Some hematological parameters and the prognostic value of CD4, CD8 and total lymphocyte counts and CD4/CD8 cell count ratio in healthy HIV sero-negative, healthy HIV sero-positive and AIDS subjects in Port Harcourt, Nigeria

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Abstract

Objective: The present study attempts to determine normal values of CD4, CD8, CD4/CD8 ratio, total WBC and differential counts, hematocrit and total lymphocyte count (TLC) in healthy HIV sero-negative and sero-positive subjects, and to assess the prognostic significance of these parameters in these subjects as compared to AIDS subjects.

Material and Methods: A total of 300 subjects (147 M, 153 F) aged between 17 and 71 years were recruited into the study. Subjects were separated according to sex and divided into three groups: Group A: healthy HIV sero-negative subjects; Group B: healthy HIV sero-positive newly diagnosed ART-naive subjects; and Group C: AIDS subjects. CD4 and CD8 counts were determined by flow cytometry; hematocrit was determined using Hawksley micro-capillary tubes; total WBC and differential counts were determined manually with the improved Neubauer counting chamber; and TLC was obtained by multiplying the percentage of lymphocytes by the total WBC count.

Results: For male subjects, significant differences were found in CD4 count, CD4/CD8 count ratio, hematocrit, total WBC and TLC, whereas for female subjects, significant differences were found only in CD4 and CD4/CD8 count ratio in the three groups of subjects. In both sexes, however, these parameters were found to be highest in healthy HIV sero-negative subjects and lowest in AIDS subjects, with HIV sero-positive subjects having intermediate values.

Conclusion: The results confirm previous reports that the CD4 count and CD4/CD8 count ratio are fairly reliable indicators of the progression of HIV infection. In addition, the results also apparently suggest that the prognostic value of CD8 count is limited and that of TLC possibly sex-dependent. The results could be of importance in our environment since previous reports have been relatively scarce. (Turk J Hematol 2008; 25: 181-6)

Key words: CD4 counts, CD8 counts, CD4/CD8 ratio, total lymphocyte count, human immunodeficiency virus (HIV), acquired immunodeficiency syndrome (AIDS).

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Introduction

Since its identification in 1981, human immunodeficiency virus (HIV) infection and the associated acquired immunodeficiency syndrome (AIDS) remain a major health burden globally. Recent estimates indicate that over 35 million people are affected worldwide, with mortality counts of over 20 million [1]. About 70% of these deaths have occurred in sub-Saharan Africa [1], where the burden of disease is high and poverty an important accomplice. In Nigeria, the current national average HIV seroprevalence rate is estimated at about 4.4%, with Rivers State predictedly having a higher rate of 5.4% [2]. However, with the relatively high cost of CD4 cell count determination, total lymphocyte count (TLC) has been suggested as an alternative in situations where facilities for CD4 cell count are not readily available or resources are limited. This is because TLC is easily obtained from routine complete blood cell counts by multiplying the percentage of lymphocytes by the WBC count [11,12]. However, a number of reports have suggested an inconsistency in the correlations between total lymphocyte and CD4 cell counts [13].

Given that, in our environment, reports on this subject are relatively scarce, the present study attempted to determine values of CD4, CD8, total lymphocyte, total WBC and differential cell counts in healthy HIV sero-negative, healthy HIV sero-positive and in persons with AIDS. The study also determined the CD4/CD8 cell count ratio and attempted to assess the possible prognostic value of these parameters using these three groups of subjects. In addition, the study attempts to establish normative values of these parameters, in our environment, for healthy HIV sero-negative and healthy HIV sero-positive subjects who have yet to commence ART. This could possibly assist Nigerian physicians with the assessment and management of HIV infection in affected individuals.

Materials and Methods

Subjects: A total of 300 subjects (147 M, 153 F; age range: 17-71 years) were recruited into the study. Subjects were separated according to sex and were further divided into three groups: Group A [controls] consisted of healthy HIV sero-negative subjects, Group B consisted of healthy HIV sero-positive
subjects, and Group C consisted of AIDS subjects. Groups B subjects were newly diagnosed subjects yet to commence ART. Groups B and C were attending the HIV clinic of a tertiary health care facility in Port Harcourt, southeastern Nigeria; Group A [control] subjects were apparently healthy staff and students of the University of Port Harcourt, Nigeria. Each control subject was examined and no evidence of acute or chronic infections or any hematologic, cardiovascular or metabolic disease likely to influence any of the hematological parameters under investigation was found. All subjects gave informed consent before recruitment into the study; ethical clearance was obtained from our institutional ethics committee. All pregnant female subjects were excluded from the study.

Methods: Five milliliters of venous blood was collected from each subject from an antecubital vein with the subject comfortably seated and with minimum stasis. The blood was immediately transferred into EDTA specimen bottles and carefully mixed. All blood specimens were collected between 9 a.m. and 12 noon each day and analyzed within 2 hours of collection.

The HIV status of each subject was determined routinely using the Chembio HIV 1/2 Stat-pak assay kit (Chembio Diagnostic Systems Incorporated, USA). The CD4 cell and the CD8 cell counts were both determined by flow cytometry using the Partec Cytoflow counter FMC system (Partec GmbH, 2006).

CD4/CD8 count ratio for each subject was obtained from the product of dividing the CD4 cell count by the CD8 cell count. Hematocrit was determined using Hawksley micro-capillary tubes centrifuged at 3000 rpm for 10 minutes; the mean of two separate readings was taken as the hematocrit value. Total WBC and differential WBC counts were determined manually using the improved Neubauer counting chamber [14]. TLC was obtained by multiplying the percentage of lymphocytes by the total WBC count [11,12].

Statistics: The results obtained are expressed as means ± standard errors of means (SEM); ranges are in parenthesis. Statistical significance was determined using the analysis of variance (ANOVA) or the Student’s t-test as appropriate. A p value less than 0.05 (p<0.05) was considered statistically significant.

Results

The results obtained from the present study for each Group are as shown in Tables 1 and 2 for male and female subjects, respectively.

Table 1 presents the ages, CD4 and CD8 counts, CD4/CD8 count ratio, hematocrit, total WBC count, percentage neutrophil, lymphocyte, monocyte, eosinophil and basophil, and TLC for the male subjects involved in the present study. ANOVA showed

<table>
<thead>
<tr>
<th>Table 1. Hematological parameters, CD4 and CD8 counts and ratio in male HIV sero-negative and sero-positive subjects and AIDS subjects.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>CD4 count (Cells/μl)</td>
</tr>
<tr>
<td>CD8 count (Cells/μl)</td>
</tr>
<tr>
<td>CD4/CD8 count ratio</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
</tr>
<tr>
<td>Total WBC count (Cells/μl)</td>
</tr>
<tr>
<td>Neutrophil count (%)</td>
</tr>
<tr>
<td>Lymphocyte count (%)</td>
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<tr>
<td>Monocyte count (%)</td>
</tr>
<tr>
<td>Eosinophil count (%)</td>
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<tr>
<td>Basophil count (%)</td>
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<tr>
<td>Total lymphocyte count (%Cells/μl)</td>
</tr>
</tbody>
</table>

Values=mean ± SEM, range in parenthesis
that significant differences existed in CD4 cell count, CD4/CD8 cell count ratio, hematocrit, total WBC count and TLC for male subjects between the three groups under consideration: healthy HIV sero-negative subjects (Group A), healthy HIV sero-positive subjects (Group B) and AIDS subjects (Group C) (p<0.05). Each of these parameters was generally the highest in the healthy HIV sero-negative subjects (Group A) and lowest in the AIDS subjects (Group C), with healthy HIV sero-positive subjects (Group B) having intermediate values.

Similarly, Table 2 presents the values of the investigated parameters in all the female subjects involved in the present study. ANOVA showed that significant differences existed only in CD4 cell count and CD4/CD8 cell count ratio for the three female groups under consideration (p<0.001). Unlike for male subjects, in female subjects, no significant differences were found in hematocrit, total WBC count and TLC between the three groups under consideration (p>0.001). However, as in male subjects, both CD4 cell count and CD4/CD8 cell count ratio were highest in the healthy HIV sero-negative subjects (Group A) and lowest in the AIDS subjects (Group C), with healthy HIV sero-positive subjects (Group B) having intermediate values.

Amongst the HIV sero-positive (Group B) subjects, 8 (21.6%) males and 26 (41.3%) females had CD4 cell counts less than 350 cells/μl. All the AIDS (Group C) subjects were found to have CD4 cell counts less than 350 cells/μl. None of the healthy HIV sero-negative (Group A) subjects had CD4 cell counts less than 350 cells/μl.

Table 2. Hematological parameters, CD4 and CD8 counts and ratio in female HIV sero-negative and sero-positive subjects and AIDS subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy HIV-negative subjects [Group A] [n= 42]</th>
<th>Healthy HIV-positive subjects [Group B] [n=63]</th>
<th>AIDS subjects [Group C] [n=48]</th>
<th>Significant differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.93±1.13 [17-71]</td>
<td>33.86±0.88 [19-71]</td>
<td>31.73±0.73 [18-65]</td>
<td>No [p&gt;0.05]</td>
</tr>
<tr>
<td>CD4 count (Cells/μl)</td>
<td>920.52±24.10 [528.00-1671.00]</td>
<td>451.46±20.23 [89.00-1377]</td>
<td>94.46±4.43 [14.00-196.00]</td>
<td>Yes [p&lt;0.05]</td>
</tr>
<tr>
<td>CD8 count (Cells/μl)</td>
<td>834.69±24.54 [326.00 - 1452.00]</td>
<td>804.27±41.86 [269.00-3943.00]</td>
<td>800.10±54.88 [58.00-5055.00]</td>
<td>No [p&gt;0.05]</td>
</tr>
<tr>
<td>CD4/CD8 count ratio (%)</td>
<td>1.23±0.04 [0.46-2.41]</td>
<td>0.67±0.04 [0.15-2.51]</td>
<td>0.19±0.02 [0.02-1.76]</td>
<td>Yes [p&lt;0.05]</td>
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<tr>
<td>Hematocrit (%)</td>
<td>33.45±0.31 [24.00-41.00]</td>
<td>30.49±0.44 [17.00-44.00]</td>
<td>30.39±0.41 [20.00-44.00]</td>
<td>No [p&gt;0.05]</td>
</tr>
<tr>
<td>WBC count (Cells/μl)</td>
<td>5.07±0.10 [3.30-7.80]</td>
<td>4.85±0.11 [2.80-9.80]</td>
<td>4.82±0.11 [2.10-8.50]</td>
<td>No [p&gt;0.05]</td>
</tr>
<tr>
<td>Neutrophil count (%)</td>
<td>64.74±0.54 [50.00-78.00]</td>
<td>61.48±0.71 [37.00-76.00]</td>
<td>61.77±0.62 [43.00-80.00]</td>
<td>No [p&gt;0.05]</td>
</tr>
<tr>
<td>Lymphocyte count (%)</td>
<td>34.33±0.56 [21.00-48.00]</td>
<td>37.94±0.75 [22.00-73.00]</td>
<td>37.46±0.62 [20.00-57.00]</td>
<td>No [p&gt;0.05]</td>
</tr>
<tr>
<td>Monocyte count (%)</td>
<td>0.24±0.04 [0.00-2.00]</td>
<td>0.17±0.04 [0.00-2.00]</td>
<td>0.21±0.03 [0.00-1.00]</td>
<td>No [p&gt;0.05]</td>
</tr>
<tr>
<td>Eosinophil count (%)</td>
<td>0.62±0.07 [0.00-4.00]</td>
<td>0.57±0.07 [0.00-6.00]</td>
<td>0.58±0.06 [0.00-2.00]</td>
<td>No [p&gt;0.05]</td>
</tr>
<tr>
<td>Basophil count (%)</td>
<td>0.12±0.03 [0.00-2.00]</td>
<td>0.02±0.01 [0.00-1.00]</td>
<td>-</td>
<td>No [p&gt;0.05]</td>
</tr>
<tr>
<td>Total lymphocyte count (%)</td>
<td>178.04±6.33 [94.0-336.0]</td>
<td>186.7±6.14 [86-489.1]</td>
<td>177.10±3.84 [80.9-277.5]</td>
<td>No [p&gt;0.05]</td>
</tr>
</tbody>
</table>

Values=mean ± SEM, range in parenthesis.

Discussion

The present study presents normative values for CD4 cell counts, CD8 cell counts, TLC and CD4/CD8 cell count ratio in healthy HIV sero-negative and healthy HIV sero-positive male and female subjects in Port Harcourt, Nigeria. Previous studies in this regard have been relatively scarce and have focused on the effects of highly active anti-retroviral therapy (HAART) on CD4 cell count [15]; on use of absolute lymphocyte count as a marker of CD4 cell count and criteria for initiating ART [16]; and on hematological parameters in HIV-infected Nigerians in Port Harcourt [17].

The CD4 cell counts obtained in the present study are in the same range as in a recent report in HIV sero-negative Nigerians [18] and are fairly similar to values reported in Caucasians [5,8], Kuwaitis [19], Indians [20], and Tanzanians [21]. However, the CD8 cell counts obtained in the present study are marginally higher than values reported for Caucasians. The non-significant differences in the CD8 cell counts between the three groups is at variance with a recent report from Zaria, northern Nigeria, in
which both CD4 and CD8 cell counts were significantly lower in patients compared to controls [22]. However, the significant differences in the CD4 cell counts seen in the present study are consistent with that report, although our values are lower than the CD8 cell counts reported in healthy controls [22].

The results of the present study suggest that sex variations apparently do exist in both the pattern of differences and possibly in the prognostic value of the parameters under investigation. For instance, although in both sexes CD4 cell count and CD4/CD8 cell count ratio consistently showed significant differences in the three groups of subjects, TLC followed a similar pattern only in males. These sex differences in both the TLC and total WBC count seen in the present study are perhaps expected based on the reported sex variations in WBC and neutrophil counts [23] and the reported cyclic variation in WBC population during the normal menstrual cycle [24]. Apparently, menstrual cyclic variations in the WBC count could possibly contribute to obscuring the pattern in females likely leading to a sex distinction. This finding would, however, require further investigation. Perhaps these cyclic variations in females could indeed account for the absence of significant differences in the hematocrit scores in females as opposed to the pattern seen in males. Cyclic changes in the hematocrit scores during the normal menstrual cycle have also been reported by the present authors in Nigerians [25] and have been similarly described in Caucasians [26].

Amongst all the parameters studied, the results of the present study apparently suggest that in our environment, the CD4 cell count and CD4/CD8 cell count ratio are fairly reliable indicators of the progression of HIV infection in both males and females. This is of possible prognostic value and confirms the findings of previous studies in this regard [27]. The results, however, also suggest that the prognostic value of the CD8 cell count is limited and that of TLC is possibly sex-dependent.

From the results of the present study, we suggest that Nigerian physicians consider both the CD4 cell count and the CD4/CD8 cell count ratio more critically in determining the immune status of persons infected with HIV. In addition, the results suggest that the usefulness of TLC in females is limited and therefore can only be used with some caution in place of both the CD4 cell count and the CD4/CD8 cell count ratio.

In conclusion, the present study attempted to report normative values for CD4, CD8, TLC, and CD4/CD8 cell count ratio in healthy HIV sero-negative and HIV sero-positive (ART naive) individuals in Port Harcourt, southeastern Nigeria. In addition, the study reports significantly higher CD4 cell and CD4/CD8 cell count ratio in healthy HIV sero-negative subjects compared to HIV sero-positive (ART naive) subjects and AIDS subjects. Our results could be of possible prognostic importance and likely assist in the management of individuals infected with HIV in our environment.

References


