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 10⁹/L, the mean leukocyte count being 4.98 x 10⁹/L. 193 (76.6%) had leukocyte count less than 6.0 x 10⁹/L. Eighty-one (32.1%) of the 252 blood donors had malaria parasitaemia. There was no significant difference between the mean leukocyte count for A. parasitaemic donors (5.04 x 10⁹/L) and that for Parasitaemic donors (4.87 x 10⁹/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; Ibanesebhior 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 antecubital 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:
1. The individual’s true leukocyte count (method A),
ii. an assumed leukocyte count of 8,006/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 parasites. 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 non-parasitaemic 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 parasitaemic donors was more than that for non-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 non-parasitaemic donors (Figure 1). The mean parasite density for method A involving true estimation of leukocyte (x = 283.07) was less than those for the assumed methods B (x = 504.69) and C (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 non-parasitaemic donors. In tropical Africa, a tendency towards leukenopia 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; Sharpe 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

<table>
<thead>
<tr>
<th>Parasitaemic</th>
<th>Parasitaemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of subjects</td>
<td>171</td>
</tr>
<tr>
<td>Leukocyte count (x 10^9/L) range</td>
<td>2.50 – 9.60</td>
</tr>
<tr>
<td>Mean (S.E)</td>
<td>5.03 (0.01)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parasite density/μL</th>
<th>True WBC count (method A)</th>
<th>Assumed WBC count 8000/μL (method B)</th>
<th>Assumed WBC count 6000/μL (method C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 499</td>
<td>68</td>
<td>51</td>
<td>59</td>
</tr>
<tr>
<td>500 - 999</td>
<td>9</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>≥ 1000</td>
<td>4</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Range</td>
<td>20 - 3920</td>
<td>40 - 8040</td>
<td>30 - 6030</td>
</tr>
<tr>
<td>Mean (S.E)</td>
<td>283.07 (5.88)</td>
<td>504.69 (11.77)</td>
<td>382.47 (8.85)</td>
</tr>
</tbody>
</table>
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 10⁹/L be adopted. This would give a closer representation of the actual count than using an assumed count of 8.0 x 10⁹/L.

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**REFERENCES**


