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A COMPARATIVE STUDY OF SERUM ADENOSINE DEAMINASE ENZYME AND SERUM FERROXIDASE ALBUMIN RATIO IN DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT

Serum adenosine deaminase enzyme and Serum ferroxidase and albumin levels were determined in 50 newly diagnosed patients of tuberculosis, as Case. Fifty age and sex matched healthy individuals were taken as controls. Mean ± SD of serum adenosine deaminase and serum ferroxidase in controls and case was found to be 22.90±3.87U/L,873.82 ± 117.44 IU/L, and 56.73±14.43 U/L, 1708.74±283.57 IU/L, respectively. Serum adenosine deaminase enzyme and Serum ferroxidase in case was significantly higher as compared to controls ($p<0.001$). The decreased levels of serum albumin in case, as compared to control was statistically significant ($p<0.001$).Serum ferroxidase: albumin ratio (Ferroxidase in International Unit per gram of albumin) in case (60.05±14.46 IU /g) was significantly higher than controls (25.02±4.5 IU/g), ($p < 0.001$) Serum adenosine deaminase enzyme good marker for diagnosis of TB but when it combine with Serum ferroxidase: albumin ratio (IU/g) it can therefore be employed as a surrogate marker to lend a hand in diagnosis and prognosis of pulmonary tuberculosis.

Keywords: Serum adenosine deaminase enzyme (ADA), Albumin, Ferroxidase, Diagnosis, Tuberculosis.

INTRODUCTION

TB is a major disease burden in India which causing a very high morbidity and mortality, leading to 3 million death annually in India. *Mycobacterium tuberculosis* bacteria have infected 9.27 million people in year 2007, globally and about 2 million incident cases of TB were in India **(1, 2).**

In addition, difficulty in diagnose to TB early is one of the main barrier in limit the spread and early treatment of this disease. A positive AFB smear and /or culture of Mycobacterium tuberculosis is gold standard for diagnosis but it is time consuming.**(3)** Usually diagnosis is based on clinical presentation, radiologic finding and positive tuberculin and BCG tests. Under such circumstances Antitubercular therapy therapy is started empirically .It therefore becomes vital to find some rapid and useful tests for the early and accurate diagnosis of tuberculosis. Various workers have tried different biochemical tests from time to time, which may help confirm the diagnosis of pulmonary tuberculosis.

Adenosine deaminase (ADA), an enzyme essential for the propagation and the differentiation of lymphocytes, particularly T-cells, so that estimation of its activity has been used for observation numerous diseases of altered immunity **(4)**. The serum activity of ADA altered in diseases that cause a cell-mediated immune reaction such as lung cancer, tuberculosis, rheumatoid arthritis and systemic lupus erythematosus. **(5, 6).**

ADA is an enzyme that catalyses the hydrolytic and irreversible deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine **(7).** This enzyme is mainly

distributed in the lymphoid tissues. It has been reflected on as a marker of T-cell activation **(8)**. Serum ceruloplasmin is a blue α-2; copper transporting globulin synthesized in liver microsomes and possesses ferroxidase activity **(9)**. It also contains an antioxidant property in serum by oxidizing ferrous iron which could otherwise act as a catalyst in generating toxic free radicals **(10)**.

Increases in the level of serum ceruloplasmin were observed in new cases of pulmonary TB either sputum positive or negative which come down with antitubercular treatment in due course of time and shows that level of serum ceruloplasmin are related with the activity of the disease process **(11)**. Albumin, a major plasma protein has been reported low in pulmonary TB **(12)**.

This study aims to find an evaluation of serum Adenosine deaminase enzyme, serum ferroxidase, albumin and ferroxidase: albumin ratio in the diagnosis of pulmonary TB and a comparison of these two tests serum ADA and ferroxidase: albumin ratio which one is better in diagnosis of Tuberculosis.

MATERIALS AND METHODS

The present study was conducted in Department of Biochemistry, Geetanjali Medical College, Udaipur, in collaboration with Department of TB. and chest, Geetanjali Medical College, Udaipur between march 2010 and January 2011 .Fifty cases of pulmonary TB were taken for the present study whose were freshly diagnosed, sputum positive .Fifty age and sex matched healthy subjects without any history of pulmonary TB were also included in the study as controls. In all cases serum adiponectin deaminase, serum ferroxidase & serum albumin levels were studied. Permission was taken from the Institutional Ethics Committee. Informed consent has been taken in English or local language if applicable.

Inclusion criteria for pulmonary TB

Cases diagnosed as a "new case" of tuberculosis; Possessing at least two positive sputum smear test positive for Acid Fast Bacilli; Radiographic abnormalities consistent with pulmonary tuberculosis, A decision by physician to treat with a full course of anti-TB Chemotherapy, non tubercular pulmonary diseases: a decision by physician.

Exclusion criteria for pulmonary TB

Patients with extra pulmonary TB and/or patients requiring surgical intervention, chronic pulmonary TB (receiving at least two courses of anti-TB treatment for more than six months), Presence of secondary immunodeficiency states: HIV, organ transplantation, diabetes mellitus, treatment with corticosteroids.

A detailed clinical history was taken and thorough physical examination was carried out in every subject. A set of investigations including three consecutive (spot-early mornings-spot) sputum samples examination, Mantoux test, Postero-anterior chest x-ray and ESR by Westergren method were carried out on study group. Serum ADA estimation was done in healthy controls, pulmonary tuberculosis patients.

Principle

Adenosine deaminase hydrolyses adenosine to ammonia & inosine. The ammonia formed further reacts with a phenol as hypochloride in an alkaline medium to from a blue color indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue colored indophenols complex formed is directly proportional to the amount of ADA present in the sample.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Ammonia} + \text{Inosine}$$

$$\text{Ammonia} + \text{Phenol} + \text{Hypochloride} \xrightarrow[\text{Medium}]{\text{Alkaline}} \text{Blue indo phenol complex}$$

This method of serum ADA estimation is based on the principle of Guisti G Galanti methods of enzymatic analyses 1974 (13). For the determination of Adenosine deaminase activity

in serum, plasma, and biological flu-ids, ADA MTB diagnostic kit from Microxpress a division of Tulip diagnostics (P) Ltd has been used.Serum albumin was estimated by Autopak kit from Roche, USA on "HITACHI 902" Clinical Chemistry analyzer. Serum ferroxidase was estimated by a Kinetic method of Somani & Ambade **(14)** on Spectrophotometer. Statistical analysis was carried out using the software program 'SPSS".

RESULTS

Control Group

Fifty normal individuals aged between 20 years - 55 years [32 Males (64.0%) and 18 females (36.0%)] were included as controls. In this group the serum ADA activity level ranged from 13.4 to 29.3 U/L with a mean value of 22.90±3.87 U/L as mentioned in Table 1. Serum ferroxidase levels in controls ranged from 690 IU/L to 1180 IU/L with a mean of 870± 117.44 IU/L units and the serum albumin were ranged from 3.0 g/dl to 4.7 g/dl with a mean of 3.5± 0.33.g/dl. The calculated ratio of ferroxidase and albumin were ranged from 17.89 IU/g to 34.70 IU/g with a mean of 25.02± 4.5 IU/g.

Case Group

Fifty TB patients aged between 19 years - 62 years [35 Males (70.0%) and 15 females (30.0%)] were included as case. Values of serum ADA were varying from 40.3U/L to 97.65 U/L and the mean ± SD were 56.73± 14.43 U/L. The mean ± SD of ferroxidase (IU)/Alb (g) ratio in case was 60.05 ±14.46 IU/L and albumin were ranged from 2.91 g/dl to 4.7 g/dl with a mean 2.91± 0.41 g/dl.

Table 1 Serum adenosine deaminase, ferroxidase (FOD), albumin (Alb) and ferroxidase: albumin ratio in controls and case

Parameters	CONTROLS	CASES	P VALUE
ADA (U/L)	22.90±3.87	56.73±14.43	<0.0001
Ferroxidase (IU/L)	873.82±117.44	1708.74±283.57	<0.0001
Albumin (g/dL)	3.53±0.33	2.91±0.41	<0.0001
FOD/Alb (IU)/(g)	25.02±4.5	60.05±14.46	<0.0001

Values are mean ± SD;

Three groups of patients according to age 0-25 yrs, 26-50 yrs and ≥ 51 yrs was formed, as shown in Table 2. The diagnosis of pulmonary tuberculosis was based on ESR, Sputum AFB, Serum ADA, Mantoux test and chest x-ray.

Serum ADA level was positive in 50 (100.0 %) ferroxidase 50 (100.0 %) and ferroxidase (IU)/Alb (g) ratio cases 50 (100%) followed by 30 (60%) in Mantoux test, 34 (68.0%) in chest x-ray, 39 (78%) in sputum AFB and 27 (54%) in ESR**.**

Table 2: Showing different parameters with positive findings in the diagnosis of Pulmonary Tuberculosis

AGE GROUP	ESR	AFB	ADA	FERROXIDASE	ALBUMIN	F/A RATIO	MT	X-RAY CHEST
0-25Yrs (n=11)	04(H)	10	11(H)	11(H)	10 (L)	11(H)	8	3
26-50Yrs (n=33)	21 (H)	26	33(H)	33(H)	30 (L)	33(H)	29	28
≥51Yrs (n=6)	2(H)	3	6 (H)	6(H)	5 (L)	6(H)	3	3
TOTAL=**50**	**27**	**39**	**50**	**50**	**45**	**50**	**40**	**34**

DISCUSSION

We evaluated 50 patients, the most prevalent complaint among the 50 subjects was cough as it was found in all (50) patients, then expectoration was found in 33(66%) patients, dyspnea in 15 (30%) patients, Heamoptysis in 9 (18%) patients, chest pain in 5(10%) patients, fever in patients 40 (80%) and toxemic manifestations in 13 (26%) patients.

Although mycobacterial culture is sensitive and standard for diagnosing tuberculosis, the time for diagnosis requires a minimum of 2-3 weeks, whereas a negative result is available after 8 to 12 weeks **(15)**. Acid fast bacilli smear, the rapid screening method for the diagnosis of pulmonary tuberculosis, is insensitive for detecting mycobacteria among tuberculosis patients **(16)**.The management of pulmonary TB becomes difficult due to this long incubation period. Patient are getting a delayed diagnosis or an unwanted administration of antituberculosis drugs if decisions taken by the clinician without culture results **(15)**.

Recently Some molecular and accurate tests are available like in vitro nucleic acid direct amplification tests (DATs) and Polymerase Chain Reactions **(17,18)**, Inspite of appearance of these a positive AFB smear and/ or culture of mycobacterium is still remain the "gold standard" for diagnosis and follow up **(15,16)**. To overcome these difficulties, we necessitate different biochemical parameters to assist in early diagnosis of pulmonary TB.

Mishra et al. assessed serum ADA levels of 51 children with confirmed tuberculosis and 20 healthy controls showing significant increase in the first group with a p-value of <0.001 **(19).**

Khalid et al. assessed the role of adenosine deaminase level in serum and Broncho Alveolar Lavage (BAL) in the diagnosis of pulmonary tuberculosis. They found that patients with pulmonary tuberculosis had significantly higher ADA level in serum and BALF than patients with non- tuberculosis lung diseases as cancer, pneumonia and normal persons (P< 0.001) **(20)**

Saeed et. al. evaluated the level of serum adenosine deaminase in association of active pulmonary tuberculosis and other infectious diseases of lung. Mean serum adenosine deaminase level in pulmonary tuberculosis (42.4±21.5 IU/ml) and other infectious diseases (38.3±23.4 IU/ml) was significantly more than controls (26.6± 8.2 IU/ml), (P<0.0001 and p<0.03 respectively), but the difference between the pulmonary tuberculosis and other infectious diseases was not statistically significant**. (21)**

Our study, the mean ± SD of serum adenosine deaminase levels in controls and case, were found to be 22.90±3.87U/L and 56.73±14.43U/L, respectively. This showed that the serum adenosine deaminase increases significantly (p<0.0001) by about 100% as compared to controls This is in accord with the studies of Mishra et al. **(19)**,Khalid et al **(20)**Saeed et. al. **(21)** who reported increased levels of serum adenosine deaminase in pulmonary TB before treatment.

Motiani (1983) analyzed Serum ceruloplasmin activity in 80 patients of pulmonary tuberculosis and in 30 healthy individuals. Serum ceruloplasmin in sputum positive patients was more than double of that in controls. Significant increase also occurred in patients with tubercular toxemia even though sputum negative. **(22)**Singhvi & Maitrya (1977), found the levels of serum ceruloplasmin in untreated patients of pulmonary tuberculosis to be increased. Levels were reduced considerably after 6 months of chemotherapy. **(23)**

In this study, the mean ± SD of serum ferroxidase levels in controls and case, were found to be 873.82±117.44 and 1708.74±283.57 IU/L, respectively. This showed that the serum ferroxidase increases significantly (p<0.0001) compared to controls. This is in agreement with the studies of Motiyani P.O. **(22),** Shinghvi & Maitra **(23)** and Sudha Rao **(24)**, who also state increased

levels of serum ceruloplasmin in new case of pulmonary TB.

Adebisi et.al. **(25)** and Batra et.al. **(26)** reported significantly decreased serum albumin levels in newly diagnosed pulmonary TB patients. Observation of above studies were in concord with our study, which showed the mean ± SD of serum albumin levels in control and case 3.53±0.33 and 2.91±0.41 g/dL, respectively indicating significant ($p<0.0001$) decrease in serum albumin levels in case as compared to controls.

Ferroxidase albumin ratio is very useful to assist in the diagnosis and therapy of pulmonary TB. The mean ± SD of ferroxidase (IU)/Alb (g) ratio in control was 25.02±4.5 while in case it was found to be 60.05±14.46. Highly significant increased ratio was found in case to the control levels ($p<0.0001$).The result of present study are in agreement with Batra et. al. study. **(26)**

Our study is unique in comparing serum ADA, ferroxidase and ferroxidase (IU)/Alb (g) ratio values in pulmonary tuberculosis, showing that serum ADA, ferroxidase and albumin estimation should be done routinely, particularly if the diagnosis of tuberculosis is in doubt. Serum ADA is better marker than ferroxidase in diagnosis of tuberculosis but ferroxidase (IU)/Alb (g) ratio can use adjunct marker in doubtful tuberculosis cases.

Serum ADA, ferroxidase and albumin can be possibly included as an alternate marker to support in the diagnosis of pulmonary TB. Its utility in the prognosis of pulmonary tuberculosis could be evaluated with follow up studies.

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