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A STUDY OF SERUM ADA ISOENZYMES AND MDA LEVELS IN TUBERCULOSIS WITH TYPE II DIABETES MELLITUS

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ABSTRACT
Tuberculosis and Type II diabetes mellitus are a major cause of morbidity and mortality in India. Diagnosis of sputum negative tuberculosis has been a challenge to the clinicians and search for reliable markers for early diagnosis has been an ongoing effort. Type II diabetes mellitus has also been diagnosed most often accidentally during routine checkup or during investigation of other disorders. Serum adenosine deaminase(ADA) levels have shown promising results as a marker for diagnosis of sputum negative TB. Serum ADA levels have also been reported to be elevated in Type II diabetes mellitus. ADA exist as two isoforms ADA1 & ADA2. The isoenzyme pattern of the elevated serum ADA is not known. Both tuberculosis & Type II diabetes mellitus are associated with increased lipid peroxidation(MDA). **Aim & Objectives**: The present study was conducted to study the relevance of serum ADA and MDA levels in Tuberculosis and Type II diabetes mellitus patients. **Materials and Methods:** Our study includes 20 normal subjects came for routine health check up in our institution and 20 patients each between ages of 20-70 years with confirmed diagnosis of TB and Type II diabetes mellitus in South indian population. **Results:** The serum ADA isoenzyme fractions and MDA levels were significantly increased in both Tuberculosis patients and Type II diabetes mellitus patients compared to control groups. **Conclusion:** Serum ADA as well as its isoenzyme levels are significantly elevated in patients with tuberculosis and Type II diabetes mellitus compared to normal controls. Oxidative damage reflected as MDA levels is positively correlated with the extent of ADA elevation in tuberculosis and Type II diabetes mellitus patients.

KEY WORDS: ADA isoenzymes, Tuberculosis, Type II Diabetes Mellitus, south indian population.

INTRODUCTION
Diabetes mellitus and Tuberculosis are a major cause of morbidity and mortality all over the world. In India around 5,00,000 mortality occur annually due to tuberculosis[1]. India is also experiencing an exponential increase in incidence of type II diabetes mellitus and is estimated to have around 87 million affected with diabetes mellitus by the year 2030[2].

Both Diabetes mellitus and tuberculosis have an association with cell mediated immune response. It is widely reported that immunological imbalance is one of the key factors associated with the metabolic disturbances in type II Diabetes mellitus.

These immunological disturbances have an association with cell mediated immune response and inappropriate T- lymphocyte function[3]. Cell mediated immune response and T- lymphocyte proliferation and function are in part regulated by Purine salvage enzyme adenosine deaminase (ADA).

ADA (EC 3.5.4.4) is a metalloenzyme that catalyses the deamination of adenosine and 2`- deoxyadenosine to inosine and 2` deoxyinosine respectively. ADA exists as two principal isoenzymes ADA1 and ADA2. ADA-1 has roughly equal affinities for adenosine and 2`-deoxyadenosine (2`-deoxyadenosine deaminase/adenosine deaminase activity ratio is 0.75). It is distributed in many tissues with high activity in lymphocytes. ADA-2 has much greater affinity for adenosine (2`-deoxyadenosine deaminase/adenosine deaminase activity ratio is 0.25). ADA-2 is found only in macrophages, which release it when stimulated by microorganisms[4]. ADA levels in various body fluids have been shown to be reliable marker for diagnosis of tuberculosis. ADA levels in serum have also been shown to be elevated in pulmonary and extra pulmonary tuberculosis and are being proposed as marker for diagnosis of tuberculosis in sputum negative cases[5,6,7].

In our current work, we incorporated the erythro-9-(2-hydroxy- 3-nonyl) adenine (EHNA) to determine the isoenzyme pattern of ADA[8]. ADA-1 is completely inhibited by erythro-9-(2-hydroxy-3- nonyl) adenine (EHNA), while ADA-2 is resistant to inhibition by EHNA. Serum ADA levels have also been reported to be elevated in type II diabetes Mellitus.

MDA a product of lipid peroxidation has been used as an index of free radical mediated damage and its levels in the serum have been shown to be elevated both in tuberculosis as well as type II diabetes Mellitus.

The present study is aimed to study the relevance of serum ADA, its isoenzymes and MDA levels in tuberculosis patients with diabetes mellitus.

MATERIALS AND METHODS

Source of data

Study group

1. Group A - Type II diabetes mellitus with tuberculosis.
2. Group B – Aged matched normal subjects

Study design

The conducted study was a cross sectional case control study.

Target Population

Adults coming to PES Institute Of Medical Sciences And Research, Kuppam, Andhra Pradesh.

Sample size

Total 60 samples were tested. Among those, 20 were TB patients, 20 were type 2 diabetes mellitus and 20 controls.

Inclusion & exclusion criteria

Inclusion Criteria

Age group 20-70 years

Clinically proven cases of tuberculosis and type II diabetes mellitus.

Exclusion Criteria

Patients with non tubercular infections.

Variable measured

1. Blood glucose by glucose oxidase peroxidase enzymatic method[1].
2. Method of estimation serum ADA isoenzymes level– colorimetric method based on the principle of Guisti G Galanti method of enzymatic analyses by using EHNA as inhibitor of ADA-1[9].
3. Estimation of HbA1c by using ion-exchange high-performance liquid chromatography (HPLC)[10].
4. Plasma MDA levels-thiobarbituric acid reagent method (Colorimetric method)[11].

Informed consent was taken from the all the patients and the study was approved by the ethical and research committee of PESIMSR, kuppam.

ESTIMATION OF ADA: The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase(POD) to generatequinone dye which is monitored in a kinetic manner calculated by ΔA/min×1180(factor).

PROCEDURE

R1	700µl
Sample (serum)	50µl First incubation (in the incubator)
Mix well and incubate for 5 minutes at 37·C in a incubator	
Add R2	300µl Second incubation (in the Analyzer)

Reference values

Serum	Normal	<30u/L
	Suspect	30U/L to 40 u/L
	Strong suspect	40U/L to 60u/L
	Positive	>60U/L

Sensitivity: The minimum detectable limit is 10 U/L.

ESTIMATION OF BLOOD GLUCOSE- by Enzymatic Colorimetric End point test (GOD -POD), by using Accucare kit.

GLYCOSYLATED HEAMOGLOBIN-HbA$_1$C: Glycosylated hemoglobin HbA$_1$C is formed non enzymaticaly by two-step reaction.

Hemoglobin A1c (%)	Degree of Glucose Control
> 8	Action Suggested
< 7	Goal
< 6	Non-Diabetic Level

ESTIMATION OF MALONDIALDEHYDE: by Thiobarbituric acid (TBA) reagent. Results were analyzed using SPSS software. Comparisons between the groups were done by ANOVA test and P value of <0.05 was considered significant.

RESULTS: The results of the study have been presented in a series of tables and graphs.

Table 1.1: Age Distribution in Control & Study Groups.

Age in yrs	TB		DM		CONTROL	
	NO	%	NO	%	NO	%
20-30	5	25	2	10	5	25
31-40	7	35	3	15	5	25
41-50	4	20	5	25	4	20
51-60	2	10	6	30	3	15
61-70	2	10	4	20	3	15
Total	20	100	20	100	20	100
Mean ±SD	40.15 ± 13.98		50.85 ± 13.44		42.85 ± 13.55	

Age distribution for control group

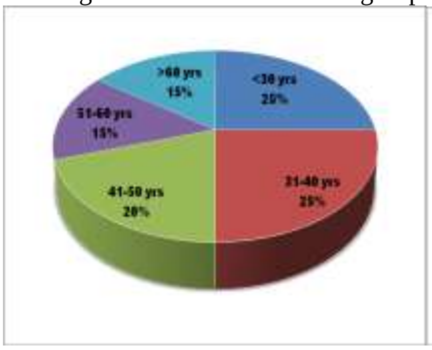

Fig 1.1

Age distribution for TB group

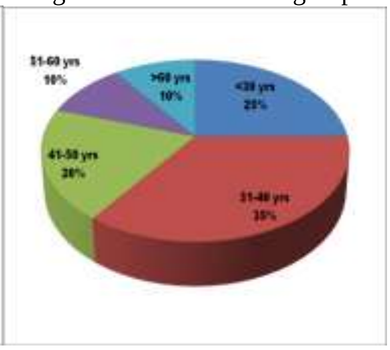

Fig 1.2

Age distribution for DM group

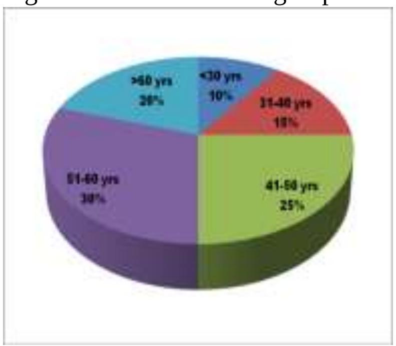

Fig 1.3

Table 1.1 & Figures 1.1 ,1.2 and 1.3 shows age distribution of the subject

Table 2.1: Gender Distribution in Control & Study Group.

SEX	TB		DM		CONTROL	
	NO	%	NO	%	NO	%
MALE	10	50	13	65	10	50
FEMALE	10	50	7	35	10	50
Total	20	100	20	100	20	100

Gender wise distribution in Control group

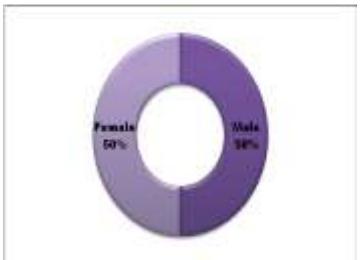

Fig 2.1

Gender wise distribution in TB group

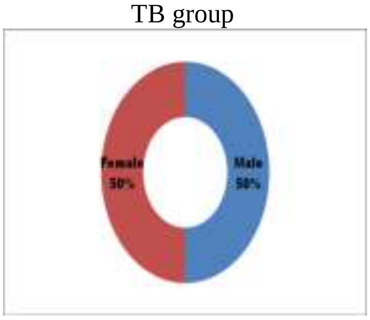

Fig 2.2

Gender wise distribution in DM group

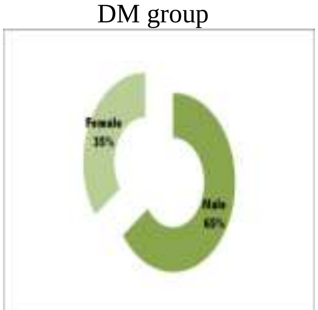

Fig 2.3

Table 2.1 & Figures 2.1, 2.2 and 2.3 shows Gender distribution of the subject.

Table 3.1: Serum ADA isoenzymes in CONTROL and TB.

STUDY VARIABLE	CONTROL (MEAN ± SD)	TB (MEAN ± SD)	p VALUE
ADA1 (U/L)	5.26 ± 1.58	10.6 ± 2.01	<0.001**
ADA2 (U/L)	14.65 ± 3.7	26.38 ± 5.34	<0.001**
TADA (U/L)	19.96 ± 3.92	36.65 ± 4.62	<0.001**
MDA (nmol/ml)	3.58 ± 0.36	6.91 ± 0.46	<0.001**

* p value >0.05, non significant **p value <0.001,strongly significant

Mean ±SD levels of TADA, ADA1, ADA2 and MDA in controls and TB patients are presented in Table 3.1 and Fig 3.2. TADA (36.65 ± 4.62) and ADA2 (26.38 ± 5.34) levels in TB patients were significantly higher (p<0.001) as compared to normals TADA (19.96 ± 3.92) & ADA1 (14.65 ± 3.7). MDA levels in TB patients (6.91 ± 0.46) were significantly higher (p<0.001) as compared to normals (3.58 ± 0.36).

Figure 3.3 shows comparison between Serum ADA Isoenzymes, FBS, HbA1c, MDA levels in CONTROL and TB groups.

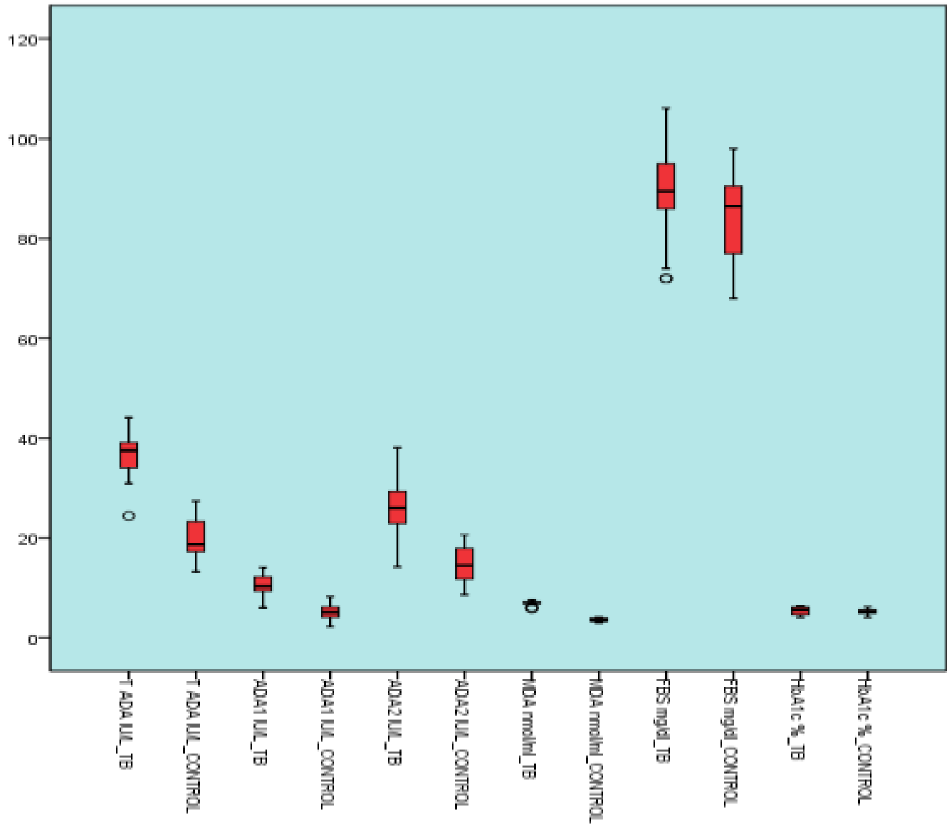

Fig 3.2

Table 4.1: Serum ADA isoenzymes, FBS,HbA1c in CONTROL and DM.

STUDY VARIABLE	CONTROL (MEAN ± SD)	DM (MEAN ± SD)	p VALUE
ADA1 (U/L)	5.26 ± 1.58	10.34 ± 2.22	<0.001**
ADA2 (U/L)	14.65 ± 3.7	23.1 ± 6.36	<0.001**
TADA (U/L)	19.96 ± 3.92	37.28 ± 5.06	<0.001**
FBS (mg/dl)	83.9 ± 8.25	188.3 ± 44.74	<0.001**
HBA1C %	5.23 ± 0.53	9.53 ± 2.32	<0.001**

* p value >0.05, non significant **p value <0.001,strongly significant

Mean ± SD levels of TADA, ADA1, ADA2, FBS, HbA1c& in controls and type 2 DM subjects are presented in table 4.1 and 4.2. FBS levels (188.3 ± 44.74) and HbA1c levels (9.53 ± 2.32) were significantly higher as compared to normal subjects with p values of <0.001.Serum ADA isoenzyme levels in type 2 DM,TADA (37.28 ± 5.06) & ADA (23.1 ± 6.36) were significantly higher as compared to normals with p value of <0.001. MDA level in DM patients (6.91 ± 0.46) were significantly higher as compared to normals (3.58 ± 0.36).

Figure 4.2 shows comparison between Serum ADA Isoenzymes, FBS, HbA1c, MDA levels in CONTROL and DM.

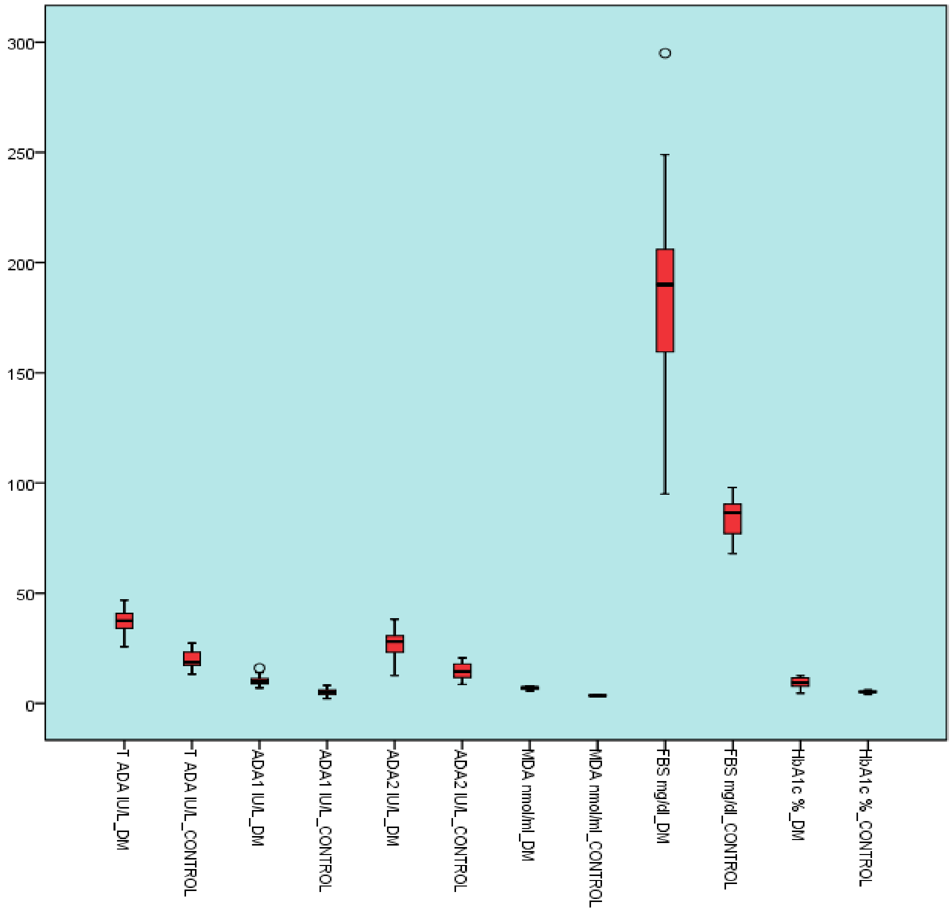

Fig 4.2

DISCUSSION

The serum ADA levels in normal subjects in this study were found to be 19.96 ± 3.92, various workers have found different values for normal human serum ADA levels, ranging from (12.492±50U/L) to (19.09±2.99U/L).[1]

In patients with TB, the ADA levels were significantly higher compared to normal controls. Serum ADA levels in patients in the present study were (36.65 ± 4.62) with range of 18.0U/L to 52.9U/L. Majority of the patients had levels in excess of 30U/L with duration of TB in excess of one year. Only in one case the TADA values were below 30U/L that was a newly diagnosed cases with duration of less than a month.

The serum ADA levels observed in our study are similar to the levels reported by other workers. Mukesh kumar et al reported serum ADA levels ranging from (32.25 to 40. 28U/L) with a mean of (38.48 ±1.56). SreenivasRao etal reported levels ranging from (35.25 to 48.50) with a mean of (40.48 ± 8.2).

ADA levels in body fluids particularly pleural fluid have been found to be reliable marker for diagnosis of TB pleural effusions[12,13]. Serum ADA levels have also been found to be elevated is non- tubercular pulmonary infections. Saeed Aminiafshar et al is a study on tubercular and non-tubercular pulmonary infections found that serum ADA levels increase in infectious disease but it cannot differentiate pulmonary TB from other infectious diseases[14].

In subjects with DM, Serum ADA levels were found to be significantly elevated compared to normal controls. Shivaprakash et al reported a significant increase in ADA levels 37.2 9.29 U/L in diabetic subjects compared to normal controls[15]. Amandeep Kaur et al reported elevated serum ADA levels in type 2 DM subjects values of (30±10.41) and (44.23±16.14) having HbA1c levels <7% &>7% respectively[16]. The serum ADA levels in the present study are similar to the values found in type 2 DM subjects with HbA1C levels > 7%. HbA1c levels in the present study in subjects with DM were > 7%.

Patients with DM have evidence of impaired cell mediated immunity, micronutrient deficiency, microangiopathy and renal insufficiency, all of which predispose to pulmonary tuberculosis. The effects are significantly more marked in diabetes patients with chronic hyperglycemia. The association could be due to non-enzymatic glycosylation of tissue proteins inducing alteration in bronchopulmonary function.

Diabetes increases by three fold the risk of person developing tuberculosis. It is estimated that diabetes is contributing to about 8% of new TB cases annually. Patients with TB are also at a higher risk of developing diabetes. TB infection is an acute stress on the individual, which is an important cause of development of impaired glucose tolerance.

The endocrine function of pancreas has also been found to be adversely affected in severe tuberculosis leading to absolute or relative insulin deficiency state. Further a family of fatty acid transporter proteins with tubercle bacilli may also lead to dysregulation of energy homostatis in TB patients. These are also reports of metabolism of antitubercular drugs like Rifampicin being affected in diabetics leading to poor response to therapy and metabolism of oral hypoglycaemic drugs being hastened by elevated cyt-p-450 activity induced by anti-tubercular drugs. Severe TB has been reported to be associated with abnormal glucose tolerance[17].

The mechanisms underlying the elevation of ADA levels in various body fluids in TB not clearly established.

ADA is an important enzyme in purine catabolism. It catalyse deamination of adenosine to inosine and deoxyadenosine to deoxyinosine. There are several forms of ADA, but the predominant ones are ADA-1 and ADA-2, which are coded by different gene loci. ADA1 is isoenzymes found in all cells, with highest concentration found in lymphocytes and monocytes, whereas ADA-2 isoenzymes are found only in monocytes[18]. ADA2 is the major component of total ADA in serum of healthy persons. It increases in biological fluids in conditions associated with stimulated activity in macrophages[19].

The present study has shown that the predominant form of ADA in the serum of patients with TB & DM is ADA-2. This finding is similar to the earlier reports that ADA2 is the major component of the activity of TADA in the serum even in healthy persons. Although ADA1 activity is higher intra-cellularly it is thought the cells preferentially release ADA2 in to circulation[20]. This preferential release may be because the cell membranes are not permeable to ADA1 or after release ADA1 may be getting rapidly degraded. ADA1 also exist in circulation in combination with a protein DPP4 and that form is referred as ADA1+CP. The exact mechanism for a predominant form of ADA2 of TADA levels in the serum are not understood.

In the present study the TADA levels in control subjects was below 26 U/L,in TB & DM patients was below 44 & 46 U/L respectively. Thus the predominant isoform of the elevated serum ADA was ADA2 in all the study groups.

The ADA levels of 30 U/L or in some reports 33U/L have been suggested as cut- off levels for diagnosis of TB. In DM the extent of elevation may vary depending upon the glycaemic status of the diabetic patient.

MDA levels have been used as good indicators of lipid peroxidation[10]. Both DM & TB are conditions where there is increased oxidative damage and consequent lipid peroxidation. The ADA may be involved in promoting oxidative damage. MDA levels showed very good correlation, both with TADA and ADA2 levels suggesting that the increased lipid peroxidation in both these (DM and TB)conditions may have association with the ADA levels.

We suggest larger studies to determine the cut-off levels that can be used for diagnosis of TB and DM.

CONCLUSION

Serum Total ADA levels as well as its isoenzyme levels are significantly elevated in patients with TB & DM. Oxidative damage reflected as MDA levels is correlated with the extent of ADA elevation in TB & DM patients.

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