Serum Ferroxidase/Albumin ratio – Diagnostic marker of Tuberculosis

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\textbf{Abstract}

The objective of this study is to incorporate serum Ferroxidase/Albumin ratio as a surrogate marker to assist in diagnosis of pulmonary tuberculosis. Forty cases of sputum for acid fast bacilli positive PTB patients compared with forty healthy controls. Serum Ferroxidase activity of Ceruloplasmin was determined by end point method using Ferrous Ammonium Sulphate Hexahydrate at 600nm. Serum Albumin was determined by Bromocresol green, dye binding method at 628nm. Mean ± SD of serum Ferroxidase and Albumin in case and control were 1616.02±216 IU/L, 3.22± 3.0g/dl and 800.87±130IU/L,4.51±3.2g/dl respectively. Serum Ferroxidase in case was significantly higher as compared to control (P<0.001). Serum Ferroxidase/Albumin ratio in case (50.06±10 IU/g) was significantly higher than control (20.21±4.0 IU/g), (P<0.001). Hence serum Ferroxidase/Albuminratio can feasibly be used as biochemical marker to assist in diagnosis of PTB.

\textbf{Keywords:} Mycobacterium, Ceruloplasmin, Acute phase response

1. Introduction

Tuberculosis is a curable infectious disease causing significant morbidity and preventable deaths worldwide. Once thought to be under control, TB is now the number one cause of infection related death world-wide\textsuperscript{1}. One third of the global population is infected with TB, of which 95% of the incidence is in developing countries\textsuperscript{2}. According to US center for disease control and prevention, laboratory criteria for diagnosis of TB include isolation of Mycobacterium TB from a clinical specimen, demonstration of MTB from a clinical specimen by Nucleic Acid amplification Test and Demonstration of Ferrous Iron to ferric iron\textsuperscript{3}. Thereby it facilitates iron binding with transferrin and inhibits iron uptake by bacilli\textsuperscript{4}.

In India broad-spectrum antibiotics are being prescribed inappropriately leading on to Multi-Drug Resistant TB. Studies have reported that MDR-TB is significantly higher among treatment failures. This can be prevented by early referral for culture. Nowadays, Radiometry using BACTEC instrument is used to reduce the diagnostic time in cultured specimens. But still, using this technique, organisms can be detected only after 7 to 8 days in smear positive patients\textsuperscript{5}.

While the treatment of TB is considered one of the most cost-effective interventions in DOTS (Directly Observed treatment, short course chemotherapy) program, we are still without a fast and simple diagnostic test that would be applicable in high-burden but resource-poor settings.

Ceruloplasmin (Cp) is an a2-globulin that contains 95% of the total copper found in serum\textsuperscript{6}. Cp is synthesized primarily by the hepatic parenchymal cells\textsuperscript{7}. Cp is an important extracellular antioxidant and free radical scavenger\textsuperscript{8}. Acting as a ferroxidase, Cp is vitally important in catalyzing the enzymatic oxidation of ferrous iron to ferric iron\textsuperscript{9}. Thereby it facilitates iron binding with transferrin and inhibits iron uptake by bacilli\textsuperscript{10}.

Albumin is the major plasma protein with molecular mass of 663KDa\textsuperscript{11}. Albumin has a single polypeptide chain of 580 amino acids with no carbohydrate side chains\textsuperscript{12}. Albumin is synthesized primarily by hepatic parenchymal cells.

Bacterial infection induces non-specific response called acute phase response. After infection with MTB, alveolar macrophages, neutrophils and granulocytes secrete pro-inflammatory cytokines into the blood stream. The liver responds to these cytokines release by producing acute phase proteins\textsuperscript{13}. During Acute phase response high level of serum Cp (Positive acute phase protein) and low level of serum Albumin (Negative acute phase protein) has been found.

Based on this the present study was conducted to find an application of serum ferroxidase activity of Cp/albumin ratio as a marker to assist in diagnosis of PTB patients.

2. Material and Methods

The present study was conducted after getting the approval from the ethical committee of Stanley medical college. It’s an age and sex matched comparative study. Forty cases of freshly diagnosed, sputum for Acid fast bacilli positive PTB patients were taken for the study. Forty healthy subjects without any history of PTB infection were also included in the study as controls. The study subjects were selected from those...
attending the TB-Tambaram Sanatorium from January’09 to April’09. The study subjects were clearly informed of the nature of the study and the blood samples were collected after getting written informed consent. The control subjects were volunteers with good health as evidenced by medical history, complete physical examination and routine laboratory tests performed before the commencement of the study.

2.1 Sample Collection

5 ml of venous blood was drawn from the subjects. Individuals fasted for 12 hours prior to sample collection. Serum samples were stored at 4°C for 1 week. The samples were analyzed for serum Ferroxidase, serum Albumin and the results were analyzed based on the data collected.

2.2 Inclusion criteria

PTB patients, both males and females between 20 to 60 yrs who were freshly diagnosed with sputum for AFB+ve, apparently healthy individuals of both sexes in the same age group were included in the study.

2.3 Exclusion criteria

PTB patients with any other active medical conditions like pleural effusion, HIV infection, Nephrotic syndrome, Bronchial asthma etc., children and Pregnant PTB patients, PTB patients with hepatocellular or renal damage. PTB patients with malignancies such as leukemia, lymphoma, breast carcinoma etc., patients not willing to give written informed consent were excluded from the study.

2.4 Quantitative estimation of Serum Ferroxidase

Serum incubated with known amount of ferrous ion in aceta buffer. Ferroxidase activity of Ceruloplasmin oxidizes ferrous ions to ferric ions. At the end of the incubation period, chromogen was added. It forms a blue colored Fe+3 complex with non-oxidized ferrous ions whose color intensity was then measured at 600nm. The difference in the Fe+3 ion concentration before and after the enzymatic reaction indicated the amount of oxidized Fe+2 ions. The amount of enzyme that converted 1µmol of substrate into product per minute was defined as one unit. Based on this principle serum Ferroxidase level was measured.

2.5 Quantitative estimation of Serum Albumin was done by bromocresol green, dye binding method.

3. Statistical methods and Results

Table 1. Mean±SD values of serum Ferroxidase, Albumin and Ferroxidase/Albumin ratio in control and case

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Ferroxidase g/dl</th>
<th>Albumin IU/L</th>
<th>Ferroxidase/Albumin IU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>800.870 ±130</td>
<td>4.51±3.2</td>
<td>20.21±4.0</td>
</tr>
<tr>
<td>Case</td>
<td>1616.02±216</td>
<td>3.22±3.0</td>
<td>50.06±10</td>
</tr>
</tbody>
</table>

Student independent t test was used to find the P value between study group and control. Serum Ferroxidase level ranges between 500 – 1012 IU/L in control and 1020 – 2102 IU/L in case. Serum Albumin level ranges between 3.50 – 4.71 g/dl in control and 2.42 – 5.40 g/dl in case. Serum Ferroxidase/Albumin ratio ranges between 13 – 28.8 IU/g in control and 39.1 – 82.9 IU/g in case. In both males and females, there was highly significant P value on comparison of the mean levels of serum Ferroxidase (P<0.001), serum Albumin (P<0.001) and serum F/A ratio (P<0.001) between case and control.

4. Discussion

Pulmonary Tuberculosis is a global disease affecting about 1/3rd of the world’s population with its attendant morbidity and mortality. The diagnosis of PTB is based primarily on the rapid and inexpensive, microscopic examination of sputum for AFB but it is limited by its poor sensitivity (40-60%)15. Mycobacterium culture is able to detect as few as 10 organisms per milliliter of sputum and overcomes many of the limitations of AFB staining but even with the use of broth-based culture systems, confirming the presence of MTB from the time of specimen collection takes at least a week15. This delays diagnosis or unnecessary administration of antituberculous drugs. Inspite of the recent in vitro nucleic acid direct amplification tests, culture results still remain the gold standard for diagnosis of PTB15. Therefore, there is a definite requirement of biochemical marker to assist in diagnosis of PTB.

With these factors taken into account, the present study was undertaken and it was found that the study group showed highly significant elevation of serum Ferroxidase/albumin ratio as compared to control. Serum F/A ratio is statistically easier to compare the PTB patients with the control rather than the individual parameters. Moreover estimation of serum F/A ratio is cheaper compared to other investigations for PTB. Therefore serum Ferroxidase/Albumin ratio may be used as a marker to assist in diagnosis of pulmonary Tuberculosis.

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