

Original Article

Serum Ferroxidase Activity of Ceruloplasmin in Pulmonary Tuberculosis

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Abstract

The objective of this study is to incorporate serum Ferroxidase as a surrogate marker of pulmonary tuberculosis (PTB). Fifty patients with PTB whose sputum samples were positive for acid fast bacilli (Group I) and 50 subjects who were completely treated for PTB (Group II) were compared with 50 healthy controls. The difference, were similarly found between male and female Group I subject and Controls in serum ferroxidase levels. Serum Ferroxidase was measured by end point method of Ozcan Erel at 600nm. Mean \pm SD of serum Ferroxidase in control, Group I and Group II were 800.87 ± 130 IU/L, 1616.02 ± 216 IU/L and 851.12 ± 201 IU/L respectively. Serum Ferroxidase in Group I was significantly higher as compared to control ($P < 0.001$) whereas in Group II it was not significant when compared with control. Serum Ferroxidase which was higher in newly diagnosed PTB patients falls back to normal level on complete treatment with ATT. Hence serum Ferroxidase activity of ceruloplasmin can be used both as a diagnostic and prognostic marker of PTB.

Key-words: Ferroxidase, Ceruloplasmin, Pulmonary TB.

Introduction

Pulmonary tuberculosis (PTB) is a global disease affecting about one third of the world's population with its attendant mortality and morbidity. PTB can be diagnosed by a thorough evaluation of history, clinical symptoms and signs, bacteriological and radiological features. But still, in quite a number of cases it becomes difficult to predict the activity of a tubercular lesion where precise information is not available particularly in developing countries like India. The diagnosis of PTB is based primarily on the rapid and inexpensive, microscopic examination of sputum for acid fast bacilli but it is limited by its poor sensitivity (40-60%).^[1] 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 Mycobacterium from the time

of specimen collection may take at least a week.^[1] Currently rapid nucleic acid amplification techniques with sensitivity of about 96% and specificity of 100% for AFB smear positive samples are commercially available.^[2] But their performance in AFB smear-negative specimens is less impressive with sensitivity ranging from 48-53%.^[3] Moreover the accuracy of this testing may be reduced by the concurrent use of anti-tuberculous drugs and inhibitors in the patient's sputum which may give false-negative result.^[3] With these factors taken into account, the present study was undertaken to evaluate a new biochemical parameter for diagnosis of active PTB infection and prognosis of PTB patients under treatment.

Material and Methods

The present study was conducted in a medical college hospital in Chennai. In this age and sex matched comparative study, fifty cases of newly diagnosed, sputum for Acid fast bacilli positive PTB patients (Group I) and fifty cases of PTB patients who were completely treated with anti-tuberculosis drug were studied. Fifty healthy subjects without any history of PTB infection were also included in the study as controls. The study subjects were selected from those attending a TB Sanatorium in Chennai. Five ml of venous blood

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Received 25th July 2015, Accepted 19th Sept 2015

were drawn from the subjects who fasted for at least 12 hours. Serum samples were stored at 4°C for one week. The samples were analyzed for serum Ferroxidase activity. The criteria for inclusion of subjects to this study were patients of either sex aged between 20 and 60 years and newly diagnosed as sputum positive TB (group I), PTB patients completely treated with anti TB drugs (group II) and apparently healthy individuals (controls)

PTB patients with active medical conditions like pleural effusion, HIV infection, Nephrotic syndrome and Bronchial asthma were excluded. Children and Pregnant PTB patients, PTB patients with hepatocellular or renal damage, PTB patients with malignancies such as leukemia were also excluded from the study. Quantitative estimation of Serum Ferroxidase was carried out by end point method of Ozcan Erel on "ERBACHEM-5" clinical chemistry analyzer at 600nm.^[4]

Statistical Analysis

ANOVA was undertaken to find the significance of differences in mean level of serum ferroxidase between the study groups. P value of <0.05 was considered as statistically different.

Results

Table 1 shows the mean value of serum ferroxidase in the studied groups. The levels are significantly higher among group I with PTB compared to group II and the control groups ($P < 0.001$) There is no significant difference in the mean values of serum ferroxidase between group II and the control

Table 1. Mean values of Serum Ferroxidase in active PTB and treated PTB subject and controls

Study group	Serum Ferroxidase I U/L (Mean \pm SD)	Range value
Group I (sputum positive PTB)	1616.02 \pm 216	1020-2102
Group II (Completely treated PTB)	851.12 \pm 201	510-1210
Control group	800.87 \pm 130	500-1012

Discussion

The mean values of serum ferroxidase in sputum positive pulmonary TB patients were twice the level of values in completely treated pul-

monary TB subjects and control subjects. This significant differences in the levels of serum ferroxidase between the groups ($P < 0.001$) indicates that it reduces to the normal levels after completing TB treatment. Ceruloplasmin (Cp) is an α_2 -globulin that contains 95% of the total copper found in serum.^[5] Each molecule of Cp contains six to eight copper atoms, most of which are tightly bound.^[6] Cp is synthesized primarily by the hepatic parenchymal cells.^[7] Acting as a ferroxidase, Cp is vitally important in catalyzing the enzymatic oxidation of ferrous iron to ferric iron. Oxidized ferrous iron binds with transferrin and thereby inhibits the iron uptake by mycobacterium tuberculosis (MTB).^[8]

Acute phase response is a non-specific response induced by MTB. After infection with MTB, pro-inflammatory cytokines, such as TNF- α and IL-1 are secreted into the blood stream by alveolar macrophages, neutrophils and granulocytes. The liver responds to these cytokines release by producing acute phase proteins.^[9] Serum Cp being positive acute phase protein, its level has been found to be elevated during acute phase response.

Once thought to be under control, TB is now the number one cause of infection related death world-wide.^[5] 95% of the TB infection incidence is in developing countries.^[10,11] India has the highest TB burden in terms of absolute number of incidence.^[12] Timely screening for TB infection is necessary to increase the chances of survival and reduce the transmission of TB in the community. Inappropriate usage of broad-spectrum antibiotics has lead to Multi-Drug Resistant TB.^[13] 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.^[14] While effective strategy to treat identical TB Patients in the form of DOTS strategy under TB control program. we are still without a fast and simple diagnostic test that would be applicable in high-burden but resource-poor settings. Hence there is a definite need of a biochemical marker that helps in diagnosis of PTB.

Estimation of serum Ferroxidase is cheaper compared to other investigations for PTB and the procedure can be completed in 5 min. Therefore serum Ferroxidase may be used both as a diagnostic and prognostic marker in pulmonary

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