Estimation of Amylase in Serum and Pleural Fluid among HIV Known Seronegative Patients with Tuberculous Pleural Effusion.

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ABSTRACT

Background: Myocardial Pleural TB is one of the most frequent extra-pulmonary manifestations of tuberculosis. Extra Pulmonary tuberculosis is the area of concern for the diagnosis of tuberculosis. Aim of this study is to estimate enzyme Amylase levels in serum and pleural fluids of HIV seronegative exudative tuberculous pleural fluids and their possible role in the diagnosis of tubercular pleural effusion. Methods: A total number of 100 patients were selected to do this study. Selected individuals were randomly included in this study as cases and controls, 50 members each. The samples of blood and pleural fluid were tested for amylase within the stipulated time, on the same day, to prevent the loss of amylase by CNP-G method.

Results: Among Group I or Cases, the mean value of serum amylase and pleural fluid amylase was 53.044 and 77.122 respectively. Among Group II or controls, the mean value of serum and pleural fluid amylase was 49.508 and 63.238 respectively.

Conclusion: Serum and pleural fluid Amylase levels indicate a coexisting pathology of pancreas, gastrointestinal tract and malignancies. According to our study, diagnosing Tuberculous pleural effusion by assessing amylase levels has less sensitivity and specificity.

Keywords: Tuberculous Pleural effusion, Serum Amylase, Pleural Amylase

INTRODUCTION

Tuberculosis (TB) is a notifiable infectious disease caused by Mycobacterium tuberculosis. Tuberculosis is more prevalent in India, which is an endemic disease. Tuberculosis is of two types clinically: Pulmonary and Extra pulmonary. Pulmonary Tuberculosis affects lungs and its lymphatics primarily, whereas Extra Pulmonary Tuberculosis can affect almost any organ in the body like pleura, meninges, intestine, reproductive tract, lymph glands, bones, joints, skin and other tissues of the body. The disease is usually chronic with varying clinical manifestations. Pulmonary Tuberculosis is the most common of TB globally (more than 85% of all TB cases).

Tuberculosis transmission occurs by airborne droplets or droplet nuclei containing tubercle bacilli. Tuberculosis is the second leading cause of death after HIV. It has become more important with the advent of the HIV epidemic, presenting in less familiar ways and pursuing a more aggressive and relentless course. It is estimated that about 1/3rd of the current global population is infected asymptomatically with tuberculosis, of whom 5 – 10 percent will develop clinical disease during their life. Tuberculous pleurisy results from Mycobacterium tuberculosis infection of the pleura. It manifests as a pleural effusion with exudative characteristics and can be found either isolated or in association with pulmonary tuberculosis (TB). Pleural TB is one of the most frequent extra pulmonary manifestations of tuberculosis. Mycobacterial proteins access this space through rupture of a sub pleural focus 6 -12 weeks after a primary infection, and rarely from a vertebral focus by direct extension.

A diagnostic approach to a patient with possible TB includes a detailed medical history, clinical examination, as well as radiological, microbiological, immunological, biochemical, molecular-biological and histological investigations. They also offer the possibility for a rapid diagnosis of active tuberculosis in patients with negative sputum smears acid-fast bacilli and enable prompt identification of drug-resistant strains of Mycobacterium tuberculosis.
directly from respiratory specimen with a high accuracy. Currently the diagnosis of pulmonary tuberculosis is based mainly on testing sputum for acid fast bacilli and radiological investigations. Extra pulmonary tuberculosis is the area of concern for the diagnosis of tuberculosis. Manifestations of pleural effusion, genital tuberculosis, tubercular meningitis and other forms cannot be diagnosed most of the times by demonstration of bacilli.

Delayed diagnosis leads to delayed treatment and prolongs the transmission of infection in the community. Late treatment can also jeopardize recovery and cure of the patient. Delays can occur at the patient level or at the level of the health care system. While it is easy to attribute delay to patients, this may not be true.

Amylases are calcium metallo-enzymes, with the calcium being absolutely required for functional integrity. However, full activity is displayed only in the presence of various anions such as chlorides, bromides, nitrate, cholate or monohydrogen phosphate, with chloride and bromide being the most effective activators.

The biochemical evaluation mainly used to classify the fluid as transudates or exudates and confirming the etiology in certain causes of pleural effusion. Aim of this study is to estimate enzyme amylase 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

A Prospective study was done from February 2014 to May 2015 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 number of 100 patients were selected to do this study. Selected individuals were randomly included in this study as cases and controls, 50 members each.

Cases - Patients presented with complaints of Pleural effusion and with known cases of HIV negative. Controls - Patients attending to chest diseases Out Patient Department without any complaints related to pleural effusion and tuberculosis.

Old cases of Tuberculosis patients, patients with renal injury/ renal failure or hepatic failure, patients suffering with typhoid or any systemic illness, transudative or undiagnosed effusions were excluded in this study. The samples of blood and pleural fluid were tested for amylase within the stipulated time, on the same day, to prevent the loss of analyte by CNP-G method (Chloro p nitrophenyl - Glucose). All samples were analyzed and cross checked in Transasia semi auto analyzer with precinorm and precipath in duplicates.

CNP-G Method: Pipette 1000 μl of reagent in to a clean and dry test tube and add 20 μl of sample to it. Mix well and aspirate the sample in to the analyze
The expected range of values for amylase activity is 23 -88 U/L.

Results were analyzed and tabulated.

RESULTS

In this study a total of 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 1].

Among Group I or cases, the mean value of serum amylase and pleural fluid amylase was 53.044 and 77.122 respectively. Among Group II or controls, the mean value of serum and pleural fluid amylase was 49.508 and 63.238 respectively [Table 2].
Sensitivity, Specificity, Positive predictive value and Negative predictive value were analyzed among cases and controls by taking the cut-off value of serum amylase and pleural fluid amylase was < 79.4 IU/L. Sensitivity and Specificity of serum amylase was 94% and 28%, where as sensitivity and specificity and significance of amylase level in serum and pleural fluid was also assessed in between cases and controls with odds ratio [Table 3].

Table 3: ROC Analysis and Odds ratio of Serum and Pleural Amylase

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Best cut off value (IU/L)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Area under ROC curve</th>
<th>Positive Predictive Value (%)</th>
<th>Negative Predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Amylase</td>
<td>&lt; 79.4</td>
<td>94</td>
<td>28</td>
<td>0.509</td>
<td>56.6</td>
<td>82.4</td>
</tr>
<tr>
<td>Pleural fluid Amylase</td>
<td>&gt; 99.8</td>
<td>0</td>
<td>82</td>
<td>0.521</td>
<td>0</td>
<td>45.1</td>
</tr>
</tbody>
</table>

ODDS Ratio

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds Ratio</th>
<th>Z statistic</th>
<th>Significance level (AREA = 0.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Amylase</td>
<td>0.2553</td>
<td>1.971</td>
<td>0.0487</td>
</tr>
<tr>
<td>Pleural fluid Amylase</td>
<td>0.2187</td>
<td>1.857</td>
<td>0.0633</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study included 50 cases of tuberculous pleural effusion and 50 controls of exudative pleural effusion of non-tuberculous origin.

Pleural fluid analysis provides a safe and accessible means for diagnosing conditions that affect the pleural space. As pleural effusions can be caused by diseases in the chest, organ dysfunction or infections below the diaphragm, drugs and systemic disease, analysis should be carried out to detect the source and type of effusion.

Conventional diagnostic tools are incapable of pinpointing the cause, so several bio-markers like ADA, interferon (IFN)-γ, a variety of tumour markers and cytokines, and C-reactive protein (CRP) have been proposed as alternative non-invasive means of establishing tuberculous etiology in cases of exudative pleural effusion.

Here in this study we have tried to evaluate the significance of Amylase in detection of Tuberculous pleural effusion. An increased pleural fluid amylase, defined as either a value greater than the upper limits of normal of the serum or a pleural fluid amylase ratio >1.0, is found in pancreatic disease, oesophageal rupture, malignancy, ruptured ectopic pregnancy, hydronephrosis and cirrhosis. Pleural fluid amylase increases in concentration because it is not cleared rapidly by the pleural lymphatics, while amylase is cleared quickly from the blood by the kidney resulting in an increased pleural fluid to serum amylase ratio.

Serum amylase may be elevated in chronic pancreatitis due to back-diffusion from the pleural space or it may be normal. An increased pleural fluid amylase concentration occurs in 10 to 14 percent of patients with a malignant pleural effusion.

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 their large study comprising of 841 cases on pleural effusions of different etiology including tuberculosis observed that pancreatic etiology is the main cause for raised amylase levels followed by malignancies. Tuberculosis cases did not show much elevation. Only 8 of 140 cases showed elevated amylase levels. Gupta KB et al in his study on amylase levels in pleural effusion observed that the serum and pleural fluid amylase levels were grossly elevated in malignant effusions compared with tuberculous, non tuberculous and non malignant effusions. Steven A Sahn et al has opined that increased pleural fluid Amylase levels greater than upper limits of normal are seen in pancreatic disease, oesophageal rupture, malignancy, pneumonia, ruptured ectopic pregnancy, hydronephrosis and cirrhosis.

CONCLUSION

Tuberculosis is the most frequent cause of exudative pleural effusion in our country, typically occurs during primary infection and tends to affect younger populations (less than 45 years old). The diagnosis is currently based on pleural tap. Serum and pleural fluid Amylase levels indicate a coexisting pathology of pancreas, gastrointestinal tract and malignancies. Pleural effusions of unknown etiology, raised serum and pleural effusion amylase levels, signifies and indicates the physician to rule out malignancy especially. According to our study, diagnosing...
Tuberculous pleural effusion by assessing amylase levels has less sensitivity and specificity.

REFERENCES

3. WHO international report 2011, pp. 3.


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