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Short Communication

Improvement in Immunological Parameters in Patients Receiving Highly Active Anti-Retroviral Therapy in Nepal

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SUMMARY: Highly active anti-retroviral therapy (HAART) has been freely available in Nepal since 2004. In the present longitudinal study, we followed two distinct cohorts of human immunodeficiency virus-infected participants, those receiving HAART and those under assessment of eligibility for HAART, during the period 2005–2007 in Kathmandu, Nepal. The median change in CD4+ T-cell count among participants receiving HAART after 12 months of the initiation of therapy was +118 T cells/μl (95% confidence interval [CI], +91 to +145 T cells/μl) and that among participants under assessment of eligibility for HAART was −74 T cells/μl (95% CI, −103 to −44 cells/μl). However, the median CD8+ T-cell count after 12 months remained stable in both the cohorts ($P > 0.05$). The CD4+/CD8+ T-cell ratio increased from 0.16 to 0.26 after 12 months of therapy ($P < 0.001$). The multivariate regression model revealed that participants >30 years of age, and injection drug users had significantly lower increases in the CD4+ T-cell count in response to therapy. We observed a high proportion of loss to follow-up after 12 months of therapy; however, the associated factors were unknown. In conclusion, we observed a significant improvement in the CD4+ T-cell count in participants receiving HAART; however, the CD4+/CD8+ T-cell ratio remained <0.5 after 12 months of treatment.

The prevalence of adult human immunodeficiency virus (HIV) infections in Nepal has been estimated to be 0.39%, with approximately 5,000 acquired immunodeficiency syndrome (AIDS) deaths per year (National Center for AIDS and Sexually Transmitted Diseases Control, 2010) (1). Highly active anti-retroviral therapy (HAART) has been freely available in Nepal since 2004. The national guidelines for anti-retroviral therapy (ART) available during this study period described three components for monitoring the therapy, namely, clinical visits, adherence, and laboratory monitoring of CD4+ T-cell counts every 6 months from the initiation of therapy. The efficacy of ART was indicated by clinical improvement and an increase in CD4+ T-cell counts, although a decrease in the viral load was mentioned; however, the viral load measurement facility was not available before 2008 in Nepal (2). Here we report the outcomes of a longitudinal observational study in the National Public Health Laboratory (NPHL), Kathmandu, from 2005 to 2007, designed to evaluate the immunological improvements or deterioration in a cohort receiving HAART and a cohort under assessment of eligibility for HAART. The study aimed to provide evidence on the impact of the HAART program in Nepal during its initial phase.

The demographic and clinical information of the participants was collected through the case files register maintained at NPHL as a part of the anti-retroviral monitoring protocol of the government of Nepal. Informed consent was obtained from all the participants. Ethical approval for the study was obtained from Tribhuvan University, Nepal. Study cohorts were followed-up until 18 months after enrollment in the study and CD4+ and CD8+ T-cell counts were taken at 6-month intervals. Although the follow-up was continued until 18 months from the date of enrollment, analysis included data until 12 months because more than 70% loss to follow-up or censoring was observed after 12 months in both the cohorts. Eligibility for HAART was assessed by the treating physicians on the basis of a CD4+ T-cell count of <200 cells/μl or according to the clinical symptoms of AIDS irrespective of the CD4+ T-cell count (2,3). In the cohort naïve to HAART, participants were censored if the CD4+ T-cell count on successive visits decreased to <200 cells/μl or the treating physicians decided to initiate anti-retroviral treatment on the basis of clinical symptoms. The anti-retroviral treatment during the study period involved a combination therapy with 2 nucleoside reverse transcriptase inhibitors (NsRTIs) plus 1 non-nucleotide reverse transcriptase inhibitor (NNRTI) or 2 NsRTIs plus 1 protease inhibitor (PI) or 2 nucleotide reverse transcriptase inhibitors (NtRTIs) plus 1 NsRTI (Abacavir) (2).

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Table 1. Characteristics of the study cohorts

Characteristic	Receiving HAART	Under assessment of eligibility for HAART
No. of participants	253	255
Age, years	31 (S.D, 9.1)	28 (S.D, 10.2)
Male (%)	161 (63.6%)	163 (63.9)
Duration since HIV diagnosis to enrolment in study, median weeks (95% confidence interval)	24 (12 to 36)	12 (6 to 36)
Injection drug use as a mode of transmission	113 (44.7%)	108 (42.4%)
Commercial sex worker as a mode of transmission	95 (37.5%)	85 (33.3%)
Proportions follow-up until 1 year	96.8%	70.6%

HAART, highly active anti-retroviral therapy; S.D, standard deviation.

Table 2. Median CD4+ T-cell count and CD4+/CD8 T-cell ratio in the study cohorts

Receiving HAART			
Variable	0 month (n = 253)	6 months (n = 253)	12 months (n = 245)
Median CD4+ T-cells/μl (95% CI)	141 (126–156) (Reference)	215 (200–230) ($P < 0.001$)	271 (248–294) ($P < 0.001$)
Median CD8+ T-cells/μl (95% CI)	901 (820–983) (Reference)	945 (889–1000) ($P = 0.13$)	975 (885–1065) ($P = 0.05$)
Median CD4+/CD8+ cell ratio (95% CI)	0.16 (0.14–0.17) (Reference)	0.24 (0.21–0.27) ($P = 0.003$)	0.26 (0.21–0.31) ($P < 0.001$)
Assessment for eligibility to HAART			
Variable	0 month (n = 255)	6 months (n = 255)	12 months (n = 180)
Median CD4+ T-cells/μl (95% CI)	357 (333–381) (Reference)	326 (294–358) ($P < 0.001$)	274 (245–304) ($P < 0.001$)
Median CD8+ T-cells/μl (95% CI)	1110 (1058–1162) (Reference)	1075 (939–1211) ($P = 0.12$)	1091 (903–1279) ($P = 0.06$)
Median CD4+/CD8+ cell ratio (95% CI)	0.33 (0.30–0.36) (Reference)	0.29 (0.27–0.32) ($P < 0.001$)	0.26 (0.23–0.29) ($P < 0.001$)

HAART, highly active anti-retroviral therapy; CI, confidence interval; n, number of participants.

We followed 253 participants receiving HAART and 255 participants under assessment of eligibility for HAART. The demographic and clinical characteristics of the participants are shown in Table 1. In this study, we quantified the immunological improvements or deterioration in terms of an increase or decrease in the CD4+ T-cell count and the CD4+/CD8+ T-cell ratio and identified factors associated with such changes. CD4+ and CD8+ T-cell counts were determined using the flow cytometry method (FACS Count, Becton Dickinson, San Jose, Calif., USA). The absolute value of CD4+ and CD8+ T-cell counts at 6-month intervals, change in CD4+ and CD8+ T-cell counts after 12 months of therapy, and the CD4+/CD8+ T-cell ratio are expressed as medians with 95% confidence intervals (CIs) around the point estimate calculated by bootstrapping using the replacement method (1,000 repetitions) (Table 2). In addition, the statistical significance of changes in immunological parameters at 6 and 12 months compared with the baseline value at 0 month was estimated using the signed-rank test and is shown in Table 2. All statistical analyses were performed using STATA 10.1 (StataCorp, College Station, Tex., USA).

Among the participants receiving HAART, the median CD4+ T-cell count was 141 cells/μl at the initiation of treatment, which increased to 271 cells/μl at the end of 12 months. The median change in the CD4+ T-cell count was +118 cells/μl (95% CI, +91 to +145 cells/μl). The CD4+ T-cell count increased from <200 T cells/μl at the initiation of therapy to ≥ 200 cells/μl in 50% of the participants. However, the median CD8+ T-cell count was stable over the study period in both the cohorts (+70 cells/μl, 95% CI, −37 to +177 cells/μl in the HAART cohort; +25 cells/μl, 95% CI, −73 to +122 cells/μl in the assessment of eligibility cohort). The median CD4+/CD8+ T-cell ratio at the start of the HAART trial was 0.16 (95% CI, 0.14 to 0.17), which increased to 0.26 (95% CI, 0.21 to 0.31) in 12 months (Table 2). In contrast, we observed a significant decrease in the CD4+ T-cell count in participants naïve to HAART and the median change in CD4+ T-cells after 12 months of enrollment in the study was −74 cells/μl (95% CI, −103 to −44 cells/μl). The CD4+ T-cell count decreased from ≥ 200 cells/μl at the start of study to <200 cells/μl in 15% of the participants (Table 2).

The average change in CD4+ T-cell counts after 12 months of commencement of HAART was modelled using the multivariate least-square linear regression method. The initial multivariate model included the covariates of age, sex, duration since HIV diagnosis to enrollment in study, and mode of transmission (i.e., injection drug use and commercial sex worker). The final model was selected by backward stepwise regression, retaining only the statistically significant covariates. The final model showed that in the HAART cohort, the increase in CD4+ T-cell counts was significantly lower in participants >30 years of age ($P = 0.018$) and par-

Table 3. Multivariate analysis of variables associated with mean change in CD4+ T-cell counts in the HAART cohort after 1 year

Characteristic	Initial model Change in CD4+ T-cell count/μl (95% confidence interval)	P	Final model Change in CD4+ T-cell count/μl (95% confidence interval)	P
Age >30 years	−58 (−107 to −10)	0.018	−56 (−102 to −10)	0.018
Male	25 (−37 to 86)	0.4	—	—
Time from diagnosis to HAART >24 weeks	40 (−5 to 86)	0.08	—	—
Injection drug user	−68 (−149 to 13)	0.09	−51 (−97 to −6)	0.028
Commercial sex worker	−5 (−75 to 66)	0.8	—	—
Intercept	171 (106 to 236)		174 (125 to 223)	—

HAART, highly active anti-retroviral therapy; intercept, the mean CD4+ T-cell count, when all co-variates are in their reference categories. Similar analysis in the cohort under assessment of eligibility for HAART showed a mean change in the CD4+ T-cell count to be −89 cells/μl (95% confidence interval, −151 to −27 cells/μl); however, none of the co-variates were significantly associated.

ticipants with injection drug use as a mode of transmission ($P = 0.028$) (Table 3). However, a similar multivariate model in patients not receiving HAART showed that there was no significant difference in the mean change in CD4+ T-cell counts across any variable.

This study revealed a significant increase in CD4+ T-cell counts in participants receiving HAART over time. However, we observed that a large proportion of the participants did not undergo regular immunological monitoring after the first year of the initiation of therapy. Within the scope of this study, it was not possible to identify specific factors responsible for such a significant loss to follow-up, although probable factors may be the centralized CD4+ T-cell monitoring facility in the capital city only, deaths, or discontinuation of therapy. In this study, we also observed that approximately 50% of the participants' CD4+ T-cell counts did not increase to the level of ≥200 cells/μl within 12 months of the initiation of therapy. This may be due to the initiation of HAART at a very late stage of AIDS or in part due to immunological non-responders (4). However, the magnitude of improvement in the CD4+ T-cell count in our study was comparable with that in similar studies conducted in India (5,6). On the basis of the time period since the diagnosis of HIV to the initiation of therapy and the baseline median CD4+ T-cell count, it is evident that the diagnosis of HIV as well as the initiation of HAART in our study in fact occurred at very late stages of the disease.

Multivariate analysis revealed that the initiation of HAART in participants >30 years of age and injection drug use as a mode of transmission were associated with lower increases in CD4+ T-cell counts compared with the reference values. This is plausible because people >30 years of age may have contracted the infection relatively earlier and injection drug users are a vulnerable group with other factors associated with relatively poor health. Poor response to HAART among injection drug users has been described in several other studies (7,8). Similarly, we observed a significant drop in CD4+ T-cell counts within a period of 12 months in the cohort under assessment of eligibility for HAART, which may be linked to late diagnosis of the disease and/or late initiation of immunological monitoring, as discussed previously.

Our study is the reflection of a real world scenario while introducing HAART in a previously unavailable area with relatively poor infrastructure for treatment monitoring. The present study has several limitations. Although the participants were followed-up for 18 months, we did not formally include the analysis of data collected after12 months because there was a significant loss to follow-up; however, if these data were included, the analysis still shows a significant increase in CD4+ T-cell counts among participans who were in the study at 18 months ($P < 0.001$) (median CD4+ T-cell count, 294 cells/μl, 95% CI, 228 to 360 cells/μl). In addition, only immunological monitoring of the patients may not be the best reflection of treatment outcomes; however, virological monitoring was not available in Nepal during this study period and we do not have data on their clinical conditions.

In conclusion, we observed a significant increase in CD4+ T-cell counts in the HAART cohort as well as a rapid decrease in CD4+ T-cell counts among a recently diagnosed HIV-infected population naïve to HAART within a 1-year follow-up period. Early initiation of HAART, a proper mechanism for better follow-up, and special attention to relatively older patients and injection drug users should be the targets for better outcomes.

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Conflict of interest None to declare.

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