ORIGINAL ARTICLE

Role of Serum Adenosine Deaminase Activity as a Prognostic Marker in HIV Patients on Antiretroviral Therapy: A Prospective Study

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Abstract:

Background: HIV infection is characterized by replication by aberrant immune activation and persistent inflammation. HIV infection is associated with loss of CD4 T cells, resulting in dysfunction of immune system. Adenosine Deaminase (ADA) has a cytokine-like costimulatory role in T cell proliferation. *Materials and Methods:* The study included 150 HIV positive patients between age group of 20-50 years from ICTC (Integrated Counseling and Testing Centre) and ART centre of Belagavi Institute of Medical Sciences, Hospital, Belagavi, Karnataka, India. ADA activity and CD4 cell count were estimated before starting any treatment and after 3 months interval of ART upto 9 months. Serum ADA activity was estimated by using colorimetric method of Giusti and Galanti. *Results and Discussion:* The study showed increased ADA activity before ART, which was gradually decreasing after 3, 6 and 9 months of ART ($p<0.001$). We observed low CD4 cell count before ART, there was steady rise of CD4 cell count after 3, 6 and 9 months of ART ($p<0.001$). After 3, 6 and 9 months of ART adenosine deaminase activity decreases as the CD4 count increases. Elevated serum adenosine deaminase activity in HIV patients is an indicator of T-cell activation. With antiretroviral therapy there is reduction in viral load and T-cell activation, which may explain the gradual decrease in ADA activity. *Conclusion:* Estimation of serum ADA activity is a simple, rapid and inexpensive test. In this study ADA activity decreased gradually with ART. Thus the study concludes that serum ADA activity may be used as a prognostic marker to monitor response to antiretroviral therapy in HIV patients in limited resource and high incidence areas.

Keywords: HIV, ART, ADA

Introduction:

Acquired Immunodeficiency Syndrome (AIDS) is one of the greatest public health and social problems threatening the human race. In 2013, there were 35 million (33.2 million–37.2 million) people living with HIV worldwide. 2.1(1.9–2.4) million new HIV infections were detected globally in 2013. The number of AIDS deaths were 1.5 (1.4–1.7) million in 2013. India has the third highest number of estimated people living with HIV (PLHA) in the world and they are about 20.89 lakh people. Free Anti-Retroviral Therapy (ART) programme was started in India in the year 2004. Since then around 1.5 lakh lives have been saved due to ART [1]. HIV infection is characterized by replication by aberrant immune activation and persistent inflammation. HIV proteins cause irregularities in signalling and apoptotic pathways; transcriptional activation and intracellular protein trafficking in HIV-infected cells are altered by negative factor (Nef) and trans-activator of transcription (Tat) proteins [2]. HIV preferentially affects CD4 T cells. CD4 count measures the degree of immunosuppression in HIV-positive patients. There is an inverse relationship between CD4 count and degree of immunosuppression. Laboratory markers used in monitoring HIV-positive patients are HIV-RNA assay (Viral load) and CD4 count. Their use is limited, because of cost and technology [3, 4].

Among the novel markers which are upcoming Adenosine Deaminase (ADA) is one. Recent studies showed a causal relationship between ADA activity and normal immune function [5]. ADA is an enzyme implicated in purine metabolism. ADA (E.C. 3.5.4.4) deaminates two nucleosides: adenosine and 2'-deoxyadenosine, producing inosine and 2'-deoxyinosine respectively. ADA has two isoenzymes ADA_1 and ADA_2. ADA_1 is found in almost all body cells, but ADA_2 coexists with ADA_1 only in monocytes-macrophages. ADA_2 and ADA_1 are coded by different gene loci. Increase of ADA_2 in monocytes-macrophages occurs when these cells are infected by intracellular micro-organisms and while the parasite is still alive [6]. ADA has a cytokine-like costimulatory role in T cell proliferation, which is independent of catalytic activity [7]. In humans, ecto ADA_1 can bind to the cell surface via dipeptidyl peptidase IV/CD26 [8], which contributes to cytokine regulation via N-terminal dipeptide cleavage and stimulates T cell proliferation by activating the CD45 receptor, which is preferentially expressed by memory T cells [9]. By interacting with CD26 on the CD4 T cell surface and with the $A_{2B}R$ on the dendritic cell (DC) surface, triggers a strong costimulatory signal for T cell activation. This ADA-mediated costimulation not only potentiates T cell proliferation but also the secretion of Th1 (IFN-γ) and proinflammatory (IL-6 and TNF-α) cytokines [7]. ADA isoenzymes may directly affect Adenosine Receptor (ADR) function by forming a complex with the receptors and changing their affinity for adenosine [10].

HIV-1 infectious particles and also the soluble envelope glycoprotein gp120 are able to inhibit (ADA) binding to CD26 on the cell surface of peripheral lymphocytes and T-cell lines [11]. Present study was aimed to evaluate the role of ADA activity as a prognostic marker in HIV patients on antiretroviral therapy. Objectives of our study were to estimate ADA activity and CD4 count in HIV patients before ART and after 3 months interval of ART up to 9 months. To compare ADA activity and CD4 count in HIV patients before and after 3 months interval of ART upto 9 months. To find out correlation between ADA activity and CD4 cell count in HIV patients.

Material and Methods:

Study design was a prospective study. The study was conducted in Department of Biochemistry Belagavi Institute of Medical Sciences (BIMS), Belagavi, Karnataka, India from June 2014 to October 2015. Study was approved by Institutional Ethics Committee. The study included 150 HIV positive patients between age group of 20-50 years with CD4 count <350cells/µl from (ICTC) Integrated Counseling and Testing Centre and ART centre of BIMS, Hospital, Belagavi. Diagnosis of HIV infection was done based on the symptoms, past history, clinical examination and confirmed with HIV tests COMBAIDS –RS Advantage-ST (HIV 1+2 immunodot test kit), HIV-1/2 Triline card test and Retrocheck HIV-WB (Rapid immunochromatographic test for HIV 1/2 antibodies). After obtaining informed written consent 4 ml of venous blood samples were collected under aseptic precautions, just before starting any treatment and after 3 months, 6 months and 9 months interval of ART. Serum was used for estimation of ADA activity and whole blood sample (EDTA as anticoagulant) was used to measure CD4 count. HIV patients with tuberculosis, cancer, Rheumatoid Arthritis (RA), psoriasis and other immunocompromised conditions were excluded from the study group. ADA Activity was estimated using colorimetric method of Giusti and Galanti [12]. ADA hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol

and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. The intensity of the blue colored indophenol complex is directly proportional to the amount of ADA present in the sample. CD4 count was measured by MEM-241 PE-conjugated monoclonal antibody to human CD4 by flow cytometric analysis [13]. All HIV positive patients were on treatment with Zidovudine (ZDV or AZT) 300mg, Lamivudine (3TC) 150mg and Efavirenz (EFV) 600mg.

Statistical Analysis:

The values obtained were expressed as Mean ± Standard Deviation. The significance of difference between the means were calculated with student't' test. Pearson's correlation coefficient was calculated to see the correlation between variables. $P<0.05$ was considered for statistical significance. Statistical software SPSS version 22 was employed for statistical analysis.

Results:

The study included 80 male HIV patients and 70 female HIV patients. Mean age of HIV patients was 38.11±7.47. Mean age of male and female HIV patients was 39.25±7.34 and 36.80±7.45 respectively. Majority of HIV patients were in sexually active age group (Table 1).

Table 1: Demographic Data

Parameter	Total	Males	Females
n	150	80	70
Age (years) (Mean±SD)	38.11±7.47	39.25±7.34	36.80±7.45

n = Number of HIV patients

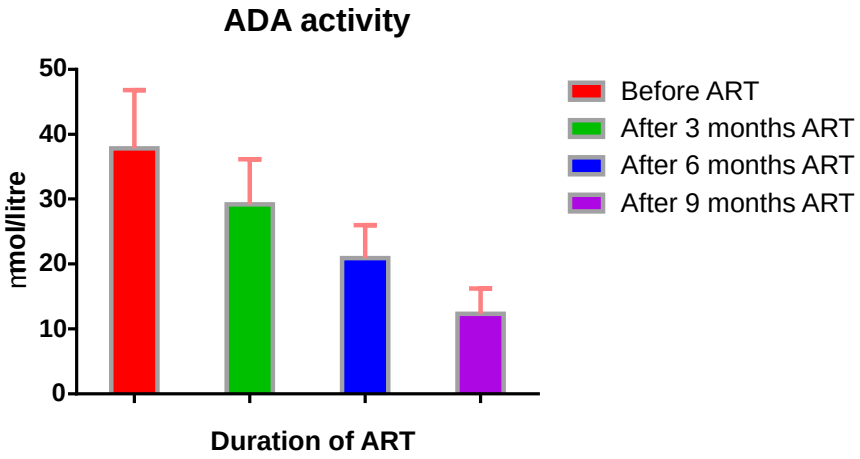

Fig. 1: ADA Activity Before and After 3, 6, 9 Months of ART in HIV Patients

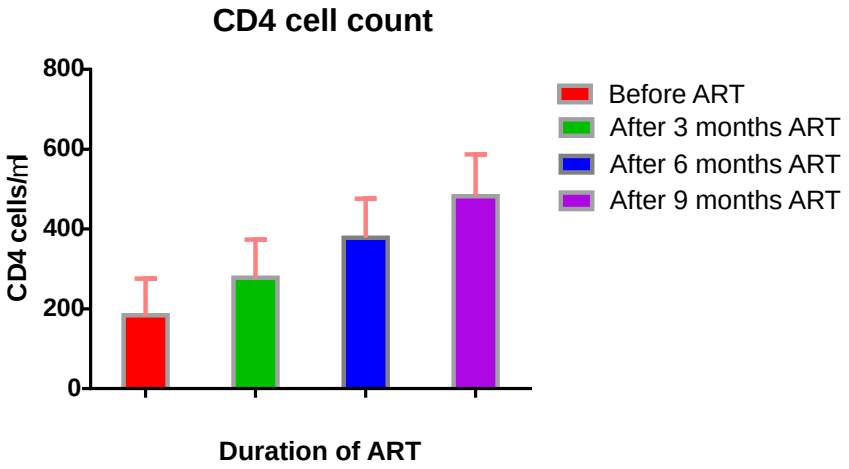

Fig. 2: CD4 Cell Count Before and After 3, 6, 9 Months of ART in HIV Patients

Table 2: ADA Activity and CD4 Cell Count in HIV Patients

Parameter	Before ART	3 months of ART	6 months of ART	9 months of ART
ADA activity(IU/L) (Mean±SD)	37.84±8.98	29.21±6.99	20.88±5.14	12.35±3.88
CD4 cell (count/µl) (Mean±SD)	183.89±91.46	277.61±95.83	378.35±98.05	482.29±104.49

Table 3: Significance of Difference of ADA Activity and CD4 Cell Count between before and After 3, 6 & 9 Months of ART in HIV Patients

Parameter	Before ART vs After 3mths.	Before ART vs After 6mths	Before ART vs After 9mths	After 3mths vs After 6mths	After 3mths vs After 9mths	After 6mths vs After 9mths
ADA activity	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
CD4 cell count	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

Table 4: Comparison of ADA Activity in Male and Female Patients

Duration of ART	Male HIV patients (n=70)	Female HIV patients (n=80)
Before ART	40.62±9.82*	35.25±6.96*
3 months of ART	31.10±7.58*	27.35±5.7*
6 months of ART	22.07±5.77*	19.69±4.15*
9 months of ART	13.18±4.29*	11.48±3.29*

**p<0.01= Significant, n = Number of subjects, all values are expressed as Mean ± Standard Deviation*

Table 5: Comparison of CD4 Cell Count in Male and Female Patients

Duration of ART	Male HIV Patients (n=70)	Female HIV Patients (n=80)
Before ART	172.95±96.38*	196.40±84.44*
3 months of ART	268.81±99.1*	287.67±90.62*
6 months of ART	366.74±102.13*	391.61±92.09*
9 months of ART	466.83±108.53*	499.97±97.47*

**p>0.05 = Not significant. n = Number of subjects, all values are expressed as Mean ± Standard deviation.*

Study observed increased ADA activity before ART, which was gradually decreasing after 3, 6 and 9 months of ART. Decrease in ADA activity at different intervals of ART was statistically significant in themselves and before ART ADA activity (Table 2, Table 3; p<0.001). Study observed low CD4 cell count before ART, there was steady rise of CD4 cell count after 3, 6 and 9 months of ART. Rise in CD4 cell count at different intervals of ART was statistically significant in themselves and before ART CD4 cell count (Table 2, Table 3; p<0.001). After 3, 6 and 9 months of ART adenosine deaminase activity decreased as the CD4 count increased. There was negligible correlation between ADA activity and CD4 cell count before ART (r=0.01). Study found that on comparison of female and male cases there were significant differences in the ADA activity and CD4 cell count. Male HIV patients showed more ADA activity than female HIV patients before ART and after 3 months, 6 months and 9 months interval of ART (Table 4). The decreased ADA activity in female cases than male cases may be due to presence of hormone estradiol which inhibits activity of ADA [14].

Cd4 cell count was higher in female HIV patients than male HIV patients before ART and after 3 months, 6 months and 9 months interval of ART, but the difference was not significant (Table 5). This may be due to pharmacokinetic differences like mean body mass indices, nutritional status, or other factors. These pharmacokinetic differences may have resulted in women having higher antiretroviral drug levels, which led to more-profound virus suppression and resulted in a

greater increase in CD4 cell count in female HIV patients [15, 16].

Discussion:

Elevated serum adenosine deaminase activity in HIV patients is an indicator of T-cell activation. With antiretroviral therapy there is reduction in viral load and T-cell activation. As a result there may be gradual decrease in ADA activity. In HIV infected individuals low ADA activity with low CD4 count indicates that there is no immunological recovery. Estimation of ADA activity helps to know the prognosis of the disease and also the status of their immunity [18]. Casoli *et al* showed that activity of the ADA enzyme during different stages of AIDS that is Lymphadenopathy Syndrome (LAS), AIDS-related Complex (ARC), full-blown AIDS and AIDS encephalopathy (AIDS enc) was significantly higher than controls [17]. Our results are in agreement with Laxmi *et al* who have shown that untreated HIV positive subjects (22.34±7.5) had significantly higher ADA activity than ART treated HIV positive subjects (13.5±3.5) concluding that ADA activity guides when to start the ART treatment regimen [18].

Study by Carrera *et al* have found that patients infected with HIV shown a significant increase in ADA activity compared with patients in the control group: 21.6±5.4 vs. 10.4±2.3 U/l ($p < 0.001$). Therapy with AZT decrease ADA activity: 21.6±5.4 vs. 15.2 ± 4.3 U/l ($p < 0.001$) with an increase in CD4 counts: 187±105 vs. 353±145/mm^3 ($p < 0.001$) [19]. Our study has shown similar findings.

Deopujari *et al* have observed that mean ADA value in HIV patient has been 87.1±36.24 with CD4 cell count >200 cells/µl (mean CD4 count 245cells/µl) as compared to 71.32±36.35 in patients with CD4 count <200 cells/µl (mean CD4 count 86cells/µl) and difference not statistically significant ($p>0.05$). There has been no correlation between ADA activity and CD4 cell count($r= -0.460$, $p=0.28$) [20], which is similar to findings of our study.

Study by Gakis *et al* has shown an increase of ADA activity in 100% of (Late- AIDS) L-AIDS group, in 64% of AIDS group, in 62% and 42% of LAS and ARC groups, respectively, and in 36% of the asymptomatic groups. ADA_2 originates exclusively from the Monocyte-Macrophage Cell System (MoMaCS) which actively releases this enzyme in the presence of live parasites in the cells' interior. It has been hypothesized that in the MoMaCS the enzyme constitutes a microbicidal mechanism independent of the respiratory burst [21]. Persistence of virus in the host in the presence of ineffective immune system clearance results in a state of chronic immune system activation. Activation of cells in the course of the immune response further favors the spread and establishment of HIV in new target CD4 helper T cells and in macrophages. In addition, virus replication is potentiated both by activation signals and by cytokines such as IL-6 and TNF-α [22]. Macrophages release large amount of ADA when they are stimulated by the presence of live HIV in their interior due to the release of type1-IFN [23]. cAMP increases in HIV infection and its degradation leads to high level of adenosine. In developing lymphocytes, adenosine may induce apoptosis through a pathway involving p53 [24]. Increase in plasma ADA is an immunogenic response towards the increase of adenosine in HIV infection by the cells [18]. In the present study we could not stage the severity of HIV infection. Low grade opportunistic infections in HIV patients may increase ADA activity.

Conclusion:

Estimation of serum ADA activity is a simple, rapid and inexpensive test. Thus the study concludes that serum ADA activity can be used as a prognostic marker to monitor response to antiretroviral therapy in HIV patients in limited resource areas where there is high incidence of HIV infection.

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