ESTIMATION OF MALARIA PARASITE DENSITY USING LEUKOCYTE COUNTS AS AN INDEX IN ADULTS VOLUNTEERS OF SOUTHWESTERN NIGERIA

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Abstract

Leukocyte counts and screening for malaria parasites were carried out on 252 apparently healthy blood donors attending a transfusion centre in Ibadan. The leukocyte count range for the donors was 2.5-9.6 x 10^9/L; the mean leukocyte count being 4.98 x 10^9/L. 193 (76.6%) had leukocyte count less than 6.0 x 10^9/L. Eighty-one (32.1%) of the 252 blood donors had malaria parasitaemia. There was no significant difference between the mean leukocyte count for Aparasitaemic donors (5.04 x 10^9/L) and that for Parasitaemic donors (4.87 x 10^9/L). Three methods (A, B and C) (A, based on true leukocyte count, B and C, based on assumed leukocyte counts of 8,000/μL and 6,000/μL respectively) were used to estimate malaria parasite density. The result of the ANOVA showed that there was no significant difference among the means of the three methods. The test for homogeneity showed a significant heterogeneity between methods A and B but homogeneity between methods A and C. Method C is a better substitute for method A than method B in the estimation of parasite density.

Keywords: Adults, asymptomatic malaria, parasite density, leukocyte counts.

1. Introduction

Several studies have been carried out to determine the prevalence of malaria parasitaemia among apparently healthy blood donors in Nigeria (Achidi *et al.*, 1995; Ibhanesebhor *et al.*, 1996; Chikwen *et al.*, 1997). Although accurate determination of parasite density requires red blood cell (RBC) count and accurate measurement of the level of RBC infected, this technique is usually not employed by researchers in the determination of parasite density (Greenwood and Armstrong, 1991). This is because aside from being time consuming and so not suitable for large-scale field studies, accurate RBC count can only be done by electronic counter which is not readily available in most research centres in Africa (Greenwood and Armstrong, 1991). Consequently, the method of choice is to multiply the number of parasites per leukocyte (WBC) in a thick film by its true leukocyte count. However, in Nigeria and other African countries most researches involving the estimation of malaria parasite density are usually based on assumed leukocyte count (Trape *et al.*, 1985; Salako *et al.*, 1990; Sowunmi, 1995; Achidi *et al.*, 1995). Two assumed leukocyte counts of 8,000 /μL and 6,000 /μL are commonly employed.

This study was carried out to:

i. determine the range of total leukocyte counts in apparently healthy blood donors
ii. determine the effect of malaria parasitaemia on leukocyte count
iii. compare the estimates of parasite density based on true leukocyte counts and assumed leukocyte counts.

2. Subjects and Methods

Two hundred and fifty-two (252) healthy blood donors (aged 18-56 years old) at the transfusion unit of Biomedics Diagnostic Centre in Ibadan, southwestern Nigeria were studied between March 2002 and July 2002. Subjects selected were those who passed the initial screening (Haematocrit or PCV) test. Participants were admitted into this study after informed consent.

2 mL of blood was obtained from each donor from an ante-cubital vein by venepuncture and collected in EDTA bottle. Measurements of haematocrit and leukocyte (WBC) were done for each donor using standard laboratory techniques (Dacie and Lewis, 1995). A thick blood film was prepared from each donor's blood sample and stained with Giemsa. The stained thick blood films were each examined for 200 high power fields under the microscope before being considered negative for malaria parasite. The number of parasites present in 200 leukocyte count was determined and the average number of parasites per leukocyte calculated. This figure was then multiplied by:

i. the individual's true leukocyte count (method A),
ii. an assumed leukocyte count of 8,000 /μL (method B), and
iii. an assumed leukocyte count of 6,000 /μL (method C) to give the respective parasite densities.

Statistical Analysis: Means were compared using ANOVA. Chi-square test was used to compare the parasite density class intervals. A P-value <0.05 was considered significant.

3. Results

The leukocyte count range was from 2.50 x 10^9/L to 9.60 x 10^9/L. The mean leukocyte count was 4.98 x 10^9/L. Eighty-one out of 252 (32%) of the blood donors were positive for malaria parasite. The leukocyte count range for parasitaemic donors was from 2.80 x 10^9/L to 9.40 x 10^9/L; the mean leukocyte count being 4.87 x 10^9/L while that for aparasitaemic donors was between 2.50 x 10^9/L and 9.60 x 10^9/l; the mean leukocyte count being 5.03 x 10^9/L. The mean leukocyte count for aparasitaemic donors was more than that for parasitaemic donors but the difference was not statistically significant (P>0.05) (Table 1).

One hundred and thirty-nine out of 252 (55.3%) of the blood donors had leukocyte count less than 5.0 x 10^9/L. Also, 193 (76.6%) had leukocyte count of less than 6.0 x 10^9/L while 59 (23.4%) had leukocyte count more than 6.0 x 10^9/L. The pattern of leukocyte distribution in parasitaemic donors was similar to that of aparasitaemic donors (Figure 1). The mean parasite density for method A involving true estimation of leucocyte ($\bar{x}$ = 283.07) was less than those for the assumed methods B ($\bar{x}$ = 504.69) and C ($\bar{x}$ = 382.47) (Table 2). However, the result of the analysis of variance (ANOVA) showed that there was no significant difference among the means. Based on the parasite density class interval, the result of test for homogeneity showed a significant heterogeneity (x^2 = 7.08; df = 2; P<0.05) between methods A and B but no heterogeneity (x^2 = 3.0; df = 2; P>0.05) between methods A and C.

4. Discussion

Thirty-two percent (32%) of the blood donors examined in this study were positive for malaria parasites. The presence of malaria parasites among semi-immune healthy persons living in an endemic area is well documented (CDC, 1999; CDC, 2000).

This study showed that the mean leukocyte count in the examined subjects was 4.98 x 10^9/L and that there was no significant difference between the mean leukocyte count of parasitaemic donors and that of aparasitaemic donors. In tropical Africa, a tendency towards leukopenia is seen in adults and most studies detect an average number of leukocytes between 5.0 x 10^9/L and 6.5 x 10^9/L (Hapwood, 1969; Ezeilo, 1971; Sharper and Lewis, 1971; Sowunmi *et al.*, 1995).

The separate counting of leukocytes per μL of the blood enables a good estimation of malaria parasite densities. However, since this method is cumbersome for mass surveys, it is necessary to adopt an average value of leukocyte count for any given locality or region.

This study showed that parasite density estimates from an assumed leukocyte count of 6.0 x 10^9/L gave closer results to those from true leukocyte count than to those from an assumed leukocyte count of 8.0 x 10^9/L. Although there was no significant difference among the means of the three methods, the result of the heterogeneity test showed that while there was no significant difference between methods A and C, the difference between methods A and B was quite significant.

Table 1: Apparently Healthy Blood Donors

	Aparasitaemic	Parasitaemic
No of subjects	171	81
Leukocyte count (x 10^9/L) range	2.50 – 9.60	2.80 – 9.40
Mean (S.E)	5.03 (0.01)	4.87 (0.02)

Table 2: Comparison of the 3 methods employed in the determination of parasite density

Parasite density/μL	True WBC count (method A)	Assumed WBC count 8000/μL (method B)	Assumed WBC count 6000/μL (method C)
1 - 499	68	51	59
500 - 999	9	20	16
≥ 1000	4	10	6
	81	**81**	**81**
Range	20 - 3920	40 - 8040	30 - 6030
Mean (S.E)	283.07 (5.88)	504.69 (11.77)	382.47 (8.85)

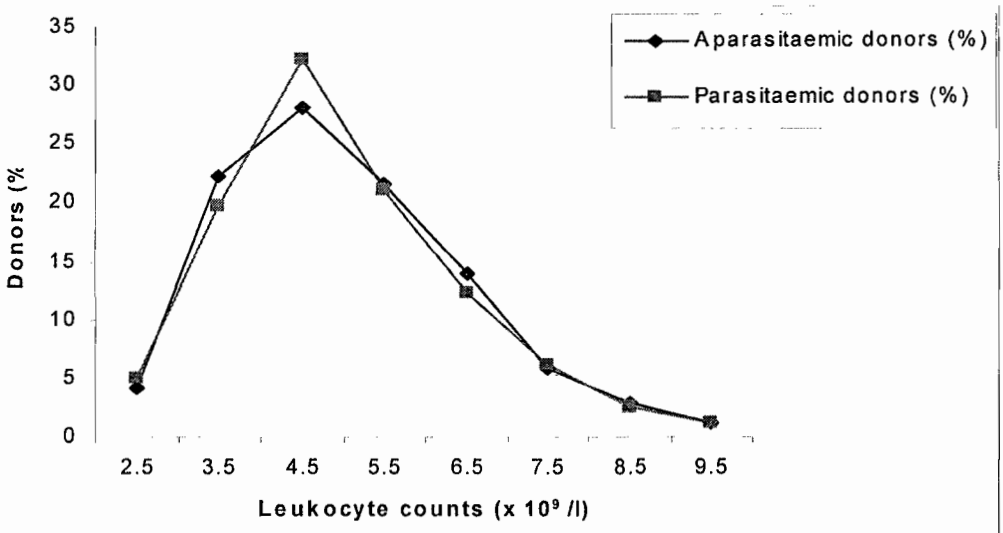

Figure 1: Distribution of leukocyte counts in blood donors

It is therefore suggested that for research involving estimation of parasite density in adults in this locality, an average leukocyte count of 6.0 x 10^9/L be adopted. This would give a closer representation of the actual count than using an assumed count of 8.0 x 10^9/L.

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REFERENCES

Achidi, E.A., Perlmann, H. and Berzins, K., 1995. Asymptomatic malaria parasitaemia and seroreactivities to *P. falciparum* antigens in Blood Donors from Ibadan, southwestern Nigeria. *Ann. Trop. Med. Parasit., 89 (6)*, 601-610.

Center for Disease Control, 1999. Malaria can be passed via blood transfusion. *Morbid. Mortal. Wkly Rep., 48*, 253.

Center for Disease Control, 2000. Global AIDS Program: Strategies in Blood safety. *National center for HIV, STD and TB Prevention Publication.*

Chikwem, J.O., Mohammed, I., Okara, G.C., Ukwandu, N.C.D. and Ola, T.O., 1997. Prevalence of Transmissible Blood Infections among Blood Donors at the University of Maiduguri Teaching Hospital, Maiduguri, Nigeria. *E. Afr. Med. J., 74 (4)*, 213-216.

Dacie, J.V. and Lewis, S.M., 1995. *Practical Haematology* (8th Ed.), Edinburgh, Churchill, Livingstone.

Ezeilo, G.C., 1971. Neutropenia in Africans. *Trop. Geogr. Med., 23*, 264-267.

Greenwood, B.M. and Armstrong, J.R.M., 1991. Comparison of two simple methods for determining malaria parasite density. *Trans. Roy. Soc. Trop. Med. Hyg., 85*, 186-188.

Hapwood, B.C., 1969.Leukocytes Levels in East Africa. *E. Afr. Med. J., 46*, 680-682.

Ibhanesebhor, S.E., Otobo, E.S. and Ladipo, O.A., 1996. Prevalence of malaria parasitaemia in transfused Donor blood in Benin City, Nigeria. *Ann. Trop. Paediat., 16 (2)*, 93-95.

Salako, L.A., Ajayi, F.O., Sowunmi, A and Walker, O., 1990. Malaria in Nigeria: a revisit. *Ann. Trop. Med. Parasit., 84*, 435-445.

Sharper, A.G. and Lewis, P., 1971. Genetic neutropenia in people of African origin. *Lancet, 2*, 1021-1023.

Sowunmi, A., Akindele, J.A. and Balogun, M.A., 1995. Leukocyte count in falciparum malaria in African children from an endemic area. *Afr. J. Med. Med. Sc., 24*, 145-149.

Trape, J.F., 1985. Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations. *Trans. Roy. Soc. Trop. Med. Hyg., 79*, 181-184.