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Original Research Article

Analysis of adenosine deaminase (ADA) Activity in HIV-positive individuals

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Abstract

Background & Objectives: Depletion of CD4+ T-lymphocytes is a hallmark of HIV infection. Therefore, CD4+ cell count is routinely done to monitor the progression of HIV infection. CD4+ cell count being costly could not be afforded everywhere in low-income countries. Thus, we aimed to ascertain the usefulness of serum levels of ADA in screening and monitoring HIV-infected patients as a simple, rapid, and inexpensive marker as compared to routinely used CD4+ cell count.

Materials and Methods: This observational analytical case-control study was performed on 150 HIV-positive patients and 50 healthy subjects. Their CD4+ cell count and Serum ADA activity were determined on Erba XL640 an automated Biochemistry analyzer. Results: were presented as Mean ± SD. Data was analyzed using SPSS-16 software.

Results: Serum ADA activity was significantly higher in HIV-positive patients than in healthy subjects ($p<0.05$). CD4+ cell counts markedly decreased in HIV-infected patients ($p<0.05$) and showed a significant inverse correlation with ADA activity ($p<0.05$). Using a cut-off level of 14.25 IU/L for Serum ADA, calculated sensitivity and specificity were 83% and 94% respectively.

Conclusions: Serum ADA activity could be considered as an alternate laboratory tool for screening and monitoring the disease progression & therapeutic outcome in HIV-infected patients as compared to routinely used CD4 cell count.

Keywords: Adenosine deaminase, Acquired immunodeficiency syndrome, Cluster differentiation, Diagnostic value, Human immunodeficiency virus, Sensitivity, Specificity

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1. Introduction

Acquired Immunodeficiency Syndrome (AIDS), a condition caused by the Human Immunodeficiency Virus (HIV), continues to be a significant public health challenge worldwide. According to the 2021 report by the National AIDS Control Organization (NACO), approximately 24.01 lakh people in India were living with HIV.[1] Among the affected regions, Maharashtra recorded the highest prevalence, with 3.96 lakh cases, followed by other states.[2]

HIV infection weakens the immune system by depleting CD4+ T lymphocytes, which play a crucial role in immune defence.[3,4] The progressive decline in these cells compromises the body's ability to fight infections, leading to severe immunosuppression and an increased risk of mortality in advanced stages of AIDS.[5] Regular monitoring of CD4+ counts and viral load is essential to assess disease progression. However, these diagnostic tests are costly, require sophisticated equipment, and need trained personnel for interpretation. This limits their usefulness & access in areas with insufficient resources.

Adenosine deaminase (EC 3.5.4.4) is a hydrolytic enzyme which carried out deamination of adenosine & deoxyadenosine nucleosides to form inosine & deoxy inosine respectively[6,7] and it has vital role in development of immune system.[8,9] Altered ADA activity has been reported in various immune related diseases like rheumatoid arthritis, systemic lupus erythematosus, tuberculosis, COPD and malignancy.[10,11]

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Some previous studies have shown increased ADA activity in HIV positive patients.[12,13] There is no evidence of such similar studies in our area so far. Hence, we carried out this study to determine the usefulness of serum ADA activity as a simple and inexpensive diagnostic marker for screening and monitoring of HIV positive patients with respect to CD4+ cell count.

2. Objective

This study aims to evaluate serum ADA activity in HIV-infected individuals and investigate its efficacy as a diagnostic biomarker. Additionally, it examines the correlation between ADA levels and CD4+ counts to determine its role in monitoring disease progression.

3. Materials and Methods

An observational analytical case-control study was conducted at the Biochemistry Department of a tertiary healthcare facility in central India from October 2021 to July 2022. Ethical approval was obtained before initiating the research. Data collection was performed with informed consent from patients visiting the ART centre of the institution.

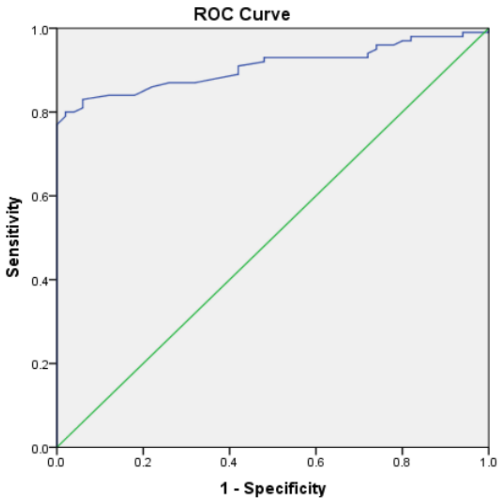
The study enrolled 150 newly diagnosed HIV-positive individuals who had not commenced antiretroviral therapy (ART) as the case group. A control group of 50 age- and sex-matched HIV-negative individuals was included for comparison. Exclusion criteria comprised prior ART exposure, alcohol dependence, tuberculosis, hepatic disorders, and cardiac or renal failure.

Blood samples were obtained under sterile conditions. Serum and plasma were separated through centrifugation at 3000 rpm for 10 minutes. Plasma samples were analyzed for CD4+ counts using flow cytometry, whereas serum samples were assessed for ADA activity using an Erba XL640 automated clinical chemistry analyzer. Both the instruments were standardized for their respective test before proceeding the samples by using positive and negative controls. BD Fluorescence-activated / assisted cell sorting (FACS Count) CD4 reagents manufactured by Becton, Dickinson and Company BD Biosciences were used for CD4+ cell counts. ADA kits were provided by Erba-Transasia Biomedicals. All biochemical analyses were performed on the day of sample collection and values were recorded.

3.1. Statistical analysis

Data was analysed by SPSS-16 software. Results were presented as mean ± SD. The unpaired t-test was applied to compare the mean values between two groups. p-value <0.05 was considered statistically significant. Receiver Operating Characteristic Curve (ROC) was constructed to establish a sensitivity-specificity relationship. Cut-off values that provided the best combination of sensitivity & specificity were determined by ROC curve analysis. Linear regression analysis was done to establish correlation between serum ADA & CD4 count.

4. Results



Graph 1: ROC curve for Serum ADA in diagnosis of HIV.

Table 1: Distribution of study participants by age and gender

Groups (N)	Gender		Age (years) Mean ± SD
	Male	Female	
Cases (150)	82	68	34.2 ± 11.9
Control (50)	25	25	33.3 ± 11.3
Total (200)	107	93	

Table 2: Serum ADA levels in study participants

Groups (N)	Serum. ADA (IU/L) Mean ± SD	p-Value
Cases (150)	20.77 ± 6.34	< 0.05
Control (50)	11.73 ± 2.48	

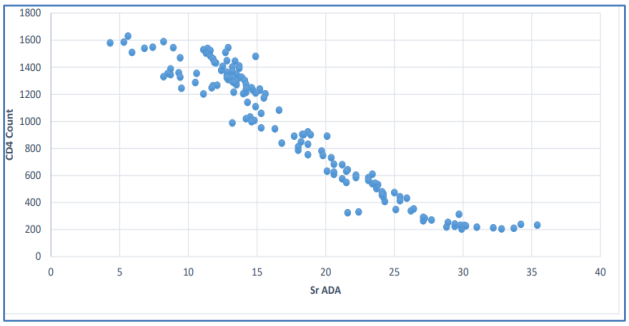
Table 3: Mean CD4 cell counts in study subjects

Groups (N)	CD4 Count (cells/µl) Mean ± SD	p-Value
Cases (150)	742.9 ± 417.1	< 0.05
Control (50)	1358.3 ± 109.9	

Table 4: Serum total ADA activity based on CD4+ cell counts

CD4 Count (cells/µl)	Number	Serum. ADA (IU/L) Mean ± SD
< 300	18	30.27 ± 2.5[a]
300 to < 700	32	23.48 ± 2.05[b]
700 to <1200	50	15.6 ± 4.03[c]
> 1200	50	11.73 ± 2.48[a,b,c]

Data with similar letters (a, b or c) in each column represents significant difference (p<0.05)



Graph 2: Correlation of CD4+ cell counts with Serum. ADA activity of all study subjects

The study found that mean serum ADA levels were significantly higher in HIV-positive individuals (20.77 ± 6.34 IU/L) compared to the control group (11.73 ± 2.48 IU/L) ($p < 0.05$). The ROC curve analysis identified a serum ADA threshold of 14.25 IU/L, yielding a sensitivity of 83% and specificity of 94%. The positive predictive value was 96.51%, whereas the negative predictive value stood at 73.44%.

CD4+ cell counts were considerably lower in HIV-positive individuals (742.9 ± 417.1 cells/µL) compared to controls (1358.3 ± 109.9 cells/µL) ($p < 0.05$). Furthermore, serum ADA activity displayed an inverse correlation with CD4+ counts ($r = -0.956$, $p < 0.05$) across different CD4+ subgroups.

5. Discussion

Adenosine deaminase (ADA) is an enzyme that plays a key role in the immune response.[14] Elevated serum ADA levels have been associated with infections such as tuberculosis, visceral leishmaniasis, and toxoplasmosis, as well as malignancies of lymphoid tissues.[15,16] Though the earlier studies reported raised level of serum ADA among HIV positive patients, its diagnostic and prognostic relevance remains debated.[17,18,19] In our study, we found that serum ADA activity was higher in HIV positive patients than in healthy subjects.

ADA is mainly found in lymphocytes (T and B cells) and monocytes**,** which are crucial for fighting infections.[20,21] In HIV infection, the immune system is continuously activated as the body tries to fight the virus. **Adenosine levels rise** due to increased cell turnover and immune response. To counteract this, the body produces **more ADA** to break down the excess adenosine and prevent immune suppression.[22,23] The current study confirms significantly elevated ADA activity in HIV-infected individuals compared to controls, aligning with previous researches.[24,25,26]

Secondly, Cyclic AMP (cAMP) is a signalling molecule inside cells that influences immune functions. In HIV-positive individuals, cAMP levels increase, which leads to higher adenosine concentrations in the body. Since ADA breaks down adenosine, its activity also increases in HIV infection as a compensatory response to prevent excessive immune suppression.[25]

In our study, a strong inverse relationship between ADA activity and CD4+ counts was established, indicating that ADA levels rise as CD4+ counts decline aligning with previous studies.[24,26,28] This correlation indicates that serum ADA activity increases by worsening of the disease. It can be concluded that as the reduction in CD4 cell count represents the stages of disease, increase of serum ADA activity might also be used to indicate progression of disease.

In present study, though we found high sensitivity & specificity of ADA assay in HIV patients which is similar to previous researches,[26,29,30] but we cannot fully replace CD4+ count with serum ADA levels. ADA and CD4+ serve distinct roles in immunological assessment. CD4+ count directly quantifies T-cell population, reflecting the immune systems functional status in HIV positive individuals.[31] In contrast, ADA is an enzymatic marker of immune activation, primarily linked to lymphocyte proliferation and differentiation. Raised ADA levels indicate heightened immune activity but do not provide direct information about T-cell depletion.[32] Thus, ADA could be used as a supplementary biomarker to CD4+ cell count in resource limited settings.

6. Conclusion

Serum ADA activity emerges as a promising, cost-effective, and easily accessible alternative to conventional HIV biomarkers. Its inverse association with CD4+ counts highlights its potential utility in monitoring disease progression and treatment response. However, larger studies are warranted to validate these findings and establish standardized ADA cut-off values for clinical application.

7. Limitation

Our study was conducted on a relatively small cohort from a single center, limiting the generalizability of our findings. Though we have excluded infectious diseases & autoimmune diseases, it is suggested that our results should be accepted with caution as we haven't confirmed the diagnosis by their specific diagnostic modality. Future studies involving larger and more diverse populations addressing confounding variables are recommended to validate our findings.

8. Conflicts of Interest

None declared.

9. Source of funding

None.

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