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Evaluation of Serum Adenosine Deaminase, Lactate Dehydrogenase and Ceruloplasmin during Anti-tuberculosis Treatment

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Abstract

Tuberculosis (TB) has afflicted various countries in the world. The aim of this study was to estimate serum Adenosine deaminase (ADA), Lactate dehydrogenase (LDH) and Ceruloplasmin in TB patients. ADA is an enzyme involved in purine metabolism and is needed for the breakdown of adenosine and for the turnover of nucleic acid in tissue. LDH is an intracellular enzyme which catalyses the oxidation of L-lactate to pyruvate, the final step in the metabolic chain of anaerobic glycolysis. Ceruloplasmin is a copper carrying α globulin which has ferro-oxidase activity and antioxidant property. It is an acute phase reactant. Total of 42 normal healthy and 50 with tuberculosis human patients were studied and their serum ADA, LDH and Ceruloplasmin levels were determined. Serum ADA, LDH and Ceruloplasmin levels were significantly raised ($p < 0.001$) in the study group during diagnosis and treatment. The serum ADA, LDH, and Ceruloplasmin levels display normal with anti-tubercular therapy and also observed normal on completion of DOTS regimen. Our study supports and concludes the fact that the serum levels of ADA, LDH and Ceruloplasmin increases in the course of TB infection and can be regarded as important determinants in diagnosis, prognosis and the extent of the infectious disease.

Keywords: *Tuberculosis; ADA; LDH; Ceruloplasmin; DOTS*

Introduction

Tuberculosis is an infectious disease which spreads through air during coughing sneezing talking and spitting by infected people with *Mycobacterium tuberculosis*. They transmit tubercle bacilli into the air. *M. tuberculosis* infection is due to inhalation of very small numbers of these bacilli [1]. More than 8 million people develop tuberculosis and about 1.8 million results into death annually, and out of which the majority of these possess latent infection [2]. Without aggressive public health measures and continued research, effective treatments or vaccine development, this treatable disease will continue to be rampant [3]. Adenosine deaminase (ADA- EC 3.5.4.4) is an enzyme involved in purine metabolism. It is needed for the breakdown of adenosine and for the turnover of nucleic acid in tissue. The activity of ADA is increased in tuberculosis [4-6]. Lactate dehydrogenase (LDH) is an intracellular enzyme found in various human tissues such as skeletal muscle, heart, liver and kidneys. It is basically a hydrogen transfer enzyme that catalyses the oxidation of L-lactate to pyruvate using nicotinamide adenine dinucleotide $(NAD)^+$ as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. LDH as a pathophysiological marker has been studied in relation to several opportunistic infections, including infection by *Pneumocystis carinii pneumonia* (PCP), tuberculosis and bacterial pneumonia [7]. Ceruloplasmin is a α-globulin synthesized in liver. Estimation of serum ceruloplasmin levels can be of value in the measurement of activity of tuberculosis [8,9]. Estimation of serum ADA, LDH and ceruloplasmin has an important role in diagnosis of tuberculosis. Therefore, the present study was designed to assess these parameters in tuberculosis before and after treatment.

Material and Methods

The total numbers of 92 subjects were included and out of which 42 normal healthy individuals and 50 patients with TB were selected from OPD, IPD and DOTS centre of MGM group of Hospitals, Navi Mumbai, India and estimation of ADA, LDH and Ceruloplasmin were carried. 31 out of 50 TB patients were evaluated for above said parameters after 2 months and 6 months of DOTS therapy. as per the Revised National Tuberculosis Control Programme (RNTCP) regimen, the patients were treated with the combination of rifampicin, isoniazid, pyrazinamide and ethambutol. Healthy subjects of both male and females were taken as control group. At three different stages i.e. at the time of diagnosis, after 2 months and 6 months of DOTS treatment blood samples were drawn from tuberculosis patients.

Adenosine deaminase (ADA) was estimated by standard colorimetric method as described by Guisti and Galanti [10]. Adenosine deaminase catalyses deamination of adenosine leading to formation of inosine and ammonia. Ammonia (NH_3) forms an intensely blue indophenol with sodium hypochlorite and phenol in alkaline solution. Sodium nitroprusside ($Na_2(Fe(ON)_5NO$) is the catalyst. The ammonia concentration thus released, deamination by ADA is directly proportional to the examination of indophenol. The reaction catalyzed by ADA is stopped at the end of incubation period by addition of phenol nitroprusside. Total serum LDH was estimated by the method given by Wootten [11]. Estimation of Ceruloplasmin was done by measuring its oxidase activity in serum by the use of o-dianisidine dihydrochloride, a colorimetric method described by Karl., *et al* [12].

Statistical analysis: 'GraphPad Quick Cals t-test calculator' was employed for determining Student's t-test and Paired t-test.

Result

Control group

Aged between 20 to 60 years [27 males (64.28%) and 15 females (35.72%)] were included as controls. In this study the mean ± SD of the ADA activity, Total LDH activity, and Ceruloplasmin activity were 19.70 ± 2.23 IU/L, 300.36 ± 28.06 IU/L, and 100.24 ± 13.19 IU/L respectively.

Study group

Among 50 newly diagnosed TB patients, 31 were subjected for enzyme activity test during treatment and on completion of therapy. In this work, 35.08 ± 2.28 IU/L serum ADA levels was calculated and a control of 19.70 ± 2.23 IU/L was measured. The serum ADA level in patients after two months and on completion (six months) of anti-tubercular therapy was found to be (26.11 ± 1.64 IU/L) and (20.37 ± 2.91 IU/L) respectively. The levels of serum LDH activity in newly diagnosed tuberculosis patients was 442.94 ± 45.85 IU/L. The level of serum LDH activity in control subjects was found to be 300.36 ± 28.06 IU/L. The mean level of serum LDH in patients after two months and after completion (six months) of anti-tubercular therapy was found to be 380.19 ± 22.96 IU/L and 314.91 ± 42.49 IU/L respectively. The levels of serum Ceruloplasmin in newly diagnosed tuberculosis patients was 184.12 ± 31.22 IU/L. The level of serum Ceruloplasmin in control subjects was found to be 100.24 ± 13.19 IU/L. The level of serum Ceruloplasmin in patients after two months and after completion (six months) of anti-tubercular therapy was found to be 155.31 ± 14.29 IU/L and 113.99 ± 17.89 IU/L respectively. No significant difference was seen in serum ADA activity in different sexes and age groups.

		TB Patients		
Particulars (IU/L)	**Controls (mean ± SD)**	**Newly Diagnosed (mean ± SD)**	**Two months of therapy (mean ± SD)**	**Completion of therapy (mean ± SD)**
ADA	19.70 ± 2.23	35.08 ± 2.28 *	26.11 ± 1.64*	20.37 ± 2.91*
LDH	300.36 ± 28.06	442.94 ± 45.85*	380.19 ± 22.96*	314.91 ± 42.49*
Ceruloplasmin	100.24 ± 13.19	184.12 ± 31.22*	155.31 ± 14.29*	113.99 ± 17.89 *

Table 1: *Comparison of serum ADA, LDH and Ceruloplasmin in control group and different study groups.*
**p < 0.001 (high statistically significant).*

Discussion

Our study revealed that the mean values of serum ADA, LDH and Ceruloplasmin were significantly higher ($p < 0.001$) in study group when compared with the control group (Table 1).

It is seen that the mean serum ADA levels were significantly increased in tuberculosis patients as compared to control subjects ($P < 0.001$). Also the mean values of serum ADA was significantly decreased in the patients after the completion of DOTS therapy ($P < 0.001$). Moreover, the observed serum ADA levels of the patients were decreased gradually after anti-tubercular therapy and reached normal levels after completion of therapy. Similar study was carried out by K Srinivasa Rao., *et al* [13]. They reported that the serum ADA values in the study group was (mean ± SD) 41.48 ± 8.02 U/L and in controls (mean ± SD) of 17.60 ± 5.17 U/L. After the 1st month of treatment there was no significant change ($p > 0.05$) in serum ADA levels 39.60 ± 0.79 U/L, however significant difference was recorded after the second month of treatment in serum ADA level (29.66 ± 0.83 U/L) and similarly at sixth month of treatment (22.12 ± 0.51 U/L) difference in serum ADA was significantly high. Zafer Kartaloglu., *et al*. [14] showed a slight elevation of serum ADA in the first month, but it decreased during treatment in parallel with the effectiveness. Meftun Unsal., *et al*. [15] revealed gradual decrease in serum ADA from 30.1± 11.7 U/L to 24.8 ± 15.6 U/L after first month of treatment and after second month 22.0 ± 10.6 U/L in limited lesion cases. And in extended lesion cases before treatment ADA values was 31.3 ± 18.3 IU/L, first and second month values were 27.5 ± 13.0 IU/L and 27.1 ± 12.2 U/L respectively.

In our study the mean serum total LDH activity was significantly increased in tuberculosis patients as compared to the control subjects ($P < 0.001$). We also found that the mean values of serum LDH activity was significantly decreased in the patients after the completion of DOTS therapy ($P < 0.001$). We observed that serum total LDH activity of the patients decreased gradually after anti-tubercular therapy and reached normal levels after completion of therapy. Similar study was carried out by Y Narsimha Reddy., *et al*. [16] but in different sample. They measured the activity of total LDH in pleural fluid of untreated TB patients and treated patients. They found that the Mean ± SD of LDH activity in the pleural fluid was significantly decreased ($P < 0.01$) in under treated cases (132.21 ± 13.22 IU/L) in comparison with untreated cases (148.65 ± 23.82 IU/L). They indicated that there was a continuous pulmonary damage due to the release of LDH and reactive oxygen species. PR Sharma., *et al*. [7] studied the activity of total serum LDH in 320 sputum negative controls and 209 sputum positive cases and revealed that the comparison of total LDH content between sputum negative controls (359.9 IU/L) and sputum positive cases (401.6 IU/L) to be significantly high ($P < 0.1$) in sputum positive cases than in sputum negative controls. Also, the sputum positive (1+, 2+ and 3+) subjects showed elevated levels of LDH, especially in sputum 3+ subjects which revealed significantly high LDH content. The high level of serum total LDH in tuberculosis patients is more likely due to mycobacterial induced tissue injury as well as other pathophysiological conditions.

The mean serum ceruloplasmin level was significantly increased in tuberculosis as compared to control ($P < 0.001$). And mean serum ceruloplasmin level was significantly decreased in the patients on the completion of DOTS therapy ($P < 0.001$) as compared to newly diagnosed patients. We observed serum ceruloplasmin levels of the patients decreased gradually after anti-tuberculous therapy and reached normal levels on completion of therapy. Also there was a significant decrease ($P < 0.001$) in the serum ceruloplasmin levels after two months of therapy as compared to the levels in newly diagnosed tuberculosis patients. PO Motiani., *et al*. [8] reported, the mean serum ceruloplasmin (680 units) among 40 patients with sputum positive for acid fast bacilli was significantly ($P < 0.001$) higher as compared to the mean serum ceruloplasmin (274 units) in control group. Similarly, the difference between the mean levels of serum ceruloplasmin in smear positive patients (680 units) and treated group (350 units) was found statistically significant ($P < 0.001$). Their finding suggests the relationship of serum ceruloplasmin level with activity of tuberculous lesion. Their finding also suggested, levels of serum ceruloplasmin were related with the activity of the disease process only but not with the extent. Singhvi and Maitrya found increased levels of serum ceruloplasmin in untreated patients of pulmonary tuberculosis. The levels were reduced considerably after 6 months of chemotherapy with Streptomycin and Isoniazid [17]. Similarly, Bachhu Singh Verma., *et al*. [18] measured serum ceruloplasmin activity in 58 patients with pulmonary tuberculosis before institution of specific chemotherapy and in 20 age and sex matched controls. They reported that the mean serum ceruloplasmin activity in tuberculosis patients (185.16 ± 55.0 U/L) was significantly higher ($P < 0.001$) as compared to the mean serum ceruloplasmin activity of normal healthy individuals (76.6 ± 10.7 U/L). Similarly, RI Cernat., *et al*. [19] demonstrated that the serum ceruloplasmin levels were significantly increased in patients with active PTB compared to the control group ($P < 0.01$). Although not shown, they observed significant correlation between ceruloplasmin and fibrinogen and between ceruloplasmin and ESR, with $r > 0.9$ at a probability level of $P < 0.01$. These observations suggest that ceruloplasmin also behaves as an acute phase reactant.

Conclusions

It was found that, levels of enzymes like Adenosine deaminase, Lactate dehydrogenase and Ceruloplasmin activities in newly diagnosed tuberculosis patients were significantly high as compared to the controls ($P < 0.0001$). The levels of these enzymes gradually decreased after anti-tuberculous therapy and reached almost normal levels on completion of therapy. It appears that the increase in the serum ADA activity in the newly diagnosed TB patients and its subsequent decrease after the DOTS therapy shows the normalization of altered T-lymphocyte turnover induced by tuberculosis. The increase in serum total LDH activity in newly diagnosed patients with active tuberculosis may be reflection of tissue injury due to the involvement of M. tuberculosis which gradually comes to the normal level after the initiation of therapy. And the increase in serum Ceruloplasmin activity suggests that increased release of stimulus to the inflammatory process may occur during the development of the infection and may also be associated with its role as an antioxidant. The present study therefore reveals the importance of determining the levels of enzymes like ADA, LDH and Ceruloplasmin in diagnosis, prognosis and the extent of Tuberculosis.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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