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Original Article

Estimation of Serum and Pleural Fluid Amylase in HIV Seronegative Patients with Tuberculous Pleural Effusion and Its Implication in The Diagnosis of Tubercular Pleural Effusion

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ABSTRACT

Introduction: *To estimate enzyme amylase levels in Serum and pleural fluids of HIV seronegative exudative tuberculous pleural fluids and their feasible position within the prognosis of tubercular pleural effusion.* ***Methods & Materials:*** *A Prospective study was done at Dept. of Biochemistry, North Bengal Medical College and Hospital, Sirajganj, Bangladesh from March 2022 to May 2023 after taking institutional ethical committee approval on exudative pleural effusion samples for detection of Tuberculosis by estimating amylase in serum and pleural fluid. A total of 100 patients were selected for this study. The selected individuals were randomly enrolled as cases and controls for this study, with 50 participants each. Amylase in blood and pleural fluid samples was tested using the CNPG method within the designated time on the same day to prevent loss of analytes.* ***Results:*** *Total 100 patients were enrolled, including 50 cases of tuberculous pleural effusion and 50 controls of nontuberculous pleural effusion. Both men and women aged between 20 and 60 years were tested. The mean age range of tuberculous pleural effusion was 35 ± 15.32 years and that of nontuberculous exudative pleural effusion was 42.2 ± 13.34 years. Of the 50 patients with tuberculous pleural effusion, 25 were male and 25 were female. Of the 50 controls with nontuberculous pleural effusion, 29 were male and 21 were female. In group I, the mean values of serum amylase and pleural fluid amylase were*

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53.044 and 77.122, respectively. In group II or controls, the mean values of serum amylase and pleural fluid amylase were 49.508 and 63.238, respectively. ***Conclusion:*** *Serum and pleural amylase levels indicate coexisting pancreatic, gastrointestinal, and malignant pathologies. Our study shows that the diagnosis of tuberculous pleural effusion by measuring amylase levels is neither sensitive nor specific.*

Keywords: *Serum Amylase, Tuberculous Pleural Effusion, Pleural Amylase*

INTRODUCTION

Tuberculosis (TB) is a notifiable infectious disorder as a result of Mycobacterium tuberculosis. In Bangladesh, TB is greater not unusualplace and endemic. Clinically, there are sorts of TB: pulmonary and extrapulmonary. Pulmonary TB normally impacts the lungs and their lymphatic vessels, whilst extrapulmonary TB can have an effect on nearly any organ withinside the frame, together with the pleura, meninges, intestines, reproductive tract, lymph nodes, bones, joints, skin, and different frame tissues[1]. As TB maximum normally impacts the economically efficient age group, development of TB has a devastating effect at the economic system of any country[2]. Early prognosis and activate remedy are important to save you contamination and its spread[3]. The diagnostic technique typically consists of an in-depth clinical history, laboratory tests, radiological, microbiological, immunological, biochemical, molecular organic and/or histological examinations. Tuberculous pleurisy results from infection of the pleura with Mycobacterium tuberculosis. It presents as an exudative pleural effusion and can occur alone or in association with pulmonary tuberculosis (TB). Pleural tuberculosis is one of the most common extrapulmonary manifestations of TB[4]. In HIV-infected patients and elderly patients with latent miliary tuberculosis, the diagnosis of pleural effusions with the presence of acid-fast bacilli in sputum may not be possible, and most pleural effusions after thoracentesis are treated on the basis of cytological examination, which is misleading. Mycobacterial proteins enter this space through the rupture of subpleural foci 6-12 weeks after primary infection[5], but also rarely through direct spread of vertebral foci[6]. The diagnostic approach in patients with possible tuberculosis includes a detailed medical history, clinical examination, radiological, microbiological, immunological, biochemical, molecular biological and histological studies. It also offers the possibility of rapid diagnosis of active tuberculosis in patients with acid-fast rod-negative sputum, allowing direct, immediate and highly accurate identification of drug-resistant strains of Mycobacterium tuberculosis in breath samples. Currently, the diagnosis of pulmonary tuberculosis is mainly performed by acid-fast bacilli testing in sputum and radiological examinations. Extrapulmonary tuberculosis is an

important area of tuberculosis diagnosis. Diseases such as pleural effusion, genital tuberculosis and tuberculous meningitis cannot be diagnosed in most cases, even if bacilli are detected. Delayed diagnosis also delays treatment, leading to prolonged community-acquired infection. It is generally accepted that the evaluation of enzymes such as adenosine deaminase, lactate dehydrogenase, alkaline phosphatase, and aminase in serum and pleural fluid is useful in the classification and diagnosis of tuberculous pleural effusions[7]. Its main function in humans is the development and maintenance of the immune system. Delay in treatment may also affect the patient's recovery and healing. Biochemical evaluation is primarily used to classify pleural fluid as an exudate or transudate and to confirm the etiology of the specific cause of pleural effusion. The aim of this study is to determine the enzyme amylase levels in serum and pleural fluid of HIV-seronegative tuberculous exudative pleural effusion type and its possible role in the diagnosis of tuberculous pleural effusion.

METHODS & MATERIALS

The prospective study was conducted from March 2022 to May 2023 at the Department of Biochemistry, North Bengal Medical College and Hospital, Sirajganj, Bangladesh, with Institutional Ethics Committee approval for exudative pleural fluid samples for detection of tuberculosis by measurement of amylase. Serum and pleural fluid had been collected. A total of 100 patients were selected for this study. The selected individuals were randomly enrolled as cases and controls for this study, with 50 participants each. 1. Cases – patients complaining of pleural effusion and known HIV negative cases. 2. Controls – patients undergoing outpatient treatment for breast disease without pleural effusion and tuberculosis related complaints.

TB patients, patients with renal impairment/renal or hepatic failure, patients with typhoid or other systemic diseases, exudative pleural effusion or undiagnosed pleural effusion were excluded from the study. Blood and pleural fluid samples were tested for amylase using the CNP-G (chloro-p-nitrophenyl-glucose) method within the specified time on the same day to prevent loss of specimens.

All samples were analyzed twice and cross-checked on a Transasia semi-automated analyzer using Precinorm and Precipath. CNP-G method: Pipette 1000 µL of reagent into a clean, dry test tube and add 20 µL of sample. Mix well and aspirate the sample into the analyzer. Expected values for amylase activity range from 23 to 88 U/L. Results were analyzed and displayed in tabular form.

RESULTS

Total 100 patients were included, among them 50 were cases of tuberculous effusion and 50 were controls of non-tuberculous effusion. Individuals with the age group of 20 - 60 years, of both sexes were studied. The

Mean age group of tuberculous effusions is 35±15.32 and of exudative pleural effusion of non-tuberculous origin is 42.2±13.34. Out of 50 tuberculous effusions patients, 25 were males and 25 were females. Out of 50 controls of non-tuberculosis effusion, 29 were males and 21 were females (**Table I**).

Table – I: Age and Sex distribution of Tuberculous and without Tuberculous Effusion Patients

Group	Age	Sex				
	Mean±SD	Male	Percentage	Female	Percentage	Total
Tuberculous effusions	35±15.32	25	25	25	25	50
Non-Tuberculous effusions	42.2±13.34	29	25	21	21	50
Total		54	25	46	46	100

Among Group I or cases, the mean value of serum amylase and pleural fluid amylase was 53.044 and 77.122 respectively. The mean value of serum and pleural fluid amylase was 49.508 and 63.238 respectively, among Group II or controls (**Table II**).

Table – II: Levels of Serum and Pleural fluid Amylase Among Cases and Controls

Groups	Serum Amylase			Pleural fluid Amylase		
	Mean	Standard Deviation	Standard Error Mean	Mean	Standard Deviation	Standard Error Mean
Cases	53.044	21.123	2.96	49.508	19.131	2.671
Controls	77.122	72.441	10.244	63.238	53.843	7.614

Sensitivity, specificity, positive predictive value, and negative predictive value were analyzed between cases and controls by setting the cutoff values of serum amylase and pleural fluid amylase at <79.4 IU/L. The sensitivity and specificity of serum amylase were 94% and 28%, respectively, while the sensitivity, specificity, and significance of serum and pleural fluid amylase levels were also evaluated between cases and controls with odds ratios (**Table III**).

Table III: ROC Analysis and Odds ratio of Serum and Pleural Amylase

ROC Analysis							
Parameter			**Best cut off value (IU/L)**	**Sensitivity (%)**	**Specificity (%)**	**Area under ROC curve**	**Positive Predictive Value (%)**	**Negative Predictive value (%)**
Serum Amylase			< 79.4	94	28	0.509	56.6	82.4
Pleural fluid Amylase			> 99.8	0	82	0.521	0	45.1
ODDS Ratio								
Parameter	Odds Ratio	Z statistic	Significance level (AREA = 0.5)					
Serum Amylase	0.2553	1.971	0.0487					
Pleural fluid Amylase	0.2187	1.857	0.0633					

DISCUSSION

Our results confirm that adenosine deaminase in serum and pleural fluid is a very good parameter for the diagnosis of tuberculous pleural effusion. The study included 50 cases of tuberculous pleural effusion and 50 controls with nontuberculous exudative pleural effusion. Pleural fluid analysis provides a safe and accessible method to diagnose diseases affecting the pleural cavity. In HIV-free endemic areas, where reactivation is the main mechanism of tuberculosis, pleural involvement has been reported in 4% of cases. These patients have a later onset of the disease, are older, and are more likely to suffer from immunodeficiency[7]. Since pleural effusions can be caused by thoracic diseases, organ dysfunction or subdiaphragmatic infections, drugs[8], and systemic diseases, analyses must be performed to determine the cause and type of pleural effusion. Since traditional diagnostic tools cannot accurately identify the cause, various biomarkers such as ADA, interferon (IFN)-γ, various tumor markers and cytokines, and C-reactive protein (CRP) have been used as alternative non-invasive means to recognize the cause. Pathogenesis of tuberculosis in exudative pleural effusions. Tuberculous empyema is an exudative pleural effusion caused by hypersensitivity to mycobacteria and their antigens. These pleural effusions are usually unilateral, small to medium in size, and rarely require emergency drainage. Elevated amylase concentrations in pleural fluid, defined as values above the upper normal limit of serum or a pleural amylase ratio of 1.0 or greater, are related with pancreatic disease[9], esophageal rupture[10], malignant disease[11], ruptured ectopic pregnancy, hydronephrosis, and cirrhosis[12]. Because amylase is not rapidly degraded

in the pleural lymphatics but is rapidly removed from the blood by the kidneys, the amylase concentration in pleural fluid increases, resulting in an increase in the ratio of pleural amylase to serum amylase. In chronic pancreatitis, serum amylase may be elevated or normal due to back diffusion from the pleural cavity[13]. Elevated amylase concentrations in pleural fluid occur in 10 to 14 percent of patients with malignant pleural effusion[14]. Diagnosis and treatment of pleural sclerosis with exude may require surgical intervention. An alternative to the use of talc slurries is pleurodesis, in which talc is injected directly into the pleural surface using video-assisted thoracoscopy. The mean and standard deviation of Amylase levels in the serum of the cases was 53.04 ± 21.12 when compared to controls which was 77.12 ±72.441 with a statistically not significant p value of more than 0.001. In pleural fluids, the mean value was 49.508 ± 19.13 in cases when compared to controls which was 63.238 ± 53.84 and the p value was also more than 0.001. The sensitivity, specificity, positive and negative predictive values were 94%, 28 %, 56.6%, 82.4% in serum and 0%, 82%, 0% and 45.1% respectively in pleural fluids. ***Victoria Villena et al.***, in a large study of 841 cases of pleural effusion of various etiologies[15], including tuberculosis, found that pancreatic etiology was the main cause of elevated amylase levels, followed by malignant tumors. The increase in tuberculous patients was not large; only 8 of 140 cases had elevated amylase levels. ***Gupta KB et al.***, in a study on amylase levels in pleural effusion[16], found that serum and pleural fluid amylase levels were significantly increased in malignant pleural effusions compared with tuberculous, nontuberculous, and nonmalignant pleural effusions. ***Stephen A. and Thern et al.*** are of the opinion that elevated amylase levels in pleural effusions above the upper limit of normal occur in pancreatic diseases, esophageal rupture, malignant tumors[17], pneumonia, ruptured ectopic pregnancy, hydronephrosis, and liver cirrhosis.

CONCLUSION

The most common cause of exudative pleural effusion in our country is tuberculosis. It typically occurs during primary infection and tends to affect the younger generation (<45 years old). Current diagnosis is based on pleural puncture. Amylase levels in serum and pleural fluid indicate coexisting pathologies of the pancreas, gastrointestinal tract, and malignant tumors. Unexplained pleural effusion and elevated amylase levels in serum and pleural fluid are signs and direct the physician to specifically exclude malignant diseases. According to our study, the diagnosis of tuberculous pleural effusion by measuring amylase levels is neither sensitive nor specific.

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Conflict of Interest

The authors declare no conflict of interest.

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