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Efficacy of Absolute White Blood Cell Count in the Determination of Malaria Parasite Density among subjects presenting to University of Calabar Teaching Hospital.Ben, S.A.¹, Useh, M.F.*² and Usanga, V.U.³

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Abstract

Malaria is a mosquito-borne infectious disease of animals and humans caused by parasitic protozoans in the genus *Plasmodium*. In 2016, there were an estimated 216 million cases of malaria in 91 countries, an increase of 5 million cases over 2015. The African region is home to 90% of malaria cases and 91% of malaria deaths. Children under 5 years and pregnant women are particularly susceptible to infection. White blood cells count (WBC) is used in the computation of malaria parasite density. Generally, in field surveys, standard WBC counts recommended by the WHO are used in the determination of malaria parasite density. Two microliter (2 μ l) and 6 μ l of capillary blood were used to make thin and thick blood smears respectively. The smears were stained with 2% Giemsa stain for 30 minutes and examined microscopically for malaria parasite. Venous blood was collected for the estimation of white blood cells count using Sysmex Automated Full blood count Analyzer. Of the 285 subjects examined, 178(62.5%) were infected with malaria. Subjects below 15 years of age had the highest prevalence rate of 26(78.8%) while subjects aged 49-59 years had the least prevalence of 7(50%). The mean (SD) WBC counts obtained in this study was 5.47 ± 1.080 ($10^9/l$), while the mean (SD) malaria parasite densities were 256.99 ± 394.271 , 289.72 ± 462.917 and $386.29 \pm 617.222 \times 10^9$ for absolute WBC count, Nigerian recommended WBC count and WHO recommended WBC count respectively. The difference in the mean malaria parasite density between Absolute WBC value and Nigerian accepted WBCs value was statistically significant ($p=0.000$). There was

also a significant difference in the mean parasite density between Absolute WBC and WHO recommended WBC value ($p=0.000$). The difference in parasite densities obtained between Nigerian accepted WBC value and WHO recommended value was also significant ($p=0.000$). Overestimation of malaria parasite density can be reduced by using the corresponding individual's WBC count to replace the $8.0 \times 10^9/l$ WBC count set by WHO for the region. The corresponding WBC count should always be estimated when malaria parasite density is required to have a precise and accurate malaria density.

Keywords: Malaria, Density, White Blood Cells.**Introduction**

Malaria is a mosquito-borne infectious disease of animals and humans caused by intracellular protozoan parasites in the genus *Plasmodium* while affects the red blood cells. Currently, there are five species of *Plasmodium* that infect man. These include; *P. falciparum*, *P. malariae*, *P. vivax*, *P. ovale* and *P. knowlesi*. Majority of deaths in Africa are principally caused by *P. falciparum*. *P. malariae* generally cause a milder form of malaria (Caraballo, 2014; WHO, 2014). *Plasmodium knowlesi* is known to cause malaria among animals but rarely cause disease in human beings. Clinical signs and symptoms usually begin 10-15 days after being bitten by the vector agents. Malaria is highly endemic in Nigeria with a stable transmission. Majority of the inhabitants (about 95%) are at high risk of the infection. Falciparum species is the predominant parasite in Nigeria. Malaria parasite is mainly

transmitted by female *Anopheles gambiae* (Enayati *et al.*, 2010). Malaria remains the leading cause of morbidity and mortality in Nigeria and in Sub-Saharan Africa mostly in children and pregnant women.

In 2010, Nigeria was reported to be responsible for over 32% of the global estimate of 665,000 malaria deaths (Roll Back Malaria, 2014). Younger children that are below five years of age and pregnant women are mostly exposed to the infection and death from malaria infection. The National Malaria Strategic Plan (NMSP) (NMSP, 2014) prescribed malaria control through three major interventions which includes, prompt diagnosis, adequate treatment and prevention of malaria transmission through integrated vector control strategy (Gollin and Zimmermann, 2007). The recommended treatment for malaria is a combination of antimalarial medications that includes an Artemisinin (WHO, 2012). Occasional doses of Sulfadoxine/Pyrimethamine (SP) are recommended in infants after the first trimester of pregnancy in areas with high incidence of malaria. Other medication may be Mefloquine, Lumefantrine or Sulfadoxine/Pyrimethamine. Quinine alongside Doxycycline can also be used if an Artemisinin is not readily available (WHO, 2010).

Malaria is diagnosed routinely by the examination of thick and thin blood films microscopically after staining with Giemsa or with rapid diagnostic test kit, which could be antigen based (Fairhurst *et al.*, 2012). Molecular biology techniques like the Polymerase Chain Reaction -PCR has been used to detect the parasite's DNA. This is not widely used in areas where malaria is highly endemic due to the complexity and high cost involved (Beare *et al.*, 2012). WBC is essential in evaluation of malaria density in malaria field trials, interventions; patients care management and drugs trials in animal and human subjects. Due to frequent lack of facilities in some malaria- endemic countries especially in Africa, the time and labour required to quantify WBCs of patients, an assumed WBCs has been set by the WHO to help in estimating parasites densities for different zones in the different continents (WHO, 2013). The aim of

the study is to assess the efficacy of using absolute White blood cells count, Nigerian and WHO recommended white blood cells count in the determination of malaria parasite density.

Methodology

Study Area

This study was carried out in the University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria. Calabar is the capital of Cross River State in the South-South geopolitical zone of Nigeria. Calabar lies between 4° 34'27" North and 6° 58'32" East. The city has a population of approximately 372,000 (NPC, 2006). Calabar lies in rainforest belt which facilitate stable malaria transmission all through the year. The study subjects were patients clinically diagnosed of malaria infections by their attending Clinicians. The major occupations of the residents of Calabar include farming, fishing and public service jobs.

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee of the University of Calabar Teaching Hospital, Calabar, following the submission of the Research Proposal of the study.

Study Population

This includes patients visiting University of Calabar Teaching Hospital with clinical features consistent with malaria.

Inclusion and Exclusion Criteria

Subjects that showed signs and symptoms of malaria infection like high fever, chills, headache and vomiting were included while those with no related symptoms were excluded.

Inform Consent

Written informed consent was obtained from all the participants before sample collection.

Determination of Sample Size

The number of samples for this research was determined using the formula by Amin *et al.* (2013). Using a malaria prevalence rate of 24% reported for Calabar by Ekong (2016).

$$n = \frac{z^2 \times P \times (1-P)}{d^2}$$

$$\begin{aligned}
 n &= \text{required sample size} \\
 d &= \text{desired absolute precision} = 0.05 \\
 P &= \text{the expected prevalence} = 24\% (\text{Ekong, 2016}) \\
 z &= 1.96 \text{ at } 95\% \text{ confidence interval.} \\
 n &= \frac{(1.96)^2 \times 0.24 \times (1-0.24)}{(0.05)^2} \\
 n &= \frac{3.84 \times 0.24(0.76)}{0.0025} \\
 n &= \frac{0.700416}{0.0025} \\
 n &= 280.1 \text{ Approximately 285 samples were collected}
 \end{aligned}$$

Collection of Blood Sample

About 2 ml of venous blood was collected into an EDTA container for the WBC, while 2ul and 6ul of capillary blood was used to make thin and thick film for the detection of malaria parasite, which is a site for more concentration of the parasite. Thin films were fixed with absolute methanol before staining. Both thin and thick films were flooded with 2% Giemsa solution for 30 minutes. The slides were washed using buffered PH 7.2 water to remove excess stain and allowed to air dry before examination. The stained slides were examined under a microscope with x100 objective lens using oil immersion. Malaria parasites were quantified using the thick blood film smear. Malaria Parasites were counted against white blood cells until 200 white blood cells were counted.

Determination of White Blood Cell Count

White blood cells count was determined using Sysmex Automated Machine, produced by Sysmex America, Inc. 1 Nelson C. White Pkwy, U.S.A.

Estimation of Malaria Parasite Density

Malaria parasites were quantified using the thick blood film smear. Malaria Parasites were counted against white blood cells until 200 white blood cells were counted. Taking the total number of WBC per microliter of blood by WHO as 8000, the malaria parasite density was determined using this formula below:

$$\frac{\text{Number of Parasites counted} \times \text{WBC standard (8000)}}{\text{Total number of WBCs counted (200)}}$$

and Nigerian recommended WBC of 6000

$$= \frac{\text{Parasites counted} \times \text{WBC standard (6000)}}{\text{Total number of WBCs counted (200)}}$$

Statistical Analysis

Data obtained from this study were analyzed using the statistical package for Social Science Program (SPSS version 22.0 Chicago). ANOVA was used to compare variation in the malaria parasite density. ($P \leq 0.05$)

Results

Table 1 showed the occurrence of *P. falciparum* infection by age of subjects. Of the 285 subjects enrolled for malaria diagnosis by microscopy, 62.5% were infected with *P. falciparum*. The most infected subjects were aged 5-15 years (78.8%), while infection was not seen among subjects greater than 70 years of age. Generally, infection declined with increasing age of subjects. There was statistically significant difference in the prevalence of malaria infection by age of subjects ($X^2=19.273$; $df=6$; $p=0.004$).

Table 2 shows the occurrence of malaria infection in the study based on the gender of subjects. Female subjects were more infected (65.3%) than males (58.3%). However the difference in prevalence of infection between both gender was statistically insignificant ($X^2=1.447$; $df=1$; $p=0.262$).

The mean absolute WBC count of the study subjects by age is shown on Table 3. The mean WBC count of the participants was $5.47(10^9/l) \pm 1.080$. The difference in the distribution of WBC count by age was not statistically significant ($F=0.715$; $p=0.638$). There was no typical pattern of increase or reduction of WBC count by age of subjects.

Table 4 shows the variations in the mean WBC counts of subjects by their infection status. The mean WBC count for subjects infected with *P. falciparum* was $5.34 \times 10^9/l \pm 1.070$, while those not infected had mean WBC counts of $5.69 \times 10^9/l \pm 1.059$. The difference in the mean WBC count between infected and uninfected subjects was statistically significant ($t\text{-test}=2.714$; $df=283$; $p=0.007$).

Table 5 compares the variation of WBC count of the study subjects by gender. The 115 males examined, had mean WBC count of $5.54 \times 10^9/l \pm 1.134$, while the 170 females had a mean WBC

counts of $5.42 \times 10^9/l \pm 1.042$. The variation in the mean WBC count according to gender differences was statistically insignificant (t-test = 0.887; df=283; p=0.376).

The variation of individual absolute WBC count by status of *P. falciparum* infection by gender is shown on Table 6. There was no significant variation in the mean WBC count obtained from those that were infected (t-test= 0.204; df= 176; p = 0.839) and those that were not infected (t-test= 0.881; df= 105; p= 0.839) with *P. falciparum* infection.

The results of estimation of malaria parasite density using absolute WBC value, Nigerian

recommended and WHO standard recommended WBC values and percentage differences in the parasite densities obtained among subjects infected with malaria by age is shown on Table 7. There was a statistically significant difference in the mean parasite count obtained between Absolute and Nigerian recommended WBC count (t-test= 4.755; df= 177; p = 0.000). The difference in the mean parasite density obtained from absolute and WHO recommended WBC count was also statistically significant (t-test= 7.378; df= 177; p = 0.000). The mean parasite density count obtained between Nigerian and WHO recommended WBC count was also statistically significant (t-test= 8.350; df= 177; p=0.000).

Table 1: Prevalence of *P. falciparum* infection by age of subjects

Age (years)	No Examined	No (%) Infected
5-10	33	26 (78.8)
16-26	69	45 (65.2)
27-37	95	54 (56.8)
38-48	54	38 (70.4)
49-59	14	7 (50.0)
60-70	13	8 (61.5)
> 70	7	-
Total	285	178 (62.5)

Table 2: Occurrence of *P. falciparum* infection by gender of subjects

Gender	No Examined	No (%) Infected
Males	115	67(58.3)
Females	170	111 (65.3)
Total	285	178 (62.5)

Table 3: Mean WBC counts of the study population by age of subjects

Age (Years)	No of Subjects	Mean W BC ($10^9/L$) \pm SD
5-15	33	5.33 \pm 1.190
16-26	69	5.51 \pm 1.052
27-37	95	5.43 \pm 1.098
38-48	54	5.57 \pm 1.143
49-59	14	5.86 \pm 0.770
60-70	13	5.23 \pm 0.927
< 70	7	5.14 \pm 0.900
Total	285	5.47 \pm 1.080

Table 4: Variation of individual's WBC counts by infection status of subjects

Infection status	No Examined	Mean WBC count ($10^9/l$) \pm SD
No with malaria parasite	178	5.34 \pm 1.073
No without malaria parasite	107	5.69 \pm 1.059
Total	285	5.47 \pm 1.080

Table 5: Variation of individual's Absolute WBC counts by gender of subjects

Gender	No Examined	Mean WBC count ($10^9/l$) \pm SD
Males	115	5.54 \pm 1.134
Females	170	5.42 \pm 1.042
Total	285	5.47 \pm 1.080

Table 6: Variation of individual's Absolute WBC counts by status of *P. falciparum* infection by gender.

Gender	No Examined	No Positive for malaria parasite	Mean WBC ($10^9/l$) \pm SD	No Not Infected	Mean WBC ($10^9/l$) \pm SD
Males	115	67	5.3 \pm 1.055	48	5.79 \pm 1.202
Females	170	111	5.32 \pm 1.088	59	5.61 \pm .929
Total	285	178	5.34 \pm 1.073	107	5.69 \pm 1.059

Table 7: Relationship between Malaria Parasite density determination and recommended WBC values by age.

No with Malaria Parasite	Mean parasite density with Absolute WBC values \pm SD	Mean parasite density with Nigerian recommended WBC (6000) values \pm SD	% deviation from Abs. WBC method	Mean parasite density with WHO recommended WBC (8000) values. \pm SD	% deviation from Abs. WBC method	No with Malaria Parasite
5-15	26	228.08 \pm 191.401	260.77 \pm 198.594	46.65	347.69 \pm 264.791	39.61
16-26	45	242.78 \pm 146.406	280.00 \pm 162.690	46.44	373.33 \pm 216.921	46.44
27-37	54	241.02 \pm 176.315	260.56 \pm 160.429	48.05	347.41 \pm 213.905	40.95
38-48	38	341.18 \pm 796.554	394.74 \pm 954.964	46.36	526.32 \pm 1271.952	39.32
49-59	7	208.57 \pm 143.112	201.43 \pm 118.382	50.83	268.57 \pm 157.843	43.71
60-70	8	181.25 \pm 79.361	213.75 \pm 98.116	45.88	285.00 \pm 130.822	38.87
Total	178	256.99 \pm 394.271	289.72 \pm 462.917	47.00	386.29 \pm 617.222	39.94

Discussion

A very high prevalence of malaria infection (62.5%) was confirmed in the study even with the ongoing national effort to eliminate malaria in Nigeria. The result obtained in this study is slightly lower than the 75% reported by Zaccheaus and Emmanuel (Zaccheaus and Emmanuel, 2013) in Calabar. The high prevalence rate of malaria reported in the study may be due to the steady or stable transmission of malaria in the area as a result of heavy rainfall, presence of stagnant water, poor drainage system, thick grasses and vegetation around residential homes and lack of constant use of malaria preventive measures like insecticide-treated nets.

The highest malaria infection rate (78.8%) was recorded among individuals aged 5-15 years. This agrees with the findings of Adu-Gyasi *et al.* (2009) which reported a higher prevalence rate of malaria in children in central Ghana. Infection rate among female subjects was (65.3%) compared to males (58.3%) ($X^2= 1.447$; $df=1$; $p=0.262$). The higher malaria infection rate in females may be due to the high involvement of females in agricultural activities and pregnancy which suppresses the immune system thereby exposing them to a higher risk of the infection.

The mean WBC count obtained in this study was $5.47 \pm 1.080 (10^9/l)$. This is slightly higher than the result obtained earlier by Jeremiah *et al.* (2007), which reported a mean WBC count of 5.1 ($10^9/l$) in subjects aged 5 to 70 years in this same locality. This may be due to the presence of other infections apart from malaria which led to a high WBC count in the study.

The finding of this study is at variance with the results of Eduardo *et al.* (2014), which reported that parasite density was overestimated using the WHO recommended WBC count only in younger children infected with malaria. This may be due to the high WBC associated with younger children. There was a statistically significant difference in malaria parasite density by age group ($X^2= 19.273$; $df= 6$; $p= 0.004$). The result of this research agrees to the earlier research conducted by Haggaz *et al.* (2014) in Central Sudan and Jalal *et al.* (2016) in Eastern

Sudan, they all reported significant differences in the mean parasite densities using absolute and WHO recommended WBC count.

In a similar study carried out by Jeremiah *et al.* (2007), a higher parasite density estimation using the assumed WBCs count of 8000 cells/ μl was obtained when compared to the parasite density obtained with absolute WBCs count, which led to overestimation of malaria parasite density in the area among the subjects. This aligns with the findings of Haggaz *et al.* (2014) which reported a high malaria parasite density estimation in malaria infected pregnant women with WHO recommended WBC count when compared to the parasite density obtained with absolute WBC count in Sudan. The mean parasite density in this study is slightly lower than the intensity reported by Zaccheaus *et al.* (2013) in the same locality. However there was a statistically significant difference in the mean parasite density between absolute and Nigerian recommended WBC count ($t= 4.755$; $df= 177$; $Pvalue = 0.000$), there was also a statistically significant relationship in the mean parasite count obtained between the absolute and the WHO recommended WBC counts ($t= 7.378$; $df= 177$; $p= 0.000$), and the difference obtained in the parasite density between Nigerian and the WHO recommended WBC count was also statistically significant ($t= 8.350$; $df= 177$; $p= 0.000$). High WBC count may give a false positive result, the need to use absolute WBC count in computation of malaria density.

WBC is very important in computation of malaria parasite density. White blood cells are manufactured in the bone marrow. Low WBC count below the normal range among subjects infected with malaria parasite may be as a result of other causes like viral infections that temporarily disrupt the work of the bone marrow, certain disorders present at birth that involve diminished bone marrow function and other infections like typhoid fever, radiation therapy, cancer and other disease conditions that damage the bone marrow. Other factors which could lead to leukopenia include; viral infections that temporarily disrupt the production of white blood cell by the bone marrow, certain disorders that are present at birth that affect bone marrow

function, cancer (or other diseases that damage bone marrow), autoimmune disorders which destroy white blood cells or bone marrow cells, severe infections that use up white blood cells faster than they are produced and medications such as antibiotics which destroy white blood cells. Lower WBC count below the normal range in subjects that were not infected with malaria parasite may be a result of other disease conditions which includes viral infections, such as a cold or influenza, chemotherapy, radiation therapy, myelofibrosis, aplastic anaemia, stem cell transplant, bone marrow transplant, HIV/AIDS, TB, enlargement of the spleen, folate deficiency and sepsis as shown on Table 6 and 7. It is agreed that the best option for computing malaria parasite densities would be to use the corresponding WBCs count for each individual, but since majority of the Laboratories in malaria endemic nations in sub-Saharan Africa may not have the capacity to estimate the WBCs count of patients for parasite density estimation, this has resulted in the immediate use of Nigerian and WHO recommended WBC counts in the estimation of malaria parasite density in the area as a substitute for individual WBC count.

Conclusion

The absolute WBC count is more accurate and reliable in estimating the actual and precise malaria parasite density. There was a statistically significant difference in the mean parasite density using the Nigerian recommended WBC value and WHO recommended WBC when compared to the mean parasite density using the absolute WBC count. The Nigerian and WHO recommended WBC value gives a high parasite density estimation, hence the need to urgently replaced it with the (individual) absolute WBC count in malaria parasite density estimation for proper patient management.

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