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Comparative Analysis of Adenosine Deaminase Level in Newly Diagnosed HIV - Positive Patients Before and After One Year of Antiretroviral Therapy

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Abstract: ***Adenosine Deaminase ADA, an inflammatory biomarker, is crucial in the immune response associated with HIV infection. This study aims to compare ADA levels in newly diagnosed HIV - positive patients before and after one year of Antiretroviral Therapy ART, and with healthy controls. Conducted at Sardar Patel Medical College, Bikaner, the observational case - control study included 50 HIV - positive patients and 50 healthy individuals. ADA levels were significantly higher in HIV - positive patients before ART compared to post - therapy and controls p - value <0.0001. The study also found a fair to substantial agreement between ADA levels and CD4 counts, with Kappa values of 0.392 and 0.766 in HIV+ patients before and after 1 - year ART. These findings suggest ADA as a potential marker for monitoring HIV progression and the efficacy of ART. The significance of this study lies in its potential to enhance the understanding of ADA as a biomarker for inflammation and immune activation in HIV - positive patients. It provides insights into the efficacy of ART in reducing ADA levels and improving immune response, thus offering a valuable tool for monitoring disease progression and treatment outcomes.***

Keywords: Adenosine Deaminase, Inflammatory Biomarker, HIV, Antiretroviral therapy, Kappa Coefficient

1. Introduction

HIV infection disrupts the immune system, leading to secondary infections and ultimately AIDS. The key features of infection due to HIV are inflammation and activation of immune system. Inflammatory biomarkers play a crucial role in the progression and pathogenesis of the disease.1 One such marker is ADA. Beyond its metabolic role, ADA is a crucial mediator of inflammation and immune response, influencing various cellular processes and disease states, including HIV infection.2 ADA influences activation of T - cell, production of chemokines and cytokines, and the overall responses of immune system. In respect to HIV ADA's activity has significant implications for disease progression and management. Therapeutic strategies targeting ADA provide promising avenues for modulating immune responses.3

Adenosine, a small regulatory molecule increases during inflammation and infection is the catabolic product of adenosine monophosphate (AMP). ADA an enzyme that catalyses and removes ammonia from adenosine and 2' deoxyadenosine and forms inosine and deoxyinosine which are toxic metabolites to the cells. ADA is present intracellularly and extracellularly. ADA is vital for the proper immune development and its function. Some studies were attributed to the increase in serum ADA (a nonspecific biomarker of T - cell activation activity in diseases where cellular mediated immunity is altered).4

As a consequence of continuous low - grade inflammation and activated immune system, a level of inflammatory biomarkers generally raised.5 ART is aimed to maintain a sustained suppression of HIV viral load, allowing for recovery of immune system. Thus, therapy results in a significant lowering of risks related to HIV infections.6

Thus, there is a requirement for initial, precise and particular biomarkers to study the progression and prognosis of disease. Assessing ADA level helps in depicting the speed of HIV replication and also assess the damage or restoration caused to immune system at the time of ART.

The purpose of this study is to investigate the changes in Adenosine Deaminase ADA levels in newly diagnosed HIV - positive patients before and after one year of Antiretroviral Therapy ART, and to compare these levels with those in healthy controls.

2. Material and Methods

An observational case control study on 50 newly diagnosed HIV+ patients and healthy subjects was conducted in Department of Biochemistry along with Department of General Medicine (ART Unit) Sardar Patel Medical College and PBM hospital, Bikaner, Rajasthan.

Study Population: Study population included newly diagnosed HIV+ clinically confirmed patients from ART Unit of Department of General medicine, PBM hospital. In control

group healthy individuals were included after a written consent taken from both patients and healthy individual.

Inclusion Criteria: Newly diagnosed clinically confirmed seropositive patients aged above 18 years were included. **Exclusion Criteria:** Below 18 years, patients having pre - existing symptoms of AIDS as stated by WHO at the start of ART therapy, having previous exposure to ART**,** having co - morbidities like diabetes mellitus, secondary infection other that HIV, inflammatory conditions, diarrhoea, rheumatoid arthritis, cancer etc., having obesity, allergic reactions or pregnancy etc, and patients who were on drugs that are known to affect concentration of ADA.

Study Participants:
Based on inclusion and exclusion criteria, a total 100 age and sex matched subjects were chosen for the study. Both patient and control group provided written consent for participation and information confidentiality was guaranteed. At the time of admission, a patient and their relative's interview was planned to collect general information and socio - demographic data. Two groups were created from study participants.

A detailed history of patients and controls like age, gender, education, occupation, monthly income, residential area, mode of transmission of viral infection, weight, type of any addiction (smoking, tobacco chewing, alcohol etc), Socio - economic status decided by modified Kuppu Swamy Scale and marital status etc were taken. Sample from patients and controls were collected after taking an informed consent.

Sampling Procedure
10 ml of blood sample were drawn by puncturing vein from both HIV+ and healthy individuals under general aseptic precautions. After collecting blood, samples of blood were aliquoted into EDTA vial for measuring CD4 count and to plain gel vial (to minimize risk of hemolysis) for serum separation to estimate adenosine deaminase. These aliquots were stored at ≤ - 20 °C in deep fridge. At time of measurement of parameters, frozen sample was thawed at room temperature. Adenosine Deaminase were estimated on Erba semi - autoanalyzer by enzymatic method.

Statistical Analysis
The mean values of different parameters in various studied groups were compared and analysed using appropriate statistical methods. Pearson correlation were employed in analysing the relationship between the ADA. Receiver operating characteristic curve were done to calculate cut - off values. Kappa coefficient of Agreement was used for measuring the degree of agreement among variables. Chi square test is used for qualitative variables. SPSS statistical software (version 24.0) was used for data analysis. Significance were considered at $P < 0.05$.

3. Observation

The average age of HIV+ patients was 35.34 years with majority (42%) were in age group 21 - 30 years followed by 36% in age group 31 - 40 years. Similarly, the mean age of healthy subjects was 34.48 years with majority (40%) in age group 31 - 40 years followed by 30% in age group 21 - 30 years. This difference in mean age among HIV+ patients and healthy subjects found to be statistically insignificant (p - value 0.6656). Overall male preponderance was present in our study with 76% males and 24% females in HIV+ group. Among healthy subjects, number of males were 86% and only 14% females. We found a statistically insignificant difference (p - value 0.2024) in gender among HIV+ and healthy subjects (Table: 1).

Table 1: Socio - demographic characteristics of HIV+ patients and healthy subjects according to age group

	HIV+ Patients (N=50)	Healthy Subjects (N=50)	P – value
Age			
(Mean±SD)	35.34±10.36	34.48±9.46	0.6656
Gender			
Male	38 (76%)	43 (86%)	0.2024
Female	12 (24%)	7 (14%)	
Residential Area			
Rural	31 (62%)	29 (58%)	0.6830
Urban	19 (38%)	21 (42%)	
Education			
Illiterate	8 (16%)	6 (12%)	0.9004
Literate	9 (18%)	10 (20%)	
Primary	20 (40%)	18 (36%)	
Secondary	9 (18%)	10 (20%)	
College	4 (8%)	5 (10%)	
Professional Degree	0 (0%)	1 (2%)	

The mean CD4+ count in HIV+ patients before initiation of ART was 178.32±83.93 cell/µl and after 1 - year ART was 293.12±102.24 cell/µl. This rise in CD4+ count in HIV+ patients after 1 - year ART was statistically significant as compared to CD4+ count before initiation of therapy (p - value <0.0001). The mean ADA in HIV+ ART naïve patients was 48.85±5.34 U/L followed by after 1 - year therapy mean ADA level becomes 28.03±3.93 U/L. In healthy subject group mean ADA level was 19.44±3.18 U/L. Here, we found a statistically significant difference in level of ADA before and after therapy compared to each other and with healthy subjects (p - value <0.0001) (Table: 2).

Table 2: Comparison of CD4+ count and serum ADA Levels amongst HIV+ ART naïve, with 1 - year ART and healthy subjects

	CD4+ Count cell/µl	ADA (U/L)
HIV+ART naïve (a)	178.32±83.93	48.85±5.34
HIV+ with 1 - year ART (b)	293.12±102.24	28.03±3.93
Healthy Subjects (c)	- -	19.44±3.18
P - value		
(a) Vs (b)	<0.0001	<0.0001
(a) Vs (c)	- -	<0.0001
(b) Vs (c)	- -	0.001

Table: 3 shows comparison of ADA level according to CD4+ count and WHO clinical stages in HIV+ patients. There is statistically high significant difference in mean ADA level before and after therapy (p - value <0.0001). Similarly, There is highly significant difference in level of ADA among different WHO clinical stages before and after follow up period (p - value<0.0001).

Table 3: Comparison of ADA level according to CD4+ count and WHO Clinical stages in HIV+ patients before and after therapy.

		ADA (Mean± SD)		p - value
		HIV+ART naïve (N=50)	HIV+ with 1 year ART (N=50)	
CD4+ count	≤200	49.57±6.32	27.16±6.47	<0.0001
	>200	42.39±3.73	28.17±6.61	<0.0001
WHO clinical stages	Stage - 1	45.87±4.13	26.83±4.59	<0.0001
	Stage - 2	46.92±4.39	31.14±11.18	<0.0001
	Stage - 3	49.2±4.19	40.7±0.0	<0.0001
	Stage - 4	51.7±4.07	42.6±0.0	<0.0001

A Pearson correlation of ADA with CD4+ count in newly diagnosed patients and after follow - up was calculated. We found that in ART naïve subjects and after 1 - year ART, ADA shows an inverse correlation with CD4+ count (r= - 0.498; P - value<0.0001 and, r= - 0.418; P - value<0.0001). After 1 - year follow - up ADA shows inverse correlation with CD4+ count and other correlation is direct (Table: 4)

Table 4: Pearson Correlation between IL - 6, IL - 4 ADA and CD4+ Count in HIV+ ART naïve and 1 - year follow - up patients.

Pearson Correlation		CD4+
ADA	HIV+ ART naïve	- 0.498
	HIV+ 1 - year therapy	- 0.418

The ROC curve at 95 CI was utilized to evaluate the accuracy of ADA and to determine cut - off points above which ADA shows further weakening of immune system. The cut - off value for ADA in HIV+ ART naïve patients is 43.8 U/L at AUC 0.858. And, At this cut - off in HIV+ patients after 1 - year follow up, ADA was found to be powerful predictor of CD4+ cell count ≤200 cell/µl with sensitivity of 82.35%, specificity 56.25%, PPV 80%, NPV 60% and accuracy 74%. And, in HIV+ patients with 1 - year ART is 31.5 U/L at AUC 0.956. And, At this cut - off in HIV+ patients after 1 - year follow up, ADA was found to be powerful predictor of CD4+ cell count ≤200 cell/µl with sensitivity of 85.71%, specificity 95.35%, PPV 75%, NPV 97.6% and accuracy 94% (**Table: 5, Fig: 1**).

Table 5: Receiver operative curve analysis to measure the prognostic accuracy of ADA against CD4+ count.

	ADA	
	ART naïve	With 1 - year ART
AUC	0.858	0.956
p - value	<0.0001	0.001
Cut off	43.8	31.5 U/L
Sensitivity	82.35%	85.71%
Specificity	56.25%	95.35%
PPV	80%	75%
NPV	60%	97.6%
Accuracy	74%	94%

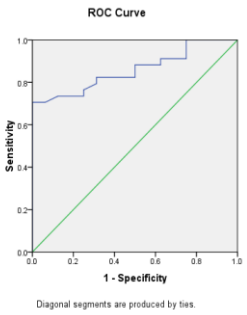

Figure 1a: Receiver operative curve analysis for evaluating Prognostic accuracy of ADA against CD4+ count in HIV+ ART naïve patients

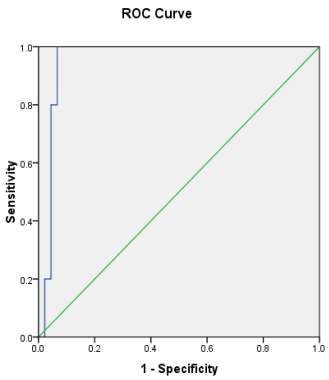

Figure 1b: Receiver operative curve analysis for evaluating Prognostic accuracy of ADA against CD4+ count in HIV+ with 1 - year ART patients.

The Kappa coefficient of agreement between CD4+ count and ADA in HIV+ART naïve patients and after 1 - year ART treatment is found 0.392 and 0.788 which shows a fair and substantial agreement between the two tests (Table: 6).

Table 6: Quantify agreement with kappa results.

		Kappa Coefficient (95 CI)
		ADA
CD4+ Count	ART naïve	0.392 (0.119 - 0.665)
	With 1 - year ART	0.766 (0.557 to 1.00)

4. Discussion

ADA being a hydrolytic enzyme is widely distributed and involved in many immune systems developing process. It plays a substantial role in development of inflammatory response and in production of cytokines.7 In HIV infection there is alteration in ADA level due to a secondary relationship with decrease in CD4+ cells.8 During

physiological conditions, level of adenosine is low in extracellular space and start increasing during disease conditions like, tissue injury, inflammation, viral or bacterial infection, hypoxia or stress. And, this increased level of adenosine regulates the functioning, proliferation and stimulation of different immune cells. And, thus by regulating the level of adenosine is an effective mechanism for limiting and resolving inflammation.9

Here, we assessed the analytical validity of ADA in HIV positive patients with a categorization as ART naïve patients and patients who had an ART therapy from last 1 - year. Additionally, these were compared with, healthy subjects. This assessment indicates that serum ADA level was markedly high in newly diagnosed HIV+ ART naïve patients compared to follow - up patients who had taken 1 - year ART treatment (p - value <0.0001) and from healthy subjects also (p - value <0.0001). ADA level was also significantly different in follow - up patients having 1 - year ART therapy and healthy subjects (p - value 0.001).

The average age of HIV+ patients was 35.34 years and of healthy subjects was 34.48 years. This difference in mean age among HIV+ patients and healthy subjects found to be statistically insignificant (p - value 0.6656). Consistent with our results **Shri et al**[10] conducted a study in Maharashtra, India and reported that incidence of HIV infection peaked at 20 - 24 years and then decreases subsequently. In **Rodrigues CP**[11] observed that the age group ranging between 30 - 39 years old is most frequently observed age group which is at greatest risk of developing HIV infection. **Akase et al**[12] reported average age of HIV patients was 36.6 ± 8.8 years. Study by **Malik et al**[13] observed that 15 - 29 years age group lack knowledge about HIV and AIDS. As a results youth or adolescent symbolize a vulnerable age group and are more susceptible to high - risk sexual conduct. The lack of adequate knowledge about HIV or AIDS precludes risk lessening through accepting enough protection and safe behaviors.

In our study male preponderance was present. This preponderance of males over females may due to the influence of the populations, medical seeking behaviors of female, gender bias and the extent of stigma.1[4] Consistent to our results study conducted by **Umesh et al**[15] and **Kaiser Ahmed Wani et al**[16] also reported male preponderance. In **Akase et al**[12] study male constituted 54.5% (48) of the study participants while 45.5% (40) were female. Study by **Dandona et al**[17] observed higher occurrence rates among males than females.

We found a statistically significant difference in level of ADA before and after therapy compared to each other and with healthy subjects also (p - value <0.0001). In line with our results **Conesa - Buendía et al**[9] also reported that serum level of ADA was dropped after 3 months of ART and markedly decreased after 12 months of ART from baseline value. Similarly, **Ipp et al**[18] also reported a very high level of ADA in serum of HIV+ patients as compared to controls. **Niedzwicki et al**[19] showed that it is the isoenzyme ADA2 which is the predominant isoenzyme found in plasma having HIV infection and this occur in the earlier stages of disease, as was demonstrated in our study. In **Abdi et al**[20] study the mean concentration of serum tADA in HIV positive and healthy subjects was 51.56 ± 12.56 U/L and 23.40 ± 11.01 U/L, respectively. In a study carried out by **Baba et al**[21] in South Africa, serum tADA activity in HIV positive subjects was 64 U/L. In the other study in India conducted by **Shah et al**[22], this activity was 11.824 U/L for the healthy group and 42.88 U/L for the patients group. On the other hand, **Poursharifi et al**[23] reported that total ADA activity in serum of healthy subjects is 14 U/L. Moreover, this decrease in ADA level in HIV+ patients after 1 - year treatment in our study may be confirmed by the facts, that were assessed in a study done on rheumatoid arthritis patients. That study suggested the role of serum ADA as biomarker in diagnosing disease and in determining disease activity.2[4] Therefore, there is a probability that similar types of effect are present in our ART - treated patients to maintain inflammatory state.

We also found that ADA was significantly high in HIV+ patients having CD4+ count ≤200 as compared to patients having CD4+ count >200 (p - value <0.0001), thus ADA shows an significant inverse correlation with CD4+ count before and after therapy. In concordance with our results **Abdi et al**[20] reported an inverse correlation between activity of total ADA and CD4+ cells; with increased CD4+ cells the mean activity of serum total ADA was decreased. **Chittiprol et al**[25] also revealed that plasma level of ADA, including level of both isoenzymes ADA1 and ADA2, also shows an inverse correlation with CD4+ cells. In another study by **Abdi et al**[26] on role of ADA in ruling out HIV mono - infection from combined infection and they found that serum total ADA concentration increases with decrease in CD4+ cells in all patients. **Khodadadi et al**[27] study also reported that level of CD4+ cells markedly reduced in all HIV patients and level of CD4+ cells showed a statistically significant negative correlation with ADA level (R2 = 0.589, p - 0.001). **Fevrier et al**[28] suggested that the level of ADA was significantly lower in subjects having concentration of CD4+ cells is highest. Therefore, the increase in activity of enzymes in compliance to a stepwise decrease in CD4+ cell counts depict that the increase in activity of total ADA leads to the worsening of disease. Hence, it is concluded that different stages of disease can be represented by decreased in CD4+ counts and progression of disease can be indicated by increased activity of total ADA.

With respect to WHO clinical stages the difference in ADA level was correlated with different WHO clinical stages between HIV+ patients before and after ART (p - value <0.0001). **French et al**[29] study also confirms that clinical staging is fair approximation and comparable with immunologic staging as a simple epidemiological and prognostic tool.

The results in our study shows that prognostic value of ADA against CD4+ count was maximum (AUC=0.858 and 0.956). We also calculated Kappa coefficient agreement among CD4+ count and ADA before and after 1 - year ART treatment. Kappa coefficient value of 0.392 and 0.766 shows a fair and substantial agreement between the ADA and CD4+ count. This means that we may depend on ADA for the prognosing the effect of ART therapy on HIV positive patients. Concordance with our results **Abdi et al**[26] also calculated Kappa coefficients of agreement among CD4+ count, total ADA and its isoenzyme ADA1, and ADA2. The

Kappa coefficient values calculated as 0.815, 0.164, and 0.824, respectively, and this shows a strong agreement among CD4+ counts and both total ADA and its isoenzyme ADA2.

5. Conclusion

This study indicates that serum levels of the inflammatory marker ADA are elevated in newly diagnosed HIV - positive patients compared to levels after one year of ART and healthy controls. This increase in level of inflammatory markers suggestive of immune activation and inflammation associated with infection. Raised level of inflammatory marker ADA demonstrates the severity of developed infection. Anti - retroviral therapy induces a moderate rise in CD4+ cells and declining of mediator of inflammation (Adenosine Deaminase) in HIV+ patients. The findings suggest that ADA could be a useful biomarker for monitoring HIV progression and the effectiveness of ART. Further research is necessary to fully understand ADAs role in HIV pathogenesis and to optimize its use in therapeutic interventions.

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