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Comparative Performance of Methods for Measuring Malaria Parasite Density in Blood Samples

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ABSTRACT

Malaria is a life-threatening parasitic disease transmitted by mosquitoes. It remains the most clinically important of the tropical diseases, widespread through the tropics. The study was done to correlate the malaria parasite density with different age ranges using WBC counts (6,000/mm3, 7,500/mm3, 8,000/mm3) as reference standard and the actual total WBC count. A total of 54 blood samples of three age ranges (< 5 years, 5-15, and > 15 years) were used. There was a significant correlation (Pearson(r) = 0.6664, P < 0.0001) between malaria parasite count and the total white blood cell count of all the age groups (< 5 years, 5-15, and > 15 years). There was a significant decrease (F = 9.988, P = 0.0002) in the total white blood cell count between patients < 5 years, > 15 years. On the other hand, when malaria parasite density for patients in the age range < 5 years using 6,000/mm3, 7,500/mm3, 8,000/mm3 and actual total white blood cell count respectively was calculated, there was a significant difference (F = 47.69, P < 0.001). The analysis of variance of patients in the age range 5-15 years also showed a significant difference (F =30.85, P < 0.0001) when different total WBC counts of 6,000/mm3, 7,500/mm3, 8,000/mm3 and the actual were used respectively. It can be concluded from the results of this study that using the average total WBC count of 8,000/mm3 was most unsatisfactory

for determining parasite density in most clinical situations. The number of parasites per total WBC and the actual WBC count was found to be the most accurate.

Keywords: Malaria, Parasite, Density, Mosquitoes, Parasitic disease

1. Introduction

Malaria is a life-threatening parasitic disease transmitted by mosquitoes. It remains the most clinically important of the tropical diseases, widespread through the tropics. The disease exacts a heavy toll of illness and death among children especially in endemic areas[1]. Malaria caused parasitic disease is seen in more than 300 million people and at least one million deaths annually[2]. Ninety percent of deaths due to malaria occur in Africa South of the Sahara mostly among young children. It kills an African child every 30 seconds[2]. Malaria is the most common cause of outpatient clinic attendance among all age groups in Nigeria and it is responsible for an estimated 300, 000 deaths yearly in children less than five years old (FMOH, 1980-1983).

The detection of malaria parasite in peripheral veins or capillary blood has always been considered an indispensable basis for the definition and diagnosis of malaria[3,4]. In the simple determination of parasite density, data collection has usually been limited to a single time point. Little is known about the natural variations in parasite density in the peripheral blood during the course of a day or a week[3,4]. In a single individual, parasite density varies spontaneously during the course of several days follow-up. Such variations can lead to an erroneous estimation of the community load of malaria infection[3-6].

High parasite densities may be observed in symptom free individuals, while scores of malaria attacks may occur among those with no detectable parasites and there is no obligate temporal correlation between the occurrence of fever and parasite density[3]. The calculation of the total number of parasites/microlitre (or mm^3) of blood requires the knowledge of the normal range of white blood cells (WBC) in various age groups. Different WBC values have been used in calculation of malaria parasite density based on assumption. The value of 8,000 WBC/mm^3 has been generally assumed[2,4,5,7-10]. Another assumed average leucocytes concentration value of 7,500 leucocytes/ mm^3 has equally been used[3]. While 6,000 leucocytes/ mm^3 has equally been employed[5,11,12]. These values may not be same in all age groups.

It is not known whether these methods give a good approximation of the parasite density. It has been observed that a common weak point in the estimation of parasite levels by counting parasites against a particular number of WBC is the (incorrect) assumption that all blood samples contain 8,000 WBC/mm^3 of blood[13]. Moreover, different age groups have varying WBC counts. Their normal ranges are infants from day one ---3 yrs: 7,500/mm^3 $\pm$ 3,500/mm^3 (Dacie and Lewis, 1985); children from 1yr -- 4yrs: 6,000 –18,000/mm^3; children between 4 -- 7years: 5,000 – 15,000/mm^3; adults: 4,000 - 11,000/mm^3[14].

2. Materials and Method

2.1. Study area and subjects

The study was conducted in the University of Nigeria Teaching Hospital, Enugu, Nigeria. Most of the inhabitants of Enugu are of the Igbo tribe and the area has a wet and rainy season. The subjects were clinically selected malaria patients from the Pediatric clinic of the UNTH.

The target populations were children less than 5 years of age, teenagers with the age bracket 5-15years and adults greater than 15 years of age.

Fifty-four (54) blood samples were collected via finger-pricking using sterile blood lancet into sterile EDTA (anticoagulant) containers.

3. Methods of Analysis

3.1. Thick blood film preparation and staining

The thick blood film preparation and staining using Giemsa method (Silverton et al., 1998).

3.2. Procedure

Thick blood films were prepared by making a blood smear with a drop of blood on a clean grease-free slide. The films were allowed to air-dry. The dried thick film was covered with 1in 10 dilution of stock Giemsa stain (filtered) with buffered distilled water pH 7.0. After 30 minutes, the stain was washed off using buffered distilled water. The back of the slide was wiped off and the slide was laced in a slide rack to dry vertically.

3.3. Examination

The leucocytes were counted in batches of 100, 200, 400, and 800, using oil immersion (x 100) objective. The malaria parasites were counted alongside each batch of leucocyte (WBC). A total of four counts for each batch were done, and the average count of malaria parasite for each batch was obtained and used in the calculation of malaria density.

3.4. Counting of total white blood cells

Total white blood cell count using Turks solution[7].

3.4.1. Procedure: About 0.02ml of anticoagulated blood from finger prick was added to 0.38ml of diluting fluid in a tube and mixed. The solution was allowed to stand for 4 minutes to lyse the red cells and tinge the white cells, a cover glass was placed on to an Improved Neubauer counting chamber. The solution containing the white cells was mixed and used to charge the counting chamber using a Pasteur pipette. The chamber was left undisturbed for 2 minutes, to allow the cells to settle, and the cells were counted using (x10) and (x40) objectives.

A total white blood cells were calculated using the counted value.

$$WBC = \frac{N \times 20 \times 10^6}{5 \times 0.1}$$

Where N = Number of cells counted

20 = The dilution factor (DF)
5mm^3 = Area counted (A)

0.1mm = The depth of the counting chamber (D)

Results expressed in /mm^3 (Silverton et al, 1998).

Normal Ranges: Infants from day one – 3yrs: 7,500/mm^3 ± 3,500/mm^3 (Dacie and Lewis, 1985).

Children from 1yr – 4years: 6,000 – 18,000/mm^3.

Children between 4 – 7years: 5,000 – 15,000/mm^3.

Adults 4,000 – 11,000/mm^3.

Determination of Parasite Densities

$$\frac{X \text{ No of parasites}}{(n) \text{ WBC}} \times \frac{s/mm^3}{1}$$

Where: X = no of malaria parasite counted

N = no of white blood cell counted per field (100, 200, 400, 0r 800).

s/mm^3 = the total WBC count (using 6,000/mm^3, 7,500/mm^3 or 8,000/mm^3).

4. Results

A total of 54 blood samples of three age groups (<5yrs, 5-15yrs and > 15years) were analysed for the malaria parasite density using different total white blood cell counts. There was a significant correlation (Pearson(r) = 0.6664, P<0.0001); see fig. 41 graph.

(Table 1) shows the mean (±SD) of total white blood cell count of the different age groups. The results show a decrease (F = 9.988, P = 0.0002) in the total white blood cell count from patients < 5 years to > 15 years.

(Table 2) represents the mean values and standard deviation of the malaria parasite density of the different age groups using the actual WBC count. The malaria parasite density was calculated after using 100, 200, 400 and 800 WBC respectively. Analysis of variance showed that there were no significant changes (F = 0.1502, P = 0.929) for age group < 5 years; F = 0.1035, P = 0.9577 for age groups 5 – 15 years and F = 0.1423, P = 0.9344 for age group > 15 years) in the parasite densities in each age group when 100, 200, 400 and 800 WNCs were counted.

(Table 3) shows the different malaria parasite density of different age groups using 6,000/ as the total WBC count. Analysis of variance showed no significant changes (F = 0.1748, P = 0.9130 for age group <5 yrs, F = 0.1429, P = 0.9330 for age groups 5 - 15 yrs and F = 0.2093, P = 0.8898 for age group >15 yrs) in the parasite densities in each age group when 100, 200, 400, and 800 WBCs, were counted.

Table 1: Showing the total white blood cell count of the different age groups.

Age	Range	Mean	Standard deviation
<5 years	5,700 – 28,800	12,980	7,412
5 – 15 years	4,000 – 19,200	8,305	4,273
>15 years	2,400 – 10,600	6,359	2,108
F = 9.988, P = 0.0002			

(Table 4) represents the malaria parasite density of different age groups using 7,500/mm^3 as the total WBC count. Its analysis of variance showed no significant changes (F = 0.174, P = 0.9135, for age group <5yrs, F = 0.1429, P = 0.9339 for age group 5 - 15 yrs and F = 0.1963, P = 0.8987 for age group >15yrs) in the parasite densities in each age group when 100, 200, 400 and 800 WBC, were counted.

Table 2: Malaria parasite density of different age groups using their actual total WBC counts.

Age	100	200	400	800	F/P values
T2 <5yrs	7,617±	7,311±	5,622±	7,892±	F = 0.1502
	10,055	9,420	4,889	13,210	P = 0.9291
T6 5 – 15yrs	9,647±	10,750±	10,970±	12,040±	F = 0.1035
	11,480	12,680	13,780	16,150	P = 0.9577
T7 >15yrs	2,550±	2,803±	2,802±	3,001±	F = 0.1423
	2,014	2,041	2,316	3,472	P = 0.9344

Table 3: Shows the different malaria parasite density of different age groups using 6,000/ as the total WBC count.

Age	100	200	400	800	F/P values
T8 <5yrs	2931±	2849±	2473±	2926±	F = 0.1748
	1858	1770	979	2771	P = 0.9130
T9 5 – 15yrs	5595±	6179±	6215±	6748±	F = 0.1420
	4433	4934	5660	6946	P = 0.9330
T10 >15yrs	2333±	2595±	2586±	2738±	F = 0.2093
	1339	1477	1689	2788	P = 0.8898

(Table 5) Show the malaria parasite density of different age groups using 8,000/mm3 as the total WBC count. Analysis of variance showed no significant changes (F = 0.1757, P = 0.9124 for age group <5yrs, F = 0.1429, P = 0.9339 for age group 5 - 15 yrs and F = 0.2090, P = 8899 for age group >15yrs) in the parasite densities in each age group when 100, 200, 400 and 800 WBC, were counted.

(Table 6) indicates the malaria parasite density for patients in the age range <5yrs using 6,000/mm^3, 7,500/mm^3, 8,000/mm^3 and the actual total WBC count respectively in the calculation. The analysis of variance showed a significant difference (F = 47.69, P <0.0001) in the malaria parasite density using different total WBC counts.

Table 4: Malaria parasite density using 7,500/mm^3 as WBC count.

Age	100	200	400	800	F/P values
T11 <5yrs	3664±	3557±	3091±	3658±	F = 0.1741
	2323	2216	1223	3464	P = 0.9135
T12 5 – 15yrs	6994±	7723±	7768±	8435±	F = 0.1429
	5541	6167	7075	8682	P = 0.9339
T13 >15yrs	2935±	3203±	3122±	3422±	F = 0.1963
	1667	1872	1999	3485	P = 0.8987

Table 5: Showing the malaria parasite density of different age groups using 8,000/mm3 as the total WBC count.

Age	100	200	400	800	F/P values
T14 <5yrs	3915±	3790±	3297±	3901±	F = 0.1757
	2479	2364	1305	3694	P = 0.9124
T15 5 – 15yrs	7460±	8238±	8286±	8997±	F = 0.1429
	5910	6578	7546	9261	P = 0.9339
T16 >15yrs	3111±	3456±	3448±	3651±	F = 0.2090
	1786	1969	2252	3718	P = 0.8899

(Table 7) Shows the malaria parasite density for patients in the age range 5 –15yrs using different total WBC counts

(6,000/mm^3, 7,500/mm^3, 8,000/mm^3 and actual total WBC count respectively). Its analysis of variance showed a significant difference (F = 30.85, P<0.0001) in the different estimations of parasite density.

Table 6: Malaria parasite density for age range (<5yrs) using different values as the total WBC counts.

No of WBC Counted	6000/mm^3	7,500/mm^3	8,000/mm^3	Actual/mm^3
100	2931±	3664±	3909±	7617±
	1858	2323	2478	10,055
200	2846±	3557±	3794±	7316±
	1773	2216	2364	9417
400	2473±	3091±	3297±	5622±
	979	1223	1305	4889
800	2926±	3658±	3901±	7892±
	2771	3464	3694	13209
	F = 47.69, P<0.0001			

(Table 8) Represents the malaria parasite density for patients in the age range >15yrs using different total WBC counts (6,000/mm^3, 7,500/mm^3, 8,000/mm^3 and the actual total WBC count respectively). The analysis of variance showed no significant difference (F = 15.25, P = 0.0002) in the estimation of parasite density.

Table 7: Malaria parasite density for the age range (5 – 15 yrs) using different values as the total WBC counts.

No of WBC Counted	6000/mm^3	7,500/mm^3	8,000/mm^3	Actual/mm^3
100	5595±4433	6994±5541	7460±5910	9617±11,432
200	6175±4934	7723±6167	8238±6578	10,752±12,678
400	6215±5660	7768±7075	8286±7546	10,974±13,780
800	6748±6946	8435±8682	8997±9261	12,039±16,150
	F = 30.85, P<0.0001			

Table 8: Malaria parasite density for the age range (>15yrs) using different value as the total WBC count.

No of WBC Counted	6000/mm^3	7,500/mm^3	8,000/mm^3	Actual/mm^3
100	2333±1339	2917±1674	3111±1786	2550±2015
200	2592±1477	3241±1846	3456±1969	2803±2041
400	2594±1704	3233±2111	3448±2252	2802±2316
800	2738±2788	3423±3485	3651±3718	3001±3472
	F = 15.25, P<0.0002			

5. Discussion

Malaria is a life-threatening parasitic disease transmitted by mosquitoes and caused by the specie of the genius plasmodium. Four species infect man; P. falciparum, P. vivax, P. ovale and P. malariae[1].

From my findings, there was a significant correlation (Pearson(r) =0.6664, P<0.0001) between malaria parasite count and the total white blood cell count of all the age groups (<5yrs, 5-15yrs and >15yrs). There was a significant decrease (F = 9.988, P = 0.0002) in the total white blood cell count from patients <5yrs to >15yrs **(Table 1)**. There was no significant change when the mean values and standard deviation of the malaria parasite density of the different age groups using the actual WBC counts (after reading 100, 200, 400 and 800 WBC respectively). Also, there was no significant variations was noticed in malaria parasite densities among different age groups using 6,000/mm^3, 7,500/mm^3 and 8,000/mm^3 and the actual WBC count respectively, there was a significant difference (F = 47.69, P<0.001). The analysis of variance of patients in the age range 5-15yrs showed a significant difference (F = 30.85, P<0.0001) when different total WBC counts of 6,000/mm^3, 7,500/mm^3 and 8,000/mm^3 and the actual were used respectively.

Those of age range >15yrs showed no significant difference. The most widely used method of parasite density determination based on the assumed average total WBC count, gave incorrect counts in malaria patients[4,15]. Assuming that counting of parasite against the WBC in the blood smear and consequent number of PRBC/WBC was correct, the probable cause of this error is the deviation of WBC counts in patients[5,15]. Therefore, this agree with Dubey, et al., 1999 that when the parasite densities were calculated based on the actual WBC counts of each patient, the error will be eliminated and more accurate parasite densities obtained[16-20].

6. Conclusion

It can be concluded from the results of this study that based on the average WBC count of 8,000/mm3 was most unsatisfactory for determining parasite density in most clinical situations. The number of parasites per total WBC and the actual WBC count was found to be the most accurate.

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7.1. Disclosure of conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

7.2. Statement of ethical approval

Ethical approval was obtained from the ethics and research committee of Asokoro District Hospital, Abuja, Nigeria, and informed consent of the patients was obtained before sample collection.

7.3. Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

7.4. Availability of data and materials

The authors declare consent for all available data present in this study.

7.5. Authors' Contribution

The entire study procedure was conducted with the involvement of all writers.

7.6. Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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