Original Article

Changes in Serum Levels of IL-6, TNF-α and CRP in People Living with HIV Following Initiation of Antiretroviral Therapy — A Longitudinal Cohort Study from a Tertiary Hospital In Eastern India

Madhuchhanda Mandal (Kayal)[1], Sumona Basu[2], Debarshi Roy[3], Anindita Debnath[4] Subhasish Kamal Guha[5]

Background and Objectives : Combination Antiretroviral Therapy (cART) is routinely used in HIV/AIDS patient care. The effect of Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) based first-line cART drug regime is observed with changes in immune activation. This study aimed to examine the effect of cART drugs (Tenofovir, Lamivudine and Efavirenz) on serum cytokine levels for HIV disease progression.

Methodology : 130 treatment-naïve HIV-infected patients were enrolled and their ART eligibility was confirmed as per National AIDS Control Organization (NACO) guidelines. Blood samples were collected for quantification of CD4+ cell count (by flow cytometry), HIV-1 Plasma Viral Load (PVL) by RT-PCR and serum cytokines (IL-6, TNF-α and CRP) by ELISA at 0, 24 and 48 weeks after treatment initiation. The treatment-naïve subjects were similarly followed up. Baseline values served as the control for both arms for statistical analysis.

Results : Patients on ART reported a significant decrease in IL-6 and CRP serum levels after 24 weeks of treatment initiation, TNF-α level showed minimal changes after 24 weeks which proceeded to increase significantly at the end of 48 weeks. The on-ART group also exhibited a substantial decrease in HIV Plasma Viral Load. CD4+ cell population showed a significant rise after 24 weeks of cART initiation.

Interpretation and Conclusion : This study on a patient population showed a negative correlation between the CD4+ cell population and serum cytokine concentration (after the administration of cART drugs). A decline in PVL supports the use of NNRTI-based cART in the management of HIV/AIDS.

[*J Indian Med Assoc* 2023; **121(9):** 20-4]

Key words **: cART, CD4+, CRP, IL-6, PVL, TNF-α.**

Initial detection of HIV through sero-surveillance in 1986 prompted a massive organized response from National AIDS Control Organization (NACO) to collaborate on HIV prevention and treatment plans with affiliate hospitals, medical centres, and NGOs[1]. The stabilising decline in HIV adult prevalence rate has been attributed to accessible free diagnostic services, integrated health care facilities such as Anti-Retroviral Therapy (ART), Parent-to Child Transmission of HIV (PPTCT) services, management of ensuing Opportunistic Infections (OIs), nutritional and psychological counselling[2].

Department of Tropical Medicine, Calcutta School of Tropical Medicine, Kolkata 700073

[1]MD, Assistant Professor

[2]MSc Molecular Biology and Human Genetics, Research Scholar, Department of Laboratory Medicine,

[3]MSc Biotechnology, Research Assistant, Department of Laboratory Medicine

[4]PhD, Demonstrator, Department of Laboratory Medicine and Corresponding Author

[5]MD, Director

Received on : 16/02/2023

Accepted on : 06/04/2023

Editor's Comment :

- Extrapolation of broader spectrum of cytokine analysis, may help us to understand newer aspects of inflammatory response in HIV infection and subsequent changes following ART administration.

HIV progresses by disrupting the immune machinery and introducing its viral DNA into the self-maintaining CD4+ memory cell population. Depletion of CD4+ and CD8+ reservoirs initiates immuno-modulation and triggers cytokine release[3]. Altering levels of circulating cytokines work synergistically to directly impact HIV proliferation and disease progression. The reorganization of the cytokine network may lead to newer and stronger cytokine bonds whose rigidity can quickly become fatal to the host body. Some researchers observed profound changes in the cytokine profile very early upon HIV infection and have drawn attention to the alteration in the co-ordination of complex immune functions. These changes are retained throughout the infection's chronic phase.

The introduction of combination antiretroviral therapy (cART) in HIV-infected individuals helps improve health by inhibiting viral replication and decreasing viremia levels in the patient[4]. It promotes immune integrity by suppressing viral replication thus improving CD4+ and CD8+ T cell proliferation and activity. The improved activities of these immune cells prolong survival and quality of life among HIV-infected persons while keeping opportunistic infections under control. Both HIV infection and antiretroviral treatment deeply influence levels of circulating inflammatory cytokines.

Our team from West Bengal carried out an observational pilot study from our Tertiary Care Hospital (Calcutta School of Tropical Medicine) to determine the baseline levels of HIV-infected individuals in comparison to age and sex-matched healthy individuals. We observed the base level elevations in IL-6, TNF-α (pro-inflammatory cytokines) and CRP in serum, after a viral attack[5]. The study was followed up to 48 weeks post-initiation of ART as per NACO guidelines.

Methods and Materials

Study Participants :

130 newly detected treatment naïve HIV-1 reactive patients were enrolled from the ART center of CSTM, Kolkata, between June-August, 2015 under NACO guidelines. The study design was approved by the Clinical Research Ethics Committee and informed consent was obtained from patients.

The study enrolled patients of both sexes, above 18 years while excluding severely ill or pregnant patients. A standardized proforma was filled up with background information of patients such as medical history, diagnosed opportunistic infections and WHO clinical staging[6] at the time of detection. The ART-treated group served as the 'case' arm and the treatment-naive group was taken as the 'control' arm for comparative studies. Both groups were followed up at 24 and 48 week-interval. Study parameters such as serum cytokine levels, CD4+ measurements, plasma viral load and incidence of opportunistic infection were recorded during admission and follow-up.

Sample Collection :

8ml of blood was collected by trained phlebotomists for measurement of cytokines, CD4+ cell count, HIV-1 RNA and biochemical analysis from all subjects. Serum and plasma were separated by centrifugation (using REMI Centrifuge- R8C, at 3000 rpm, 10 minutes). Samples were labelled and stored in cryovials at -20°C, limited to a single freeze-thaw cycle.

CD4+ T Cell Count :

CD4+ T cell count was done in BD-FACS Calibre (Serial No-E97300192) in absolute numbers and percentages and was analyzed using BD Cell Quest software.

Detection of Plasma HIV-1 Viral Load (PVL) :

HIV-1 RNA Viral Load from each study participant was recorded by Cobas TaqMan 48 by following the Abbott Real-time HIV-1 assay[7] (Abbott Molecular Inc, USA). The viral load represents the presence of viral nucleic acids in plasma and is analogous to the HIV-1 disease progression.

Serum Cytokine Estimation :

Cytokine Sandwich ELISA was conducted to specifically detect and quantify the concentration of soluble cytokines in the sample. The cytokines (IL-6, TNF-α) and CRP were measured from the serum of all subjects using ELISA kits (Ray Biotech, USA).

Laboratory Investigations :

Routine biochemical laboratory tests were performed on collected plasma supernates for estimation of lipid profile, liver function, urea, creatinine and fasting blood glucose. These tests were conducted by an Autoanalyzer machine (ERBA-EM-360) according to the manual (TransAsia Biochemical Ltd).

Statistical Analysis :

Baseline data was converted into a Microsoft Office 2007 Excel spreadsheet and revised for outliers/errors. This data was then imported into Graph Pad Prism (version 6.0, San Diego, CA, USA) for statistical analysis. Parametric data were shown as mean ±SD and compared using the unpaired t-test. In all the analyses, p-values $\leq$0.05 were considered statistically significant.

Results

Socio-demographic Characteristics :

The enrolled subjects comprise females (n=68, 52.3%), males (n=59, 45.3%) and transgender (n=3,2.3%), where the mean age was 34±10 years. The majority of the participants belonged to the Kolkata district (66.1%) and self-reported addictions to tobacco and alcohol were found in a majority of male patients (n=96, 73.8%). Their marital status revealed 81(62.3%) were married, 35 (26.9%) were unmarried and 14 (10.7%) were widows/widowers (Table 1).

Clinical Observations :

Out of the total enrolled patients, 77(59.2%) were assigned WHO clinical staging with Stages 1 & 2, and 53 patients (40.8%) were in Stages 3 & 4 (Table 1). Following NACO guidelines, 63 enrolled patients were

immediately initiated on an ART regimen and were given fixed-dose combinations of tenofovir (300mg), lamivudine (300mg) and efavirenz (600mg). Follow-up of this cohort was performed at 24- and 48 weeks post-treatment initiation.The treatment naïve group (51) was similarly followed up every 24 weeks. By the end of our study, 69 patients enrolled on the ART regimen and 27 remained treatment naïve.

Serum Cytokines and their associated CD4+ and Patient Viral Load :

The baseline cytokines (IL-6, TNF-α, and CRP) were recorded and treatment naïve participants did not show statistically significant improvement in any of the parameters (CD4+ T cell count, PVL, IL-6, TNF-α and CRP) when compared to their baseline values. Conversely, the on-ART patients reported an increase in their CD4+ T cell count and decreased PVL after 24 weeks and a significant decrease in plasma CRP, IL-6 and TNF-α ($p<0.05$) in comparison to their baseline levels (Figs 1-3).

Table 1 — *Baseline particularities of subjects(treatment naïve) at point of enrolment (n=130)*

Variable		Frequency(N)	Percentage(%)
Gender	Female	68	52.3
	Male	62	47.7
Age	Median ± IQR	(34±10)years	
	Range	(18-70)years	
	>34years	82	63
	<34years	48	37
Educational Status	Literate	86	66.1
	Illiterate	44	33.9
Addiction	H/O Smoking	96	73.9
	H/O Alcohol	73	56.2
	H/O both Alcohol and smoking	69	53
Marital status	Married	81	62.3
	unmarried	35	26.9
	widow and widower	14	10.7
Clinical staging	Stage 1 & 2	77	59.2
	Stage 3 & 4	53	40.8
Active Tuberculosis	Present	33	25.3
	Absent	97	74.6
Other co-infections	Present	47	36.1
	Absent	83	63.8
CD4+ cell count (cells/μL)	Median ± IQR	(365 ±354.2)cells/μL	
	Range	(9-1335)cells/μL	
HIV-1 Plasma Viral Load (log_{10}copies/ml)	Median ± IQR	(4.5±1.2) copies/ml	
	Range	(1.3-6.2) copies/ml	

Table 2 — *Number of patients who were "Lost to Follow-Up" (LFU) and mortality cases during 48 weeks of study period*

Duration(after enrolment)	LFU (number)	Mortality (number)
At 24 weeks follow-up	11	06
At 48 weeks follow-up	14	03
Total	25	09

At 48 weeks, elevated levels of TNF-α in serum (pg/ml) marked a significant increase as compared to their baseline data. The patients receiving ART treatment had increased CD4+ cell count and decreased levels of serum IL-6, CRP and PVL upon follow-up at 48 weeks. Plasma viral load of 3 subjects were 'below-detection' level after our study enrolment and ART initiation. At 24 weeks follow-up, 32.6% (30 out of 92), had undetectable PVL which increased to 41 % (33 out of 80) at 48 weeks follow-up (assuming good drug compliance).

Opportunistic Co-Infections :

Our baseline observations indicated the presence of co-infections (Pulmonary/ extra-pulmonary TB) in

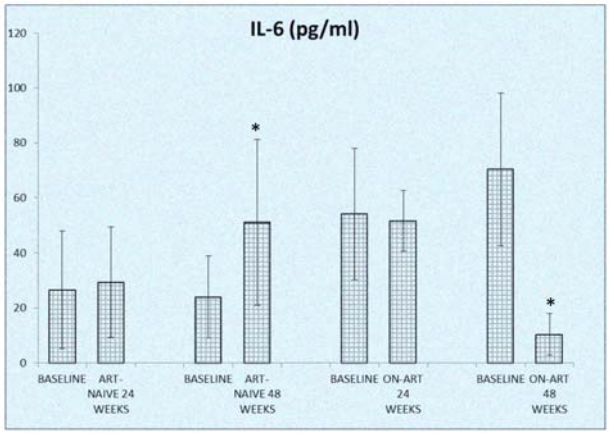

Fig 1 — Changes in serum IL-6 level in subjects, who continued to be ART- naïve after 24/48 weeks of enrolment. Also, changes in serum IL-6 level in subjects after 24/48 weeks of ART initiation (as per NACO guidelines)
*P value according to unpaired t-test (significance level ≤0.05).
**Values are in mean±SD

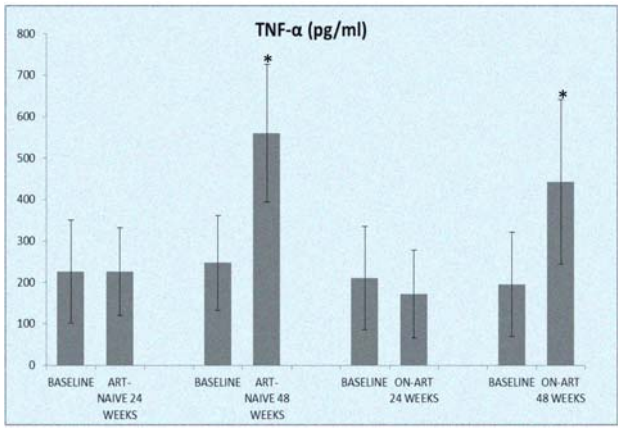

Fig 2 — Changes in serum TNF-α level in subjects, who continued to be ART- naïve after 24/48 weeks of enrolment. Also, changes in serum TNF-α level in subjects after 24/48 weeks of ART initiation (as per NACO guidelines)
*P value according to unpaired t-test (significance level ≤0.05).
** Values are in mean±SD

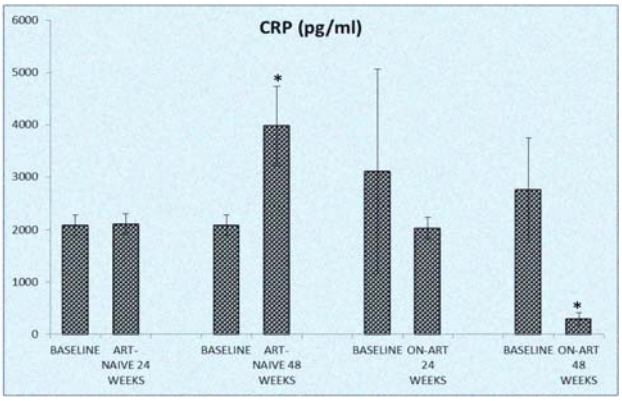

Fig 3 — Changes in serum CRP level in subjects, who continued to be ART- naïve after 24/48 weeks of enrolment. Also, changes in serum CRP level in subjects after 24/48 weeks of ART initiation (as per NACO guidelines)

*P value according to unpaired t-test (significance leveld"0.05).
** Values are in mean±SD

36(28%) of the enrolled. After 48 weeks of follow-up of the HIV/TB (Tuberculosis) on ART and anti-TB drugs, the CD4+ cell count increased significantly compared to their treatment naïve counterparts. PVL, IL-6, TNF-α and CRP levels were also significantly diminished in this cohort (Table 3). The presence of candidiasis (oropharyngeal and esophageal) in 13 patients were also noted.

Deaths and LFU :

25 patients were lost in follow-up within 48 weeks of enrolment along with 9 cases of mortality. Their deaths were augmented by the presence of O.Is (n=7, 77.8%). They also presented with low CD4 count (<±50 cells/mm^3) and high detectable PVL (>106 copies/ml). One outlier case was registered with a higher CD4+ count (≥700 cells/ mm^3).

Discussion

Surveillance efforts undertaken by National AIDS Control Programme (NACP) have revealed a consistently declining trend in the disease prevalence, incidence of new infections and mortality due to AIDS since 2010[8]. A similar falling trend is presented by epidemiological analyses in West Bengal, with 62,000 AIDS-related deaths; contributing 6% to the nation's HIV burden[9]. Our study was carried out to record changes in serum levels of selected inflammatory cytokines with attention to classic HIV detection and prognosis parameters after 24 and 48 weeks, to draw baseline comparisons.

IL-6, a well-known indicator of persistent inflammation as a result of HIV infection has been linked to morbidity and mortality events. Active studies have demonstrated consistently low levels of plasma IL-6 in virologically suppressed individuals undergoing treatment. Our study recorded a slight decline in IL-6 in on-ART group ((with CD4+ cell count ≤200 cells/ mm^3) after 24 weeks as opposed to elevated IL-6 levels observed in the treatment naïve group (with CD4+ cell count >500 cells/mm^3). However, after 48 weeks, the on-ART group displayed a considerable decline in their IL-6 population compared to their baseline levels with CD4+ cell count restoring up to >350 cells/mm^3. Anomalies were noted in two separate cases where IL-6 levels declined before ART initiation[10] (CD4+ cell count -240/mm^3 and 315/mm^3. Elevated IL-6 levels can serve as an early prognostic marker for HIV and timely ART intervention can minimize inflammation[11].

TNF-α, a pro-inflammatory cytokine in the TNFR pathway is a hallmark promoter of HIV-1 infection[12], where levels of plasmic TNF-α were observed to be much higher in ART-naïve patients than in patients' on-ART[13]. Our study recorded minute changes in TNF-α levels of the on-ART group even after 20 weeks of treatment as opposed to a significant jump after 48 weeks. Controlled viremia aided by ART did not effectively reduce the TNF-α level even after a rise in CD4+ levels, as per our surveillance data. The treatment-naive group also exhibited a significant elevation in TNF- α level (higher than the on-ART group) in comparison to its baseline level.

CRP levels of the untreated group display a marked increase at the end of 48 weeks compared to the on-ART group which records a drastic fall in their levels at 48 weeks post-treatment initiation. Low levels of CRP in HIV-infected patients can potentially serve as a predictor for longevity and a prognostic marker for the prevalence of opportunistic co-infections and cardiovascular morbidities[14].

Our study exposed extrapulmonary tuberculosis as the primary co-infection and

Table 3 — *Changes in cytokines, immunological and virological parameters in HIV/TB co-infected patients who started on ART and ATD (baseline versus 48 weeks FU)*

Variable	Baseline values of treatment- naive HIV/TB patients (n=19)**	48 weeks following ATD and ART (n=19) **	p- value*
IL-6 (pg/ml)	46.93±62.90	18.08±5.04↓	0.01 (S)*
TNF-α (pg/ml)	457.09±231.61	232.86±137.10↓	0.007 (S)*
CRP (pg/ml)	2519.89±1718.55	1507.78±869.81↓	0.004 (S)*
CD4 cell count (cells/µL)	131.72±184.00	324.25±141.01↑	0.006 (S)*
HIVPVL (log_{10} copies/ml)	5.12±0.582	3.98±1.075↓	0.03 (S)*

*P value according to unpaired t-test (significance level (S) ≤0.05).
** Values are in mean ±SD

mucocutaneous candidiasis and extrapulmonary TB as common OIs. Of the 9 subjects who succumbed, 7 were on-ART and had acquired OIs, with 4 of them infected with TB (TB meningitis/Lymph Node TB) and were receiving CAT 1 ATD (Category 1 Anti Tubercular Drug). 6 deaths were reported before the completion of 24 weeks of our study, making extrapulmonary TB a bad prognostic factor.

We inferred that the coordinated regimen of both ART and TB drugs worked in tandem to suppress inflammation. Our study detected a few cases of Elite Controllers (ECs)[15] (n=23) where despite maintaining low viremia levels, elevation in inflammatory biomarkers continued, signaling the presence of Long-term Non-Progressors (LTNPs).

Conclusion

Being one of the few regional studies drawing on associations between the influence of ART and the altering levels of inflammatory markers[16], our study was supported by clinical monitoring of treatment adherence and frequency of co-infections. However, a further extrapolation on a wider population of the Indian gene pool, with a broader spectrum of cytokines analysis, may bring forward and unwind newer aspects of inflammatory networking in hosts after HIV infection and subsequent ART administration. Besides ART, a careful individualized approach supported with strategies that focus on minimizing the impact of co-morbidities[17] can further strengthen the study.

References

1 Kumar S — AIDS and COVID-19 infections: impact on vulnerable Indian population. *New Microbes and New Infections* 2021; **42:** 100903.

2 National AIDS Control Organization -Sankalak: Status of National AIDS Response (Third edition, 2021)http://naco.gov.in/sites/default/files/Sankalak_Status_of_National_AIDS_Response_Third_Edition_2021.pdf (accessed Sep 25, 2022): 23-32.

3 Deeks SG, Overbaugh J, Phillips A, Buchbinder S— HIV infection. *Nature Reviews Disease Primers* 2015; **1(1):** 1-22.

4 Pau AK, George JM — Antiretroviral therapy: current drugs. *Infectious Disease Clinics* 2014; **28(3):** 371-402.

5 Debnath A, Mandal M, Choudhuri S, Saha MK, Guha SK— Baseline Levels of Interleukine-6, Tumor-Necrosis Factor-Alpha and C-Reactive Protein in Treatment Naive Human Immunodeficiency Virus Infected Patients-A Study from a Tertiary-Care Hospital in Eastern India. *Current Biomarkers (Formerly: Recent Patents on Biomarkers)* 2016; **6(2):** 124-32.

6 UNAIDS — Report on the global AIDS epidemic, 2013. https://www.unaids.org/en/resources/documents/2013/20130923_UNAIDS_Global_Report_2013 (accessed Sep 20,2022).

7 Scott LE, Crump JA, Msuya E, Morrissey AB, Venter WF, Stevens WS — Abbott RealTime HIV-1 m2000rt viral load testing: manual extraction versus the automated m2000sp extraction. Journal of Virological Methods 2011; **172(1-2):** 78-80.

8 National AIDS Control Organization -Sankalak: Status of National AIDS Response (Third edition, 2021)http://naco.gov.in/sites/default/files/Sankalak_Status_of_National_AIDS_Response_Third_Edition_2021.pdf (accessed Sep 25,2022): 23-32

9 Ganguly S, Chakraborty D, Goswami DN — HIV/AIDS epidemic in West Bengal: An overview. *Journal of Family Medicine and Primary Care* 2018; **7(5):** 898.

10 Hattab S, Guihot A, Guiguet M, Fourati S, Carcelain G, Caby F, *et al* — Comparative impact of antiretroviral drugs on markers of inflammation and immune activation during the first two years of effective therapy for HIV-1 infection: an observational study. *BMC Infectious Diseases* 2014; **14(1):** 1-9.

11 Trovato M, Ruggeri RM, Sciacchitano S, Vicchio TM, Picerno I, Pellicanò G, *et al* — Serum interleukin-6 levels are increased in HIV-infected patients that develop autoimmune disease during long-term follow-up. *Immunobiology* 2018; **223(3):** 264-8.

12 Pasquereau S, Kumar A, Herbein G — Targeting TNF and TNF receptor pathway in HIV-1 infection: from immune activation to viral reservoirs. *Viruses* 2017; **9(4):** 64.

13 Behrens NE, Wertheimer A, Love MB, Klotz SA, Ahmad N — Evaluation of HIV-specific T-cell responses in HIV-infected older patients with controlled viremia on long-term antiretroviral therapy. *PLoS ONE* 2020; **15(9):** e0236320.

14 Chenciner L, Symonds M, Dissanayake O, Hunter A, Burns F, Miller RF — Lymphocyte-CRP-ratio and CRP-albumin-ratio as potential inflammation markers in adults with HIV. *Journal of Acquired Immune Deficiency Syndromes* 2022; **13:** 10-97.

15 Gurdasani D, Iles L, Dillon DG, Young EH, Olson AD, Naranbhai V, *et al* — A systematic review of definitions of extreme phenotypes of HIV control and progression. *AIDS* 2014; **28(2):** 149-62.

16 Amoani B, Sakyi SA, Barnie PA, Karen Pomeyie K, Aniagyei W, *et al* — Effect of ART on Cytokine Profile amongst HIV Patients: A Systematic Review and Meta-Analysis. *Focus Med Sci J* 2021; **7(3)**.

17 Kumbale CM, Voit EO — Toward Personalized Medicine for HIV/AIDS. *Journal of AIDS and HIV Treatment* 2021; **3(2):** 37.