take place. Occasionally, however, it was difficult to tell the difference between the mature trophozoites and the gametocytes of *P. vivax* and between *P. malaria* trophozoites and rounded *P. falciparum* gametocytes. Also it was not possible to distinguish between the late trophozoites and gametocytes of *P. malaria* in thick films, but the need to know whether

gametocytes could be present in the blood was usually confronted to a *P. falciparum*.

However, the routine examination of a thick blood films was based on the examination of 100 good fields. **That is a slide was pronounced negative only after no parasite was found in 100 fields**; some further 100 fields were examined if parasite were present before a final identification of species made. This ensured there was little or no possibility of a mixed infection when more than one species was present in the blood film being overlooked, though *P. falciparum* is known to be the major cause of malaria infections in Africa. The technique in which the thick film examination was done was as follows:

Step 1: The slide was focused using objective 10 and the reading area was selected. A part of the film that was well stained, free of staining debris, and well populated with white blood cells was selected. The film was well made and of even thickness such that there was no problem but poor quality films needed to be searched extensively.

Step 2: The immersion oil was placed on the thick film.

Step 3: The x100 oil immersion objective was then swivelled over the selected portion of the blood film.

Step 4: The oil immersion objective was then lowered until it touched the immersion oil.

Step 5: The blood film was then examined, by moving along the edges of the thick film, then moving in a lateral movement and so on.

Step 6: In order to determine whether the blood film was positive or negative for malaria, continued examination for 20 to 30 fields was made. If any doubtful diagnosis were made, more fields (up to 100) were examined. The findings were then recorded at the end of the examination on an appropriate record form and the results were included with a parasite count.

THE PARASITE COUNT

Parasite counts for the blood film were necessary for the following reasons: the physician could want to know how severe the malaria was and whether the malaria parasites were responding to anti-malarial treatment given. The parasite counts were especially important in *P. falciparum* infection as they are known to be potentially fatal. The district health officer could need to know the severity of malaria infection being seen on the local health facilities. Also, the data could be needed for special purposes, such as testing the sensitivity of parasites to anti-malarial drugs. Several methods of determining the number of parasites; among them the "number of parasite per micro litre of blood" was used as described, below:

- **Parasite per Micro litre (ul) of Blood**

This was a practical method of adequate accuracy. It was based on the number of parasites per ul of blood in a thick film; these being counted in relation to a predetermined

number of leukocytes [18]. All the white blood cells (WBC) and parasites were counted on 20 to 30 fields, then the following formula was applied [19]:

$$\frac{Number\ of\ parasites}{Number\ of\ WBC} \times 8000 = \text{Number}$$

of parasites per microlitre of blood (Parasites/ul)

- **Parasites per White Blood Cells on Thin Blood Films**

Here, the number of white blood cells (WBC) and parasites could be counted on 20 to 30 fields, and the following formula used to obtain the number of parasites per WBC (WHO, 2007).

Number of Parasites / Number of white blood cells x 1000

- **Parasites per Red Blood Cell on Thick Blood Films**

All the red blood cells (RBC) and parasites could be counted on 20 to 30 fields and the formula below used to obtain the number of parasites per 1000 RBC (WHO, 2007).

$$\frac{Number\ of\ parasite}{Number\ of\ \text{red blood cells}} \times 1000$$

PROCEDURE FOR DETERMINATION OF FULL BLOOD COUNT

This was done using the counter (Abx MICROSOT, 1995 RAC 028 Ind., Montpellier; France). It was a 15 parameters counter which did RBC counts, WBC count, HCT, MCV, haemoglobin, Differential count, platelet count, and the other parameters such as MCH, MCHC and RDW were calculated automatically. Briefly, the principle of measurement was based on the variation of resistance and impudence induced by the passage of cells through a calibrated micro-orifice. In the process of measurement each sample was diluted with electrolyte diluents (charge conductor) whose conductivity was known to quite different from that of the cells. The diluent was aspirated through a calibrated micro-orifice and the electrical resistance between the 2 electrodes increased proportionally to the volume of the cells. For instance, in RBC measurement;

According to Ohm's law;

V=IR   where; V = Voltage, I = Current and R = Resistance

Since I remained constant, R increased every time a red blood cell passed through the micro-orifice, and V then also increased proportionally to the volume of the voltages requiring the use of an amplification circuit which increased the voltages providing room for the electrical analyser (also needed to eliminate the background noise) to analyse the results.

The dilution used for RBC was 12ul of blood sample mixed with 2.2 ml of diluent giving a dilution fraction of 1/148 or a dilution factor of 184; 27.5ml. The dilution was then mixed further with 3ml of the diluent giving a dilution fraction of 1/20 or a dilution factor of 20 and the microprocessor analysed the cells.

STATISTICAL ANALYSIS

Data generated was analysed manually and differences were considered significant at P-value ≤ 0.05.

RESULTS

DEMOGRAPHIC DATA

The study was conducted within February and March 2008 at the Limbe Provincial Hospital Laboratory. A total of 100 patients were recruited made up of 33% males and 67% females. The ages of the patients ranged from 0.4 year (4 months) to 85 years with a mean (±SD) age of 29.602 (±18.138) years and all the 100 patients recruited were positive for *Plasmodium falciparum* malaria (Table 4.1).

The subjects were stratified into the following age groups; <15 years (25 subjects), 15 to 30 years (29 subjects), 31 to 45 years (31 subjects) and > 45 years (15 subjects) (Table 4.1).

MALARIA PARASITE DENSITY

Parasite load was fairly low, ranging from ≤500 parasites per microlitre of blood to >1500 parasites per microlitre of blood with a mean (±SD) of 680.40 (±1105.59) parasite per microlitre of blood. As far as age was concern, it was observed that the age group 31 to 45 years had the highest occurrence of malaria infections with a generally low parasitaemia, whereas, the age group <15 years had the highest occurrence of subjects with the highest parasitaemia of >1500 parasites per microlitre of blood. In addition, the age group >45 years had the lowest occurrence of malaria infections also with a generally low parasitaemia. However, there was no statically significant difference in the occurrence of malaria infection in the various age groups (P- value = 0.19).

DISTRIBUTION OF MALARIA PARASIT DENSITY ACCORDING TO AGE GROUPS

The age group 30 to 45 years had the highest infection rate. Many of the cases had parasitaemia of ≤500 parasites/µl of blood, and a majority of them (90%) had parasitaemia ≥320 parasitaemia/µl of blood. There was no statistically significant difference in the parasite density among the different ages (Table 4.1).

Table 4.1: Distribution of Malarial Parasite Density According to Ages

Parasite density (N° of parasites / µl of blood	Age groups (years)				
	< 15	15-30	31-45	> 45	Total
≤ 500	10	18	22	11	61
501-1000	10	10	08	03	31
1001-1500	01	00	01	01	03
>1500	00	01	00	00	05
Total	25	29	31	15	100

$X^2 = 9.63$ d.f = 9   P=0.108

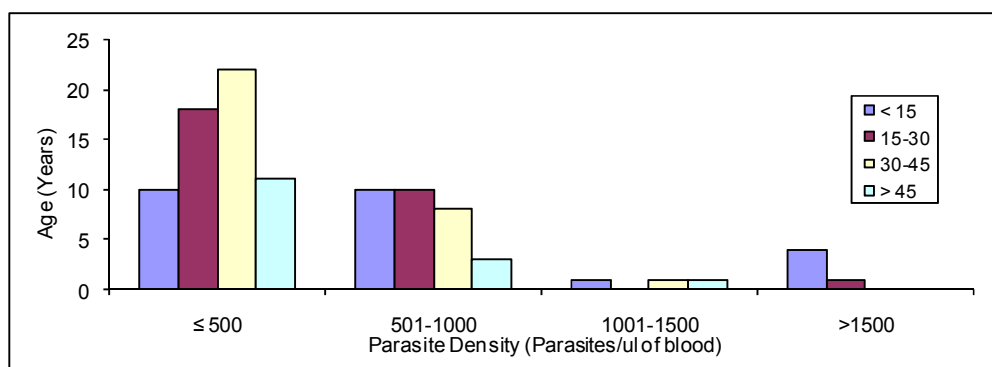PARASITE DENSITY BY AGES OF SUBJECTS AT LIMBE PPROVINCIAL HOSPITAL

Figure 11. Variation of Parasite Density with Age

4.3 MALARIA PARASITE DENSITY ACCORDING TO SEX

It was observed that, there was generally high occurrence of malaria infection in females than males, leading to a higher prevalence of malaria parasitaemia in females. In addition, females had the highest occurrence with the highest parasitaemia of >1500 parasites/µl of blood. However, there was no statistically significant difference (Table 4.2).

Table 4.2: Malaria Parasite Density Distribution Influenced by Sex

Parasite density (N° of parasites / µl of blood	Sex		Total
	Males	Females	
≤ 500	18	43	61
501-1000	10	21	31
1001-1500	03	00	03
> 1500	02	03	05
Total	33	67	100

$X^2 = 6.546$ d.f = 3   P = 0.088

4.4 DISTRIBUTION OF MALARIA PARASITE DENSITY AS INFLUENCED BY BOTH AGE AND SEX

It was observed that in all the various age groups, females had the highest prevalence of malaria parasitaemia also with the highest occurrence with the highest parasitaemia >1500 parasites/µl of blood. There was no statistically significant difference (Table 4.3).

4.5 VARIATION OF BLOOD CELL COUNTS FROM THE NORMAL COUNTS BASED ON SEX

Generally, more patients were observed with relatively low red blood cell counts and haemoglobin concentrations with many females than males. In addition none of the sexes had [Hb] and RBC greater than normal.

Table 4.3: Malaria Parasite Density as Influenced by Both Age and Sex

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Parasite density/µl of blood	Age								Total
	< 15		15-30		31-45		> 45		
	Sex								
	M	F	M	F	M	F	M	F	
≤ 500	06	04	02	16	06	16	04	07	61
501-1000	02	08	05	05	02	06	01	02	31
1001-1500	01	00	00	00	01	00	01	00	03
>1500	02	02	00	01	00	00	00	00	05
Total	11	14	07	22	09	22	06	09	100

$X^2 = 32.14$ d.f = 21   P = 0.057

It was observed that, there was an increase in the white blood cell counts in females than in males. Moreover, a normal mean cell volume and mean cell haemoglobin was observed in many of the patients of both sexes, though the highest occurrence of low mean cell volume count was observed in males and high mean cell volume count in females However, there was no statistically significant difference in the blood cell indices in the both sexes (Table 4.4).

Table 4.4: Variation of Blood Cell Counts from the Normal Based on Sex

Blood cell Indices	Sex								X^2	d.f	P-value
	Male				Female						
	N	Low	Normal	High	N	Low	Normal	High			
WBC	33	02	25	06	67	09	48	10	1.290	02	0.795
RBC	33	17	16	00	67	34	33	00	0.005	02	0.999
[Hb]	33	20	13	00	67	40	27	00	0.008	02	0.998
(MCV)	33	10	18	05	67	09	45	13	4.093	02	0.351
(MCH)	33	14	16	03	67	19	39	09	2.053	02	0.674

Normal values: WBC → (3.5-10.0) x 10^3/mm^3; RBC → (3.8-5.8) x 10^6/mm^3 [Hb] → (11.0-16.5) g/dl
MCV → (80-97) µm^3 MCH → (26.5-33.5) pg **N = Number per group**

VARIATION OF BLOOD COUNTS FROM THE NORMAL COUNTS BASED ON AGE

It was observed that the age group <15 years had highest white blood cell counts and the age groups 31 to 45 years and >45 years had the lowest white blood cell counts. In addition, all the age groups were observed to have fairly low red blood cell and haemoglobin concentration. Moreover, the age group <15 years was observed to have low mean cell volumes and mean cell haemoglobin counts, hereas the other age groups had fairly normal counts (Table 4.5).

Table 4.5: Variation of Blood Cell Counts from the Normal Counts Based on Age

Blood cell Indices	Age															
	< 15				15-30				31-45				> 45			
	N	Low	Normal	High	N	Low	Normal	High	N	Low	Normal	High	N	Low	Normal	High
WBC	25	01	13	11	29	05	20	04	31	03	28	00	15	02	12	01
RBC	25	11	14	00	29	18	11	00	31	15	16	00	15	06	09	00
[Hb]	25	19	06	00	29	20	09	00	31	15	16	00	15	06	09	00
MCV	25	11	14	00	29	07	16	06	31	02	19	10	15	01	12	02
MCH	25	17	08	00	29	11	14	04	31	04	19	8	15	01	14	00

N = Number per category

DISTRIBUTION OF BLOOD CELL INDICES WITH THE AGES OF SUBJECTS AT THE LIMBE PROVINCIAL HOSPITAL
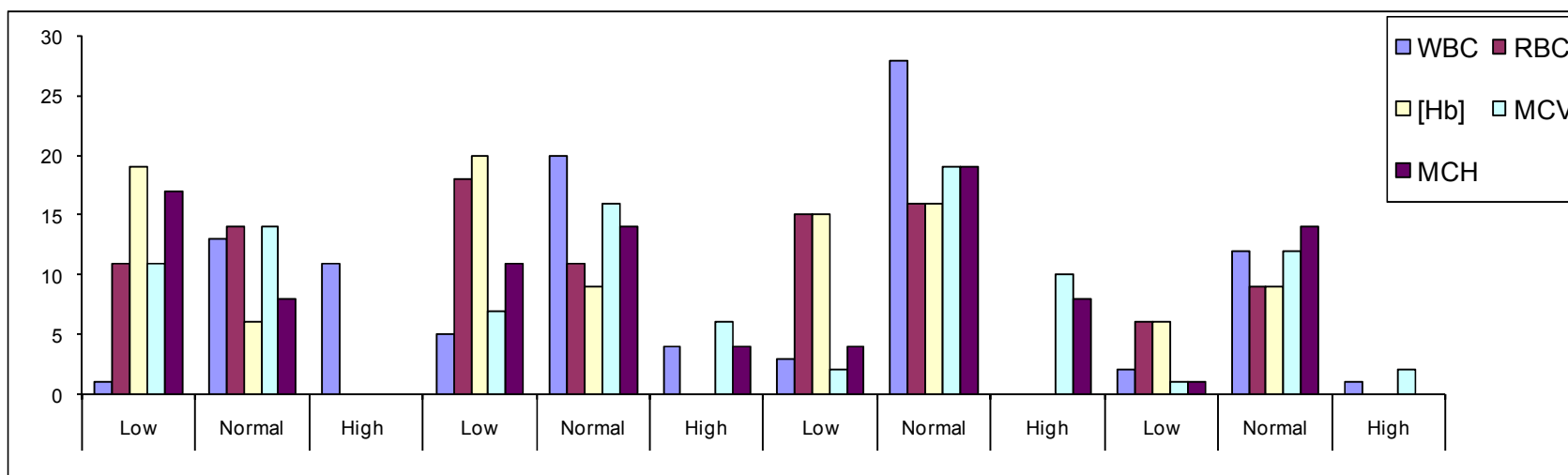

Figure 12. Variation of Blood Cell Counts from the Normal Counts with Age

VARIATION OF THE DIFFERENTIAL WHITE BLOOD CELL COUNTS AND PLATELET COUNTS FROM THE NORMAL COUNTS

From observation, it was found that, more than 70% of the subjects had a fairly normal differential white blood cell counts and platelet counts. However, 25% of the subjects had lower lymphocyte and monocyte counts whereas, 12% showed up with high granulocyte counts (Neutrophils, Basophiles and Eosinophils). The platelets count was observed to be equally distributed with equal percentages of occurrence with high and low counts (Table 4.6).

Table 4.6: Variation of the Differential White Blood Cell Counts and Platelet Counts from the Normal Counts

Differential white cell count and platelet	N	Low	Normal	High
Lymphocytes	100	21%	65%	14%
Monocytes	100	30%	62%	08%
Granulocytes (Neutrophils, Basophils, Eosinophils)	100	06%	82%	12%
Platelets	100	13%	74%	13%

Normal values: Lymphocytes → (1.2 - 3.2) x 10³/mm³   Monocytes → (0.3 - 0.8) x 10³/mm³

Granulocytes → (1.2 - 6.8) 10³/mm³   Platelets → (150 - 390) 10³/mm³

4.8 HAEMOGLOBIN AND ANAEMIA STATUS

Sixty subjects were observed to have haemoglobin levels <11g/dl, which was an indication of anaemia. Forty subjects were observed to be non-anaemic based on the above classification. Levels of haemoglobin ranged from 3.2g/dl to 15.6g/dl (±SD), with a mean (±SD) value of 9.962(±2.667) g/dl, and the overall prevalence of anaemia was observed to be 60%. People classified with anaemia had values between 3.2 to 10.8g/dl, which correspond and indicated mild anaemia. The mean haemoglobin concentration (10.160 (±3.129) g/dl) in males (P-value> 0.05) was fairly similar to that of females (9.566 (±3.432) g/dl).

DISTRIBUTION OF HAEMOGLOBIN WITH RESPECT TO PARASITE DENSITY AND BASED ON THE VARIOUS AGE GROUPS

Based on observation, anaemia was mild. Mild anaemia subjects had lower haemoglobin levels than non-anaemic subjects. The mean haemoglobin levels for mild-anaemic subjects was as follows based on the various age ranges: 7.6 (±2.2)g/dl in <15 years subjects; 8.1 (±1.8) g/dl in 15 to 30 years subjects; 9.3(±2.0)g/dl in 30 to 45 year old subjects and 8.1 (±2.0) g/dl in > 45 years subjects. It was observed that the anaemia status decreased from <15 years to 30 to 45 years old subjects and then increased in >45 years old subjects. In the other hand, it was observed that, parasitaemia decreased from <15 years old subjects. However, in the non-anaemic subjects, it was observed that haemoglobin concentrations were fairly constant and normal, while the parasite density was

observed to be highest in <15 years subjects, higher in >45 years subjects and high in 15 to 30 years and 30 to 45 years old subjects (Table 4.7).

Table 4.7: Distribution of Haemoglobin with Respect to Parasite Density and Based on the Various Age Ranges

Groups	Mean haemoglobin (g/dl)				Mean parasite density/ul of blood			
	< 15	15-30	31-45	> 45	< 15	15-30	30-45	> 45
Anaemic subjects	7.6 (±2.2)	8.1 (±1.8)	9.3 (±1.5)	8.1 (±2.0)	1221.1 (±2315.9)	582 (±495.4)	533.3 (±231.6)	440 (±131.5)
Non-anaemic subjects	12.5(±0.5)	12.3(±0.9)	12.4(±1.2)	12.8(±1.7)	1156.7 (±1301.6)	417.8 (±125.1)	441.3 (±160.4)	533.3 (±243.3)

4.10 RELATIONSHIP BETWEEN PARASITAEMIA AND ANAEMIA STATUS

A majority of the subjects who were anaemic had parasite densities of ≤500 parasites/µl with about 90% of them presenting parasite densities of ≥320 to 640 parasites/µl of blood. Also, the majority of those who were non-anaemic had parasite densities ≤ 500 parasites/µl of blood (Table 4.8).

Table 4.8: Relationship between Parasitaemia and Anaemia Status

Parasite density categorization	Anaemia status		Total
	Anaemic	Non-anaemic	
≤ 500	33	28	61
501-1000	22	09	31
1001-1500	02	01	03
> 1500	03	02	05
Total	60	40	100

$X^2 = 2.495$ d.f = 3   P-Value = 0.697

DISTRIBUTION OF PARASITE DENSITY AND BLOOD CELL INDICES ACCORDING TO ANAEMIA STATUS

It was observed that, the mean (±SD) parasite density in the anaemic subjects was higher than in the non-anaemic subjects. The mean (±SD) haemoglobin concentration, mean cell volume, mean cell haemoglobin and red blood cell counts were higher in non-anaemic patients than in anaemic patients. The mean (±SD) total white blood cell counts was higher in the anaemic patients.

Based on the differential white blood cell counts, there was a fairly equal lymphocyte and monocyte counts in both the anaemic and non-anaemic patients, a fairly higher granulocyte (Neutrophil, Basophile Eosinophil) count was observed in the non-anaemic patients. Finally, a higher mean (±SD) platelets count was observed in anaemic patients (Table 4.9).

Table 4.9: Influence of Anaemia Status on Parasite Density and Blood Cell Indices

Parameters	Anaemic		Non-anaemic	
	N	Mean ± SD	N	Mean ± SD
Parasite density	60	758 (± 1353.6)	40	564 (± 554.8)
[Hb]	60	8.3 (± 1.9)	40	12.5 (± 1.2)
MCV	60	86.2 (± 15.4)	40	91.1 (± 10.4)
MCH	60	27.5 (± 4.6)	40	28.9 (± 4.0)
RBC	60	3.1 (± 0.8)	40	4.4 (± 0.6)
WBC	60	6.8 (± 4.5)	40	6.7 (± 0.6)
Lymphocytes	60	2.2 (± 1.9)	40	2.2 (± 1.2)
Monocytes	60	0.4 (± 0.4)	40	0.4 (± 0.2)
Granulocytes	60	4.0 (± 3.1)	40	4.1 (± 3.4)
Platelets	60	275.5 (± 123.1)	40	257.4 (± 85.9)

4.12 CORRELATION BETWEEN PARASITE DENSITY AND BLOOD CELL INDICES

After correlation analysis was performed based on the null (Ho) and alternative (Ha) hypothesis:

- Ho: Presence of significant correlation between parasitaemia and blood cell indices.
- Ha: Absence of significant correlation between parasitaemia and blood cell indices.

It was observed that, there was a positive correlation between the parasite density and white blood cell count (P-value = 0.317). A positive correlation was also observed with red blood cell count (P-value = 0.05).

A negative correlation was observed with the haemoglobin concentration, mean cell volume, and mean cell haemoglobin with a very significant correlation with haemoglobin concentration (P = 0.397).

There was a significant correlation with the mean cell volume and mean cell haemoglobin (P = 0.131 and 0.103) respectively. There was a positive correlation with the lymphocyte, monocyte and granulocyte counts (Eosinophil, Basophil and Nitrophils) (P = 0.388, 0.419 and 0.344) respectively.

There was no correlation between malaria parasite density and platelet count (P > 0.5). Thus from data in table 4.10, The H1 Hypothesis was rejected.

Table 4.10: Relationships between Parasite Density and Blood Cell Indices

Parameters	n	Mean ± SD	ΣX	ΣX^2	$\Sigma X_1 X_2$	R	r^2	Sr	tcal	d.f	ttab	P-value
Parasite density	100	680.4(±1105.6)	68040	167307200	-	-	-	-	-	-	-	-
WBC	100	6.8(±4.1)	678	6284.96	496307.6	0.08	0.006	0.1	0.769	98	2.000	P=0.429 P>0.025
RBC	100	3.6(±0.9)	360.65	1396.3641	265577.2	-0.19	0.04	1.891	1.891	98	2.000	P=0.058 P>0.025
[Hb]	100	9.9.(±2.7)	996.2	10628.34	665074	-0.04	0.002	-0.432	-0.432	98	-2.000	P=0.693 P>0.025
MCV	100	88.2(±13.8)	8816.6	796076.76	5765256	-0.16	0.03	-1.553	-1.553	98	-2.000	P=0.112 P>0.025
MCH	100	28.1(±4.4)	2808.2	80812.4	1829872	-0.17	0.02	-1.669	-1.669	98	-2.000	P=0.091 P>0.025
Lymphocyte	100	2.2(±1.7)	222	765.86	159718	0.05	0.001	0.473	0.473	98	2.000	P=0.621 P>0.025
Monocytes	100	0.4(±0.3)	41.2	27.68	29268	0.03	0.001	0.340	0.340	98	2.000	P=0.767 P>0.025
Granulocytes	100	6.8.4(±4.1)	404.6	2655.4	298456	0.07	0.004	0.655	0.655	98	2.000	P=0.489 P>0.025
Platelets	100	23.2(±290.1)	2324.2	8385465	15664420	0.4	0.2	4.413	4.418	98	2.000	P=0.0001 P>0.025

P<0.025 $\Longrightarrow$ Reject H1: P>0.025 $\Longrightarrow$ Accept Ho

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DISCUSSION AND CONCLUSION

There was a generally fair low parasite count observed ranging from ≤500 to >1500 parasites per micro litre of blood. This was similar to the findings reported from Yaounde, on low parasitaemia in malaria positive patients at the University Centre Hospital (CHU) [7] and to that reported from Buea on asymptomatic malaria and haemoglobin levels in primary school pupils [18]. The highest parasite rate for the study was observed in subjects of the age group 31 to 45 years made up of 31%, although there was no significant variation in parasite rates in the different age groups, but a significant variation was observed in the parasite density. It was observed that the age group <15 years had the highest frequency, harbouring parasite loads of >1500 parasites per micro litre of blood with the age groups 31 to 45 years and >45 years with a frequency of zero. This could be as a result of low immunity to malaria by the <15 years old children and a stronger immunity in the older age groups.

This study was carried from February to March, a period of transition from the dry to the rainy season in Limbe, though with scanty rainfall, high humidity and high temperatures. During this period, the climatic conditions provide habitats for breeding and the temperature favours rapid growth and development of the Anopheles mosquito vectors. The topography of Limbe presents a hilly zone, and lowland with the presence of the sea and many forest and bushy areas, makes drainage difficult at the road sides, around settlements and houses providing many breeding sites for the vector. These factors probably could be the cause of the high transmission rate in the town. Based on the occurrence and the density of parasite by sex it was observed that the female sex was more exposed than the males. Since the Limbe Regional Hospital provides services to patients from other villages around Limbe, and many women there are mainly farmers, the high occurrence of parasitaemia in women could be as a result of their work exposure to the forest and bush farm lands which are sites which promote the vector breeding.

Based on the haemoglobin levels recorded in patients, the prevalence of anaemia was 60% and anaemia was mild in most cases, though a few cases presented with severe anaemia mainly children and pregnant women. A previous study carried out in Yaounde (CHU) reported a prevalence of 57% with mild anaemia [7]. Another previous study carried out in Buea District reported a prevalence of 78% for anaemia in hospitalized children with acute malaria (unpublished data). In CHU, the mean haemoglobin level was 10.58g/dl and in the Buea District, it was 7.8g/dl. In this study a mean haemoglobin concentration of 9.9g/dl was observed; all indicating mild anaemia.

Anaemia in acute falciparum malaria has been reported to be caused by increased

destruction of both parasitised and non-parasitized erythrocytes and decreased erythropoiesis. In addition, RBC could be increased due to reticulocytosis following haemolysis, whereas the MCH and MCV could be lowered due to an increased ratio of red cell volume in relation to the haemoglobin content (Table 4.10).

As far as white blood cells and platelets are concerned, little was known about the changes that occur in malaria positive patients. A measurement of WBC and platelets of 230 healthy children from a Tanzanian community, 1369 children admitted to hospital with symptomatic malaria and 1461 children with other medical conditions. Children with malaria had higher WBC compared with the community controls and leucocytosis was strongly associated with the younger age, deep breathing anaemia and death. The WBC was not associated with a positive blood culture [19].

In this study, fairly low lymphocyte and monocyte counts were independently associated with morbidity and a fairly high granulocyte count was observed. A platelet count of less than $150 \times 10^3/mm^3$ of blood was found in 13% of the subjects and was associated with age and parasite density.

Based on observation and basic assumption, the majority of patients and parents of children that took part in this study, could be placed among those who earn above average income. This is because most of them are civil servants, business men and farmers. This could account for the general well being of the subjects. Furthermore, many patients are familiar with the signs and symptoms of malaria, its treatment, prevention and control.

The introduction of serological methods of testing malaria such as the para sight F test, and the optimal assay for *P. falciparum* now enables patients to get quick results and promptly attack the disease before it reaches the chronic level [20]. Also the use of the arthemisinin combination therapy and impregnated mosquito bed nets have brought in much improvement in the prevention, treatment and control of malaria.

To conclude, from statistical correlation analysis, the following was observed:

A negative correlation between the parasite density and the age of the subjects; the parasite density and the haemoglobin concentration; mean corpuscular volume; and the mean cell haemoglobin.

A positive correlation between the parasite density and the WBC; RBC and the white blood cell differential count.

In addition, it was observed that no correlation exists between the parasite density and the platelets count and the parasite density did not seem to have any significant effect on the blood cell counts.

CONCLUSION

Heavy parasite loads in the body destroy the red blood cells, reticulocytosis and lead to anaemia. The number of eosinophils increased with an increase in the parasite load.

Hence, malaria and anaemia are two important problems facing patients at the Limbe Provincial Hospital especially children <15 years of age. Females were also observed to have a high prevalence of *Plasmodium falciparum* infection especially those pregnant than men, and subjects who presented with anaemia generally had higher parasite load compared to their non-anaemic counterparts.

   All anaemic patients should under go check up for malaria parasites and treated promptly when positive.

   Control Programme should be organised that would regularly check up children and pregnant women attending the Hospital against malaria to avoid chronic and severe infections which may lead to anaemia.

Acknowledgement

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