Introduction: In developing countries, the standard methods used to monitor HIV disease progression and therapy response are clinical assessment, CD4+ T lymphocyte count measurement, and plasma viral load (PVL) quantification. These tests require expensive equipment and skilled technicians, so monitoring HIV in resource-limited countries remains challenging as few laboratories can offer these tests free of cost.

Methodology: Newly diagnosed HIV seropositive subjects (n = 130) were categorized into three study groups: CD4 counts <200 cells/µl (group A, 43 subjects); 200-500 cells/µl (group B, 44 subjects); and >500 cells/µl (group C, 43 subjects). At recruitment, PVL estimation was performed for group A subjects only, who were then initiated on highly active antiretroviral therapy (HAART) and were followed up after six months for evaluation of response to HAART by measuring the CD4 counts and PVL. Groups B and C were followed up after six months to monitor disease progression by measuring only CD4 counts.

Results: Among group A subjects, a rise in the median CD4 counts after six months of HAART was observed. At baseline, PVL ranged from 2636 to >750,000 copies/ml with a median PVL at baseline of 165,000 copies/ml. At follow-up, 90% of the study subjects had undetectable levels of viraemia. Among group B and C subjects, a fall in the CD4 counts at follow-up was observed.

Conclusions: CD4 count is a powerful tool to determine response to antiretroviral therapy (ART) and monitor disease progression in HIV/AIDS. PVL is important to assess response to ART, especially in immunovirologic discordant responses.

Key words: CD4+ T lymphocyte count (CD4 count); HIV; highly active antiretroviral therapy (HAART); plasma viral load (PVL)


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with absolute counts, the impact of antiretroviral therapy (ART) on the predictive value of the CD4 count, the role relative to other markers such as viral load, the derivation of scores to predict AIDS diseases, and the use of CD4 count as a surrogate endpoint, among others. Moreover, CD4 count does not reflect activation of the immune system, particularly in cases of immunovirologic discordant responses. In spite of all this, it continues to be a mainstay of monitoring for both untreated and treated patients [8,9].

The value of HIV-RNA quantification (Plasma HIV-1 viral load) as a prognostic marker has long been established. An approximately inverse relationship to the CD4 count and survival time has been observed in around 80% of patients [10,11]. Higher HIV-RNA levels are associated with more rapid decline of CD4+ T-cells, assisting prediction of the rate of CD4 count decline and disease progression [12,13]. Treatment response has been strongly linked to the baseline HIV-RNA level. Monitoring viral load is critical to assessing the efficacy of ART [14].

It has been shown by many Indian and foreign studies that CD4 counts fall and viral loads rise after successful antiretroviral therapy, whereas CD4 counts fall progressively in patients without HAART as the disease advances [15-17]. However, in some groups of patients, viral load decreases without appropriate immunologic recovery after HAART. Conversely, other patients respond immunologically to HAART without an important suppression in viral load. These two scenarios are known as discordant responses [18-21].

Our study aimed to analyze disease progression and therapy response in newly diagnosed HIV seropositive subjects in a tertiary care centre in North India.

**Methodology**

The study was performed in the Department of Microbiology, Maulana Azad Medical College, New Delhi, from September 2007 to March 2010. A total of 130 newly diagnosed adult HIV seropositive subjects were enrolled. The study group was comprised of 88 males and 42 females over the age of 18 years. Informed consent was obtained from each participant prior to enrollment. The subjects were registered with the ART clinic of the hospital, staged according to the WHO clinical staging [22], and were then referred for CD4 count testing.

At the time of recruitment, blood samples were collected for CD4 counts in K3 EDTA (liquid) Vacutainer tubes (Becton, Dickinson and Company, Franklin, NJ, USA). All study subjects were categorized into three study groups as follows: CD4 counts fewer than 200 cells/µl (group A, 43 subjects); 200 to 500 cells/µl (group B, 44 subjects); and more than 500 cells/µl (group C, 43 subjects). Blood sample for PVL estimation was obtained for group A subjects only, at recruitment.

CD4 counts were determined by the FACSCount system (Becton, Dickinson and Company, San Jose, CA, USA). Plasma viral load (PVL) was estimated using an Amplicor HIV-1 Monitor Test, version 1.5 (Roche Diagnostics, Branchburg, NJ, USA) by the standard procedure (Detection limit 400-7, 50,000 copies/ml).

Group A cases were initiated on HAART including two nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI) per National AIDS Control Organization recommendations [22]. All subjects (40 subjects) with haemoglobin counts higher than 8g/dl were started on zidovudine, lamivudine and nevirapine. Subjects who were anemic at the time of recruitment (3 subjects) or those who developed severe anemia (2 subjects) during the course of the above-said regimen were given a combination regimen of stavudine, lamivudine and nevirapine. These cases were followed up after six months for evaluation of response to HAART by measuring the CD4 counts and PVL. Group B and C cases were followed up after a period of six months to monitor disease progression by measuring the CD4 counts only. Forty-two age- and sex-matched HIV-negative healthy controls were also included for comparing CD4 counts with HIV-positive individuals.

To detect whether there was a significant difference in the levels of CD4 counts between baselines and follow-up, the Wilcoxon signed rank test was applied. The adopted significance level for statistical inference was 5% (p value < 0.05).

**Results**

The median age of the HIV-seropositive study subjects was 32.5 years (IQR 24.2-40.8) with a male to female ratio of 2.1:1. The predominant age group in males and females was 26 to 30 years. The most common mode of acquiring infection was heterosexual contact (85.38%) followed by intravenous drug abuse (9.2%). The majority (75%) of the study subjects were from urban areas. Thirty-one percent of the males and 42% of the females were illiterate. Three percent of the study subjects had a positive VDRL (venereal disease research laboratory) test for syphilis, 6.9%
were positive for Hepatitis B surface antigen (HBsAg), and 2.3% were positive for anti-Hepatitis C virus (HCV) antibodies.

The median values of CD4 counts were significantly higher (p < 0.5; 0.001) in the healthy control group as compared to HIV-positive study subjects (805 cells/µl; IQR 645-1063 versus 279 Cells/µl; IQR 154-482) at baseline.

Eighty of the 130 subjects recruited in our study reported for follow-up, and there were 15 fatal cases. Seven of these deaths were attributed to complications caused by AIDS and 4 to tuberculosis. Cause of death could not be ascertained in 4 cases because of lack of proper medical facilities in the rural villages where these patients lived; therefore, data on the cause of the terminal illness in these patients could not be collected. Thirty-five cases were lost to follow-up, which could be explained by fact that patients who were relatively symptom free did not feel the need to report to the ART clinic for follow-up after six months. Furthermore, some patients moved from their local residence to their native place to avoid stigma associated with the disease. Among group A subjects (n = 43), twenty-six (60.5%) subjects reported for follow-up and the adherence rate was greater than 95%. Nine (20.9%) subjects died within six months (four due to tuberculosis and five due to AIDS), and eight (18.6%) were lost to follow-up. Among group B subjects (n = 44), thirty-four (77.3%) subjects reported for follow-up, three subjects (6.8%) died within six months (two due to AIDS and case not known in one case), and seven subjects (15.9%) were defaulters. Among group C subjects (n = 43), twenty (46.5%) reported for follow-up, three subjects (7%) died within six months (cause unknown), and 20 subjects (46.5%) were defaulters.

Table 1 shows the change in the CD4 counts of the subjects in the groups A, B and C at follow-up after six months in relation to the WHO clinical stage of subjects at baseline. The number of subjects in the four different stages is also shown in the three groups.

In group A, there was a statistically significant rise in the median CD4 counts after HAART (Table 1). Out of the 26 subjects who reported for follow-up,
PVL was performed only for 20 subjects due to limited resources. For these subjects at baseline, PVL ranged from 2,636 to more than >750,000 copies/ml with a median PVL at baseline of 165,000 copies/ml. At baseline 75% of subjects with CD4 count ≤ 50 had a viral load of greater than 100,000 copies/ml (range 140,000- >750,000 copies/ml) (Table 2). At follow-up, the viraemia level was undetectable (i.e., viral load of less than 400 copies/ml) in 90% of the study subjects (Table 3). Three of the group A cases showed immunological failure according to National AIDS Control Organisation (NACO) guidelines. Out of these, two cases exhibited a fall in CD4 levels after six months of HAART (195 cells/µl at baseline to 88 cells/µl at follow-up and 110 cells/µl at baseline to 94 cells/µl at follow-up), while one case had CD4 levels persistently below 100 even after completing six months of HAART (5 cells/µl at baseline to 40 cells/µl at follow-up). Two of these cases with immunological failure showed undetectable viraemia at follow-up after six months of HAART, while one showed a viral load of 1,187 copies/ml (the case with CD4 counts of 195 cells/µl at baseline fell to 88 cells/µl at follow-up). One of our cases showed a viral load of 407 copies/ml at follow up (>750,000 copies/ml at baseline) but responded well immunologically (CD4 counts of 142 cells/µl at baseline rising to 252 cells/µl at follow-up). Therefore, immunovirologic discordant response was noted in three of our cases (15%). Among group B, there was a fall in the level of CD4 counts at follow-up which was not statistically significant. Among group C, a statistically significant fall in the CD4 counts was observed at follow-up (Table 1).

Table 2. Relation of CD 4 count with plasma viral load at baseline (n = 20)

<table>
<thead>
<tr>
<th>CD 4 COUNT (cells/µl)</th>
<th>Number (%) of cases with PVL in copies/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 10,000</td>
</tr>
<tr>
<td>≤ 50</td>
<td>0</td>
</tr>
<tr>
<td>51-100</td>
<td>1 (5)</td>
</tr>
<tr>
<td>101-150</td>
<td>1 (5)</td>
</tr>
<tr>
<td>151-199</td>
<td>1 (5)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3 (15)</td>
</tr>
</tbody>
</table>

Conclusion

CD4 count is a powerful tool to determine response to HAART and also to monitor disease progression in newly diagnosed HIV-seropositive subjects. The drawback of this test is that it does not
pick up non-responders to first-line ART with 100% certainty. Therefore, to prevent unnecessary switches to second-line ART, immunological failure should not be interpreted as failure to first-line ART without considering a viral load test. Unfortunately, in a country such as India where the disease burden is concentrated in rural areas, this may not be feasible as PVL estimation is not widely available. In 2008, NACO piloted a national strategy for the provision of free second-line ART in India; thus it is necessary to provide free-of-cost PVL estimation facilities in a large number of centers in India.

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References


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