Original Article

Serum ceruloplasmin albumin ratio as a biochemical marker to assist the diagnosis and prognosis of pulmonary tuberculosis patients

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ABSTRACT

Background: Pulmonary tuberculosis (PTB) is the most prevalent communicable disease worldwide, caused by *Mycobacterium* TB. Inflammatory response is mediated by acute phase reactants of which ceruloplasmin is a positive phase reactant and albumin, a negative phase reactant. **Aim:** To estimate and to compare the serum level of ceruloplasmin, albumin (C/A) and its ratio in newly diagnosed TB patients, patients on treatment, cured patients, and normal healthy individuals. To determine its role in the diagnosis and prognosis of the PTB patients. **Settings and Design:** A cross-sectional study was conducted at TB hospital attached to KAPV Medical College, Tiruchirappalli, over a period of 3-month. **Materials and Methods:** 100 individuals which includes 75 TB patients with sputum-positive pulmonary tuberculosis and 25 healthy controls were taken for the study and divided into 4 groups. Group 1 - newly diagnosed patients, Group 2 - patients on treatment, Group 3 - cured patients, and Group 4 - normal healthy individuals. Serum ceruloplasmin was estimated by RAVINS method. Serum albumin was measured by BCG method. Statistical analysis was done by SPSS-16 software. **Results:** Mean value of C/A ratio in Groups 1-4 were 21 ± 7.69, 19 ± 4.4, 3.2 ± 0.38, and 4.3 ± 0.52, respectively. C/A ratio is significantly increased ($P < 0.01$) in newly diagnosed TB patients when compared to normal individuals. The C/A ratio in cured patients is significantly decreased when compared to newly diagnosed patients ($P < 0.01$). **Conclusion:** In spite of recent advances and newer techniques, biochemical parameters which include C/A ratio can be used as a marker to assist in the diagnosis and prognosis of the disease.

Key words: Acute phase reactant, Albumin, Ceruloplasmin, Tuberculosis

INTRODUCTION

Pulmonary tuberculosis (PTB) is the most common communicable disease and a more prevalent in India compared to rest of the world. As the WHO estimation, tuberculosis prevalence in India per lakh population was 2011 in 2013, and it is a great financial burden to the country.[1] According to the district TB unit (DTU) statistics, during the first ½ year (January to June)of 2015, in total 739 new smear positive pulmonary tuberculosis patients were identified in Tiruchirappalli district. PTB is caused by *Mycobacterium* TB, which is an aerobic bacilli.

The inflammatory response is usually identified by the acute phase reactants. Acute phase reactants are proteins whose blood levels are altered in infection, inflammation, and injuries.[2] Two types of acute phase reactants are there in our body, positive and negative phase reactants. Ceruloplasmin being a positive reactant, its level will be elevated in infections whereas albumin, a negative reactant, the level will be decreased in infection.[3]

Ceruloplasmin is an acute phase protein, synthesized primarily by the hepatic parenchymal cells.[4]

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Macrophages and lymphocytes also produce a small amount of ceruloplasmin. Ceruloplasmin is an α2-globulin that contains approximately 95% of the total serum copper, giving it a blue. Each molecule contain 6-8 copper atoms. It is synthesized as apoceruloplasmin, which is biologically inactive. Copper is added by intracellular ATPase enzyme. Carbohydrate side chains are subsequently added in the endoplasmic reticulum of hepatocytes. Copper appears to be essential for the normal folding of the polypeptide chain and attachment of normal carbohydrate side chains. After getting incorporated with copper and carbohydrate side chains, it becomes Holoceruloplasmin.

It acts as an extracellular antioxidant, also known as Ferroxidase I.[5] It also acts as a host defence mechanism by its radical scavenging a copper donor activity. Increased plasma ceruloplasmin levels are associated with the generation of oxidant products O^{-2} and H_2O_2. Oxidation of ferrous ion leads to superoxide ion formation which finally 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.

Sialic acid is slowly removed from ceruloplasmin by tissue and plasma neuraminidases resulting in exposure of the penterminal galactose residue on the CHO side chains. Once a critical number of galactose residues are exposed, the protein is rapidly removed by galactose receptors of the hepatic parenchymal cells and catabolise.

Albumin the most abundant plasma protein, synthesized by hepatocytes has 585 amino acids. It has a heart-shaped three-dimensional structure stabilized by 17 intrachain S-S bonds. 50-60% weight of all plasma proteins is by albumin and the main function of albumin is to maintain the colloid osmotic pressure.[6] Recent evidence indicates that albumin may provide antioxidant protection by functioning as a serum peroxidase in the presence of reduced glutathione, which is an intracellular antioxidant catabolism occurs mainly by pinocytosis and lysosomal degradation of protein into amino acids. Epidemiological data consistently show that reduced levels of serum albumin is associated with increased mortality.[7]

This ceruloplasmin albumin (C\A) ratio is expected to be elevated in newly diagnosed TB patients, decline progressively in patients under treatment and becomes normal in patients who are declared as cured.

Aim

The aim of the present study was to estimate serum ceruloplasmin, albumin level and its ratio (C/A ratio) and to determine its role to assist in the diagnosis, and prognosis in the PTB patients.

MATERIALS AND METHODS

After getting approval from Institutional Ethical Committee, a cross-sectional study was conducted in 100 individuals which includes 75 TB patients at TB hospital attached to KAPV Medical College, Tiruchirappalli and 25 healthy controls over a period of 3-month. Patients with sputum positive pulmonary tuberculosis were taken for the study. They were divided into four groups.

Groups	Study population	Total no.
1	Newly diagnosed	25
2	On treatment	25
3	Cured	25
4	Controls	25

Newly, diagnosed group represents patients who are sputum positive and have not, yet started treatment or treatment duration of <1 month.

Sputum positive group includes patients who show at least one positivity, among the two consecutive sputum smears collected:

On treatment group: Patients who have taken treatment for more than a month.

Cured: Initially sputum positive patients, who completed treatment of 6 months and had negative smear result on at least two occasions (one at the time of treatment completion).

This study involves both sexes and age ranges between 18 and 45 years. TB patients associated with HIV, renal disorders, bronchial asthma were excluded from the study.

Serum ceruloplasmin was estimated by RAVINS (para-phenylenediamine) method.[8] Serum albumin was measured by BCG method. We have also measured blood urea, serum creatinine to rule out renal disease and total protein level to assess the nutritional status in all the study groups. Statistical analysis was done by SPSS-16 software with appropriate statistical tests.

RESULTS AND OBSERVATIONS

The mean value of serum ceruloplasmin, serum albumin, and C/A ratio of Groups 1-4 are shown in Table 1. C\A ratio is significantly increased ($P < 0.01$) in newly diagnosed TB patients when compared to normal individuals.

The C/A ratio in patients on treatment is not significantly decreased ($P = 1$) when compared with

Table 1: Comparison of mean and SD of C/A and its ratio among study groups

Variables	Mean±SD (*n*=25)			
	Group 1	Group 2	Group 3	Group 4
Ceruloplasmin (mg/dL)	67±21	63±17	44±12	42±8
Albumin (g/dL)	3.2±0.45	3.1±0.58	3.2±0.38	4.3±0.52
C/A ratio	21±7.69	19±4.4	13.5±3.5	9.6±1.9

C/A: Ceruloplasmin albumin, SD: Standard deviation

Table 2: Comparison of mean and SD of blood urea, serum creatinine and total protein among study groups

Variables	Mean±SD (*n*=25)			
	Group 1	Group 2	Group 3	Group 4
Urea (mg/dL)	30.7±12.2	30.0±7.2	35.6±7.9	25.8±2.6
Creatinine (mg/dL)	0.9±0.2	0.89±0.20	0.912±0.18	0.95±0.18
Total protein (g/dL)	6.6±1.2	6.5±0.9	5.9±0.33	6.9±0.4

C/A: Ceruloplasmin albumin, SD: Standard deviation

newly diagnosed. The C/A ratio in cured patients is significantly decreased when compared to newly diagnosed($P < 0.01$) but at the same time, the statistical difference between cured and normal persons is also significant ($P = 0.036$), which means the C/A ratio has started to decrease but not yet reached the levels of normal individuals.

The maximum values of ceruloplasmin obtained in Groups 1-4 are 110, 110, 70, and 67 mg/dL, respectively, and the minimum values are 30, 32, 30, and 32 mg/dL respectively. The maximum values of albumin in Groups 1-4 are 4.2, 4.4, 3.8, and 5.5 g/dL and the minimum values are 2.5, 2.4, 2.4, 3.4 g/dL. The ceruloplasmin value well correlates with C/A ratio. Patients with high ceruloplasmin value have increased C/A ratio, whereas patients with low ceruloplasmin value have low C/A ratio.

We have a statistical significance of $P < 0.01$ for total protein between Groups 1 and 3, and Groups 3 and 4 (Table 2). There is no statistical significance between groups for blood urea and serum creatinine except Groups 3 and 4 which shows a significant difference in blood urea level. We have found no correlations between age, sex with C/A ratio, whose $P > 0.05$.

DISCUSSION

Tuberculosis is the most prevalent disease in India and the major cause for mortality and morbidity. Though sputum AFB staining is commonly used for diagnosing tuberculosis, it is not the confirmatory test. In spite of the recent *in vitro* nucleic acid PCR tests, *Mycobacterium* TB culture is the gold standard test for diagnosis.[9] However, it will take minimum of 7 days from the collection of sample.[10] This will unnecessarily delay the diagnosis time. Hence, biochemical marker is definitely required to assist in the diagnosis of PTB.

In our study, C/A ratio is high in newly diagnosed patients than the normal control persons with high statistical significance ($P < 0.001$). These findings correlate well with the study by Anuradha et al ($P < 0.001$).[3] and Cernat *et al*.[7]

Wong[11] and Grange[12] were reported that significant increase in ceruloplasmin, α-1 antitrypsin and haptoglobulin in PTB patients as compared to normal individuals.

Ramesh *et al*.[13] has reported that statistical significance in C/A ratio between newly diagnosed and cured patients ($P = 0.001$) as well as newly diagnosed and patients on treatment ($P = 0.001$). Serum C/A ratio in our study was significantly high in the newly diagnosed patients when compared to the cured patients ($P < 0.01$), but no significant change in patients on treatment when compared to newly diagnosed patients.

C/A ratio of cured patients was significantly decreased when compared to newly diagnosed patients ($P < 0.01$). This observation was in agreement with the studies of Batra *et al*.[14]

There is a statistically significant difference between cured and normal healthy controls. This can be explained that there could be some delay in the decrement of C/A ratio when compared to microbiological diagnosis.

Serum albumin level in newly diagnosed TB patients is low when compared to normal individuals. This is evidenced by the study of Karyadi *et al*.[15]

Serum albumin level does not show much significant difference in Groups 1-3. Hence, the variations in the C/A ratio is mainly due to changes in the serum Ceruloplasmin level.

CONCLUSION

Hence, this serum C\A ratio can be used as a marker to assist in the diagnosis of TB to increase the sensitivity of the sputum smear study, and it can also be used in the assessment of prognosis of the disease.

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