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ORIGINAL RESEARCH

Assessment of ratio of serum SOD and whole blood glutathione peroxidase in diagnosis of tuberculosis

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ABSTRACT

Background: The main cause of tuberculosis is Mycobacterium tuberculosis (MTB). Free radicals and reactive oxygen species (ROS) are produced during tuberculosis infection. The present study was conducted to assess ratio of serum SOD and whole blood glutathione peroxidase in diagnosis of tuberculosis.

Materials & Methods: 60 subjects were divided into 3 groups of 20 each. Group I were healthy subjects, group II were suffering from respiratory tract infection (RTI) or bronchial asthma or chronic obstructive pulmonary disease (COPD) or bronchiectasis or bronchial carcinoma and group III were tuberculosis patients. In all, superoxide dismutase and GPx was measured.

Results: SOD (U/mL) before and after treatment in group I was 125.2 and 120.4, in group II was 140.6 and 130.5 and I group III was 1286.4 and 984.2 respectively. The difference was significant (P< 0.05).GPx (U/L) before and after treatment in group I was 8624.8 and 8512.0, in group II was 4825.2 and 7246.8 and I group III was 974.2 and 2640.6 respectively. The difference was significant (P< 0.05).

Conclusion: The serial measurement of ratio is useful to monitor the sensitiveness of M. tuberculosis toward drug therapy and diagnose drug-resistant cases. The measurement of the ratio of serum SOD to whole blood GPx might help in the early diagnosis of TB.

Key words: blood GPx, serum SOD, TB

INTRODUCTION

The main cause of tuberculosis is Mycobacterium tuberculosis (MTB). The high lipid content of this pathogen is the reason for many of its unique clinical characteristics.[1]One third of the world population has latent Mycobacterium tuberculosis. HIV/ AIDS epidemics have increased the burden of tuberculosis by enhancing the rate of tuberculosis acquisition and activation of latent MTB to active Mycobacterium tuberculosis.[2]

Though there is a considerable increase in detecting tuberculosis (TB) cases, there is still a gap globally between the new cases reported in 2019 (about 7.1 million) and the estimated

incident cases in 2019 (about 10.0 million). This large gap is stated to be due to underreporting and under-diagnosing of TB cases.[3]

Free radicals and reactive oxygen species (ROS) are produced during tuberculosis infection. Free radicals are cytotoxic and need to be removed by efficient antioxidant system. Consequently, enhanced ROS and free radical production may lead to imbalance in the host antioxidant capacity. This may cause oxidative stress and lipid peroxidation.[4]Research study had been performed to make the assay for glutamine synthetase (GS) and superoxide dismutase (SOD) in the serum of TB patients, both pulmonary TB (PTB) and extra-pulmonary TB (EPTB). These two leaderless proteins are released extracellularly by Mycobacterium tuberculosis (M. tuberculosis).[5] The present study was conducted to assess ratio of serum SOD and whole blood glutathione peroxidase in diagnosis of tuberculosis.

MATERIALS & METHODS

The present study comprised of 60 subjects of both genders who agreed to participate in the study.

Data such as name, age, gender etc. was recorded. They were divided into 3 groups of 20 each. Group I were helthy subjects, group II were suffering from respiratory tract infection (RTI) or bronchial asthma or chronic obstructive pulmonary disease (COPD) or bronchiectasis or bronchial carcinomaand group III were tuberculosis patients.

5 ml blood samples were collected from the subjects by venipuncture. After centrifugation, the serum was transferred to the respective clean and sterile Eppendorf tube. All the samples were stored at 2–4°C until further analysis.Serum SOD was measured by using a reagent kit of Randox Labs Ltd, USA. Superoxide dismutase was measured from the degree of inhibition of this reaction and expressed as units/mL (U/mL). The concentration in whole blood was measured by using a reagent kit of Randox Labs Ltd., USA. The concentration of the enzyme in the whole blood was expressed as units/liter (U/L).Results were tabulated and assessed statistically. P value less than 0.05 was considered significant.

RESULTS

Table I Distribution of patients

Groups	Group I	Group II	Group III
Status	Control	TB control	TB
M:F	10:10	12:8	11:9

Table I shows that group I had 10 males and 10 females, group II had 12 males and 8 females and group III had 11 males and 9 females.

Table II Serum superoxide dismutase (SOD) activity before and after treatment

Groups	Before	After	P value
Group I	125.2	120.4	0.94
Group II	140.6	130.5	0.05
Group III	1286.4	984.2	0.01

Table II, graph I shows that SOD (U/mL) before and after treatment in group I was 125.2 and 120.4, in group II was 140.6 and 130.5 and I group III was 1286.4 and 984.2 respectively. The difference was significant (P< 0.05).

Graph I Serum superoxide dismutase (SOD) activity before and after treatment

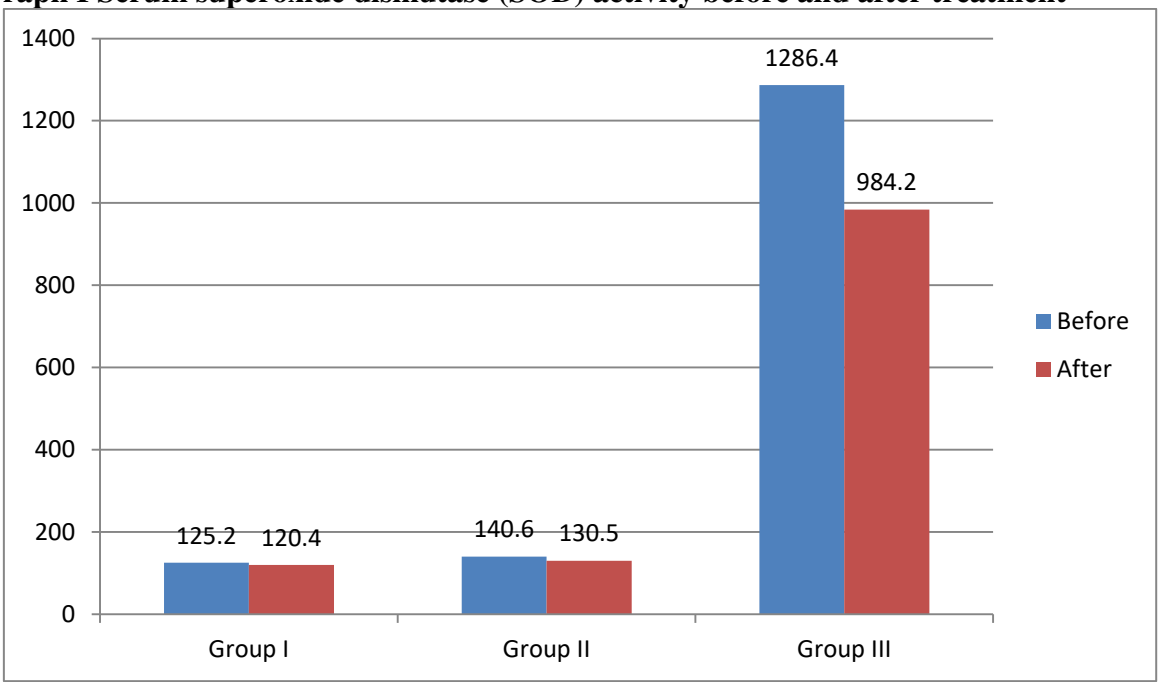
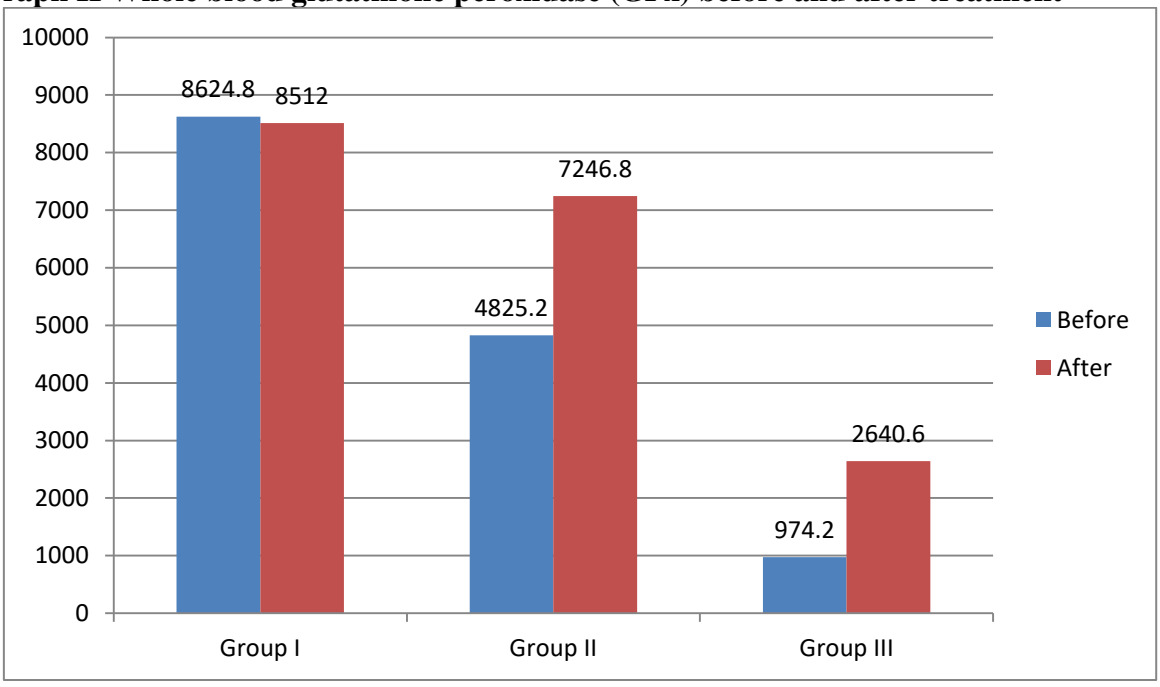


Table III Whole blood glutathione peroxidase (GPx) before and after treatment

Groups	Before	After	P value
Group I	8624.8	8512.0	0.91
Group II	4825.2	7246.8	0.02
Group III	974.2	2640.6	0.04

Table III, graph II shows that GPx (U/L) before and after treatment in group I was 8624.8 and 8512.0, in group II was 4825.2 and 7246.8 and I group III was 974.2 and 2640.6 respectively. The difference was significant (P< 0.05).

Graph II Whole blood glutathione peroxidase (GPx) before and after treatment



DISCUSSION

The higher requirement of L-glutamine for M. tuberculosis is for the formation of a poly-L-glutamate/glutamine cell wall structure contributing 10% of cell wall mass.[6] The high GS level allows the enhanced growth of pathogenic mycobacteria under restrictive conditions in vivo. For M. tuberculosis, iron is the obligate cofactor for at least 40 different enzymes including SOD as encoded in its genome.[7] Again, only pathogenic mycobacteria can survive and proliferate inside macrophages whereas M. tuberculosis survives nutrient starvation by using the β-oxidation pathway.[8] The phagosome with the help of its membrane-bound NADPH oxidase system reduces O_2 to superoxide anion ($O_2^{\cdot}$) and oxidative burst is initiated. The imbalance between oxygen-derived reactive oxygen species (ROS) and the antioxidant system to scavenge it, will develop oxidative stress.[9]The present study was conducted to assess ratio of serum SOD and whole blood glutathione peroxidase in diagnosis of tuberculosis.

We found that group I had 10 males and 10 females, group II had 12 males and 8 females and group III had 11 males and 9 females. Chattopadhyayet al[10] in their study the participants were divided into three groups: Normal control; 2-Lung disease control and 3-TB patients (3A-pulmonary and 3B-extrapulmonary). The serum SOD and whole blood GPx activity were measured spectrophotometrically for all participants initially. Both of these parameters were assayed again after 1 month's usual additional treatment for groups II and III.The ratio as calculated in TB patients is >9 and 8 times, respectively, than those of normal and lung disease control subjects. With anti-TB drug therapy for 1 month, there was a significant decrease in the ratio.

We observed that SOD (U/mL) before and after treatment in group I was 125.2 and 120.4, in group II was 140.6 and 130.5 and I group III was 1286.4 and 984.2 respectively. GPx (U/L) before and after treatment in group I was 8624.8 and 8512.0, in group II was 4825.2 and 7246.8 and I group III was 974.2 and 2640.6 respectively. Chinedu et al[11] in their study 251 individuals consisting 120 treatment naïve individuals with active TB [26 (TB+HIV+) and 12 malaria parasite (MP) and TB (TB+MP+) co-infection, 82 HIV negative (TB+HIV−)], 26 Latent TB (LTB) and 105 apparently healthy control (AHC) were studied. TB infection was determined by ZiehlNeelsen sputum smeared microscopy and GeneXpert. MP was confirmed by Giemsa staining technique, HIV by immuno-chromatographic method. The mean levels of vitamins E, C and selenium were significantly lower in individuals with TB infections compared with AHC ($p<0.05$).

Rajopadhye SH et al[12]assessed the oxidative stress markers in the HIV-TB and TB patients. Samples from 50 healthy volunteers were used as controls. Serum was assessed for pro-oxidant markers such as Nitric Oxide (NO), Thiobarbituric Acid Reactive Species (TBARS), C-Reactive Protein (CRP), superoxide anion. Antioxidant markers such as catalase and Superoxide Dismutase (SOD) were assessed. Total serum protein, was also assessed.Among the pro-oxidants, serum NO levels were decreased in TB group while no change was seen in HIV-TB group. TBARS and CRP levels showed significant increase in both groups; superoxide anion increased significantly in HIV-TB group. Catalase levels showed decreased activities in TB group. SOD activity significantly increased in HIV-TB but not in TB group. The total serum proteins were significantly increased in HIV-TB and TB groups. The values of Control cohort were with the normal reference ranges.

CONCLUSION

Authors found that the serial measurement of ratio is useful to monitor the sensitiveness of M. tuberculosis toward drug therapy and diagnose drug-resistant cases. The measurement of the ratio of serum SOD to whole blood GPx might help in the early diagnosis of TB.

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