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Research Article

ADENOSINE DEAMINASE ACTIVITY IN HIV POSITIVE CASES

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Abstract: Adenosine deaminase (ADA) converts adenosine and deoxyadenosine to toxic metabolites inosine and 2'deoxyinosine. ADA is responsible for the maturation and proper function of cell mediated immunity. Depletion of CD4 T-lymphocytes in HIV infection which is a hallmark makes a pavement for a variety of diseases and malignancies. To ascertain the ADA activity in before ART and after ART treated HIV-positive patients and its variation in comparison to controls within this locality. Total 50 HIV positive subjects and 27 HIV Seronegative healthy individuals were recruited into the study. CD4 counts were estimated to the HIV positive subjects and ADA activity was assessed to both the group individuals. In general the study 'p' value of ADA activity demonstrated highly significant when controls vs before treatment (ART) HIV positive subjects and controls vs after treatment (ART) HIV positive subjects were compared. Among untreated HIV positive subjects had significantly higher ADA activity [males (27.7±5.5 U/L vs females (14.6±4.2 U/L), P < 0.0001] than ART treated [males (14.3±3.4 U/L vs females (10.4±2.5 U/L), P < 0.0001]. In HIV infected individuals low ADA activity with low CD4 count is dangerous. HIV Seropositive subjects should be constantly assessed the ADA activity to know the prognosis of the disease and also the status of their immune status more prominently males. Nevertheless ADA activity also guides when to start the ART treatment regimen. Hence helpful in extending the life span of HIV infected individuals to some extent.

Keywords: Adenosine deaminase, Anti-Retroviral Therapy, Cluster Difference 4.

INTRODUCTION

Human macrophages are implicated in a variety of pathological events in HIV infection the property of which is a depletion of cluster difference 4 (CD4) cells that make a pavement for a variety of diseases and malignancies.

Adenosine, a small regulatory molecule [1] increases during inflammation and infection [2] is the catabolic product of adenosine monophosphate (AMP). Adenosine deaminase (ADA E.C.3.5.4.4) catalyses and removes ammonia from adenosine and 2' deoxyadenosine and forms inosine and deoxyinosine [3] which are toxic metabolites to the cells. ADA is present intracellularly and extracellularly [3,4]. ADA1, ADA2 and ADA1 and ADA complexing protein [5] are the three isoenzyme forms of human ADA. The actives of ADA1 and ADA2 are very much essential to the cells in maintaining homeostasis because former helps in protection of the cell from metabolites [6,7] and the latter is responsible for the differentiation of monocytes to macrophages [6,8]. ADA is vital for the proper immune development and its function [9]. Some studies were attributed to the increase in serum ADA (a nonspecific marker of T-cell activation activity in diseases where cellular mediated immunity is altered [5,10]. ADA also stimulates release of excitatory amino acids and is essential to the coupling of adenosine receptors and heterotrimeric G proteins [11]. This triggers a number of signal transduction molecules (CAMP-PKA, ERK-MAPK pathways, Hypoxia inducible factors (HIF1α and HIF 2α) and transcription factors when coupled to A2B, A2A (adenosine receptors) which results in gene expression and differentiation [12]. Activation of ADA Receptors depends on the local concentration of adenosine[1].

The objective is to ascertain the ADA activity in before treatment and after treatment (ART) in HIV-positive patients and its variation in comparison to controls within this locality. The hypothesis was based on whether ART has any effect on the ADA activity and CD4 cell count in HIV Seropositive subjects. However to our knowledge few studies have been performed to investigate the changes in serum ADA activity in HIV infection in accordance to CD4 count. Some studies showed there will be an increase in ADA activity in HIV infection [13]. In our study we observed a significant decrease in ADA activity among ART treated HIV positive subjects than before treatment (ART).

MATERIAL AND METHODS

Group design, participants and blood collection:

The study was conducted in Mamata medical college and general hospital, Khammam, Andhra Pradesh, India during July to December 2010. The institutional ethics review board approved study

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protocols and forms and each study individually provided informed written consent. For this study 50 HIV seropositive cases were recruited along with 27 healthy individuals as controls obtained from hospital and student population. For confirmation and diagnosis of HIV infection we followed the guidelines of NACO (2003) recommendations for HIV testing. The subjects tested positive for HIV infection, nonsmokers and not on ART were incorporated into the study. Subjects who were on multivitamins, antioxidants, obesity, anti-hypertensive and anti lipidaemic drugs were excluded. Assessment of CD4 count was done by flow cytometry. HIV subjects were divided based on centers for disease control and prevention classification (CDC) classification into three groups [14]. From the same set of sample subjects again blood was collected after four months of ART.

Venous blood samples were drawn from HIV positive subjects into plain and K_3-EDTA containing tubes for serum and plasma extraction. Serum and plasma were separated by centrifugation at 3000rpm for 10 minutes. Plasma EDTA aliquots were used for CD4 count and serum was used for estimation of adenosine deaminase. ADA Kits were obtained from Tulip Diagnostics, Coral crest Biosystems, Goa, India. ADA hydrolases 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. Analysis was performed on the day of collection.

Statistical analysis:

The student "t" was used to compare the mean values between two groups when the data normally distributed while Mann-Whitney rank sum test when non normally distributed. When two or more groups tested Kruskal-Wallis One Way Analysis of Variance on ranks and for multiple comparisons versus control group Dunn's method was used. Multiple pairwise comparisons (Holm-Sidak test) were used for ages in different groups. P values of < 0.05 were considered statistically significant.

RESULTS

As the disease progressed the BMI (body mass index) of HIV infected cases also decreased and much appreciated in HIV cases before ART treatment. BMI compared between healthy controls, before and after treatment (ART) HIV positive cases a statistical difference of p 0.000 was observed. The BMI of HIV infected study sample after treated with ART there was a significant difference when compared versus the same sample before treatment.

The median CD4 count before treatment (ART) in HIV positive cases were 305 cells/Cu mm and after four months of treatment (ART) the CD4 count of HIV positive cases was 368 cells/ Cu mm. There was a statistical difference in CD4 count (<0.001) when compared before treatment (ART) and after treatment (ART) in HIV positive cases (paired t test). When comparing CD4 count with control versus each group of before treatment (ART) and versus after treatment (ART) HIV positive cases [Table 2] the significance was <0.001. When CD4 count compared between controls, before ART and after ART in HIV cases (Table 1) the difference was <0.05.

Among the HIV infected positive patients we observed a significant increase of the ADA as the CD4 cell count decreased in untreated patients' i.e. before starting the ART treatment. Adenosine deaminase variable [Table 1] when compared between controls, before ART treatment and after ART treatment HIV positive cases the difference was P <0.001. Significance was observed (< 0.001) when compared between controls and before ART, control and after ART and before ART and after ART HIV positive cases. Comparing the healthy controls ADA (Table 1) versus each group (Table 2) ADA of before treatment (ART) groups and after four months of treatment (ART) the significant difference was <0.001 (student "t" test except controls versus group III). Similarly in between controls and all groups of before ART and after using ART HIV cases the difference was <0.001. When multiple group comparisons versus controls were analyzed statistical difference was <0.05 except control versus group III.

Table.1: Different variables in healthy HIV seronegative controls, before ART and after ART in HIV positive Cases

Variable	Controls (n-27)	Before treatment (ART) HIV Cases (n= 50)	After treatment (ART) HIV Cases (n= 50)
Age	22.34±7.5	39.44±10.66	39.44±10.66
Sex	15M:12F	27M:23F	27M:23F
BMI (Kg/m²)	25.2 ± 1.9	21.8±1.9	24.1±1.76
CD4 cells/cu mm	814± 66	305.8± 172.3	368.1± 173.8
Adenosine Deaminase	5.22± 2.5	22.34± 7.5	13.5± 3.5

Table.2: Variables according to CD4 cell count[14] in HIV positive cases.

Variable	Group I (n=12) >500 CD4 cells/cumm	Group II (n=19) 499-200 CD4 cells/cumm	Group III (n=19) <199 CD4 cells/cumm	P value
Age	31.5±9.0	41.9±10.1	41.8±10.1	=0.05
Before ART CD4 cells/cu mm	545.5±35.1	356.9±84.5	135.1±39.0	<0.001
After ART CD4 cells/cu mm	579±29.1	425.8±83	177±55.6	<0.05
Before ART Adenosine Deaminase	14.3±3.1	20.8±4.9	28.5±6.5	<0.05
After ART Adenosine Deaminase	9.5±2.5	13.2±3.1	14.7±3.0	=0.05

DISCUSSION

Our study found that males showed more ADA activity than females. Untreated HIV positive subjects had significantly higher ADA activity [males (27.7±5.5 U/L vs females (14.6±4.2 U/L), P < 0.0001] than treated (ART) [males (14.3±3.4 U/L vs females (10.4±2.5 U/L), P < 0.0001]. One possible mechanism for the decreased ADA activity in females than males is due to the presence of hormone estradiol which inhibits the activity of ADA [15].

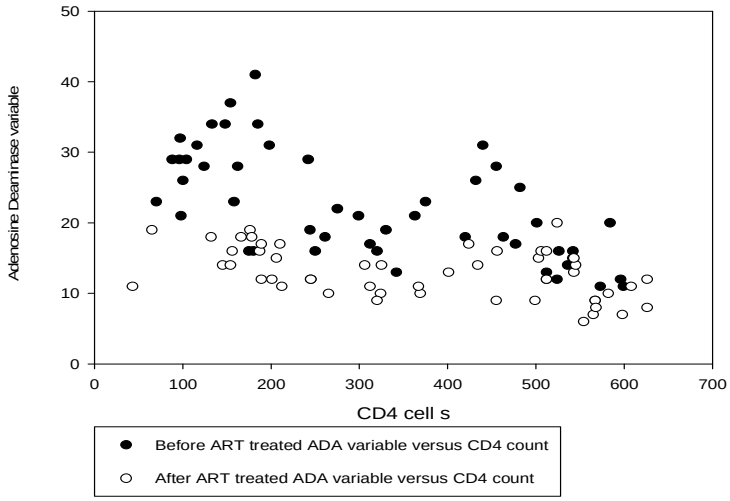
In other studies where ADA2 was found to be increased in HIV positive subjects [16, 17]. Increase in plasma ADA is an immunogenic response towards the increase of adenosine in HIV infection by the cells. In before treatment (ART) or untreated HIV positive subjects, the T-cells stimulated with HIV proliferate and produce lymphokines (IFN-g and IL-2), capable of activating macrophages [20]. Macrophages have been shown to harbor large amounts of HIV for a long time without being killed, thereby serving as a reservoir of HIV [18]. Consequently, 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 [19]. Secondly as cAMP increases in HIV infection its degradation leads to high level of adenosine in the body. So to nullify this ammoniacal nucleic acid effect, ADA converts adenosine into inosine by removing ammonia from adenosine.

After ART treatment adenosine deaminase activity decreases as the CD4 count increases. This is due to the fact that after ART the HIV viral particles diminishes in number and the activation or immunogenic response also decreases resulting in decreased ADA activity.

Out of 50 cases 3 cases shown reduced amount of ADA activity, CD4 cell count and weight as after treatment (ART). The mean duration of HIV infection among them is 12.1 years. During the catabolism of adenosine there is increased production of free radicals. Apart from this, in HIV infection, will be increased production of free radicals. This construction probably can mutate the ADA gene resulting in less production. This leads to accumulation of toxic metabolites (adenosine) that result in immunodeficiency. These things account probably the subject is in a severe immunocompromised state as the high level adenosine and IFN gamma inhibits ADA activity. Activation rejection of T cell lymphocyte causes decreased ADA activity and the cell mediated immunity is in jeopardy.

Figure 1 shows the scattered values of adenosine deaminase activity before treatment (ART) and after treatment (ART) in HIV positive subjects in accordance to their CD4 count respectively. There is an increase in CD4 count and decrease in ADA activity when HIV positive individual when on ART treatment. This proves that the ADA can be a good index in knowing the prognosis of HIV Seropositive subjects.

Figure.1: Correlation of CD4 cell count versus ADA activity before ART and after ART in HIV positive cases



CONCLUSION

Our study demonstrated higher ADA activity as the CD4 count decreases in HIV positive untreated subjects and lower ADA activity after the treatment (ART). HIV positive subjects should be frequently assessed ADA activity to know the prognosis of the disease and also to know the status of their immune status, especially males. However ADA activity also guides when to start the ART. Hence helpful in extending the life span of HIV infected individuals to some extent. Unless there is an effective antidote to the abnormal signals that are generated in HIV infection [20] from free radicals due to the catabolism of adenosine which are responsible for dysregulated pathological cytokines and also in probable mutagenesis of the ADA gene till then there would no validation to the HIV infected suffering humanity.

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