Establishment of T-lymphocyte subset reference intervals in a healthy adult population in Chennai, India


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Received April 4, 2007

Estimation of CD4+ T-lymphocytes continues to be an important aspect for monitoring HIV disease progression and response to antiretroviral therapy. Most of the diagnostic laboratories often rely on western text books for CD4+ T-lymphocyte reference values, which could often be unreliable for usage in local settings. Therefore, we attempted to establish the reference values for T-lymphocyte subsets among healthy adults in a cross-sectional study carried out at the YRG Centre for AIDS Research and Education (YRG CARE) in Chennai, south India, in 213 (84 female and 129 male) healthy, HIV-1/2 seronegative adults as volunteers. Whole blood specimens were processed for CD4+, CD8+ T-lymphocyte estimation and haematological parameters. The established range of CD4+ T-lymphocyte counts for men and women were 383 - 1347 cells/µl (mean 865 and median 845 cells/µl) and 448 - 1593 cells/µl (mean 1021 and median 954 cells/µl), respectively. Women had significantly higher absolute CD4+ T-lymphocyte counts ($P<0.001$) and CD4+/CD8+ T-lymphocyte ratio as compared to men. The established normal range of CD4+ T-lymphocyte % was 21 - 59 (mean 40.2 and median 40.1). The influence of age was not observed in any of the parameters except CD4+/CD8+ T-lymphocyte ratio with the >45 yr age group. Further studies with greater sample size may be required to define the staging of HIV disease in relation to the normal CD4 T-lymphocyte count in the general population.

**Key words** CD4+ T-lymphocyte - India - reference range

The depletion of CD4+ T-lymphocyte population in the blood is widely believed to be a better predictor of disease progression in acquired immunodeficiency syndrome (AIDS). Therefore, the estimation of CD4+ T-lymphocyte continues to play an important role in the monitoring of HIV disease progression and response to antiretroviral therapy (ART). Available evidences suggest that the variations in CD4+ T-lymphocyte could depend on certain important factors namely environment, ethnicity, genetic differences and dietary patterns in addition to age and gender. Although a few studies have been conducted in India at diverse locations in the past, most of the laboratories still depend on western textbook references, which are believed to be unreliable for usage in local diagnostic settings. Further, studies have shown that variations...
exist in relation to reference values of T-lymphocyte subsets, and warrants estimation measures be carried out in quality control established and good clinical laboratory practice (GCLP) certified laboratories. Hence, we established reference ranges for the adult population of Chennai, south India, in a prospective study carried out between March and August 2005, at the YRG Centre for AIDS Research and Education (YRG CARE) in Chennai, India.

In the present study, we enrolled 213 laboratory confirmed healthy HIV-1/2 seronegative volunteers to establish the reference interval of CD4+ and CD8+ T-lymphocyte subsets. The volunteers were invited to participate in the study through distribution of handouts and e-mails circulated to various organizations. The protocols involving human subjects were approved by YRG CARE’s Institutional Review Board prior to conducting the research. The study subjects comprised apparently healthy adults (age range 18-56 yr) from the general community. Before enrollment, the protocol was explained in detail and written informed consents were obtained from the volunteers. A study questionnaire was administered that included details viz., marital, extra-marital relations and medical history. Subjects with a history of connective tissue disorders; chronic illnesses like diabetes mellitus, hypertension, chronic renal disease, severe allergies, recent past immunization, steroid therapy in the past three months, antibiotic usage four weeks prior to enrollment, blood or blood product transfusion in the past 6 months, those with either a history of alcohol intake, smoking or strenuous exercise were excluded. In addition, each participant was examined to investigate certain physiological parameters like blood pressure, basal metabolic index (BMI), X-ray and electrocardiogram (ECG) before enrollment. Female subjects that claimed to be pregnant, with a questionable pregnancy status or within 5 days of last menstruation were ruled out by collecting blood only at specified hours in the morning (between 08.00-10.00 h). The blood specimens were also tested for certain infectious conditions; HIV1/2 antibody determination by double ELISA (Abbott HIV-1/2, Murex Biotech, Kent, UK and HIV Uni-form II Ag/Ab, Biomérieux, The Netherlands), rapid plasma reagin (RPR) (Span Diagnostics, Surat, India), Treponema pallidum passive agglutination (TPPA) (Serodia, Fujirebio, Japan), hepatitis B virus surface antigen (HBsAg) (Hepanostika HBsAg Unifom II, Biomérieux, The Netherlands) and anti-hepatitis C virus (HCV) by ELISA (Abbott Murex, South Africa). Persons reactive to any of these conditions were excluded.

The study initially enrolled 227 participants as per the National Committee for Clinical Laboratory Standards, 2000 (NCCLS), guidelines. Two subjects were excluded due to abnormalities seen in ECG/X-ray, 7 due to infectious conditions namely, syphilis (n=1), HBV (n=4) and HCV (n=2). Further, five subjects were also excluded due to antibiotic use during the study tenure.

The data generated were analyzed using the Statistical Package for Social Sciences software, version 13.0 (SPSS, IL, USA). Mann-Whitney U test was used to compare the distribution of lymphocyte subsets between the genders. To determine parameters that needed an upper and lower boundary (double boundary); the central 95 per cent of the distribution was employed. Reference interval was calculated from the mean 1.96 standard deviation (SD). For non-normally distributed analytes, the reference intervals were calculated non-parametrically from the central 95th percentile.
Of the total 213 participants (age range 18-56 yr) (mean and SD; 29.8 ± 7.6 yr), 129 (60.6%) were male and 84 (39.4%) were female. Ninety five per cent of the participants were Tamil speaking and the remaining were Telugu and Urdu speaking. The established CD4+ T-lymphocyte % and CD8+ T-lymphocyte % were 21-59 (mean 40.2) and 18-43 (mean 30.8), respectively. The distribution of CD4+ and CD8+ T-lymphocyte subset values and CD4/CD8+ T-lymphocyte ratios based on age and gender is presented in the Table. The established reference range of CD4+ T-lymphocyte count for men and women were 383-1347 cells/µl and 448 - 1593 cells/µl, respectively. The mean CD4+ T-lymphocyte count in men and women were 865 cells/µl (median 845 cells/µl) and 1021 cells/µl (954 cells/µl), respectively. Women had significantly (P<0.001) higher absolute CD4+ T-lymphocyte count and CD4:CD8+ T-lymphocyte ratio than males. However, there was no significant difference in CD8+ T-lymphocyte count with regards to gender. The established normal range of CD4+ T-lymphocyte % was 21 - 59 (mean 40.2 and median 40.1).

Lymphocyte subset values in HIV-seronegative individuals are reported to be affected by race, geographical location, gender, circadian changes, and physical exercise, and a wide variation in counts have also been reported world wide\(^5-9\). Researchers have reported a mean CD4+ T-lymphocyte count of 1036 cells /µl in healthy non-smokers\(^7\), while others have reported a median of 868 cells/µl in San Francisco, USA\(^18\). These studies clearly suggest the possibility of variation within the same ethnic group. Several reports on reference ranges of blood lymphocytes for the western population as well as for countries outside the western hemisphere are also available namely, Malaysia, Singapore, China, Uganda and Ethiopia\(^{18-22}\). Based on reported variations in normal values of lymphocyte subsets, there are questions whether CD4+ T-lymphocyte count thresholds used in western countries are appropriate for India. Though the ranges of lymphocyte sub-populations in Indians have been reported\(^{10-15}\), the information is based upon relatively small number of blood samples confined to certain geographical areas. It is also of interest to know how our target population differs from other populations studied across the world, and whether such differences need to be taken into account while interpreting data with regard to the immune status of such individuals in the Indian setting.

We observed differences in CD4+ T-lymphocyte reference intervals from other previously established studies from southern India\(^10\). We found that the ranges established among men and women were 383-1347 cells/µl and 448-1593 cells/µl, respectively. The findings of lower level of CD4+ T-lymphocyte in our study and those from Asia\(^{19-21}\) as compared with the western world\(^{2,18,23}\) indicates the requirement of newer staging criteria in India for treating HIV disease. Although some efforts\(^{10,13}\) have been initiated to revise the new staging criteria, more prospective studies need to be done, so that the population like ours with inherently low CD4+ T-lymphocyte counts would avail important clinical implications. Further, the significant differences in CD4+ T-lymphocyte values among the genders indicate that the use of a separate reference range (male and female) for the clinical interpretation

### Table. Age and gender distribution of absolute CD4+ and CD8+T-lymphocyte counts and CD4/CD8+ T-lymphocyte ratios in healthy adult population in Chennai, south India

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>Gender</th>
<th>Mean CD4</th>
<th>Mean CD8</th>
<th>Mean CD4/CD8 ratio</th>
<th>Mean CD4</th>
<th>Mean CD8</th>
<th>Mean CD4/CD8 ratio</th>
<th>Mean CD4</th>
<th>Mean CD8</th>
<th>Mean CD4/CD8 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>782</td>
<td>721</td>
<td>1.21</td>
<td>1011</td>
<td>694</td>
<td>1.51</td>
<td>911</td>
<td>729</td>
<td>1.36</td>
</tr>
<tr>
<td>18 - 25</td>
<td>Female</td>
<td>726</td>
<td>694</td>
<td>1.35</td>
<td>866</td>
<td>638</td>
<td>1.36</td>
<td>1090</td>
<td>730</td>
<td>1.58</td>
</tr>
<tr>
<td>26 - 30</td>
<td>Overall</td>
<td>890±599</td>
<td>708±481</td>
<td>1.34±1.02</td>
<td>914±472</td>
<td>729±533</td>
<td>1.36±0.93</td>
<td>981±585</td>
<td>813±663</td>
<td>1.33±0.86</td>
</tr>
<tr>
<td>31 - 35</td>
<td></td>
<td>880±543</td>
<td>704±517</td>
<td>1.32±0.77</td>
<td>880±543</td>
<td>704±517</td>
<td>1.32±0.77</td>
<td>926±550</td>
<td>730±540</td>
<td>1.38±1.00</td>
</tr>
<tr>
<td>36 - 45</td>
<td></td>
<td>865</td>
<td>738</td>
<td>1.28</td>
<td>842</td>
<td>667</td>
<td>1.38</td>
<td>956</td>
<td>704</td>
<td>1.38</td>
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<tr>
<td>&gt;45</td>
<td></td>
<td>856</td>
<td>704</td>
<td>1.38</td>
<td>956</td>
<td>704</td>
<td>1.38</td>
<td>129</td>
<td>730</td>
<td>1.38</td>
</tr>
</tbody>
</table>

*Mean CD4 & CD8 values are given in cells/µl
*P<0.001 compared to male
may be more appropriate, although many treatment guidelines have not considered the difference of CD4+ T-lymphocyte count in different genders. Similarly, CD4+ T-lymphocyte % values obtained were also different from other reports. Although our study proposes the range of 21-59 per cent, the other study has indicated a lower level of 31.8-34.2 per cent. However, our results pertinent to upper and lower ranges are comparable to certain north Indian and Asian studies excepting one recent north Indian study, where researchers have shown a lower CD4+ T-lymphocyte range of 17.6 per cent. Our study has certain limitations, especially the smaller sample size and the confinement to a single geographical area that restricted the performance of certain other descriptive investigations. Therefore, this necessitates a multi-centric approach to evaluate and compare the performances of healthy subjects in regards to T-lymphocyte subsets.

In conclusion, the establishment of normal ranges with the local population is a helpful tool to clinicians for the better clinical management of HIV disease in southern India. Future cohorts with greater sample size may be required to define the staging of HIV disease in relation to the normal CD4+ T-lymphocyte count subsets in the local general population.

Acknowledgment

Authors thank the clinical and laboratory staff of YRG CARE, Chennai for their assistance and Shri Rajakumar for helping in searching relevant literature.

References


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