(e) ISSN Online: 2321-9599

(p) ISSN Print: 2348-6805

ORIGINAL ARTICLE

Alteration of adenosine deaminase activity and lipid peroxidant (MDA) in serum and pleural fluid for diagnosis of pulmonary tuberculosis

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ABSTRACT:
Background: Tuberculosis is one of the commonest chronic infectious diseases; highly endemic in India kills five lakh patients every year. Oxidative Stress plays important role in inflammatory & degenerative diseases including pulmonary tuberculosis. There is hardly any one study available in literature correlating oxidative stress, lipid profile values and antioxidant status together with the pulmonary tuberculosis; so we decided to conduct this study. **Methods:** Study group included newly diagnosed 50 cases of pulmonary tuberculosis and control group included 50, age and sex matched healthy volunteers and employees. All the subjects were subjected to complete physical and systemic examinations, routine investigations including Sputum for AFB by Ziehl-Neelsen staining, AFB culture and Chest x-ray and special tests like Erythrocyte sedimentation rate (ESR), Malondialdehyde (MDA) and Adenosine deaminase (ADA) and findings recorded and statistically analysed. **Results:** In the study group with 33 males and 17 females, we found Serum MDA mean ± SD 2.91±0.99; Serum ADA 38.15±13.47 while The mean levels of pleural fluid MDA and ADA in tubercular patients were found to be 1.65±0.53 n mole/ml and 56.88±22.1 U/L respectively. While in controls with 61 males and 39 females, these values were 1.72±0.45 n mole/ml (MDA), 20.15± 6.70 U/L (ADA) respectively. **Conclusion:** Tuberculosis effect more males (66%) than females (34%). Malondialdehyde (MDA) and Adenosine deaminase (ADA) were found statistically significantly higher in study group when compared with control, ($p < 0.001$). Antioxidant plays important role for prevention of pulmonary Tuberculosis.
Key words: - Tuberculosis, Malondialdehyde, Adenosine deaminase, Lipid peroxidation, Oxidative stress.

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This article may be cited as: Chauhan AS, Thakur M, Gahlot G, Vyas RK. Alteration of adenosine deaminase activity and lipid peroxidant (MDA) in serum and pleural fluid for diagnosis of pulmonary tuberculosis. J Adv Med Dent Scie Res 2017;5(3):162-168.

INTRODUCTION

Tuberculosis is one of the commonest chronic infectious diseases and highly endemic in India and five lakh patients die every year (Gupta B K et al., 2013; Vinay Bharat et al., 2013). It usually affects lungs but cases of extra-pulmonary tuberculosis are not rare. Delay in diagnosis and in initiating treatment results in poor prognosis and squeal in up to 25% of cases (Gupta B K et al., 2013; Gupta B K et al., 2010). Pulmonary Tuberculosis (PTB) can be confirmed by sputum examination and diagnosed easily but diagnosing extra-Pulmonary TB becomes frequently difficult, since the specificity and sensitivity of non-invasive methods is very low. Several workers have estimated the specificity and sensitivity of Adenosine Deaminase (ADA) and found out its reliability (Gupta B K et al., 2010; Gupta B K et al., 2010). Several biochemical reactions occur in human body during health and disease; as a result of these essential reactions, there is formation of highly reactive oxygen species (ROS) which consist of free radicals (FR). In reactions with FR, bio-molecules undergo oxidation and through donation of their own electrons, they themselves become new secondary radicals that continue radical chain reactions and support spatial and time-dependent oxidative stress (OS) propagation and consequently lead to the cell/ tissue damage (Halliwell B 1994). In healthy conditions at the cellular level, there is a critical balance that exists between the FR generation and the various antioxidant defence mechanisms. But during certain disease processes there is a huge imbalance between these two mechanisms resulting in OS, hence this condition is characterized by disturbance in the pro-oxidant – antioxidant balance in favour of the former, which leads to a potential harm to the cell (Sies H 1985). ROS can damage proteins, lipids, nucleic acids and other cellular components under oxidative stress conditions (Valentine JS et al., 1998). OS plays an important role in inflammatory & degenerative diseases like pulmonary tuberculosis (De Oliverira et al., 1994).

Mycobacterium tuberculosis (M. tuberculosis) is an intracellular pathogen which grows and replicates in the host macrophages. The pathogen activates the invaded macrophages and results in free radical burst (Wiid IS et al., 2004; Mc Garvey J.A et al., 2004). These FR induce lipid peroxidation (LP), a chain process which affects polyunsaturated fatty acids (PUFA) mainly localized in cell membranes, in which end products such as malondialdehyde (MDA) is generated (Janero DR. 1990). MDA is itself responsible for some of the damaging effects of free radicals on Deoxyribonucleic acid (DNA) and on cell membranes (Penn ZJ et al., 1996). High levels of lipid peroxidation products like MDA is seen in advanced tuberculosis and can be measured in the blood as a parameter of oxidative stress (Kwiatkowska S et al., 1999). There are number of studies available in the literature where different researchers have tried to find out the level of oxidative stress, lipid profile values and antioxidant status separately in pulmonary tuberculosis (PTB) patients, there is hardly any one study available in literature correlating these three parameters together with the disease; so we decided to conduct this study (Kwiatkowska S et al., 1999).

Lipid Peroxidation converts poly unsaturated fatty acids present in cell membrane to the primary product of Lipid peroxides and to secondary metabolites such as malondialdehyde and thus, causing cell injury and death via DNA strand breakage and membrane damage (Halliwell B. and Chirico S. (1993). Malondialdehyde (MDA) is the important marker of lipid peroxidation. Adenosine deaminase is an enzyme involved in purine metabolism (Spencer N et al., 1968). ADA catalyses irreversible hydrolytic deamination of adenosine to produce inosine and ammonia(Martin D.W. et al. (1981). Adenosine deaminase is secreted by T-lymphocytes and macrophages during infection, so ADA is marker in chronic inflammatory conditions such as tuberculous pleural effusions (Selvakumar N et al., 1991) Normal serum and pleural fluid level of ADA is < 30 U/L. Increased in various forms of Tuberculosis making it a marker for tuberculosis of lungs with pleural effusion. In the present study we measured levels of MDA, ADA in normal control and subject groups.

MATERIAL AND METHODS

SAMPLE SELECTION CRITERIA

The class which was clinically suspicious of other infection diseases besides pulmonary tuberculosis like infectious mononucleosis, typhoid, viral hepatitis, HIV infections and malignant tumor were not included in this study as this disease can also affect serum and pleural fluid ADA levels. Patients suffering from diseases of heart, liver, kidney, skeletal muscles and RBCs, which tend to alter MDA levels, were also excluded from the study.

Subjects were divided into two groups.

Group A = 50 Healthy subjects as control.

Group B = 50 Pulmonary Tuberculosis subjects.

ESR, MDA, ADA determination were done in pulmonary tuberculosis subjects as well as in healthy subjects. Confirmation of Pulmonary Tuberculosis patients by sputum smear, Mauntox test determine the disease status.

COLLECTION OF SAMPLES

(a) Blood sample: - Venous blood (5ml) was withdrawn and transferred to clean dry centrifuge tube. Blood was allowed to clot at room temperature and centrifuged.
(b) Pleural fluid – 0.9ml of pleural fluid was taken in a test tube containing 10ml of mixture of 0.05 ml of glycerol and 0.05 ml of ethylene glycol.

Analytical grade chemicals, standard were used and following estimation were done.

(1) Erythrocyte sedimentation Rate (ESR)
(2) Determination of Malondialdehyde (MDA)
(3) Determination of Adenosine deaminase activity (ADA).

ESR ESTIMATION

By Westergen's method

1 part of anticoagulant (3.8% tri sodium citrate solution) + 4 parts of Blood, filled the pipette with blood by sucking till the O mark and clamped it vertically in the tube Read the upper level of red cells exactly after one hour. It is expressed as the fall of RBC's in mm at the end of first hour (mm/hr).

ESTIMATION OF MALONDIALDEHYDE (MDA)

MDA concentration was estimated as reactive substances by a thiobarbituric acid assay method11. Reagent used in TCA-TBA-Hcl-Prepared by dissolving 15% w/v Tri chloro acetic Acid and 0.375/w/v thio barbituric acid in 0.25 N-Hcl and to make 100 ml. 0.4ml of serum 0.6ml TCS-TBA-Hcl reagent was mixed well and kept in boiling water bath for 10 min. after cooling add 1.0 ml freshly prepared IN NaOH so as to eliminate centrifugation Absorbance of pink color was measured at 535nm against blank calculated by 16.0 X O.D. 535 n moles/ml.

2.5. Estimation of ADA –Kit method:-

(a) ADA – MTB reagent L1= phosphate buffer
(b) ADA-MTB reagent (l2) =Adenosine reagent
(c) ADA – MTB reagent (L3) = Phenol reagent
(d) ADA-MTB reagent (L4) = Hypochlorite reagent.
(e) ADA – MTB standard(S) = ADA standard

STATISTICAL ANALYSIS

The data obtained for various parameters was subjected to statistical analysis. Arithmetic mean and standard deviation were calculated of all the parameters studied, to compute 't Values' (student's t-test) On the basis of t values 'p values' (probability) were determined to make out the

significance of variance between the mean values of individual parameters among the two groups of the subjects studied.

RESULT AND DISCUSSIONS

It is observed that the mean ESR level was found to be 4.10 ± 2.07mm in first hour with a range of 1.0 to 9.0 mm in control group. The mean ESR level was significantly raised to 20.86± 7.01 with the range of 9.0 to 36.0mm in first hour in study group of pulmonary Tuberculosis. The rise was statistically significant as evident by P-value (P<0.001) fig (1)

The mean serum malondialdehyde (MDA) concentration was found to be 1.72±0.45 with a range of 0.64 to 2.56 nmole/ml in healthy control subjects. These results resembled with the observation made by Madhav, L et al., 2007. [**Table:1; (Figure:1)**]. The serum MDA level was increased to 2.91±0.99 nmole/ml with a range of 1.44 to 4.8 nmole/ml in pulmonary tuberculosis group. The increase was statistically significant as compared to that of control group as evident by p-value (p<0.005). The results of present study are in close collaboration with the findings of Madhav, L et al., 2007**.** It might be possible that increased oxidative stress and decreased antioxidant activity in patients of pulmonary tuberculosis resulted increased lipid peroxidation leads to increased MDA concentration as reported by Tesfaye, M et al., 2003.

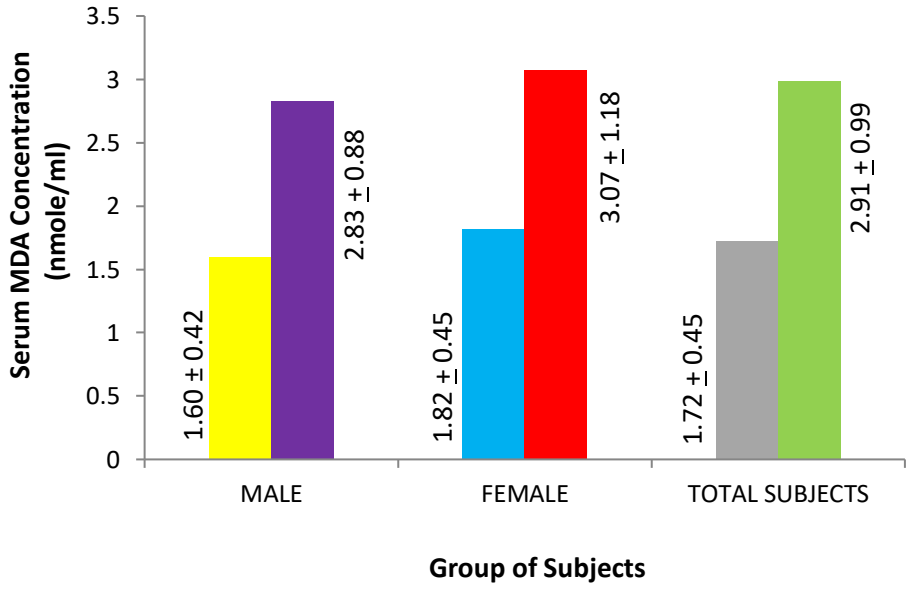
TABLE- 1: Serum MDA concentration (nmole/ml) in healthy control and pulmonary tuberculosis patients (Study Group)

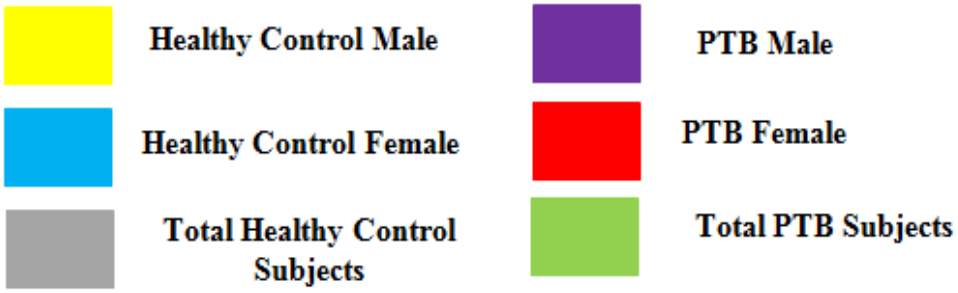
	Male		Female		Total	
	Control Group	Study Group	Control Group	Study Group	Control Group	Study Group
Mean	1.60	2.83	1.82	3.07	1.72	2.91
SD	0.42	0.88	0.45	1.18	0.45	0.99
Range	1.1 - 2.6	1.1 - 4.8	0.6 - 2.6	1.4 - 4.8	0.6 - 2.6	1.4 - 4.8
SE	0.09	0.16	0.09	0.28	0.06	0.14
't'	6.98		4.31		7.76	
p-value	< 0.001				< 0.005	
Statistical Significance	HS**				S*	

**S = Significant*

***HS = Highly Significant*

Fig (1): Serum MDA concentration (nmole/ml) in healthy control and pulmonary tuberculosis (PTB) patients





It is revealed from **Table: 2** that the mean pleural fluid MDA concentration was found to be 1.65±0.53 nmole/ml with a range of 0.64 to 3.04 nmole/ml in the present series of study. These results are in close agreement with the founding of Gupta, K.B., 2002. The increased concentration of MDA in pleural fluid might be due to decrease in cellular immunity.

TABLE- 2: Comparison of Serum MDA (nmole/ml) and PF-MDA (nmole/ml) levels in pulmonary tuberculosis patients (Study Group)

	Male		Female		Total	
	Serum MDA	PF-MDA	Serum MDA	PF-MDA	Serum MDA	PF-MDA
Mean	2.83	1.61	3.07	1.72	2.91	1.65
SD	0.88	0.55	1.18	0.50	0.99	0.53
Range	1.44-4.8	0.64-3.0	1.4-4.8	0.64-2.5	1.4-4.8	0.64-3.0
SE	0.15	0.09	0.28	0.11	0.14	0.07
't'	6.63		4.45		7.93	
p-value	< 0.001					
Statistical Significance	HS**					

PF= Pleural Fluid
***HS = Highly Significant*

It has been observed from **Table: 3** and **Figure:2** that the mean serum Adenosine Deaminase (ADA) level was found to be 20.15±6.70 U/L with a range of 13.3 to 47.1 U/L in normal control subjects. The results of present series of study resembled with the findings of Kelbel C et al., 1995.

TABLE- 3: Serum ADA (U/L) concentration in healthy control and pulmonary tuberculosis patients (Study Group)

	Male		Female		Total	
	Control Group	Study Group	Control Group	Study Group	Control Group	Study Group
Mean	21.55	38.42	18.96	37.67	20.15	38.15
SD	8.45	12.17	4.58	15.90	6.70	13.47
Range	13.3- 47.1	17.8 - 68.7	13.3 - 31.2	14.0 - 80.0	13.3 - 47.1	14.0 - 80.0
SE	1.76	2.15	0.88	3.72	0.94	1.90
't'	6.09		4.89		8.53	
p-value	< 0.001					
Statistical Significance	HS**					

***HS = Highly Significant*

The serum ADA level was found to be 38.15±13.47 U/L with a range of 14.0 to 80.0 U/L in the pulmonary tuberculosis patients. The results of present study are in close agreement with the finding of Blake J. and Berman P. 1982. The increase concentration of serum ADA in tubercular patients as compared to that of control was statistically significant as evident by p-value ($p<0.001$) [**Table:3; (Figure:2)**]. It might be due to decreased cellular immunity in pulmonary tuberculosis [Paliwal R. and Shah, K.V., 1998] also reported that the plasma ADA activity is higher in disease where cellular immunity is impaired.

Fig (2): Serum ADA concentration (U/L) in healthy control and pulmonary tuberculosis (PTB) patients

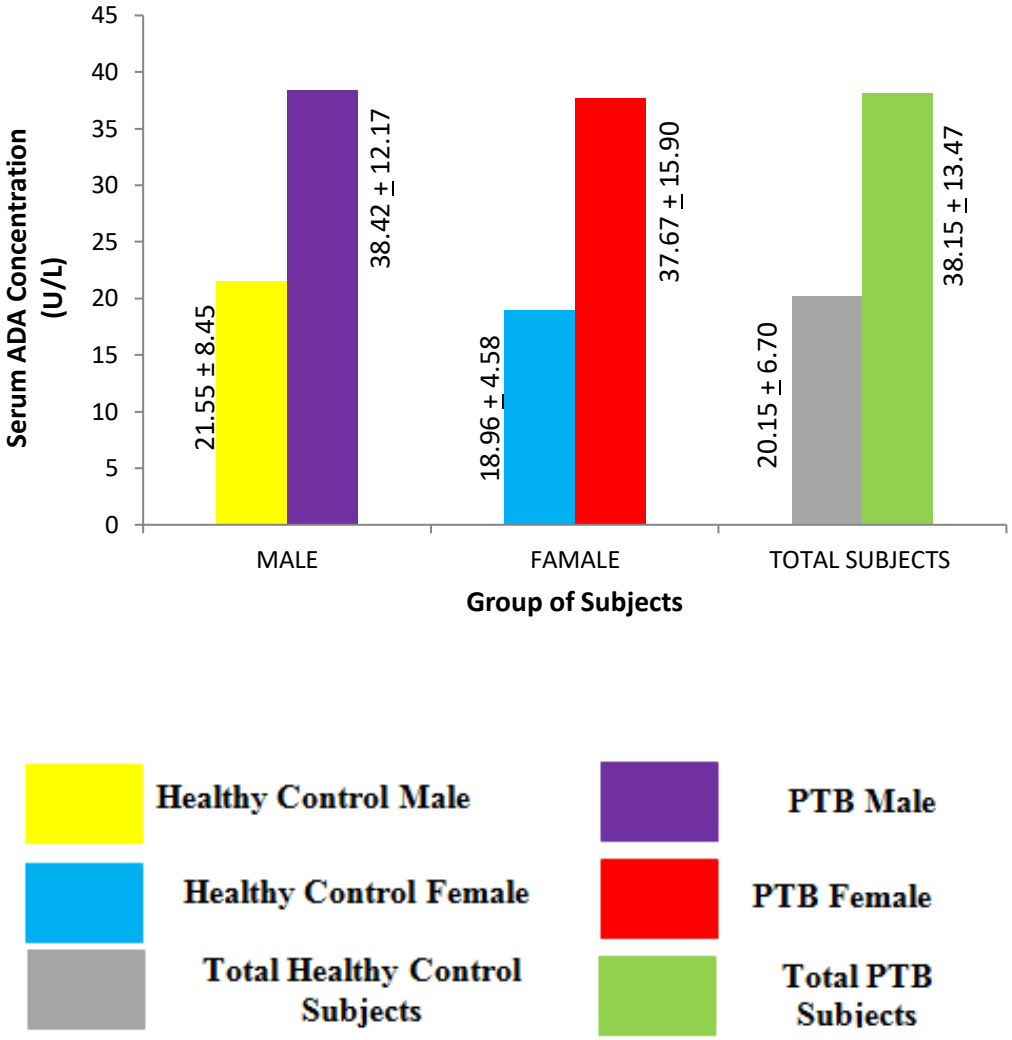


Table: 4 indicates the mean pleural fluid ADA level was found to be 56.58±22.21 U/L with as range of 16.25 to 94.32 U/L in the present series of study. These results are in collaboration with the finding of Mathur et al (2006)[26].The decreased cellular immunity in tuberculosis resulting increased pleural fluid ADA concentration as observed in the present series of study.

TABLE- 4: Comparison of Serum ADA (U/L) and PF-ADA (U/L) levels in pulmonary tuberculosis patients (Study Group)

	Male		Female		Total	
	Serum ADA	PF-ADA	Serum ADA	PF-ADA	Serum ADA	PF-ADA
Mean	38.42	56.08	37.67	57.48	38.15	56.58
SD	12.17	21.95	15.90	23.27	13.47	22.21
Range	17.8 - 68.7	16.2 - 94.3	14.0 - 80.0	16.7 - 92.6	14.0 - 80.0	16.2 - 94.3
SE	2.15	3.88	3.81	5.48	1.92	3.14
't'	4.18		3.58		5.22	
p-value	< 0.001					
Statistical Significance	HS**					

PF=Pleural Fluid

***HS = Highly Significant*

It is evident from **Table:5** that mean serum MDA and ADA levels were found to be 2.91±0.99 nmole/ml and 38.15±13.47 U/L respectively. The serum MDA and ADA levels were increased significantly as shown by p-value (p<0.001).

TABLE- 5: Comparison of Serum MDA (nmole/ml) and Serum ADA (U/L) levels in pulmonary tuberculosis patients (Study Group)

	Male		Female		Total	
	Serum MDA	Serum ADA	Serum MDA	Serum ADA	Serum MDA	Serum ADA
Mean	2.83	38.42	3.07	37.67	2.91	38.15
SD	0.88	12.17	1.18	15.90	0.99	13.47
Range	1.4 - 4.8	17.8 - 68.7	1.4 - 4.8	14.0 -80.0	1.4 - 4.8	14.0 -80.0
SE	0.15	2.15	0.28	3.81	0.14	1.92
't'	16.55		9.05		18.25	
p-value	< 0.001					
Statistical Significance	HS**					

***HS = Highly Significant*

The mean levels of pleural fluid MDA and ADA in tubercular patients were found to be 1.65±0.53 nmole/ml and 56.88±22.21 U/L respectively (**Table:6**). The PF-MDA and PF-ADA levels were raised significantly in tubercular patients; might be due to reduced immunity in these patients.

TABLE- 6: Comparison of PF-MDA (nmole/ml) and PF-ADA (U/L) levels in pulmonary tuberculosis patients (Study Group)

	Male		Female		Total	
	PF-MDA	PF-ADA	PF-MDA	PF-ADA	PF-MDA	PF-ADA
Mean	1.61	56.08	1.72	57.48	1.65	56.58
SD	0.55	21.95	0.50	23.27	0.53	22.21
Range	0.6 - 3.0	16.2 - 94.3	0.6 - 2.6	16.7 - 92.6	0.6 - 3.0	16.2 - 94.3
SE	0.09	3.88	0.11	5.48	0.07	3.14
't'	14.45		10.17		17.49	
p-value	< 0.001					
Statistical Significance	HS**					

PF= Pleural Fluid

***HS = Highly Significant*

CONCLUSION

- The serum MDA and ADA concentration was found to be increased significantly in tubercular patients as compared to that of control group; might be due to increased oxidative stress associated with reduced cellular activity.
- A positive correlation was recorded between the increase of serum MDA and ADA concentration in tubercular patients because tubercular patients possessed oxidative stress along with decrease cellular immunity.
- When serum MDA and ADA concentration were correlated with that of control group, a negative correlation was recorded; this might be due to the fact that serum MDA and ADA concentrations are independent to that of normal control level but dependent on the severity of the disease.
- The pleural fluid MDA and ADA concentration was found to be raised in tubercular patients; might be due to reduced immunity level in disease state.
- A positive correlation was observed between pleural fluid MDA and pleural fluid ADA concentration in pulmonary tuberculosis. This might be due to increased oxidative stress resulting decreased cellular immunity in tubercular patients.

ACKNOWLEDGEMENTS

The research was supported by Principal S.P. Medical College and controller of attached hospitals, Bikaner and all indoor and outdoor patients making it possible for me to conduct this work in this institution. I am extremely grateful to Principal & controller and my department.

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