ORIGINAL ARTICLE

Serum Ceruloplasmin Albumin Ratio as A Marker of Treatment Response in Pulmonary Tuberculosis

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ABSTRACT

BACKGROUND: The study was conducted to observe treatment response in pulmonary tuberculosis and to see diagnostic efficiency of ceruloplasmin and albumin ratio between pulmonary tuberculosis and multi drug resistant tuberculosis. MATERIALS AND METHODS: It was a longitudinal case control study. 50 diagnosed cases of pulmonary tuberculosis before starting treatment (group PTB-1) were followed after 2 months of treatment (group PTB-2). 50 diagnosed case of multi drug resistant tuberculosis (group MDR-TB). 50 age and sex matched healthy controls were taken and every candidate was examined for ceruloplasmin albumin ratio. RESULTS: Result (mean ± SD) of ceruloplasmin albumin ratio (mg of Ceruloplasmin/gm of Albumin) in the Control group was 5.23 ± 0.67, in PTB-1 was 9.32 ± 2.25, in the PTB-2 group was 7.46 ± 1.32 and in MDR TB group it was 9.365 ± 1.643. Hence it was seen that Ceruloplasmin albumin ratio was significantly higher in PTB-1 group compared to Control with p value <0.001. Ratio was lower in PTB-2 (2 months after treatment) compared to PTB-1 (before starting treatment) and it was statistically significant with p value <0.001. there was no significant difference in ratio was observed between PTB-1 and MDR TB group. CONCLUSION: It showed that the Ceruloplasmin albumin ratio increases after Tuberculosis and then it gradually comes down towards the normal level after treatment. Serum Ceruloplasmin albumin ratio (mg of Ceruloplasmin/gm of Albumin) can therefore be incorporated as a surrogate marker to assist in diagnosis as well as prognosis of pulmonary tuberculosis.

Key Words: MDR-TB, ceruloplasmin, pulmonary tuberculosis, resistance.

INTRODUCTION

Tuberculosis (TB) is a major cause of death despite being a curable infectious disease. The disease caused by Mycobacterium Tuberculosis (MTB), has affected mankind for over 5000 years. The bacilli was discovered more than a century back by Sir Robert Koch in 1882 and effective drugs for treatment have been available for more than half a century, yet it continues to be a leading cause of morbidity and mortality1. One third of the world’s population is infected with TB bacilli, i.e. have latent TB, and of those 10% have a life time risk of developing to active disease1.

Tuberculosis (TB) is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. In 2012, 8.6 million people fell ill with TB and 1.3 million died from TB globally. Though India is the second-most populous country in the world one fourth of the global incident TB cases occur in India annually. In 2012, out of the estimated global annual incidence of 8.6 million TB cases, 2.3 million were estimated to have occurred in India. Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top three causes of death for women aged 15 to 44. In 2012, an estimated 530 000 children became ill with TB and 74 000 HIV-negative children died of TB. TB is a leading killer of people living with HIV causing one fifth of all deaths2.

The global TB epidemic like situation is further aggravated by HIV infection and emergence of drug-resistant tuberculosis2. A particularly dangerous form of drug-resistant TB is multidrug-resistant TB (MDR-TB), which is defined by WHO as the disease caused by TB bacilli resistant to at least isoniazid and rifampicin, the two most prescribed anti-TB drugs3,4. It is a serious threat to TB control programme and requires new guidelines for its management5. About 450 000 people
developed MDR-TB in the world in 2012. More than half of these cases were in India, China and the Russian Federation. It is estimated that about 9.6% of MDR-TB cases had XDR-TB. 1,2

Diagnosis of tuberculosis in Revised National Tuberculosis Control program (RNTCP) is still based on sputum AFB microscopy and Culture is considered as gold standard.4 To improve the diagnosis of TB, more rapid diagnostic techniques have been investigated in recent years. The main transporter of copper, α2-globulin Ceruloplasmin is a multifunctional enzyme.5 High serum Ceruloplasmin levels were observed in patients of pulmonary TB (PTB)6. Albumin, a major plasma protein of 69 KD has been reported low in pulmonary TB7. Various studies on serum Ceruloplasmin and albumin in pulmonary tuberculosis have shown that Ceruloplasmin/albumin ratio assist in the diagnosis and monitoring therapy of pulmonary TB8.

This study was conducted to observe how Ceruloplasmin/Albumin ratio respond to treatment in pulmonary tuberculosis patients and how this help in early detection of treatment failure which may be due to MDR TB.

MATERIALS AND METHODS

Study subjects: The study was conducted jointly in the Departments of Biochemistry and Microbiology, Lady Hardinge Medical College and Associated Hospitals, New Delhi and Department of Chest clinic, Lok Nayak Hospital, New Delhi, India after getting approval from the Institutional Ethical Committee of Lady Hardinge Medical College, Delhi University, New Delhi, India. This study was done after obtaining written informed consent from the enrolled subjects included in our study. A total of 150 subjects were enrolled in our study and they were divided into three groups. 50 patients of newly diagnosed pulmonary TB (PTB group), 50 patients of newly diagnosed MDR TB (MDR TB group) and 50 age and sex matched healthy control after excluding inflammatory diseases (Control group).

Study design: This study was a longitudinal case control study. Blood sample were collected from healthy control (after excluding TB and other inflammatory disease), MDR-TB patients before starting treatment and PTB patients twice, first before starting treatment (PTB-1) and at the end of intensive phase of treatment that is after 2 months of treatment (PTB-2). Blood sample were processed and serum samples were separated and stored. Methods of measurements: All patients and healthy control were subjected to detailed history and clinical examination. The blood sample in plain vacutainer was allowed to clot at room temperature. It was then centrifuged at 3000 rpm for 10 minutes to separate serum samples were stored at -20°C for further batch analysis for serum ceruloplasmin and serum albumin level.

Tests were carried out in fully automated clinical chemistry analyzer, Beckman (SYNCHRON CX9) using standard reagents/kits. For serum albumin (gm/dl) analysis bromocresol green (BCG) method was used. For estimation of serum Ceruloplasmin (mg/dl) level quantitative immunoturbidimetric method was used which is based on principle of turbidimetric reaction which occurs between the anticeruloplasmin polyclonal antiserum and its corresponding antigen in optimal pH condition and in the presence of polyethylene glycol polymer (PEG). The turbidity of the immune-complex is proportional to the concentration of ceruloplasmin in the examined sample which is end point reaction and reading was taken at 340nm wavelength. Based on estimated values mg of Ceruloplasmin/gm of albumin was calculated in each subject.

Statistical evaluation: The data obtained were analysed by using statistical tests ANOVA, independent t-test and paired t-test for comparison between groups and p value was calculated, p value < 0.05 was taken as a significant using Statistical Package of Social Sciences (SPSS) version 17.
RESULTS

Table: 1 Ceruloplasmin / Albumin ratio (C/A) in Control and Study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Mean ± SD (mg/gm)</th>
<th>SE of mean</th>
<th>p value compared to Control</th>
<th>p value compared to PTB-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>5.226 ± 0.671</td>
<td>0.095</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PTB</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PTB-1</td>
<td>50</td>
<td>9.321 ± 2.250</td>
<td>0.318</td>
<td>&lt;0.001*</td>
<td>-</td>
</tr>
<tr>
<td>PTB-2</td>
<td>50</td>
<td>7.465 ± 1.322</td>
<td>0.187</td>
<td>&lt;0.001*&lt;1</td>
<td>&lt;0.001*&lt;1</td>
</tr>
<tr>
<td>MDR</td>
<td>50</td>
<td>9.365 ± 1.643</td>
<td>0.232</td>
<td></td>
<td>0.913</td>
</tr>
</tbody>
</table>

Fig-1: Ceruloplasmin / Albumin in Control and Study groups.

Demographic and clinical profile: In our study the maximum numbers of patients (70%) were in the age group of 20-40 years and it was more common in male (56.66%) than female (43.33%). Most of the patients presented with complain of cough with sputum, weight loss, fever and haemoptysis.

Serum analysis: In our study we calculated ceruloplasmin and albumin ratio for all the subjects results is shown in table-1 and graphical presentation in figure-1. We applied statistical test ANOVA between all the groups and we found difference in mean of ceruloplasmin/albumin in all the groups to be statistically significant with p value < 0.001. It was observed that Ceruloplasmin/Albumin levels were significantly raised in PTB and MDR cases as compared to control group. Independent t-test was applied to compare the difference in mean of ceruloplasmin/albumin between the Control group and the Study groups PTB-1, PTB-2 and MDR, which was found to be statistically significant (p<0.001). However no statistically significant difference was observed in the results between PTB-1 and MDR groups (p=0.913). Paired t-test was used to analyse treatment response in PTB patients the difference in mean of ceruloplasmin/albumin before (PTB-1) and after (PTB-2) starting treatment was observed to be statistically significant (p<0.001). After 2 months of anti tuberculosis treatment (ATT) ceruloplasmin/albumin levels were found to decrease significantly, but were still higher as compared to the control group.

DISCUSSION

According to Revised National Tuberculosis Control Programme (RNTCP), TB is diagnosed based on sputum microscopy and chest X-ray. For the treatment, patients are divided into two categories base on site and extent of disease, Category-I (New cases of pulmonary and extra pulmonary tuberculosis), Category-II (Sputum smear-positive Relapse, Failure and Default). First time diagnosed patients; treatment is given according to category I. Patients who come after treatment failure, relapse or default of category I are given treatment according to category II. Patients who fail to respond even to category II are suspected to have MDR-TB. So MDR-TB is not a clinical diagnosis. At present the common protocol for diagnosis of MDR-TB has been subjecting the culture isolates to antibiotic sensitivity test which may take 3-8 weeks time depending on the direct or indirect diagnostic approach. Thus, these techniques have a major limitation with respect to time taken in arriving at a diagnosis which is a huge drawback in MDR-TB patients as it has a very rapid course and may be fatal. So there is requirement of techniques which diagnose MDR TB early and it should be less complex and easily available as other techniques like PCR based Line Probe Assay and DNA sequencing which diagnosed MDR TB early but the techniques are more complex and costly which makes it’s unavailability at primary and secondary health care centers. Ceruloplasmin is an acute phase protein and is usually elevated in chronic infections and inflammation. Albumin is negative acute phase reactant that’s why it
Serum Ceruloplasmin Albumin Ratio as A Marker of Treatment Response in Pulmonary Tuberculosis

should decrease in infection and inflammation. Ceruloplasmin and Albumin changes in different direction so if we take ratio of Ceruloplasmin / Albumin (mg / gm) then change will magnify so its diagnostic value will be increase so ratio of ceruloplasmin and albumin is considered better indicator than serum ceruloplasmin or Albumin alone as observed that difference will be much higher than individual acute phase reactant. In our study, we calculated Ceruloplasmin / Albumin ratio (mg of Ceruloplasmin / gm of Albumin) and it was 5.226 ± 0.671 in control, 9.321 ± 2.250 in PTB-1, 7.465 ± 1.322 in PTB-2 and 9.365 ± 1.643 in MDR. Thus Ceruloplasmin / Albumin almost double in PTB and MDR and it gradually come back to normal level. Difference in control and study groups was statistically significant (p value <0.001). Difference in PTB-1 and PTB-2 groups was statistically significant with p value <0.001. This study is comparable with study by Batra et al8 2007, in which Serum Ceruloplasmin and albumin was estimated. Serum Ceruloplasmin / Albumin ratio was found higher in PTB patients compare to controls, while it shows that it decrease after treatment. However no statistically significant difference was obtained in the results between PTB-1 and MDR groups (p=0.913). This shows inability of ceruloplasmin/albumin to differentiate between PTB and MDR-TB. Significant difference in PTB-1 and PTB-2 shows that ceruloplasmin/albumin can be used to study treatment response in pulmonary tuberculosis patients. Any deviation that is ceruloplasmin/albumin not responding to treatment may suggest treatment failure, relapse, irregular treatment or emergence of resistance to antitubercular drugs. So it can be used to see the treatment response in PTB patients at primary and secondary centres where higher and complex diagnostic facility is not available as it is less costly and its easy availability. But there was no statistically significant difference observed between PTB-1 and MDR groups so ceruloplasmin/albumin ratio cannot differentiate MDR TB from PTB. Ceruloplasmin and albumin are non specific acute phase reactant so they cannot be used to diagnose TB; it only supports other diagnostic methods for TB.

CONCLUSION

There is no simple screening method with high sensitivity for detection of resistance in *M.tuberculosis* in PTB patients. Early detection of drug resistance among patients of PTB aiding in initiation of immediate alternate therapy and prevent spread of MDR *M.tuberculosis*. Our study showed that the ceruloplasmin albumin ratio increases after Tuberculosis and then it gradually comes down towards the normal level after treatment. Serum Ceruloplasmin albumin ratio (mg of Ceruloplasmin/gm of Albumin) can therefore be incorporated as a surrogate marker to assist in diagnosis as well as prognosis of pulmonary tuberculosis, for measuring response to ATT and for early detection of drug resistance. It can also be used to support diagnosis of *M.tuberculosis* infection. But its role in diagnosis and differentiation of PTB and MDR-TB is nil.

REFERENCES

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