Oxidative Stress Markers and Selenium Levels of Pulmonary Tuberculosis Patients in Some Dot Centers in Port Harcourt

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Original Research Article

ABSTRACT

Severe oxidative stress has been reported in Tuberculosis (TB) patients, and this is associated with increased production of free radicals due to phagocyte respiratory burst, malnutrition, and poor immunity. This study investigated the levels of Malondialdehyde (MDA), Superoxide dismutase (SOD) and Selenium (SEL) levels in naïve Tuberculosis (TB) patients and TB patients on treatment. The study comprised of a total of 100 TB patients made up of 57 TB patients on treatment with anti-tuberculosis therapy and 43 naïve TB patients not on treatment. MDA and SOD were analyzed using Enzyme linked Immunosorbent assay technique (ELISA). While selenium and tuberculosis were detected by atomic absorption spectrophotometer and Gene Xpert respectively. The MDA level in TB patients on treatment was significantly higher ($p<0.0011$) than the MDA level of naïve TB patients. Also, the SOD level of TB patients on treatment was significantly lower ($p<0.0426$) than the SOD level of naïve TB patients. While the levels of Selenium in TB patients on treatment was significantly higher ($p< 0.0002$) than the selenium level of naïve TB patients. The study showed an increase in oxidative stress due to low levels of antioxidants and high lipid peroxidation due to increased MDA levels in TB patients on drugs. Therefore it might be appropriate to include antioxidant supplementation, either through nutrition or antioxidant therapy in the treatment regimen of TB patients; this may signify an innovative line of management of TB patients to quick recovery.

Keywords: Antioxidants; selenium; Malondialdehyde; tuberculosis; free radicals; oxidative stress.

1. INTRODUCTION

Tuberculosis is a serious public health challenge, which is enhanced by some factors such as socioeconomic status, sanitation, malnutrition, crowded living conditions and resistance to TB drugs [1]. [2] reported a prevalence of tuberculosis as 33.5% in Port Harcourt.

Oxidative stress is a situation that occurs in biological systems when there is a disruption of the balance between antioxidants and free radicals. Free radicals or oxidants are usually produced during normal metabolism in the body, and they perform some useful functions at low concentrations [3]. The human system has a mechanism that counterbalances the effects of oxidants; this mechanism is known as the anti-oxidant system. Antioxidants can be divided into enzymatic and non-enzymatic. Enzymatic antioxidants include Superoxide dismutase, glutathione peroxidase, catalase, heme oxygenase-1, thioredoxines and so on, while the non-enzymatic antioxidants include vitamins C

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and E and uric acid [3]. An imbalance in the effects of Free radicals or Reactive Oxygen Species (ROS) and these antioxidants in favour of ROS results in oxidative stress [4].

Selenium is a fundamental dietary trace element that enters the food chain through plants, which absorb it from the soil[5] Selenium is taken up with the diet mainly as selenium ion acids[6].

It is believed that selenium exerts its effect through its incorporation into selenoproteins. Glutathione peroxidase was the first selenoprotein to be discovered 30 years ago, and selenium is now found to be an essential component of at least four glutathione peroxidase [7].
Oxidative stress has been reported in individuals suffering from tuberculosis and this has been attributed to poor nutrition and also altered immunity [8]. Mycobacterium tuberculosis is capable of inducing the generation of ROS by activating phagocytes – mononuclear and polymorphonuclear – that have antimicrobial activity. Consequently higher generation of ROS which may be a physiological mechanism for the body's defence against TB may lead to damage to the lungs [9]. There have been reports of an increased level of free radicals in the pathogenesis of TB. [10].

Oxidative stress in tuberculosis (TB) may be due to tissue inflammation, poor dietary intake of micronutrients, and release of free radicals from macrophages and side effects of anti-TB drugs. Availability of antioxidants to neutralize the harmful effects of the generated free radicals may be one of the factors determining the severity of the disease [11].

Selenium is a vital trace element, which is important in immunity against microorganisms. However, blood selenium levels are lower in patients who suffer pulmonary tuberculosis and TB-HIV infection [12]. Higher mortality among TB infected patients is related to low levels of blood selenium [13].

Tuberculosis infection causes the generation of ROS, which in turn cause peroxidation of Lipids [14]. The process involves unsaturated fatty acids found in localized cell membranes, forming an end product known as Malondialdehyde (MDA). These products of Lipid peroxidation can be measured in blood because they diffuse from the site of inflammation [14].

The present study was conducted to assess the levels of these oxidative parameters and selenium in Tuberculosis patients.

The assessment of oxidative stress markers is not in the regimen in the management of TB patients. This has affected the outcome of TB patients' thereby increasing mortality rate [15] Hence, measurement of oxidative stress markers may be an index of monitoring response to treatment in tuberculosis management [16].

Evaluating the levels of oxidative stress markers and Selenium in TB patients as part of management may help reduce the severity of the disease and also will improve their quality of life.

2. MATERIALS AND METHODS

2.1 Subject Characterization and Design

This cross-sectional study was carried out on 100 patients with pulmonary tuberculosis between ages 20-60 years who were attending three DOT Centers in Port Harcourt metropolis, in Rivers state, Nigeria.

This comprised of a case group who were patients with 43new Pulmonary TB cases or naïve TB cases and case group of 57patients of established cases of pulmonary tuberculosis on treatment.

2.2 Selection/ Exclusion Criteria

The sampling frame was based on three DOT centres in the Port Harcourt Metropolis. It comprised of 136 male and female subjects between 20 to 60 years of age attending the DOT Centers for diagnoses and treatment. The 36 subjects that were TB negative were excluded.

Negative TB patients, HIV positive TB patients, pregnant women, and very ill patients were excluded from the study. Also, patients suffering from other pulmonary diseases apart from TB, those with complications such as diabetes mellitus, hepatic disease, renal failure and endocrine disorder, pregnancy, and history of smoking were also excluded from the study.

2.3 Sample Collection and Storage

Blood samples were collected from the subjects by vein puncture. Ten millilitres of blood was collected from the subjects with a sterile hypodermic needle and dispensed into a lithium heparin sample container and mixed gently. The

heparinised sample was separated using Mega centrifuge 1.0 Heraus instrument at 5,000 rpm for 5 minutes. Plasma samples were separated into plain test tubes and stored frozen at -20°C until the time of determination of Malondialdehyde (MDA), Superoxide dismutase (SOD) and Selenium following standard operating procedures. The sampling frame was within seven (7) days. Sputum samples produced by the patients were analysed with Gene Xpert for TB daily.

2.4 Laboratory Methods and Procedures

Malondialdehyde (MDA), Superoxide dismutase (SOD) and Selenium level were measured quantitatively by the sandwich-enzyme linked immunosorbent assay (ELISA) method as described by Elabscience Biotechnology Company Limited (China). TB in sputum samples was diagnosed by Gene Xpert.

2.5 Ethical Consideration

Ethical clearance was obtained from the Rivers State Hospitals Management Board, and informed consent was sort from the patients and only those who gave consent participated in the study. A structured questionnaire on demographic data was administered to all participants.

2.6 Statistical Analysis

Data generated were analysed using statistical package for social sciences (SPSS), and results were presented as mean ± standard deviation, inferences were deduced using Independent t-test, ANOVA, Tukey multiple comparisons and Correlation coefficient. Results were considered statistically significant at 95% confidence interval ($p<0.05$).

3. RESULTS

3.1 Oxidative Stress Markers and Selenium Levels of TB Subjects on Treatment and Naive TB Subjects

The details of the mean and standard deviations of oxidative stress markers and selenium levels of TB subjects on treatment and TB naive subjects are shown in Table 1. Descriptive analysis demonstrated that, in the group of 57 TB subjects on treatment, the mean and standard deviation of MDA level is 9.85 ± 5.1, while in the group of 43 naïve TB subjects the mean and standard deviation of MDA level is 1.39 ± 1.33. When the level of MDA in naïve TB cases was compared with the MDA levels in TB subjects on treatment, the MDA level was significantly higher in TB patients on treatment compared to naïve TB patients ($P = 0.0011$).

The mean and standard deviation of SOD level for TB subjects on treatment and naïve TB subjects was 16.62 ± 7.8 and 25.07± 12.8 respectively. When the level of SOD in naïve TB cases was compared to the SOD levels in TB subjects on treatment, the SOD level was significantly lower in TB patients on treatment compared to naïve TB patients ($P = 0.0426$).
Mean and standard deviation of Selenium level TB subjects on treatment and naïve TB subjects were 1.31 ± 0.68 and 0.71 ± 0.20 respectively. When the level of SEL in naïve TB cases was compared with the SEL levels in TB subjects on treatment, the Selenium level was significantly higher in TB patients on treatment compared to naïve TB patients ($P = 0.0002$).

3.2 The Levels of Oxidative Stress Markers and Selenium Levels in Relation to Gender of TB Patients on treatment

The details of the mean and standard deviations of oxidative stress markers and selenium levels of TB subjects on treatment in relation to gender are shown in Table 2. When the level of MDA level in female TB subjects on treatment was compared to male TB subjects on treatment, there was no significant difference in females compared to males ($p = 0.2614$). The sex of the TB subjects on treatment had no effect on their MDA levels.

A similar presentation was observed for the level of SOD level ($p = 0.5571$) and selenium level ($p = 0.682$), when female TB subjects on treatment were compared to male TB subjects on treatment, the investigation showed that there is no significant difference in females compared to males.

3.3 The Mean and Standard Deviations of MDA, SOD and SEL Levels of Naive TB Subjects according to Age Ranges

The details of the comparison of MDA, SOD and SEL levels in TB subjects are shown in Table 3. Among the age ranges that were compared, there was no significant difference in the MDA and SOD ($p= 0.203$ and $p= 0.254$ respectively).

However, when the level of SEL in naïve TB subjects was compared among the age ranges, there was a significant difference (p = 0.0099). The investigation showed that the SEL level significantly reduced with an increase in the age of naive TB subject.

Table 1. Mean and standard deviations of oxidative stress markers and selenium levels of TB subjects on treatment and naive TB subjects

	MDA (nmo/L)	SOD (U/mL)	SEL (mg/L)
TB patients on Treatment n=57	9.85 ± 5.1	16.62 ± 7.8	1.31 ± 0.68
Naive TB patients n=43	1.39 ± 1.33	25.07± 12.8	0.71 ± 0.20
p-values	0.0011	0.0426	0.0002
t-start	-3.3376	1.759	-6.212

Table 2. Mean and Standard Deviations of Oxidative Stress Markers and Selenium Levels of Males and Female TB Subjects on Treatment in Relation to Gender

	MDA (nmo/L)	SOD (U/mL)	SEL (mg/L)
Female TB patients on treatment n=31	12.28 ± 6.65	16.07 ± 8.71	1.34 ± 0.79
Male TB patients on treatment n=26	6.97 ± 3.04	17.28 ±6.68	1.27 ± 0.55
p-values	0.2614	0.5571	0.682
t-start	1.143	-0.5906	0.4106

Table 3. Mean and standard deviations of MDA, SOD and SEL Levels of Naive TB Subjects according to Age Ranges

Age ranges	MDA nmo/L	SOD U/mL	SEL(mg/L)
16-25yrs naive TB n=11	1.21 ± 0.36	18.05 ± 4.05	0.89 ± 0.26
26-35yrs n=6	1.26 ± 0.30	28.75 ± 22.61	0.69 ± 0.13
36-45yrs n=11	2.22 ± 0.70	22.36 ± 12.74	0.61 ± 0.17
46-55yrs n=8	0.93± 0.37	16.88 ± 8.82	0.64 ± 0.16
56-65yrs n=7	1.03 ± 0.8	16.6 ± 1.48	0.69 ± 0.09
p-values	0.2032	0.2546	0.0099
F-values	1.566	1.394	3.862

3.4 Association between MDA, SOD & SEL and Duration of Treatment

Details of the Correlation matrix and regression plots for MDA, SOD and SEL and the duration of treatment of the TB subjects are shown in table 4 and Figs. 1-6. The association between MDA in TB subjects on treatment and duration of treatment showed a positive correlation (R^2 = 0.6109, p-value = 0.0001). The association between SOD levels in TB subjects on treatment and duration of treatment showed a negative correlation (R^2 = -0.8153, p-value = 0.0001). The association between SEL levels in TB subjects on treatment and duration of treatment showed no significant correlation with duration of treatment (R^2 = 0.0355, p-value = 0.7931). Correlation analysis between MDA and SOD in TB subjects on treatment was a negative correlation (R^2 = -0.4496, p-value = 0.00005). The association between MDA and SEL in the TB subjects on treatment showed no correlation (R^2 = 0.037791, P value = 0.7802). The association between SOD and SEL in the TB subjects on treatment showed no significant correlation. (R^2=0.014091 P=0.9171).

R^2= 0.610898
P= 0.0001

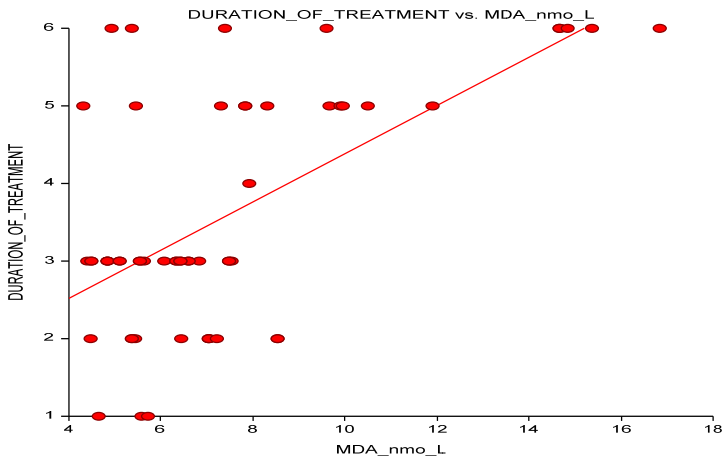

Fig. 1. Correlation Plots of Duration of Treatment and MDA Values of TB Subjects on Treatment

R^2= -0.81535
P= 0.0001

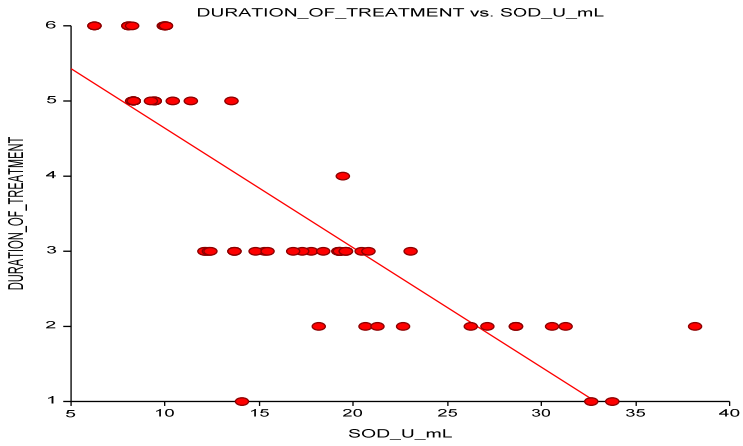

Fig. 2. Correlation plots of the duration of treatment and SOD values of TB subjects on treatment

Table 4. Association between MDA, SOD & SEL and Duration of Treatment

	Duration Of Treatment	MDA (nmo/L)	SOD (U/mL)	SEL(mg/L)
MDA(nmo/L)	0.6109*			
SOD (U/mL)	-0.8153*	-0.4497*		
SEL(mg/L)	0.0355	0.0378	0.0141	

R^2= 0.035511
P= 0.7931

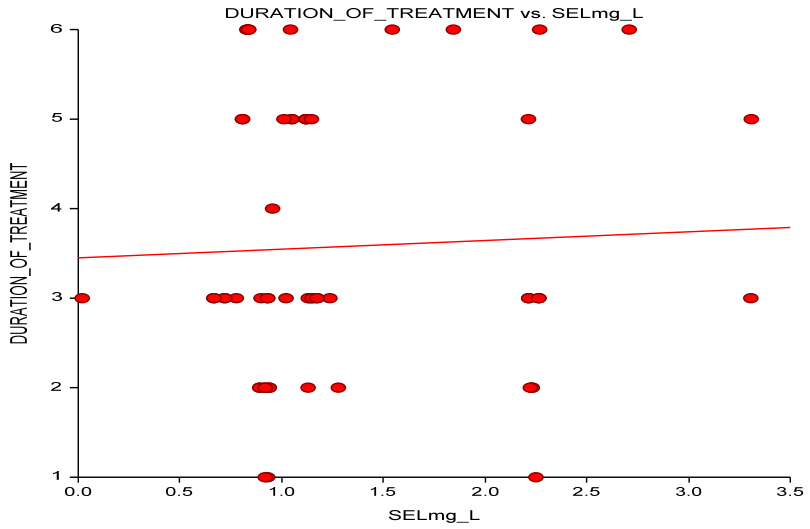

Fig. 3. Correlation Plots of Duration of Treatment and Selenium Values of TB Subjects on Treatment

R^2= -0.44968
P= 0.0005

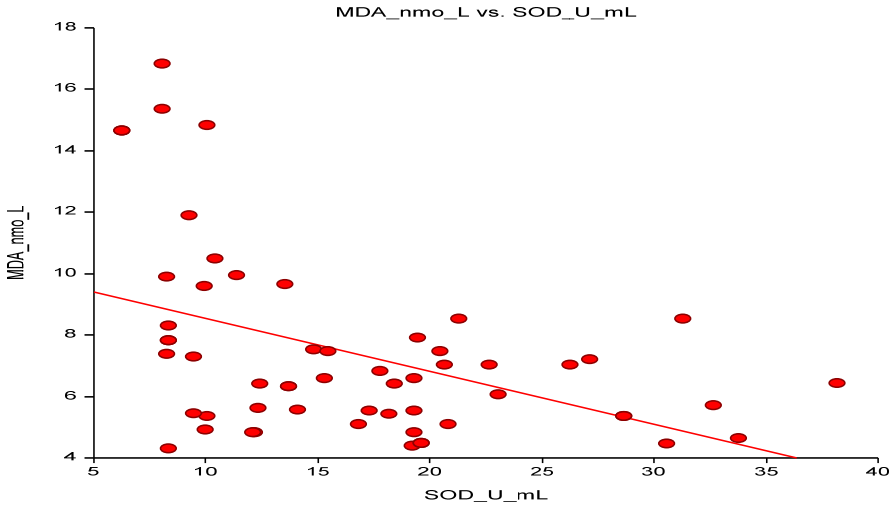

Fig. 4. Correlation plots of MDA and SOD values of TB subjects on treatment.

R^2 =0.037791
P=0.7802

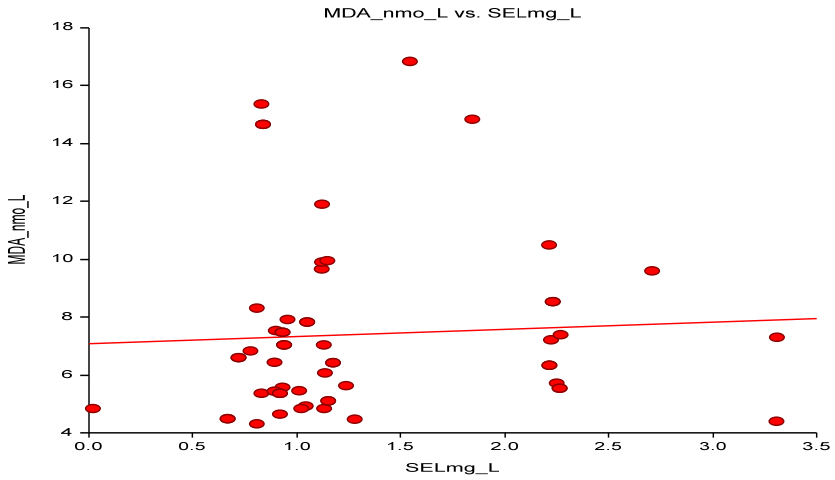

Fig. 5. Correlation plots of MDA and Selenium values of TB subjects on treatment

R2=0.014091
P=0.9171

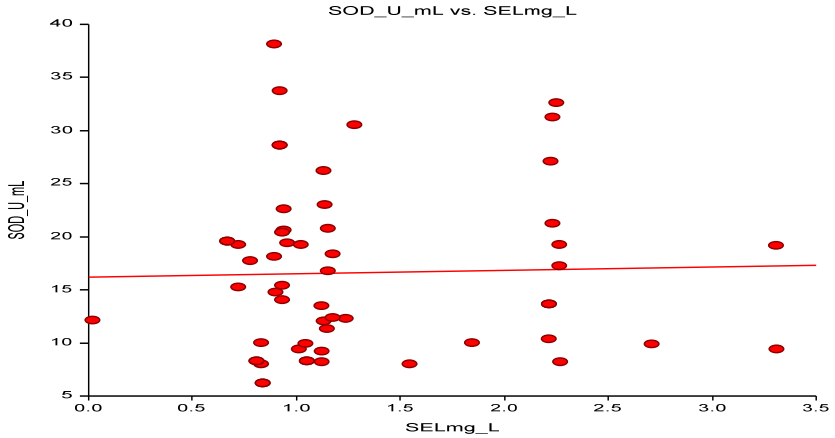

Fig. 6. Correlation plots of SOD and Selenium values of TB subjects on treatment

4. DISCUSSION

The intensity of oxidative stress in TB is not only assessed by the production of free radicals (lipid peroxidation product like MDA) and antioxidant enzymes like SOD but also by Selenium levels in TB patients. Selenium is an essential part of some antioxidant enzymes like Glutathione peroxidase.

Our findings of significantly higher selenium levels in TB subjects on treatment compared to naïve TB subjects in this study is in agreement with the work of [17], who observed an increase in selenium levels in TB patients with or without HIV co-infection after 2 months of anti-TB therapy. The increased selenium in TB patients on treatment is also in line with the study carried out by [18], they reported a higher selenium level in TB patients after 60 days of treatment with anti-tuberculosis drugs. This can be as a result of the improvement in the amount of antioxidant enzymes with selenium as a vital part and possibly improved nutrition. In the present study, we also found that the serum level of selenium in naïve TB patients was significantly lower compared to the counterpart on drugs. A study in China done by [19), showed similarly significantly low levels of molybdenum, zinc, copper, and selenium in the serum of tuberculosis patients. [19,20], also reported that serum levels of selenium in patients with active pulmonary tuberculosis are low when compared with healthy cases. A study carried out by [21], reported that

patients with active tuberculosis have lower concentrations of various micronutrients including selenium in blood. In the study by [18], the TB group had a lower selenium level than the control group. Also in a survey conducted in Korea, it was reported that patients with TB showed significantly lower zinc and selenium levels than healthy controls [22]. Recently a study by [23] also found out that the serum level of selenium in TB patients was significantly lower compared to healthier controls. Selenium is an important component of anti-oxidative enzymes such as glutathione peroxidase (GPx) that is crucial in the protection of host cells from oxidative damage in the inflammatory response and clearing tuberculosis. The antioxidant activity of selenium can alter mycobacterium DNA. To avoid DNA damage, M. tuberculosis has developed effective mechanisms to interrupt the activity of GPx. The impaired GPx can result in selenium deficiency in TB patients [24]. The reduced selenium concentrations were also associated with the severity of anaemia, which is common in active tuberculosis patients. It is thus recommended that selenium deficiency might contribute to anaemia through increased oxidative stress in tuberculosis patients. According to one group of authors, a two-month intervention with vitamin E and selenium supplementation reduced oxidative stress and increased the total antioxidant capacity in patients with treated pulmonary tuberculosis [25]. Selenium has direct antioxidant activity as a result of its incorporation into selenoproteins, such as glutathione peroxidases, thioredoxin reductase, and some isoforms of methionine sulfoxide reductase. Selenium is an essential trace element which is important in immunity against microorganisms. However, blood selenium levels are lower in patients who suffer pulmonary tuberculosis and TB-HIV infection [26]. Higher mortality among TB infected patients is related to low levels of blood selenium [27]. Consumption of Selenium-Vitamin E supplements has been highly recommended due to its potential to decrease the reactive oxygen species and increase antioxidant activities in pulmonary tuberculosis patients [28].

Our findings of significantly higher MDA in TB patients on treatment compared to naïve TB patients and significantly lower SOD in TB patients on treatment compared to naïve TB patients is at variance with some studies by [29,30, 16 &31], they reported a reduced MDA in TB patients on treatment compared to TB cases not on anti-tuberculosis drugs and also increased SOD levels in TB patients on treatment compared to naïve TB cases. According to [29] free radicals have been associated with the development of lung fibrosis that may be a long term development of pulmonary tuberculosis oxidative stress could play a pathogenesis of anti-tubercular drug induced hepatotoxicity and lower levels of plasma glutathione and higher levels of MDA may be due to oxidative stress ensuing from anti-tubercular drug. In case of tuberculosis, the lipid peroxidation (LPO) stimulation accomplished by a sharp reduction of SOD enzyme activity is observed. The finding in the present study may also be as a result of either no accurate assessment of the level of treatment relating to the duration of treatment or the cases are rifampicin resistant which is in line with the study by [11], he found that Plasma concentrations of Hydrogen peroxide, MDA and protein carbonyl were significantly higher among rifampicin resistant subjects compared with AFB positive and AFB negative groups and the plasma SOD activity was also significantly reduced among rifampicin resistant subjects compared with AFB positive and AFB negative groups. We were not able to verify duration of treatment and also confirm if the cases were rifampicin resistant cases this is one of the limitations of this research. The findings of significantly high MDA corroborates with a previous study reported, that serum MDA concentration, were significantly higher in patients with TB than in healthy Ethiopian control subjects [32].

This study showed a significant a negative correlation between MDA and SOD in TB subjects on treatment, that is as the MDA increases the SOD decreases, this is in accordance with the observation by [29], which is an increased MDA and reduced SOD in tuberculosis patients on treatment. This negative correlation between MDA and SOD is also in agreement with the study by [31], they found a significant correlation between high MDA levels and low SOD levels. [32], suggesting increased use of ROS as a vital contributing factor to reducing the concentration of antioxidants in TB patients. Malnutrition leads to decreased supplementation of antioxidants and better ROS generation, this leads to increased utilisation of these compounds and that may signify a pathogenic loop that results to noticeably enhance oxidative stress during tuberculosis.

We extended these findings by showing that the level of Superoxide dismutase, an enzymatic anti-oxidant was seen to be non-significantly lower in female naïve TB patients when compared with the male naïve counterparts [33].

The levels of selenium levels were significantly reduced with an increase in age.

5. CONCLUSION

In the present study, it was observed that free radical activity was increased with increased MDA and the antioxidant level was low in TB patients on treatment, and this indicates that there is oxidative stress. TB patients are not able to produce enough antioxidants to cope with the increased free radicals (oxidative stress) in them and also the levels were significantly higher in TB patients on treatment this indicates that the levels of antioxidants that selenium is incorporated into increases with the treatment, and this reduces oxidative stress. Hence, it appears that co-administration of suitable antioxidant supplementation is required to protect TB patients from free radical attack, as blind supplementation of antioxidants with anti-tubercular drugs in tuberculosis patients may cause more harm than benefit [34]. One of the limitations of our study is that we did not evaluate serum selenium levels of the apparently healthy individuals. Moreover, food consumption and nutritional status of people should be evaluated, as there are several reports that reported malnutrition among the healthy population.

CONSENT

Informed consent was sort from the patients and only those who gave consent participated in the study a structured questionnaire on demographic data was administered to all participants.

ETHICAL APPROVAL

Ethical clearance was obtained from the Rivers State Hospitals Management Board.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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