

## Study on CD4 cell responses in HIV infected subjects in Nepal

BR Tiwari,<sup>1</sup> P Ghimire<sup>2</sup> and S Malla<sup>3</sup>

<sup>1</sup>Nepal Red Cross Society, Central Blood Transfusion Service; <sup>2</sup>Central Department of Microbiology, Tribhuvan University and <sup>3</sup>National Public Health Laboratory, Kathmandu, Nepal

Corresponding author: Mr. Bishnu Raj Tiwari, NRCS, Central Blood Transfusion Service, Kathmandu, Nepal, e-mail: bishnu\_117@yahoo.com

### ABSTRACT

HIV infection is characterized by gradual deterioration of immune function mainly the CD4 cells. This study was conducted with the objectives to evaluate the kinetics of CD4 cell depletion and duration of HIV infection and the role of ART in improving CD4 cell levels specifically in Nepalese HIV patients. During April 2005 to March 2006, all together 220 blood samples collected from 110 HIV patients visiting National Public Health Laboratory (NPHL), Kathmandu, were analyzed for CD4 cell count using standard protocol. CD4 cell count before and after starting of anti-retro viral therapy showed significant association ( $P < 0.05$ ). The results of this study clearly indicated that antiretroviral therapy has been playing a role in maintenance CD4 cell counts in HIV infected patients.

**Keywords:** HIV infection, CD4 cell count, ART, Nepal.

### INTRODUCTION

HIV infection is characterized by a gradual deterioration of immune function. Most notably, crucial immune cells called CD4 cells are disabled and killed during the typical course of infection.<sup>1</sup> HIV is persistent and ultimately progressive in the vast majority of untreated hosts, there is increasing evidence that HIV have specific cellular immune responses playing a major role in determining the tempo of viral replication and thus the clinical outcome of infection.<sup>2</sup>

In humans, evidence supporting a protective effect of cellular immune responses in HIV infection is less direct. Long-term nonprogressive infection has been associated with both strong virus-specific CTL and with robust *gag* p24-specific CD4 cell proliferative responses.<sup>3</sup> Indeed, some studies have reported a direct inverse correlation between viral load and HIV-specific T-cell responses in untreated HIV-infected subjects.<sup>4</sup>

CD4 cell count is used to determine how well the immune system is working in people who have been diagnosed with HIV. The pattern of CD4 cell counts over time is more important than any single CD4 cell value because the values can change from day to day. The CD4 cell pattern over time shows the effect of the virus on the immune system. CD4 cell counts generally decrease as HIV progresses untreated HIV-infected subjects.<sup>4</sup>

This study was designed to understand the CD4 cell depletion kinetics and role of anti-retroviral therapy (ART) in maintaining CD4 cell count based at National Public Health Laboratory (NPHL) where HIV/AIDS patients are referred for CD4 cell evaluation before starting and during ART.

### MATERIALS AND METHODS

This was a cross-sectional descriptive study conducted during April 2005 to March 2006, based at NPHL with the voluntary involvement of HIV seropositive (HIV +) Nepalese males and females. The study population comprised of 110 HIV visiting NPHL, Kathmandu, during the study period. At least two blood samples from each case were collected (taking all due precautions) at the interval of six months. Information on antiretroviral therapy and other treatments were collected by filling a questionnaire from the participants. An informed consent taken from each of the participant included.

Three milliliters of blood samples from each subject were collected in K<sub>3</sub>EDTA. The collected samples were subjected for CD4 cell count within an hour of collection following standard aseptic procedures. CD4 cell count was done using dedicated Flow Cytometry Absolute Cell Count System at NPHL using standard protocol<sup>5</sup> employing standard quality control system. The results of the test were recorded in excel-sheet and analyzed using standard statistical tools as required.

All subjects were further categorized based on the 1993 revised classification system for HIV infection by CD4 cell count categories (<200, 200-499 and >500 cells/mm<sup>3</sup>).<sup>6</sup> The subjects having the CD4 cell count less than 200/mm<sup>3</sup> were selected for the course of ART.

### RESULTS

A total of 220 blood samples from 110 HIV+ (62 males and 48 females) were analyzed. Out of total 110 HIV+, 52.0% were under ART and the remaining 48.0% were not (Table-1). The status of CD4 cell count before and after 6 months of receiving ART and subjects not under ART are shown in Fig. 1. The mean CD4 cell count in

**Table-1:** Distribution of HIV patients taking antiretroviral therapy

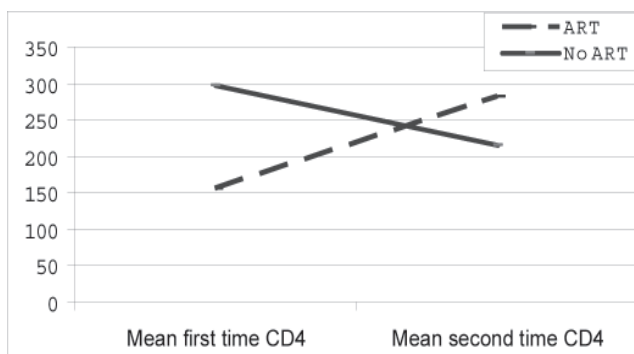
Subjects	No of patients		Total
	Male	Female	
Taking ART	30	27	57
No taking ART	32	21	53
Total	62	48	110

patients at first visit to NPHL was 155/mm<sup>3</sup> which was seen to be increased (297/mm<sup>3</sup>) after six months of ART. On the other hand, among the subjects not receiving ART, the mean CD4 cell count dropped from 281/mm<sup>3</sup> to 214/mm<sup>3</sup> in six months interval. The mean CD4 cell count before and after ART values were statistically significant at P<0.05% level. There was no significant difference in the pattern of CD4 cell count change before and after ART in both the sex.

**DISCUSSION**

The antiretroviral drug Zidovudine was introduced in 1986 for the treatment of HIV/AIDS.<sup>7</sup> Over the next few years, also other antiretroviral drugs such as nucleoside reverse transcriptase (NsRTIs), non-nucleoside reverse transcriptase (NNRTIs) and protease inhibitors (PIs) were introduced and at present, three or more ART drugs are recommended worldwide for the treatment of HIV+.<sup>8</sup> HIV/AIDS patients are rapidly increasing in Nepal with a concentrated epidemic in certain specific population.<sup>9</sup> Keeping in view of this fact, antiretroviral treatment in Nepal was introduced in 2004.<sup>7</sup>

Higher number of male participants represented the total HIV infected population in Nepal.<sup>10</sup> However, irrespective of gender, most of them were aware on their HIV infection status. Most of the participants had mid level education (ten class and above) and acquired counseling. This may be the reason for their knowledge about HIV infection and the immune status.



**Fig 1:** Comparison of CD4 cell count in HIV infected subjects receiving ART and no ART

Much understood fact regarding CD4 cell depletion, highest T-cell activation states, T-cell dynamics, and HIV-specific T-cells in HIV infection is derived from the analysis of peripheral blood lymphocytes.<sup>11,12</sup> This CD4 cell depletion and effectiveness of ART in treatment of HIV+ in context of Nepal is not yet understood. This study, therefore, was conducted to understand CD4 cell depletion kinetics and effectiveness of ART among Nepalese. In this study, the mean CD4 cell count in patients at first visit to NPHL (155/mm<sup>3</sup>) increased (297/mm<sup>3</sup>) significantly after six months of ART. This finding indicated that the treatment was effective. Gullick *et al*<sup>13</sup> and Pakkar *et al*<sup>14</sup> reported that the gradual CD4 cell count rise are likely to reflect the generation of new cells by peripheral expansion of pre existing T-cell clones or generation of thymically derived naïve cells among ART patients. However, the CD4 cell count did not attained above 500 cell/mm<sup>3</sup> and we are not in position to tell what could be the result had the cases were followed for longer period. However, Hunt *et al*<sup>15</sup> reported the continuous increase of CD4 cell count among the HIV/AIDS subjects who were receiving highly active antiretroviral therapy (HAART). Our finding was in agreement with the result reported by Bosch *et al*<sup>11</sup> and Crystal *et al*.<sup>12</sup> For the best of our knowledge this is the first report on CD4 cell response to HIV+ in Nepal.

Among the HIV+ subjects not receiving ART the mean CD4 cell count was 281/mm<sup>3</sup> during the first CD4 cell monitoring. The CD4 cell count continuously and significantly decreased to 214/mm<sup>3</sup> in six month interval. The finding of the present study was in agreement with the findings of Fauci<sup>16</sup> and Pantaleo *et al*.<sup>17</sup> We have not elucidated the mechanisms of such depletion of CD4 cell count. However, several investigators reported that the CD4 cell count decreased due to the disruption of the cell membrane as HIV buds from the surface or the intracellular accumulation of heterodisperse RNAs and unintegrated DNA.<sup>18,19,20</sup> Hoxie *et al*<sup>21</sup> also suggested that intracellular complexing of CD4 cell and viral envelope products can result in cell killing. Similarly, other investigators proposed that the untimely induction of a programmed cell death as an additional mechanism for CD4 cell loss in HIV infection.<sup>22</sup>

The CD4 cell count of HIV+ subjects showed that there is 24.0% drop in 6 months who have not receiving ART. This requires further study to understand its progression in a year or two. For a country like Nepal, opportunistic infection are common due to pollution, unhygienic living condition and exposure to pollens and other agricultural materials; avoiding opportunistic infection may prolong the life span of the HIV infected people.<sup>23</sup> Result of the study showed that ART taken in time is able to maintain

the CD4 cell count thus preventing from opportunistic infections. Further continuation and extension of such work is required to understand better kinetics of CD4 cell depletion in Nepalese HIV+.

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