



Serum Ceruloplasmin Albumin Ratio As A Prognostic Marker in Pulmonary Tuberculosis Patients.

KEYWORDS

tuberculosis, ceruloplasmin, albumin

Payal C. Gamit

Tutor, biochemistry, Government Medical college and SSG hospital, Baroda, Gujarat.

Margeyi M. Mehta

Assistant Professor, biochemistry, Government Medical college and SSG hospital, Baroda, Gujarat.

A.T.Leuva

Dean, medical college, Government Medical college and SSG hospital, Baroda, Gujarat.

ABSTRACT BACKGROUND: Pulmonary tuberculosis caused by mycobacterium tuberculosis is communicable disease of global importance. India has the highest number of TB cases in the world and it is leading cause of death because of its high mortality and morbidity due to the emergence of multi-drug resistant TB strains and HIV infection which reactivates latent TB making it lethal. Moreover, failure to diagnose TB early remains one of the primary hurdles in preventing the spread of this disease. While the treatment of TB is considered one of the most cost-effective interventions in DOTS (Directly Observed treatment) program, we are still without a fast and simple diagnostic and prognostic test that would be applicable in high-burden but resource-poor settings.

In tuberculosis, the albumin content decreases while the globulin like Ceruloplasmin increases leading to high ceruloplasmin albumin ratio. The albumin fraction has been increased and the ceruloplasmin proved to decrease towards normal in the course of treatment. To help for an early detection and prognosis the serum ceruloplasmin albumin ratio can be of great help in a tertiary care hospital like SSG Hospital Baroda.

METHOD: The study was carried out at the Clinical Chemistry Laboratory at S.S.G. Hospital and Medical College, Baroda. Ethical Clearance was obtained from the Institutional Ethics Committee for Human Research, Medical College and S.S.G. Hospital, Baroda.

In this Prospective cohort Study, 40 newly diagnosed pulmonary tuberculosis patients in the age group of 15 to 75 years attending to Pulmonary medicine OPD of S.S.G. hospital and patients from Urban health centre, Vadodara were selected as a case for study of serum ceruloplasmin albumin ratio and same patients were followed up after completion of treatment. For controls 40 age and sex matched healthy volunteers were taken.

Serum ceruloplasmin was estimated by turbidimetric immunoassay and Serum albumin was estimated by bromocresol green method on fully automated biochemistry analyzer.

RESULTS: MEAN \pm SD for S.CERULOPLASMIN, S.ALBUMIN and S.CERULOPLASMIN ALBUMIN ratio in newly diagnosed pulmonary TB patients and pulmonary TB patients after completion of treatment were 86 ± 10 , 3052 ± 284 , 0.028 ± 0.004 and 45 ± 6 , 4325 ± 494 , 0.01 ± 0.002 respectively.

p value for S.CERULOPLASMIN, S.ALBUMIN and S.CERULOPLASMIN ALBUMIN ratio were <0.0001 .

CONCLUSION: There is a definite requirement of economic biochemical parameters in assisting the diagnosis and follow up of Pulmonary Tuberculosis specially in the lower socioeconomic strata.

Therefore Serum ceruloplasmin albumin ratio may be used as a marker to assist diagnosis, effectiveness of treatment and prognosis of Pulmonary Tuberculosis patients.

Introduction:

Pulmonary tuberculosis is communicable disease of global importance. It is caused by mycobacterium tuberculosis¹. India has the highest number of TB cases (1.98 millions cases out of global 9.4 millions cases) in the world and it is leading cause of death because of its high mortality and morbidity due to the emergence of multi-drug resistant TB strains and HIV infection which reactivates latent TB making it more severe². Moreover, failure to diagnose TB early remains one of the primary hurdles in curtailing the spread of this disease³. 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 and prognostic test that would be applicable in high-burden but resource-poor settings⁴.

Albumin is one of the most important serum proteins produced in the liver. It represents 50 to 60% by weight of all plasma proteins⁵. Ceruloplasmin is an acute phase protein

which is normally synthesized in the liver. Ceruloplasmin is an α_2 -globulin. Ceruloplasmin is an important extracellular antioxidant⁶.

In chronic infectious diseases like tuberculosis, the Albumin (Negative acute phase protein) content decreases while the globulin like Ceruloplasmin (Positive acute phase protein) increases leading to high ceruloplasmin albumin ratio. The albumin fraction was increased and the ceruloplasmin proved to decrease towards normal in the course of treatment⁷.

There are several studies showing high ceruloplasmin albumin ratio in pulmonary tuberculosis which becomes normal after completion of treatment^{3,4}.

Material and methods:

The study was carried out at the Clinical Chemistry Laboratory from May to December 2014 at Shree Sayajirao General Hospital and Medical College, Va-

dodara. Ethical Clearance was obtained from the Institutional Ethics Committee for Human Research, Medical College and S.S.G. Hospital, Baroda.

Study Tool:

Newly diagnosed pulmonary tuberculosis patients attending to Pulmonary medicine OPD of S.S.G. hospital and patients from Urban health centre, Vadodara were selected as subject for study of serum ceruloplasmin albumin ratio.

Study Design:

Informed consent of subjects and controls included in the study was obtained for participation in study and for blood collection. Detailed medical history of the subjects including personal data, present complaints and complication, treatment history, past history, family history and personal history was taken.

Inclusion criteria:

For cases: 40 newly diagnosed patients of pulmonary tuberculosis in the age group of 15 to 75 years (both males and females) without any other systemic disorders. Male patients were follow uped after completion of treatment.

For controls: 40 healthy volunteers in the age group of 15 to 75 years (both males and females) coming to OPD for routine medical check up.

Exclusion Criteria:

- Pulmonary infections other than Tuberculosis
- Patients with Renal disorders
- Patients with Liver disorders
- Pregnancy
- Female patient taking oral contraceptive (OC) pills

The following investigations were done:

- Serum ceruloplasmin
- Serum Total Protein
- Serum Albumin
- Serum Total Bilirubin
- SGPT (ALT)
- Random blood sugar
- Serum Urea
- Serum Creatinine

Estimation of Serum ceruloplasmin was done by Turbidimetric Immunoassay method on Fully Automated Biochemistry Analyzer-MIURA.

Estimation of Serum albumin was done by BCG method on Fully Automated Biochemistry Analyzer-MIURA.

And then ratio of serum ceruloplasmin and serum albumin was calculated.

1.3 Theory and Calculation:

Ceruloplasmin is a copper containing α_2 -globulin, a glycoprotein with enzyme activities. The primary physiological role of Ceruloplasmin involves plasma redox reactions. Acting as a ferroxidase, it is vitally important in regulating the ionic state of iron in particular, oxidizing Fe^{+2} to Fe^{+3} . The ferroxidase action of ceruloplasmin allows it to inhibit iron dependent lipid peroxidation. Ceruloplasmin also binds copper ions and can inhibit copper stimulated hydroxyl radical formation and lipid peroxidation⁹.

Mycobacteria can induce reactive oxygen species (ROS) production by activating phagocytes and although there are an important part of the host defense against Mycobacteria, enhanced ROS generation may promote tissue in-

jury and inflammation⁹. Ceruloplasmin being acute phase protein, increases in PTB patients.

Albumin is mainly synthesised in liver. Rate of synthesis is approx.14.0 gms/day. Concentration of albumin decreases in: severe protein calorie malnutrition (PCM), liver diseases like cirrhosis of liver, (albumin synthesis impaired), nephrotic syndrome (albumin is lost in urine)¹⁰. It also decreases in chronic infections like Tuberculosis.

Calculation:

Estimation of serum ceruloplasmin and serum albumin was carried out in patients as well as in controls. Statistical analysis was done by using t-test to find out significance of difference between two groups and correlation coefficient to find out statistical correlation between two variables and its significance. Interpretation was done according to p-values as follows:

$p < 0.0001$ was considered highly significant

$p < 0.05$ was considered significant

$p \geq 0.05$ was considered not significant

1.4 Result :

Table 1: Comparisons of Serum ceruloplasmin and Serum albumin and ceruloplasmin albumin ratio in newly diagnosed Pulmonary TB patients with Controls

	CONTROL	NEWLY DIAGNOSED PULMONARY TB PATIENTS	P VALUE
S.CERULOPLASMIN (mg/dl)	36±6	86±10	P<0.0001
S.ALBUMIN (mg/dl)	4200±390	3052±284	P<0.0001
S.CERULOPLASMIN: ALBUMIN (RATIO)	0.009±0.001	0.028±0.004	P<0.0001

This table shows that Serum Ceruloplasmin is significantly high in newly diagnosed pulmonary TB patients than control patients (P<0.0001). While serum albumin is significantly low as compared to control patients (P<0.0001). So serum ceruloplasmin albumin ratio is significantly high in newly diagnosed pulmonary TB patients than control patients (P<0.0001).

Table 2: Comparisons of Serum ceruloplasmin and Serum albumin and ceruloplasmin albumin ratio in patients of Pulmonary TB after completion of treatment with Controls

	CONTROL	PULMONARY TB PATIENTS AFTER COMPLETION OF TREATMENT	P VALUE
S.CERULOPLASMIN (mg/dl)	36±6	45±6	P<0.0001
S.ALBUMIN (mg/dl)	4200±390	4325±494	P>0.05 (P=0.2128)
S.CERULOPLASMIN: ALBUMIN (RATIO)	0.009±0.001	0.01±0.002	P<0.05 (P=0.0059)

This table shows that Serum Ceruloplasmin is comparatively high in pulmonary TB patients after completion of treatment than control patients (P<0.0001). Serum ceruloplasmin albumin ratio is significantly high in pulmonary TB patients after completion of treatment than control patients (P<0.05).

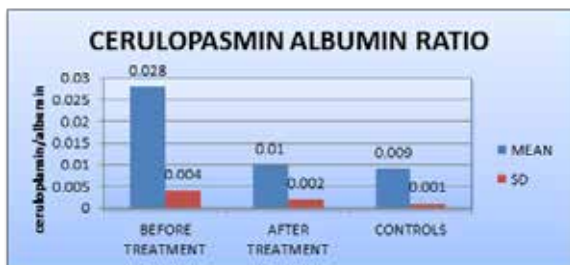
Table 3: Comparisons of serum ceruloplasmin and serum albumin and ceruloplasmin albumin ratio in newly diagnosed patients of Pulmonary TB with patients of pulmonary TB after completion of treatment.

	NEWLY DIAGNOSED PULMONARY TB PATIENTS	PULMONARY TB PATIENTS AFTER COMPLETION OF TREATMENT	P VALUE
S.CERULOPLASMIN (mg/dl)	86±10 (Range:69.2-110.4)	45±6 (Range:33.6-56.1)	P<0.0001
S.ALBUMIN (mg/dl)	3052±284 (Range:2300-3400)	4325±494. (Range:3600-5100)	P<0.0001
S.CERULOPLASMIN: ALBUMIN (RATIO)	0.028±0.004 (Range:0.020-0.038)	0.01±0.002 (Range:0.007-0.015)	P<0.0001

From above tables it is evident that Serum Ceruloplasmin & Serum ceruloplasmin albumin ratio is significantly high in newly diagnosed pulmonary TB patients than pulmonary TB patients after completion of treatment (P<0.0001). Serum albumin is significantly low in newly diagnosed pulmonary TB patients than pulmonary TB patients after completion of treatment (P<0.0001).

Serum Ceruloplasmin/Albumin in study groups:

Serum ceruloplasmin albumin ratio is significantly high in newly diagnosed pulmonary TB patients and it start decreasing with treatment of pulmonary TB. The ratio is highly significant when compared with control patients (P<0.0001).



1.5 Discussion:

Ceruloplasmin is an acute phase protein which is normally synthesized in the liver. Increased plasma ceruloplasmin levels are associated with generation of oxidant products i.e., O_2 & H_2O_2 . Oxidation of ferrous ion leads to superoxide ion leads to peroxidative damage. Ceruloplasmin due to its ferroxidase activity can catalyze the oxidation of Fe^{2+} with concomitant production of H_2O from O_2 and acts as an acute phase reactant.

Albumin is one of the most important serum proteins produced in the liver. It represents 50 to 60% by weight of all plasma proteins. Infection induces a reduction in plasma albumin level¹¹.

Present study was carried out, at Clinical Chemistry Laboratory in S.S.G. Hospital and Medical College, Vadodara, to measure the level of Serum ceruloplasmin, serum albumin and serum ceruloplasmin albumin ratio was calculated in Pulmonary TB patients. This prospective cohort study consisted of 80 subjects; 40 were patients with pulmonary TB (age ranges: 15-75 years with mean age 39.6±16.7) including 26 males (65%) and 14 females (35%) and 40 controls (age ranges: 15-75 year with a mean age 35.5±9.2) including 28 males (70%) and 12 females (30%) were eval-

uated.

In this present study 40 known cases of newly diagnosed Pulmonary TB patients were subjected to Serum Ceruloplasmin and Albumin tests. These same 40 patients were followed after completion of treatment and again Serum Ceruloplasmin and Albumin were measured. Serum ceruloplasmin and albumin ratio is calculated from data and it is compared with data from 40 healthy controls.

Findings of the present study are as follows:

Mean value of Serum Ceruloplasmin Albumin ratio in newly diagnosed pulmonary TB patients is 0.028±0.004 compared to Mean value of Serum Ceruloplasmin Albumin ratio after completion of treatment is 0.01±0.002 and 0.009±0.001 in Controls, which is also highly significant (p<0.0001).

Several studies have shown association between serum ceruloplasmin, serum albumin and serum ceruloplasmin albumin ratio in Pulmonary TB:

In 1989, P .O. Motiani et al. observed that significant rise of serum ceruloplasmin in sputum positive pulmonary TB, as compared to sputum negative with radiological extent of disease¹².

In 1990, Chandra Immanuel et al. has studied acute phase proteins in PTB. There was a significant decrease with treatment in the concentrations of C-reactive protein, ceruloplasmin, haptoglobin and α_1 -acid glycoprotein¹³.

Rohini K et al. in 2010 has conducted study on 35 diagnosed cases of PTB. They estimated levels of micronutrients, heptoglobin and ceruloplasmin and found that micronutrients are significantly low while heptoglobin and ceruloplasmin were found to be increased significantly in tuberculosis patients as compared with healthy volunteers¹⁴.

In 2011, R. I. Cernat et al. conducted study on 47 subjects diagnosed with

active PTB and 170 healthy control. Copper and ceruloplasmin levels were increased in patients with active PTB compared to the control group (P < 0.01)¹⁵.

Ramesh, Rakesh Mudaraddi et al. in 2012 were determined serum ceruloplasmin and albumin levels in 75 patients of tuberculosis. They found Serum ceruloplasmin albumin ratio decreases after taking antituberculous treatment. Therefore serum ceruloplasmin albumin ratio can be used as marker to assist the diagnosis, treatment and prognosis of pulmonary tuberculosis patients¹⁶.

Ali AA et al. in 2013 has conducted study on 60 confirmed TB cases. Also 20 apparently healthy subjects were used as control. The results showed that there significant (p < 0.05) increasing in Ceruloplasmin-Albumin ratio in TB patients as compared to control¹⁷.

In another study of 2012, Dr. Zia H. Khan et al. has conducted study on 50 patients of pulmonary TB. A gradual significant decreased in gamma globulin level was observed after one month treatment. Which reaches to control level on six months post treatment as improvement occurred. This study is a valuable guide in deciding upon the duration of therapy necessary for individual cases⁷.

In recent study of 2014, Dr. G. Anuradha et al. has conducted case control study on 40 cases of sputum for acid fast bacilli positive PTB patients and 40 healthy controls. Serum Ferroxidase in case was significantly higher as compared to control (P<0.001). Serum Ferroxidase/Albumin ratio in case was significantly higher than control (20.21±4.0 IU/g), (P<0.001). Hence serum Ferroxidase/Albumin ratio can feasibly be used as biochemical marker to assist in diagnosis of PTB⁴.

1.6 Conclusion:

- Findings of present study indicates that
- Serum ceruloplasmin level increases in Pulmonary TB because it is acute phase protein
- Serum albumin decreases in chronic infections like Pulmonary TB.
- So if Serum ceruloplasmin albumin ratio is calculated in Pulmonary TB patients (before and after treatment), it serves as a diagnostic and prognostic marker for the patients.
- In spite of recent in vitro nucleic acid direct amplification tests and culture of the Mycobacteria in a specimen for diagnosis and prognosis of Pulmonary Tuberculosis, there is a definite requirement of biochemical parameters in assisting the diagnosis and follow up of Pulmonary Tuberculosis.
- Therefore Serum ceruloplasmin albumin ratio may be used as a marker to assist diagnosis, effectiveness of treatment and prognosis of Pulmonary Tuberculosis patients.
- Serum ceruloplasmin albumin ratio after 1 month, 2 months, 3 months, 4 months, 5 months and 6 months of antituberculous drugs is advisable to see the effectiveness of the treatment

International Journal of Tuberculosis and lung diseases. Serum trace metal and ceruloplasmin variability in individuals treated for Pulmonary Tuberculosis. 2011; 15(9):1239-45.

16. Ramesh , Rakesh Mudaraddi, Ravindra Maradi. Research Journal of Pharmaceutical, Biological and Chemical Sciences. Serum ceruloplasmin albumin ratio as a biochemical marker to assist the diagnosis, treatment and prognosis of Pulmonary Tuberculosis patients. 2012; 3 (2) : 494-99.
17. Ali AA, Abdulrazaq MS AL-Saadi Mak. International Journal of Biotechnology and Allied Fields, September. Ceruloplasmin albumin ratio as a biochemical maker for the diagnosis of Tuberculosis. 2013; 1(9): 414-18.

References:

1. Adedapo K. S, Arinola O. G, Ige O.M, Adedapo A.D.A, Salimonu L.S. African Journal of Biomedical Research. Combination of Reduced Levels of Serum Albumin and Ipha-2-Macroglobulin Differentiates Newly Diagnosed Pulmonary Tuberculosis Patients from Patients on Chemotherapy. 2006; 9: 169 – 72.
2. RNTCP, Training module for Medical practitioners ; central TB division; December 2010.
3. Hitender Singh Batra, Parduman Singh Babu Lal Somani, Ashish Gupta, Sangeetha Sampath and Vivek Ambade. Indian Journal of Clinical Biochemistry. Serum ferroxidase albumin ratio as amarker in pulmonary tuberculosis. 2007 ; 22 (2) : 106-08.
4. G. Anuradha, V. Amudavalli, K. Pramila. International Journal of Biomedical And Advance Research. Serum Ferroxidase/Albumin ratio – Diagnostic marker of Tuberculosis. 2014 ;05 (02): 106-08.
5. Emmanneal Bourdon, Nadine Loreau, Denis Blache. FASEB Journal. Glucose and free radicals impair the antioxidant properties of serum albumin. 1999; 13: 233-44.
6. Carl A. Burtis, Edward R Ashwood, David E. Bruns. Amino acids and proteins. Tietz Fundamentals of Clinical Chemistry 6th edition. Pp: 286-16.
7. Dr. Zia H. Khan, Shankar S. Warke. International Journal of Pharmaceutical Research & Allied Sciences. Effect of Antituberculosis Drugs on Levels of Serum Proteins in Pulmonary Tuberculosis Patients. 2012 ;1 (3): 94-100.
8. M.N.Chatterjee, Rana Shinde. Textbook of medical biochemistry. 8th edition; 2012: 98-101.
9. K. Frank Austen. Tuberculosis. Harrison's principles of internal medicine. 17th edition; Mc grew hill. 2008;
10. Robert K Murray, David Bender et al. Plasma proteins and Immunoglobulins. Harper's illustrated biochemistry. 29th edition; 2012: 632-40.
11. M F Badrawy, M. Farid et al. Serum ceruloplasmin estimation as an index of disease activity in Pulmonary Tuberculosis. Science and medical journal of cai medical syndrome. 1990; 2(4) :101-11 .
12. P O Motiani, SK Luhadia et al. Ind Journal of Tuberculosis. Serum ceruloplasmin levels in relation to activity of Pulmonary Tuberculosis. 1983; 30: 152-55.
13. Chandra Immanuel, G.S. Acharyulu M. Kannapiran, R Segaran and G. Raghupati Sarma. Acute phase proteins in Tuberculosis patients. Indian Journal of Chest Disease & All Science. 1990; 32(1): 15-23.
14. Rohini K , Srikumar P S et al. International Journal of Biological and Medical Research. Alteration in the levels of serum micronutrients in tuberculosis patients. 2013; 4(1): 2958-61.
15. R. I. Cernat, T. Mihaescu M. Vornicu, D. Vione, R. I. Olariu, C. Arsene.