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Diagnositic Utility of Serum Adenosine Deaminase and Liver Function Tests in Tuberculosis and Hiv Patients

KEYWORDS: Adenosine Deaminase, Liver function test, HIV, AIDS, TB

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ABSTRACT *Adenosine deaminase is a catabolic enzyme, of the purine catabolic pathway. The function of ADA also is related to the development of immune system and cell differentiation in human. The global incidence of tuberculosis (TB) has sharply increased particularly in areas where HIV and tuberculosis are both prevalent. The Adenosine deaminase activity was found to be elevated in the fluid sample of most of the suspected cases of Tuberculosis and HIV. But it has been observed that in the patients presented with typical clinical picture of tuberculosis infection and HIV, the adenosine deaminase activity in serum is much above the reference range. The Adenosine deaminase activity along with the lymphocyte to Neutrophils ratio, total leucocytes count and protein electrophoresis can be used as a diagnostic test for the diagnosis of HIV and HIV plus tuberculosis infection. The study consists of 37 patients with HIV infection, 33 patients with HIV and tuberculosis and 43 patients who suffered from tuberculosis and 25 normal subjects. All the serum samples were analyzed for ADA, Serum Total Proteins, Albumin, Globulin, Serum Alkaline Phosphatase, Serum Alanine Transaminase, Serum Aspartate Transaminase and Serum Total Bilirubin. HIV patients had significantly higher ADA levels compared to normal controls. TB Patients also had significantly higher ADA and Total Leucocyte count when compared to normal controls. Comparison of normal subjects with patients suffering with both HIV and TB showed that the patients had significantly higher ADA and Alkaline phosphatase levels.*

INTRODUCTION

Though India is the second-most populous country in the world, India has more new TB cases annually than any other country. In 2011, out of the estimated global annual incidence of 9 million TB cases, 2.3 millions were estimated to have occurred in India, accounting for approximately one fifth of the global incidence. [1]

Similarly there were approximately 34 [31.4–35.9] million people across the world living with HIV in 2011. Sub-Saharan Africa is the most affected region, with nearly 1 in every 20 adults living with HIV. Sixty nine per cent of all people living with HIV are living in this region. There is no cure for HIV infection. However, effective treatment with antiretroviral drugs can control the virus so that people with HIV patients can enjoy healthy and productive lives. [2]

India has the third largest number of people living with HIV/AIDS. As per the 2008-09 HIV estimates, there are an estimated 23.9 lakh people currently living with HIV/AIDS in India with an adult prevalence of 0.31 percent in 2009. [3]

Among the states, Manipur has shown the highest estimated adult HIV prevalence (1.40%), followed by Andhra Pradesh (0.90%), Mizoram (0.81%), Nagaland (0.78%), Karnataka (0.63%) and Maharashtra (0.55%). Besides these states, Goa, Chandigarh, Gujarat, Punjab and Tamil Nadu have shown an estimated adult HIV prevalence greater than national prevalence (0.31%).

Tuberculosis is the commonest opportunistic infection among people living with HIV and in several instances HIV and TB co-exist. The possibility of HIV infection in cases of tuberculosis and Vice-Versa, should be considered at all times. In 2011 worldwide 430,000 people were estimated to have died of TB and HIV co-infection, in addition to the 990,000 people who died from TB alone. It has been seen that AIDS with a co-infection of tuberculosis exist more predominantly in the lower economic group of people.

In resource-poor settings, smear negative TB is difficult to diagnose and also difficult to exclude especially in HIV infected patient. [4]

There are a few numbers of tests available for the diagnosis of tuberculosis, such as Adenosine deaminase activity, polymerase chain reaction, Interferon gamma and Lysozyme. There is also microbiological confirmation of the microorganisms i.e. culturing of the fluid obtained, looking out for the Acid fact bacilli in the sputum or fluid. The various tests for the diagnosis of tuberculosis, Polymerase Chain Reaction (PCR), is expensive and is not found to be more sensitive to pleural fluid.

Thus the only test left is Adenosine deaminase Activity (ADA). This is a single test which is sensitive and specific and at the same time inexpensive and easy to perform [5].

Adenosine deaminase (ADA) is a hydrolase enzyme, polymorphic and actively participates in the metabolism of adenine nucleotides. This enzyme catalyzes hydrolytic de-amination of adenosine and deoxyadenosine to inosine and deoxyinosine respectively; in this process ammonia is released. ADA modulates the concentration of adenosine which is both a metabolic precursor for nucleic acids (intracellular) and significant signaling molecule involved in the regulation of various physiological processes. Lymphoid tissue has 10 to 20 time's higher adenosine deaminase concentration than the other tissues. Looking into the role of this enzyme in the differentiation of lymphoid cells and maturation of monocytes to macrophages and taking note of the lack of sufficient information regarding the levels of biochemical parameters in tubercular and HIV subjects, the present study was initiated.

Adenosine deaminase test can be used for early TB detection where TB is endemic or other diagnostic means are expensive in adult population. Adenosine deaminase analysis is a simple and inexpensive colorimetric test that can be performed on serum and body fluids. Since 1978, ADA it has been used in the diagnosis of tuberculous effusion by Puras,

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et al 1978; Ocana, et al 1993; Petterson, 1984; Fontana, et al 1988 [6 - 9].

The ADA measurement is used commonly in European and Asian countries where these is a higher incidence of tuberculosis. ADA is also being considered as a diagnostic tool for HIV [10].

Mycobacterium tuberculosis is not the source of Adenosine deaminase activity. However increased Adenosine deaminase activity in biological fluids from tuberculosis and HIV positive patients might be due to the interaction of the mycobacterium and HIV with the host factors [11-13].

There for it is felt pertinent to find out the usefulness of ADA measurement in serum along with liver function tests for prediction of HIV & TB coinfection.

MATERIAL & METHODS

Patients included in this study were from the OPD and indoor wards of Shree Krishna Hospital. Age and sex matched normal subjects were selected from the staff members of Shree Krishna Hospital and Pramukh Swami Medical College as control. Liver function tests and serum ADA were analyzed in the following groups of patients from blood collected in plain tube following standard venipuncture procedure.

The study comprised of four groups:

- Patients with HIV disease without associated tuberculosis (No: 37)
- Patients with HIV disease with associated tuberculosis (No: 33)
- Tuberculosis patients without HIV infection (No:43)
- Control subjects (No:25)

Serum Adenosine Deaminase was estimated by the method suggested by Giusti and Gilani [14]. All other parameters e.g. Serum Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase, Total Bilirubin, Total proteins Albumin and A/G Ratio were analyzed from serum on semi-automated chemistry analyzer standard methods. Suitable statistical methods were used to interpret the data. Both male and female patients were included in the study.

RESULTS

The results of the study are depicted in Tables 1 and Table-2.

TABLE-1

	ADA IU/L mean ±SD	ALP IU/L Mean ±SD	Total Protein gm/dl Mean ±SD	Albumin gm/dl mean ±SD	Globulin gm/dl Mean ±SD	A/G ratio Mean ±SD	ALT IU/L Mean ±SD	AST IU/L Mean ±SD	Total Bilirubin mg/dl mean ±SD	WBC Cell/ cumm Mean ±SD
HIV	47.64 ± 8.63	147.39 ± 89.49	9.65 ± 0.64	3.78 ± 0.7	3.82 ± 0.681	1.55 ± 0.32	47.35 ± 9.14	59.54 ± 8.13	0.9 ± 0.75	7100 ± 4619.16
TB	61.84 ± 26.45	185.94 ± 48.24	6.9 ± 0.54	4.16 ± 0.66	2.73 ± 0.53	1.46 ± 0.49	26.58 ± 7.44	30.12 ± 7.95	0.69 ±0.56	11451.6 ± 5263.05
HIV+TB	87.7 ± 26.76	171.5 ± 49.71	7.39 ± 0.49	3.78 ± 0.7	2.8 ± 0.75	1.01 ± 0.42	29.9 ± 11.18	32.9 ±10.91	0.62 ± 0.22	6905.29 ± 3841.0
Control	14.42 ± 6.72	142.4 ± 32.75	7.63 ± 0.56	4.43 ± 0.35	3.07 ± 0.77	1.5 ± 0.65	28.88 ± 8.65	29.48 ± 8.65	0.27 ± 0.11	6569.23 ± 2212.83

ALP– ALKLINE PHOSPHATASE
TPR – TOTAL PROTIEN
ALB – ALBUMIN
GLO – GLOBULIN
A/G- ALBUMIN GLOBULIN RATIO

ALT – ALANINE TRANSAMINASE (SGPT)
AST – ASPARTATE TRANSAMINASE (SGOT)
TBI – TOTAL BILLIRUBIN
WBC – TOTAL LEUCOCYTE COUNT

TABLE-2 Statistical analysis: P Values

	ADA	ALP	TPR	ALB	GLO	A/G	ALT	AST	TBI	WBC
Control Vs HIV	<0.001	>0.05	>0.05	<0.01	<0.001	<0.001	>0.05	>0.05	<0.01	>0.05
Control Vs TB	<0.001	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.001
Control Vs HIV+TB	<0.001	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
HIV Vs TB	<0.05	<0.05	>0.05	>0.05	<0.001	<0.001	<0.05	<0.05	>0.05	<0.001
HIV Vs HIV+TB	<0.001	>0.05	>0.05	>0.05	<0.001	<0.001	>0.05	<0.05	>0.05	>0.05
HIV+TB Vs TB	<0.001	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.001

From the above table no. 1 & 2 it is obvious that the serum ADA levees were significantly higher in tuberculosis, HIV and also in HIV with tuberculosis as compared to normal subjects. The same was true for ALP also. The maximum, ADA level was found in HIV-tuberculosis co-infection group. The total Leucocyte count is significantly high in tuberculosis but within reference range in subjects having HIV and tuberculosis.

DISCUSSION

Identifying pulmonary tuberculosis in patients with negative sputum smear results is a diagnostic challenge. Considering the low yield of smear and culture in pulmonary tuberculosis, non-microbiological methods may provide new tools for diagnosis. The determination of adenosine deaminase levels is used as one of the tests to prove tuberculosis. There is elevation in serum concentrations of ADA that could be used as a screening test [15].

Mohammad Abdi et al [16] reported mean and standard deviation of ADA for HIV positive and healthy groups were 51.56 ± 12.56 U/L and 23.40 ± 11.01 U/L, respectively. The main source of serum total ADA activity results from ADA2 isoenzymes and its concentration was greater than ADA1 (43.46 ± 12.58 U/L and 16.45 ± 9.78 U/L for ADA2 versus 8.11 ± 5.28 U/L and 6.33 ± 3.93 U/L for ADA1). One explanation could be that the isoenzymes ADA-2 which contribute significantly to total ADA in diagnosing pulmonary TB are found mainly in the monocytes [17] which are not significantly affected in HIV patients compared to CD4 T-lymphocytes. In our study the result shows ADA activity in HIV positive and control group

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were 47.64 ± 8.63 and 14.42 ± 6.72 respectively.

Shahla Afrasiabian [18] found that the average of serum ADA in TB patients group and control group was 20.88 (±5.97) and 10.69 (±2.98), respectively. Agarwal *et al* [19] reported that ADA level as 15.3 (±0.23) in healthy people, 19 (±0.68) in non-pulmonary TB cases, and 38.48 (±1.56) in pulmonary TB patients. In our study the level of serum ADA in TB patients and in healthy group was 61.84 ± 26.45 and 14.42 ± 6.72 respectively.

Raj B et al (1985) [20] measured, ADA activities in pleural fluid and serum of patients suffering from tuberculosis, lung cancer and pneumonia. They reported a significantly high ADA level in patients with tuberculosis than that of normal controls and patients of lung cancer and pneumonia.

Sinha P K et al (1985) [21] reported a high ADA activity in pleural effusion of patients with tuberculosis, when compared with other in practice lab-procedure for diagnosing tubercular effusion like mycobacterium culture and histopathology. The estimation of ADA activity is sensitive, simple, rapid and least invasive. In our study the HIV patients had significantly higher ADA levels compared to normal controls. TB Patients also had significantly higher ADA and Total Leucocytes count when compared to normal controls. Comparison of normal subjects with patients suffering from both HIV and TB showed that the patients had significantly higher ADA and Alkaline phosphatase levels. When patients of HIV were compared with TB patients the HIV patients had significantly higher serum globulin levels. The serum ADA level was significantly higher in patients having both HIV & TB infections when compared to TB patients alone. ADA levels were significantly higher when both TB and HIV coexisted in the same patient in comparison to patients having TB alone. The Total Leucocytes count was significantly more in TB patients.

Alatas et al [22] reported significant decrease in the activities of ADA after treatment in the serum of the patients with pulmonary tuberculosis. Human Immuno deficiency virus, which is a retro virus, causes the breakdown of the body's immune system, leaving the afflicted persons vulnerable to a host of life threatening opportunistic infections, neurological or unusual malignancies. Recent update on AIDS cases shows there are about 22.6 million people in the world who are living with HIV / AIDS. HIV and TB form a lethal combination, each speeding the other's progress. HIV weakens the immune system. Someone who is HIV positive and infected with TB bacilli is many times more likely to become sick with TB than someone infected with TB bacilli that are HIV-negative. In our study we found that serum ADA level increase in both TB and HIV patients but the highest was found in coinfection with TB and HIV.

CONCLUSION:

Use of ADA activity for diagnosis of TB depends on prevalence of Tuberculosis. Where TB is endemic or diagnostic procedures are expensive, ADA appears to be a useful test for early TB diagnosis. If ADA level is highly elevate in Tuberculosis patients, along with normal Alkaline phosphatase and total Leucocytes counts will suggest coinfection with HIV and should be thoroughly evaluate for it.

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