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Original Research Article

# Tuberculous Pleural effusion diagnosis by detection of Alkaline Phosphatase in Serum and Pleural fluid

**Shyam Prasad B.R[1], Mahesh G[2], Tagore R[3], Uma Devi M[4]**
[1]M.D, Assistant Professor, Department of Biochemistry, Government Medical College, Ananthapuram.
[2]M.D, Assistant Professor, Department of Forensic Medicine, Government Medical College, Ananthapuram.
[3]M.D, Professor, Department of Biochemistry, Siddhartha Medical College, Vijayawada.
[4]M.D, Professor, Department of Biochemistry, Osmania Medical College, Hyderabad.

***Corresponding author**
Dr. B.R. Shyam Prasad M.D
Email: swetha.kiraan@gmail.com

**Abstract:** Tuberculous Pleural effusion is one of the most frequent causes of exudates in large series of pleural effusions in immunocompetent patients. The Aim of this study is to estimate enzyme Alkaline Phosphatase levels in serum and pleural fluids of HIV seronegative exudative Tuberculous Pleural fluids and their possible role in the diagnosis of tubercular pleural effusion. Serum and Pleural fluid samples were collected from symptomatic cases of pleural effusion, with known HIV status negative and also from Non Tuberculous pleural effusion patients attending General medicine and TB and Chest Department of Government Medical College and Hospital at Ananthapuram for one year. Alkaline phosphatase was estimated by p-Nitrophenol method - kinetic. The Mean value of serum and pleural fluid alkaline phosphatase among HIV negative patients with tuberculous effusion was 95.7±51.3 and 83.5±36.9 respectively. Alkaline phosphatase was also assessed among serum and pleural fluid of non-tuberculous pleural effusion patients and the mean value of serum ALKP was 140.96±54.5 and pleural fluid ALKP was 140.4±38.9. Serum alkaline phosphatase levels were not of significant p value in this study. Earlier studies on alkaline phosphatase were also of not any significance in the diagnosis of tuberculous pleural effusion.
**Keywords:** Alkaline Phosphatase, Tuberculous Pleural effusion, Serum, Pleural fluid

## INTRODUCTION

Tuberculosis is causing major impact on public health globally, whereas India is the most endemic for Tuberculosis cases. Tuberculosis is a specific infectious disease caused by Mycobacterium tuberculosis. Tuberculosis is the leading cause of morbidity and mortality from an infectious disease, mostly confined to developing and underdeveloped countries.

Tuberculosis spread by airborne droplets or droplet nuclei from a person with Pulmonary TB. Transmission often occurs indoors, where droplets and droplet nuclei can stay in the air for a long time [1].

Tuberculosis is causing much problem worldwide both in rural and urban areas because of droplet spread, offering more resistance to human immune system and arising as a Panbiotic resistance bacterium (Total resistance to Tuberculosis drugs). The progression of TB has disastrous effects on the economy as the disease most often affects the economically productive age group [2]. The control of disease epidemic is also become very crucial.

Tuberculous Pleural effusion is one of the most frequent causes of exudates in large series of pleural effusions in immunocompetent patients. If we exclude patients with underlying pulmonary disease, TB is the most common cause of pleural exudates in many areas of the world [3]. Pleural disease now more commonly represents reactivation than primary infection and is present in approximately 7 percent of cases of active pulmonary TB.

There were an estimated 12 million prevalent cases of tuberculosis in 2010. This is equivalent to 178 cases per 100000 populations. There were an estimated 650000 cases of multi drug resistant tuberculosis among the world's prevalent 12 million cases of tuberculosis [4].

Tuberculosis is a treatable disease as specific drugs are available. To halt the advance of tuberculosis, an important first step is to suspect and diagnose the disease early so that treatment and other control measures can be undertaken.

Alkaline Phosphatase (ALP) catalyzes the alkaline hydrolysis of a large variety of naturally occurring and synthetic substrates. Divalent ions, such as Mg2+, Co2+, and Mn2+, are activators of the enzyme, and Zn2+ is a constituent metal ion.

Diagnostic difficulties can occur with extra pulmonary tuberculosis which is diagnosed usually by an invasive procedure. This study was planned to evaluate the Alkaline Phosphatase enzyme in the serum and pleural fluid in the classification and diagnosis of tubercular pleural effusions.

The Aim of this study is to estimate enzyme Alkaline Phosphatase levels in serum and pleural fluids of HIV seronegative exudative Tuberculous Pleural fluids and their possible role in the diagnosis of tubercular pleural effusion.

## MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry, Government Medical College & Hospital, Ananthapuram. Patients were appraised for the purpose of study and written consent was taken prior to commencement of study. Ethical clearance was obtained from the ethical clearance committee of this institution.

Serum and Pleural fluid samples were collected from symptomatic cases of pleural effusion, with known HIV status negative and also from Non Tuberculous pleural effusion patients attending General medicine and TB and Chest Department of Government Medical College and Hospital at Ananthapuram from January 2013 to December 2013.

100 patients were included in this study and were selected randomly, considered as two groups.

- Group I - Cases - Tuberculous pleural effusion patients: 50
- Group II - Controls - Non Tuberculous pleural effusion patients: 50

### Inclusion criteria

- X – Ray findings suggestive of exudative pleural effusion
- Pleural effusions with HIV status negative
- Sputum positive for AFB with pleural effusion
- New cases of tuberculosis pleural effusion

### Exclusion Criteria

- HIV positive case
- Old cases of Tuberculosis
- Renal Injury/Failure
- Known cases of typhoid or any systemic illness
- Transudative effusions
- Cases of hepatitis and Liver failure
- Undiagnosed pleural effusion

Random Unhemolysed Serum and Pleural fluid Samples were processed immediately after collection and were analyzed for Alkaline Phosphatase. These samples were cross checked in Systronics UV spectrophotometer, spectrophotometer and Transasia semi auto analyzer with precinorm and precipath in duplicates.

Serum and pleural fluid alkaline phosphatase was estimated by p-Nitrophenol method - kinetic [5].

### Procedure

Pipette 1000 µl of freshly reconstituted alkaline phosphate reagent in a clean dry test tube and add 20 µl of serum. Both are mixed well and read in an analyzer at 405 nm by kinetic method with a lag time and kinetic interval of 60 seconds.

### Linearity

The procedure is linear up to 1000 U / L. for higher values the sample should be diluted with normal saline and the assay should be repeated. The final result should be multiplied by the dilution factor.

### Reference range

Serum Alkaline Phosphatase (adults):
Males - 40 to 125 U / L.
Females - 42 – 98 U / L.
(Levels up to 3 times of this may be normal in children).

All the results were entered into spread excel sheet and analyzed.

## RESULTS

In this study 50 cases with tuberculous effusion and 50 cases without tuberculous effusion were included. Among 100 selected individuals, 54 were males and 46 were females. The mean age of Tuberculosis pleural effusion was 35±15.32 and of Non Tuberuclous effusion was 42.2±13.34 (Table No:1).

**Table-1: Showing the Age and Sex distribution of studied population**

| Group | Age | Sex | | | | Total |
|---|---|---|---|---|---|---|
| | Mean ± S.D | Male (n) | Percentage | Female (n) | Percentage | |
| Tuberculous effusions | 35±15.32 | 25 | 25% | 25 | 25% | 50 |
| Non Tuberculous effusions | 42.2±13.34 | 29 | 29% | 21 | 21% | 50 |
| Total | - | 54 | 54% | 46 | 46% | 100 |

The Mean value of serum and pleural fluid alkaline phosphatase among HIV negative patients with tuberculous effusion was 95.7±51.3 and 83.5±36.9 respectively. Alkaline phosphatase was also assessed among serum and pleural fluid of non tuberculous pleural effusion patients (without tubercular etiology) and the mean value of serum ALKP was 140.96±54.5 and pleural fluid ALKP was 140.4±38.9.

**Table-2: Serum and Pleural fluid ALKP among Tuberculous and Non Tuberculous effusion patients**

| Groups | Serum | | | Pleural Fluid | | |
|---|---|---|---|---|---|---|
| | Mean | Standard Deviation | Standard Error Mean | Mean | Standard Deviation | Standard Error Mean |
| Tuberculous effusion | 95.792 | 51.394 | 6.9743 | 83.506 | 36.904 | 5.0544 |
| Non Tuberculous effusion | 140.96 | 54.568 | 7.7171 | 140.464 | 38.918 | 5.5039 |

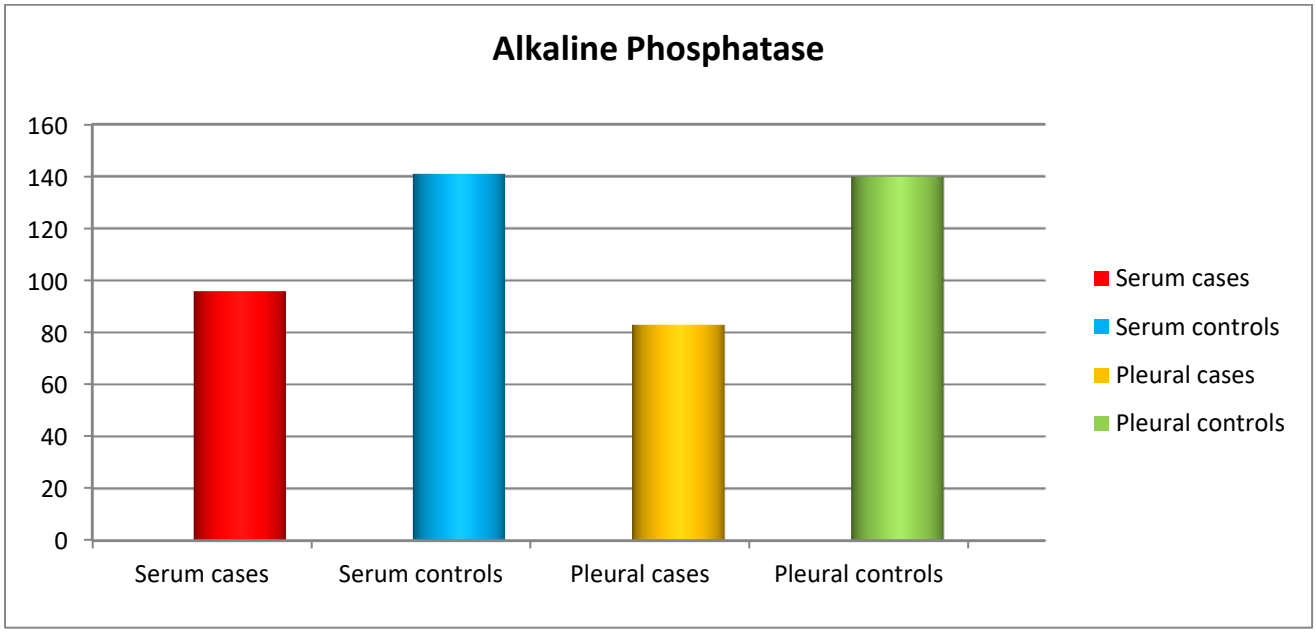


**Fig-1: Diagrammatic representation of Alkaline Phosphatase levels among serum and pleural fluid samples**

After assessing Alkaline Phosphatase among serum and pleural fluid samples, they were compared in between cases and controls. ROC analysis was done. Odds ratio was also assessed to know the significance of ALKP as diagnostic test among tubercular pleural effusion patients.

ROC analysis shown sensitivity and specificity of serum ALKP was 68% and 80% and pleural fluid ALKP was 88% and 92%. In ROC analysis it was estimated that pleural ALKP shown better sensitivity and specificity, when compared with serum ALKP.

Odds ratio assessment shown that both Serum and Pleural ALKP were extremely statistically significant when compared among cases and controls. Pleural ALKP shown high statistical significance (<0.0001) when compared to serum ALKP (0.0003).

**Table-3: ROC Analysis and ODD's Ratio assessment**

| ROC Analysis | | | | | | |
|---|---|---|---|---|---|---|
| Parameter | Best cut off value (IU/L) | Sensitivity (%) | Specificity (%) | Area under ROC curve | Positive Predictive Value (%) | Negative Predictive value (%) |
| Serum Alkaline Phosphatase | < 98.4 | 68 | 80 | 0.792 | 78.3 | 74.1 |
| Pleural fluid Alkaline Phosphatase | < 105 | 88 | 92 | 0.890 | 91.7 | 73.4 |
| ODDS RATIO | | | | | | |
| Parameter | Odds Ratio | | Z statistic | | Significance level (AREA = 0.5) | |
| Serum Alkaline Phosphatase | 0.1758 | | 3.633 | | 0.0003 | |
| Pleural fluid Alkaline Phosphatase | 0.0767 | | 4.886 | | < 0.0001 | |

## DISCUSSION

Comparing the significance of conventional testing for the diagnosis of tuberculous pleural effusion, the ADA-activity in the pleural fluid is clearly superior to all of them. But, in this study we took a chance to evaluate alkaline phosphatase significance in tuberculous effusion.

ALP activity is present in most organs of the body and is especially associated with membranes and cell surfaces located in (1) the mucosa of the small intestine and proximal convoluted tubules of the kidney, (2) in bone (osteoblasts), (3) liver, and (4) placenta. Although the exact metabolic function of the enzyme is not understood, it appears that ALP is associated with lipid transport in the intestine and with the calcification process in bone.

The mean and standard deviation of age of cases and controls were 35 ± 15.32 and 42.2 ± 13.34 in controls. This is in accordance with the incidence of tuberculosis which showed the age of tuberculosis pleural effusion as common in adults [7].

The mean and standard deviation for the variable in serum of cases is 95.79 ± 51.39 with a p value of < 0.001 which is significant when compared to controls which is 140.96 ± 54.568. The mean and standard deviation of ADA in pleural fluid of cases is 83.51 ± 36.90, with a p value of <0.001 which is significant when compared to controls whose mean and standard deviation is 140.46 ± 38.918.

Pleural fluid and serum alkaline phosphatase levels are significantly decreased in tuberculous pleural effusions. The sensitivity for serum ALP obtained is 68%, specificity is 80%, positive predictive value is 78.3% and negative predictive value is 74.1%. The same for pleural fluid were 88%, 92%, 91.7% and 73.4% respectively.

Significance of Alkaline phosphatase levels to differentiate tuberculous effusions from other causes of exudates were limited [8] and were increased in exudative pleural effusions when compared to transudates.

Mushtaq A lone *et al.* [9] in his study on pleural effusions had obtained an ALP level of > 75 in 72 exudates and < 75 in 12 exudates studied. They had obtained a sensitivity of 100 & and specificity of 85.71 % in diagnosis of exudates. The positive predictive value was 58.62% and negative predictive value was 100%. They opined that alkaline phosphatase levels to differentiate tuberculous effusions from other causes of exudates was limited and were increased in exudative pleural effusions when compared to transudates.

K.B.Gupta *et al.* [10] in their study on exudates of various etiologies had obtained a mean pleural fluid level of 88.53 ± 31.27 in exudates with a statistical significance of p < 0.05. They obtained a sensitivity and specificity of 85% and 75 % respectively. The positive and negative predictive values were 92% and 63%, they had opined that the increased levels are seen in exudates when compared to transudates and can be used complimentarily with lights criteria in classification of exudates.

F Carion *et al.* [11] in their study on exudates of different aetiology of which 72 cases were of tuberculous origin obtained a mean and standard deviation of 54.6 ± 34.3 in tuberculous effusions and 55.3 ± 31.6 in non tuberculous effusions. They did not find any significance in tuberculous pleural effusions.

However the study done by Jadhav *et al.* [8] observed a mean and standard deviation of 140.36 ± 43.21 in the serum of tuberculous pleural effusions and 140.6 ± 32.80 in non tuberculous groups. In pleural fluids of the two groups the mean and standard deviations were 124.66 ± 58.69 and 60.83 ± 56.51. The

mean and standard deviation of pleural and serum ratio were 0.906 ± 0.370 in tuberculous group and 0.390 ± 0.280 in non tuberculous group. The p values were significant <0.0001 in pleural fluids and p / s ratio and < 0.981 in serum. They concluded that pleural fluid ALP levels and the ratio of serum and pleural fluid ratio can be used in the differentiation of tuberculous and non tuberculous pleural effusions. However the study recommended further studies.

## CONCLUSION

In a developing country of high tuberculosis incidence like India, a diagnosis of TB should always be considered when there is failure to respond to the conventional antimicrobial (not anti tubercular) therapy or the patient presents with unusual manifestation.

Serum alkaline phosphatase levels were not of significant p value in this study. Earlier studies on alkaline phosphatase were also of not any significance in the diagnosis of tuberculous pleural effusion.

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