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A STUDY OF SERUM ADA ISOENZYMES AND MDA LEVELS IN TUBERCULOSIS WITH AND WITHOUT TYPE 2 DIABETES MELLITUS

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ABSTRACT

Tuberculosis and type 2 diabetes mellitus are a major cause of morbidity and mortality in India. Diagnosis of sputum negative TB has been a challenge to the clinicians and search for reliable markers for early diagnosis has been an ongoing effort. Type 2 DM 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 –ve TB. Serum ADA levels have also been reported to be elevated in DM. ADA exist as two isoforms ADA1 & ADA2. The extent of elevation in ADA when both TB & DM are coexisting are not known. The isoenzyme pattern of the elevated serum ADA is not also known. Both TB & DM are associated with increased lipid peroxidation. MDA levels have been shown to be elevated in TB as well as DM. However the extent of elevation when TB & DM are coexisting has not been studied. **Aims & Objectives:** The present study was conducted to study the relevance of serum ADA levels in diagnosis of TB when type 2 DM is coexisting and alteration in isoenzyme patterns both in TB and type 2 DM. **Methods & Materials:** The study subjects included 20 patients each with confirmed diagnosis of TB, DM and coexisting DM & TB and age matched controls. To study serum MDA levels in TB, DM and coexisting DM & TB. **Results:** The serum ADA isoenzyme fractions and MDA levels were increased in TB, DM patients, but the levels were significantly higher when both TB & DM were coexisting(<0.05) compared to control groups. **Conclusion:** Serum ADA as well as its isoenzyme levels are equally elevated in patients with TB as well as DM. In patients with coexisting TB & DM, the elevations both in TADA as well as ADA2 as much higher as compared to TB or DM alone. Oxidative damage reflected as MDA levels is correlated with the extent of ADA elevation in all the three groups of patients.

KEYWORDS: ADA isoenzymes, Tuberculosis, Type II Diabetes Mellitus, erythro-9-(2-hydroxy-3- nonyl) adenine (EHNA), south indian population, MDA.

INTRODUCTION

Diabetes mellitus and Tuberculosis are a major cause of morbidity and mortality world over. In India around 500000 deaths occur annually due to TB.[1] India is also experiencing an exponential increase in incidence of type 2 diabetes mellitus and is estimated to have around 87 million affected with diabetes mellitus by the year 2030.[2] Coexistence of Diabetes mellitus and Tuberculosis is found to occur at a proportion of 25-45% of diabetes having pulmonary TB and 20-37% of TB patients having Diabetes mellitus.[3] 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 2 Diabetes mellitus. These immunological disturbances have an association with cell mediated immune response and inappropriate T- lymphocyte function.[4] 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 ionosine and 2` deoxyionosine 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.[5] ADA levels in various body fluids have been shown to be reliable marker for diagnosis of TB. ADA levels in serum have also been shown to be elevated in pulmonary and extra pulmonary TB and are

being proposed as marker for diagnosis of TB in sputum –ve cases.[6,7,8] In our current work, we incorporated the erythro-9-(2-hydroxy- 3-nonyl) adenine (EHNA) to determine the isoenzyme pattern of ADA.[9] Serum ADA levels have also been reported to be elevated in type 2 diabetes Mellitus. In view of the increased total ADA levels both in TB and type 2 DM, total ADA levels as marker of TB diagnosis needs careful evaluation in view of the increased prevalence of coexistence of both TB and DM.

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 TB as well as type 2 DM. However to our knowledge there are no studies carried out on MDA levels in whom TB and type 2 DM are coexisting.[10] The present study is an attempt in these directions. While TADA levels have been shown to be elevated both in TB & DM there have been no reports of the isoenzyme pattern of the increase in ADA levels either in TB or in DM. The present study is aimed at finding if there are any differences in the isoenzyme pattern of the elevated ADA levels between TB and DM. ADA1 is completely inhibited by erythro-9-(2-hydroxy-3- nonyl) adenine (EHNA), while ADA2 is resistant to inhibition by EHNA.

MATERIALS AND METHODS

Source of data

Study group

1. Group A - Type 2 diabetes mellitus without tuberculosis.
2. Group B - Tuberculosis without type 2 diabetes mellitus.
3. Group C - Both Tuberculosis and type 2 diabetes mellitus (co-existing TB& DM).
4. Group D – Aged matched normal subjects

The conducted study was a cross sectional case control study. Adults coming to PES Institute Of Medical Sciences And Research, Kuppam, Andhra Pradesh were the target population. Sample size was 80. Among those, 20 were type 2 DM, 20 were TB with Diabetes mellitus, 20 were TB without Diabetes mellitus and 20 controls.

Inclusion & exclusion criteria

Inclusion Criteria

- Age group 20-70 years
- Clinically proven cases of type2 diabetes mellitus and tuberculosis

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– using kit and colorimetric method based on the principle of Guisti G Galanti method of enzymatic analyses by using EHNA as inhibitor of ADA1.[14]
3. Estimation of HbA1c by using ion-exchange high-performance liquid chromatography (HPLC).[15]
4. Plasma MDA levels-thiobarbituric acid reagent method (Colorimetric method).[16]

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 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 $\Delta A/min \times 1180$(factor).

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

GLYCOSYLATED HEAMOGLOBIN-HbA_1C: Glycosylated hemoglobin HbA_1C is formed non enzymaticaly by two-step reaction.

ESTIMATION OF MALONDIALDEHYDE: by Thiobarbituric acid (TBA) reagent Results were analyzed using SPSS software Version 21. 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		TB & DM		CONTROL	
	NO	%	NO	%	NO	%	NO	%
20-30	5	25	2	10	0	0	5	25
31-40	7	35	3	15	6	30	5	25
41-50	4	20	5	25	3	15	4	20
51-60	2	10	6	30	6	30	3	15
61-70	2	10	4	20	5	25	3	15
Total	20	100	20	100	20	100	20	100
Mean ±SD	40.15 ± 13.98		50.85 ± 13.44		50.8 ± 9.91		42.85 ± 13.55	

Age distribution for control group

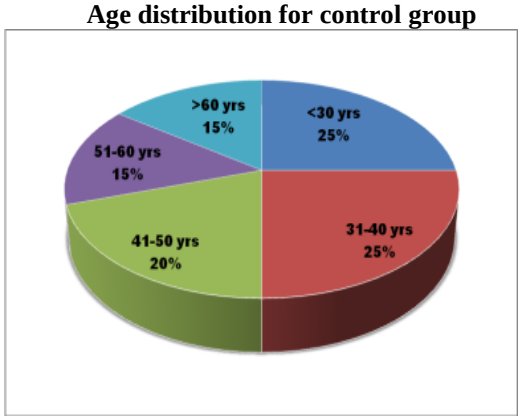


Fig 1.1

Age distribution for DM group

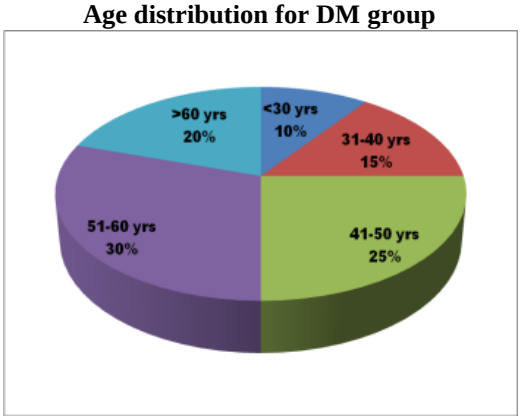


Fig 1.2

Age distribution for TB group

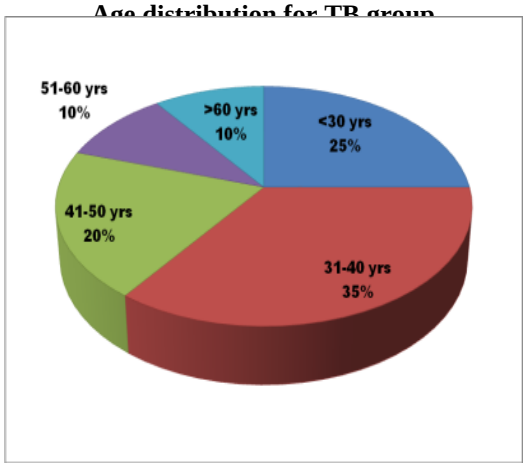
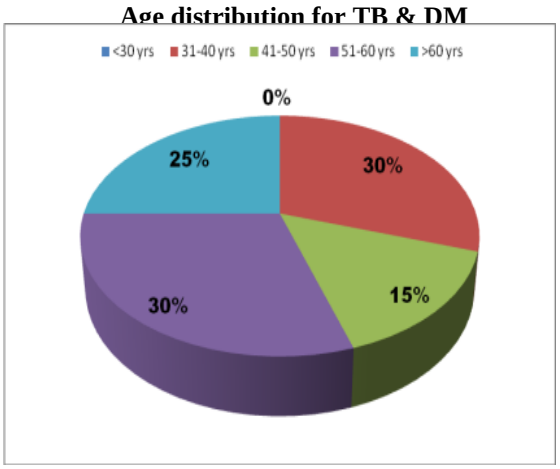


Fig 1.3

Age distribution for TB & DM



Figs 1.4

Table 1.1 & Figures 1.1 to 1.4 shows age distribution of the subject in each groups.

Table 2.1: Gender Distribution in Control & Study Group.

SEX	TB		DM		TB & DM		CONTROL	
	NO	%	NO	%	NO	%	NO	%
MALE	10	50	13	65	15	75	10	50
FEMALE	10	50	7	35	5	25	10	50
Total	20	100	20	100	20	100	20	100

Gender wise distribution in Control group

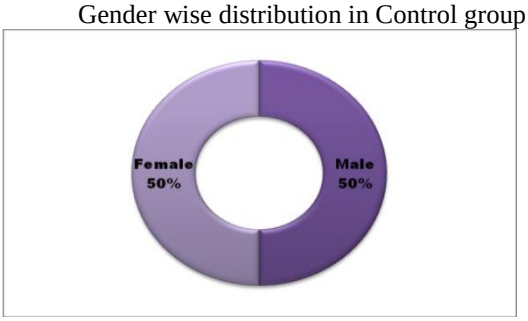


Fig 2.1

Gender wise distribution in DM group

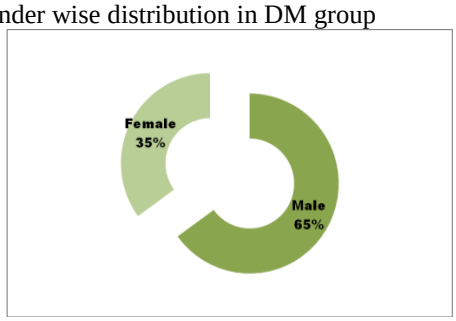


Fig 2.2

Gender wise distribution in TB group

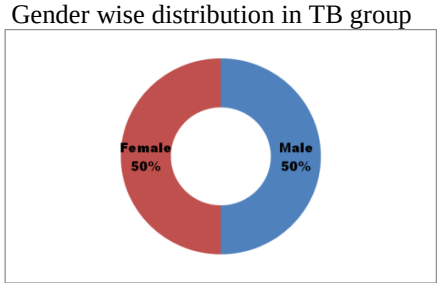


Fig 2.3

Gender wise distribution in TB & DM group

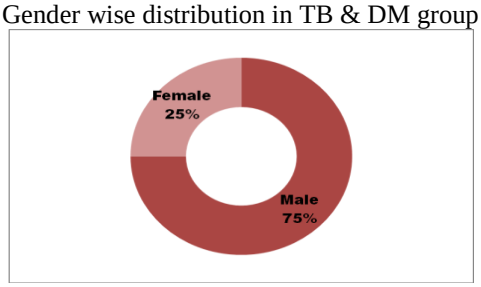


Fig 2.4

Table 2.1 & Figures 2.1 to 2.3 shows Gender distribution of the subject in each groups.

Table 3.1: Serum ADA Isoenzymes, FBS,HbA1c, MDA levels in CONTROL and TB groups:

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**
FBS (mg/dl)	83.9 ± 8.25	88.65 ± 9.13	0.937*
HBA1C %	5.23 ± 0.53	5.42 ± 0.82	0.985*
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, FBS, HbA1c and MDA in controls and TB patients are presented in Table 3.1 and Fig 3.2. FBS and HbA1c levels were similar in both the groups. 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).

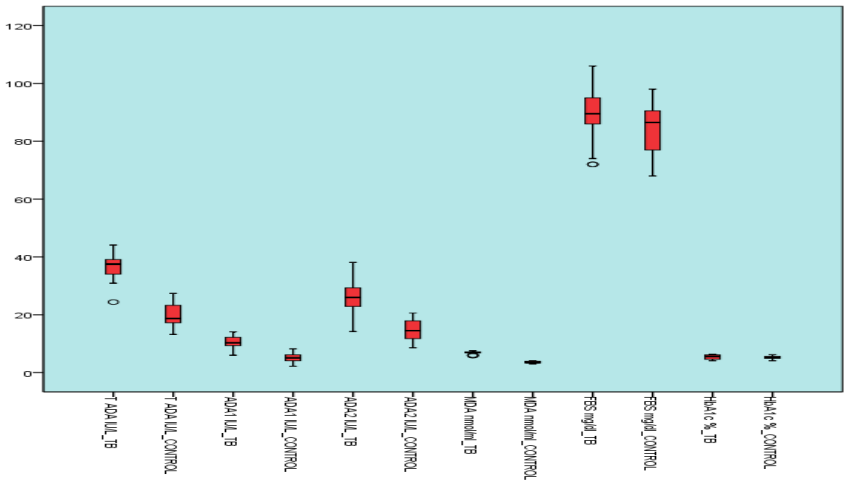


Figure 3.2: shows comparison between Serum ADA Isoenzymes, FBS, HbA1c, MDA levels in CONTROL and TB groups.

Table 4.1: Serum ADA isoenzymes, FBS, HbA1c, MDA levels 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**
MDA (nmol/ml)	3.58 ± 0.36	6.95 ± 0.63	<0.001**

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

Mean ± SD levels of TADA, ADA1, ADA2, FBS, HbA1c& MDA in controls and type 2 DM subjects are presented in table 4.1 and figure 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).

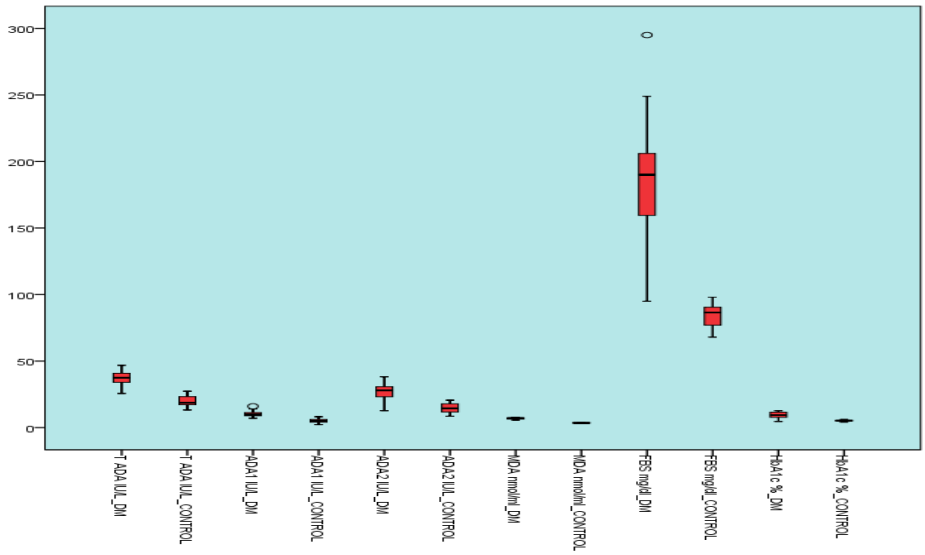


Figure 4.2 shows comparison between serum ADA isoenzymes, FBS, HbA1c, MDA levels in CONTROL and DM.

Table 5.1: Serum ADA Isoenzymes, FBS,HbA1c, MDA levels in CONTROL and Co-existing TB & DM.

STUDY VARIABLE	CONTROL (MEAN ± SD)	BOTH (TB+DM) (MEAN ± SD)	P VALUE
ADA1 (U/L)	5.26 ± 1.58	10.03 ± 1.93	<0.001**
ADA2 (U/L)	14.65 ± 3.7	39.12 ± 11.38	<0.001**
TADA (U/L)	19.96 ± 3.92	51.2 ± 10.4	<0.001**
FBS (mg/dl)	83.9 ± 8.25	170.3 ± 44.42	<0.001**
HBA1C %	5.23 ± 0.53	9.15 ± 2.36	<0.001**
MDA (nmol/ml)	3.58 ± 0.36	12.16 ± 1.46	<0.001**

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

Mean±SD levels of TADA, ADA1, ADA2, FBS, HbA1c & MDA in controls & subjects with co-existing TB & DM are presented in Table 5.1 & Fig 5.2. FBS levels (170.3 ± 44.42) &HbA1c levels (9.15 ± 2.36) were significantly higher as compared to normal subjects with a p value of <0.001. Serum ADA levels, TADA (51.2 ± 10.4) & ADA2 (39.12 ± 11.38) were also significantly higher (p<0.001) as compared to normals. MDA level in co-existing TB & DM patients (12.16 ± 1.46) were significantly higher as compared to normals (3.58 ± 0.36).

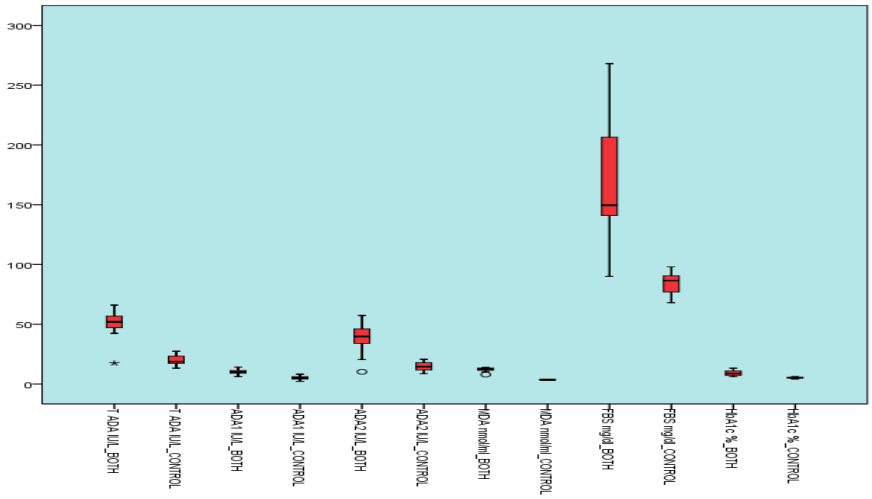


Figure 5.2 shows comparison between serum ADA isoenzymes, FBS,HbA1c, MDA levels in CONTROL and Co-existing TB & DM.

Table 6.1: Serum ADA Isoenzymes, FBS, HbA1c, MDA levels in TB and DM.

STUDY VARIABLE	TB (MEAN ± SD)	DM (MEAN ± SD)	P VALUE
ADA1 (U/L)	10.6 ± 2.01	10.34 ± 2.22	0.976*
ADA2 (U/L)	26.38 ± 5.34	23.1 ± 6.36	0.99*
TADA (U/L)	36.65 ± 4.62	37.28 ± 5.06	0.99*
FBS (mg/dl)	88.65 ± 9.13	188.3 ± 44.74	<0.001**
HBA1C %	5.42 ± 0.82	9.53 ± 2.32	<0.001**
MDA (nmol/ml)	6.91 ± 0.46	6.95 ± 0.63	0.99*

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

Table 7.1 and Fig 7.2 show comparison of Serum TADA, ADA1, ADA2, FBS, HbA1c & MDA levels between subjects with TB and DM. Both FBS (188.3 ± 44.74) and HbA1c levels (9.53 ± 2.32) were significantly higher in subjects with DM alone as compared to subjects with TB (88.65 ± 9.13 and 5.42 ± 0.82). Serum ADA isoenzyme levels were similar in both the groups, not statistically significant. MDA level were similar in both the groups.

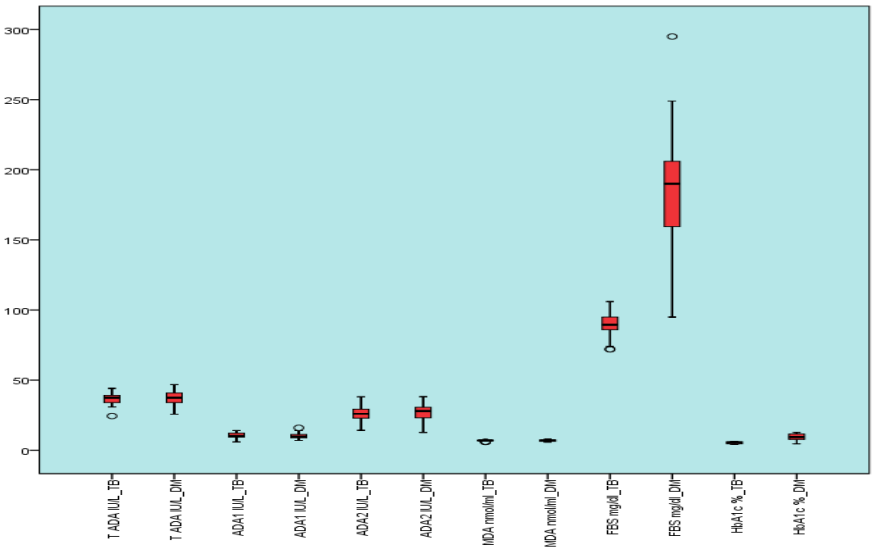


Figure 6.2: shows comparison between serum ADA Isoenzymes, FBS, HbA1c, MDA levels in TB and DM.

Table 7.1: Serum ADA isoenzymes, FBS, HbA1c, MDA levels in TB and Co-existing TB & DM.

STUDY VARIABLE	BOTH (TB & DM) (MEAN ± SD)	TB (MEAN ± SD)	p VALUE
ADA1 (U/L)	10.03 ± 1.93	10.6 ± 2.01	0.793*
ADA2 (U/L)	39.12 ± 11.38	26.38 ± 5.34	<0.001**
TADA (U/L)	51.2 ± 10.4	36.65 ± 4.62	<0.001**
FBS (mg/dl)	170.3 ± 44.42	88.65 ± 9.13	<0.001**
HBA1C %	9.15 ± 2.36	5.42 ± 0.82	<0.001**
MDA (nmol/ml)	12.16 ± 1.46	6.91 ± 0.46	<0.001**

Table 7.1 and Fig 7.2 shows comparison between mean ± SD values of serum TADA, ADA1, ADA2 levels, FBS, HbA1c & MDA in subjects with TB and coexisting TB and DM. FBS and HbA1c levels were significantly higher in subjects with coexisting TB and DM as compared to subjects with only TB. ADA isoenzyme levels in subjects with coexisting TB and DM, TADA (51.2 ± 10.4) & ADA2 (39.12 ± 11.38) were also significantly higher as compared to subjects with only TB, TADA (36.65 ± 4.62) &ADA2 (26.38 ± 5.34). MDA level in subjects with coexisting TB and DM (12.16 ± 1.46) were significantly higher compared to subjects with DM alone (6.91 ± 0.46).

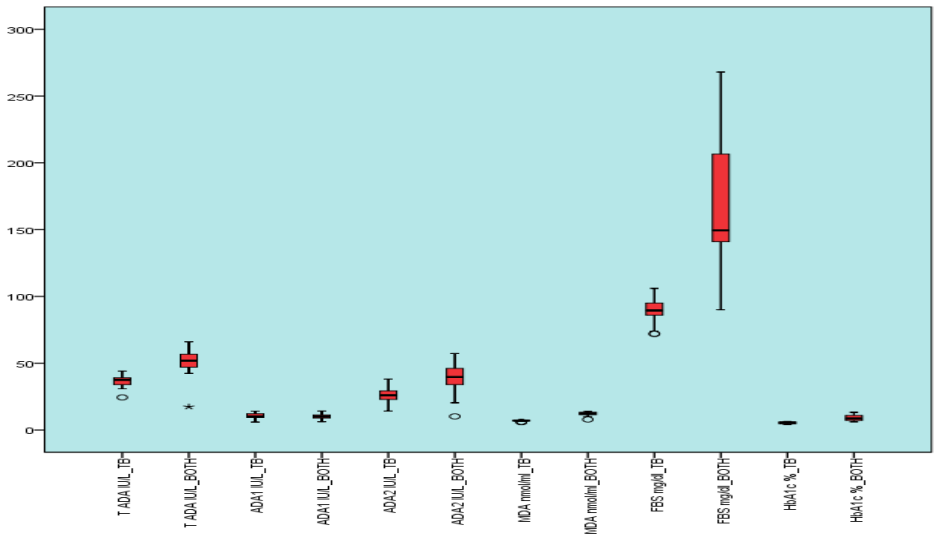


Figure 7.2 shows comparison between serum ADA isoenzymes, FBS,HbA1c, MDA levels in TB and Co-existing TB & DM.

Table 8.1: Serum ADA isoenzymes, FBS, HbA1c, MDA levels in DM and Coexisting TB & DM.

STUDY VARIABLE	BOTH (MEAN ± SD)	DM (MEAN ± SD)	P VALUE
ADA1 (U/L)	10.03 ± 1.93	10.34 ± 2.22	0.957*
ADA2 (U/L)	39.12 ± 11.38	23.1 ± 6.36	<0.001**
TADA (U/L)	51.2 ± 10.4	37.28 ± 5.06	<0.001**
FBS (mg/dl)	170.3 ± 44.42	188.3 ± 44.74	0.295*
HBA1C %	9.15 ± 2.36	9.53 ± 2.32	0.898*
MDA (nmol/ml)	12.16 ± 1.46	6.95 ± 0.63	<0.001**

Table 8.1 and Figure 8.2 shows comparison of serum TADA, ADA1, ADA2, FBS, HbA1c & MDA values between subjects with DM and coexisting DM and TB. FBS and HbA1c levels were similar in both groups. Serum ADA isoenzyme levels were also found to be significantly higher in subjects with coexisting TB and DM, TADA (51.2 ± 10.4) & ADA2 (39.12 ± 11.38) as compared to subjects with DM alone TADA (37.28 ± 5.06) & (23.1 ± 6.36). MDA level in subjects with coexisting TB and DM (12.16 ± 1.46) were significantly higher compared to subjects with DM alone (6.95 ± 0.63).

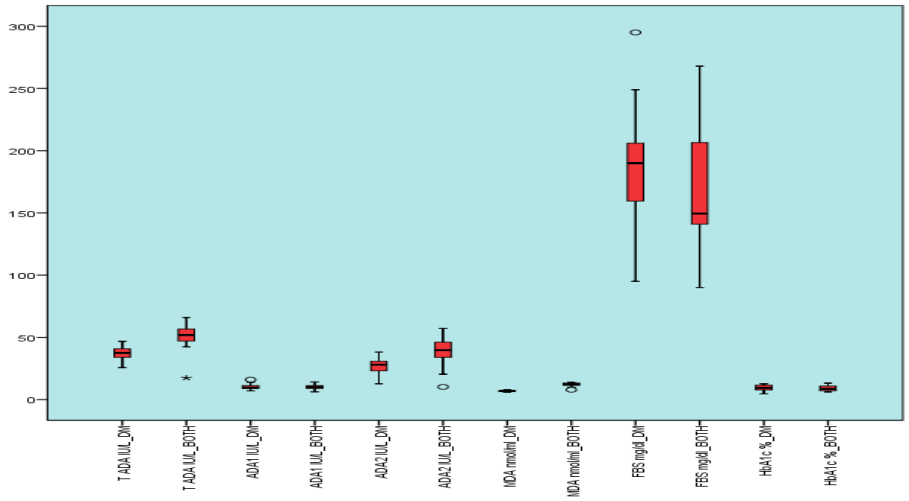


Figure 8.2 shows comparison between serum ADA isoenzymes, FBS, HbA1c, MDA levels in DM and Coexisting TB & DM.

Table 9.1: Serum ADA Isoenzymes, FBS, HbA1c, MDA levels in all study groups.

STUDY VARIABLE	CONTROL (MEAN ± SD)	TB (MEAN ± SD)	DM (MEAN ± SD)	BOTH (MEAN ± SD)
ADA1 (U/L)	5.26 ± 1.58	10.6 ± 2.01	10.34 ± 2.22	10.03 ± 1.93
ADA2 (U/L)	14.65 ± 3.7	26.38 ± 5.34	23.1 ± 6.36	39.12 ± 11.38
TADA (U/L)	19.96 ± 3.92	36.65 ± 4.62	37.28 ± 5.06	51.2 ± 10.4
MDA (nmol/ml)	3.58 ± 0.36	6.91 ± 0.46	6.95 ± 0.63	12.16 ± 1.46

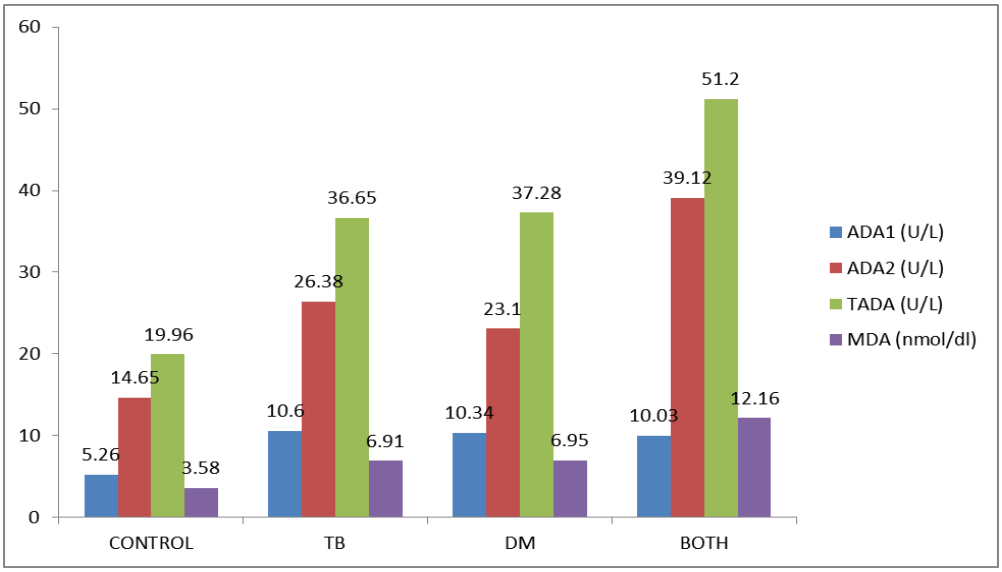


Fig 9.2.

Table 9.1 and Figure 9.2 shows comparison of serum TADA, ADA1, ADA2& MDA values between all study groups. Serum TADA levels were found to be significantly higher in subjects with coexisting TB and DM (51.2 ± 10.4), and ADA2 also elevated as compared to subjects with DM and TB alone. MDA level also significantly higher in subjects with coexisting TB and DM (12.16 ± 1.46) compared to subjects with DM and TB. ADA1 levels were higher in all the 3 group of patients compare to control subjects but there was no significant differences between any of the groups.

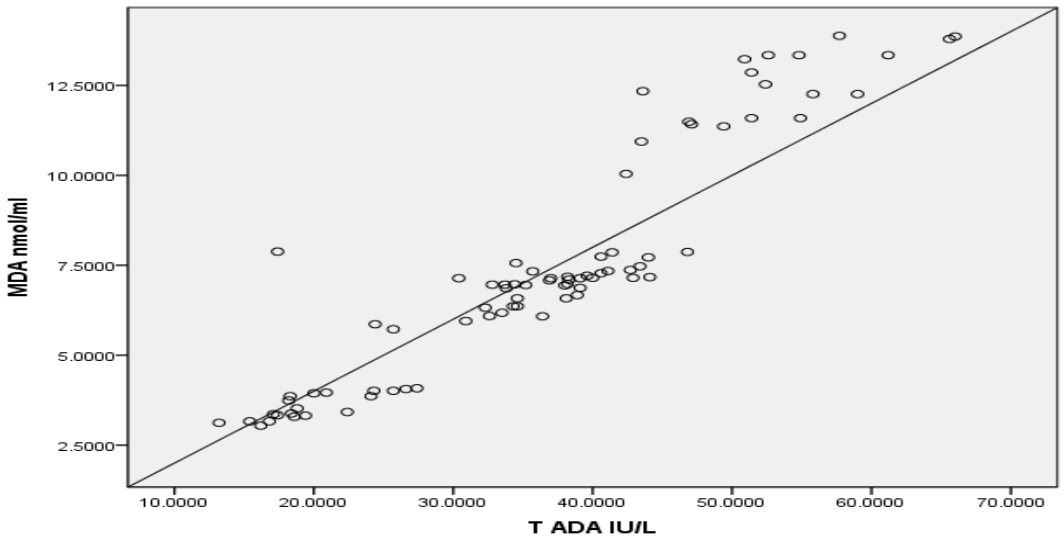


Figure 10.3: Shows Comparison between MDA and ADA2 levels in all the study groups.

Fig 10.2
The Figure 10.2 shows correlation between MDA &TADA levels. There was a good correlation with r value of 0.93 between MDA & TADA.

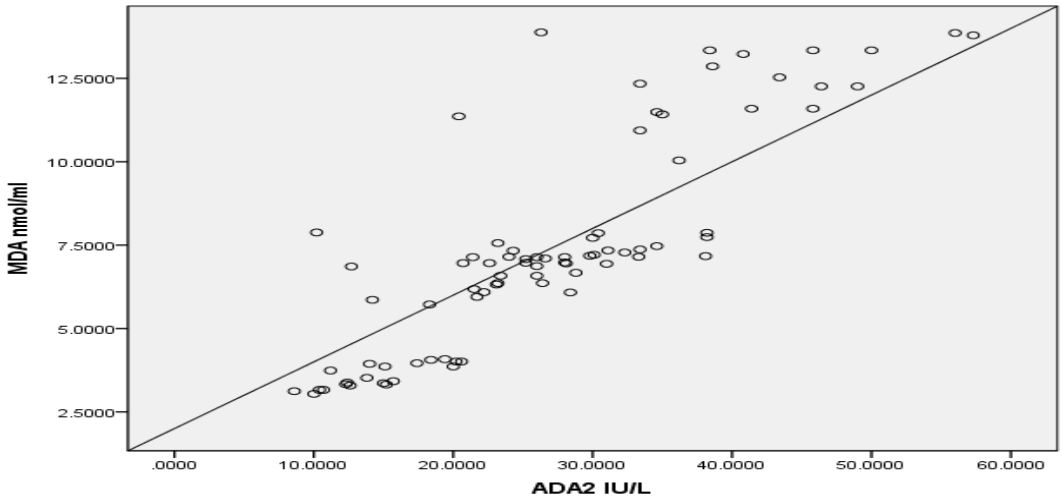


Figure 10.3: Shows Comparison between MDA and TADA levels in all the study groups.

Fig 10.3
The Figure 10.3 shows correlation between MDA &TADA levels. There was a good correlation with r value of 0.85 between MDA & ADA2.

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). Sreenivas Rao 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.[17,18] 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.[19]

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.[11] 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[97]. 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%. In subjects with co-existing TB & DM, both FBS &HbA1c levels were higher compared to subjects with only TB or DM alone. This finding is in line with suggestions that glycaemic controls will be poor when TB & DM coexist.

Majority of the subjects present in the study had type 2 DM for varying length of the time and had contacted TB infection recently. Increased susceptibility of patients with diabetes to develop TB infection has been attributed to neutrophil dysfunction and production of important cytokine IL -1beta& TNF-alpha were significantly lower in patients with poor glycaemic control. 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 antitubular 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[21]. Therefore a multiciplicity of factors appear to predispose diabetics to develop TB and TB patients to develop impaired glucose tolerance or frank diabetes.

Serum TADA & ADA2 levels in subjects in with coexisting TB & DM were also elevated (51.2 ± 10.4)&(39.12 ± 11.38) ranging from 35-85U/L. The elevation in serum ADA levels in subjects with coexisting TB & DM were significantly higher compared to those seen in subjects with TB alone (36.65 ± 4.62) & (26.38 ± 5.34) and also in subjects with DM alone (37.28 ± 5.06) & (23.1 ± 6.36). The glycaemic status in subjects with TB & DM was also poorer compared to subjects with either TB alone or DM alone.

The mechanisms underlying the elevation of ADA levels in various body fluids in TB or in DM are not clearly established. ADA is an important enzyme in purine at catabolism. It catalyse deamination of adenosine to inosine and deoxyadenosine to deoxyinosine. These are several forms of ADA, but the predominant ones are ADA1 and ADA2, which are coded by different gene loci. ADA1 is isoenzymes found in all cells, with highest concentration found in lymphocytes and monocytes, whereas ADA2 isoenzymes are found only in monocytes[65]. ADA2 is the major component of the activity of total ADA in the serum of healthy persons. It increases in biological fluids in conditions associated with stimulated activity in macrophages.[18]

The present study has shown that the predominant form of ADA in the serum in patients with TB, DM or coexisting TB & DM is ADA2. 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.[13]

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 but in patients with coexisting TB & DM was below 65 U/L much higher than the level found in other groups. In coexisting TB & DM, ADA2 levels were 51U/L much higher than those found in patients with TB & DM alone. Thus the predominant isoform of the elevated serum ADA was ADA2 in all the study groups. The 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. Both DM & TB are conditions were 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 conditions may have association with the ADA levels. In view of the elevation in TADA as well as ADA2 levels both in DM & TB, it is suggested that use of ADA either TADA or as isoenzyme fractions of ADA

needs to be reviewed particularly when both TB & DM are coexisting. We suggest larger studies to determine the cut-off levels that can be used for diagnosis of TB in the presence of DM.

BIBLIOGRAPHY

1. K. Srinivas Rao, H.Anandkumar, B.M.Rudresh, T. Srinivas, K.HarishBhat biomedical research, 2010; 2: 189-194.
2. Raman Kutty V, Joseph A, Soman CR. High prevalence of type2 DM in an urban settlement in Kerala. India Ethn health, 1999; 4: 231-9.
3. K.R.Lsuryakirani, Santhakumarai & R. Lakshmikumari Ind.J Tub, 1998; 45-47. Ind-J-Tub, 1998; 45: 47-48.
4. M.Shivaprakesh, S.Chennaih, YSR Murtyhy, E.Anjaiah, S.AnandRao, C.Suresh JIACM, 2006; 7(2): 114-17.
5. L. valdes, E.Sanjose, D. Alvarez, j>M.Valle Eur. Resp. J, 1996; 9: 747-751.
6. Ungerer JP, Oosthuizen HM, Retief JH, Bissbort SH. Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions. *Chest*, 1994; 106: 33.
7. Cristalli G, Costanzi S, Lambertucci C *et al*. Adenosine deaminase: functional implications and different classes of inhibitors. *Med Rev*, 2001; 21: 105–28.
8. Ungerer JP, Oosthuizen HM, Bissbort SH, Vermaak WJ. Serum adenosine deaminase: isoenzymes and diagnostic application.*Clin Chem*, 1992; 38: 1322–6.
9. Ungerer JPJ, Oosthuizen HM, Bissbort SH, Vermaak WJH. Serum adenosine deaminase: isoenzymes and diagnostic application. Clin Chem, 1992; 38: 1322–1326.
10. Raman Kitty V, Joseph A, Soman CR. High prevalence of type2 DM in an urban settlement in Kerala. India Ethn health, 1999; 4: 231-9.
11. M Shiva prakash, S Chennaih, YSR Murthy, E Anjaiah, S Anand Rao, C Suresh. JIACM, 2006; 7(2): 114-117.
12. Dong-Ho Han, Polly A. Hansen, Lorraine A. Nolte, and John O. Holloszy. Removal of Adenosine Decreases the Responsiveness of Muscle Glucose Transport to Insulin and Contractions. Diabetes, 1998; 47: 1671-1675.
13. Davis S, Abuchowski A,Park YK, Davis FF. Alteration of the circulating life and antigenic properties of bovine adenosine deaminase in mice by attachment of polyethylene glycol. Clin. Rxp. Immunol, 1981; 46: 649-652.
14. Kwiatkowska S, Piasecka G, Zieba M, Piotrowski W, Nowak D. Increased serum concentrations of conjugated dienes and malondialdehyde in patients with pulmonary tuberculosis. *Respir Med*, 1999; 93(4): 272-276.
15. Method of estimation serum ADA isoenzymes level– using kit and colorimetric method based on the principle of Guisti G Galanti method of enzymatic analyses, New York, 1974; 1092-1099.
16. American Diabetes Association. Standards of Medical Care for Patients with Diabetes Mellitus. Diabetes Care, 2001; 24(Suppl. 1): 33–43.
17. Placer ZA, Linda L, Crushman JBC,. Estimation of product of lipid peroxidation in biochemical system.Annals Biochem, 1966; 16: 359-64.
18. Lakkana Boonyagars & kiertiburanakul, J infect Dis Antimicrobial Agents, 2010; 27: 111-118.
19. Yash.p.Kataria & Imtiaz Khurshid, Chest, 2001; 120(2): 334-336.
20. Saeed Aminiafshar et al, Tanaffos, 2004; 3: 19-23.
21. Amandeep Kaur et al, JCDR, 2012; 6: 252-256.