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The Non-Enzymatic Antioxidant and Level of Oxidative Stress of Tuberculosis Patients in Selected Treatment Center in Addis Ababa Ethiopia

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Abstract

Introduction: Non-enzymatic antioxidants are good scavengers of free radicals preventing their overproduction there by reducing the level of oxidative stress. This work was undertaken at Saint Peter TB specialized hospital and Tekle Haimanot health center from March 2012 to May 2013. Aim: To determine changes in Non-Enzymatic Antioxidants and level of oxidative stress of tuberculosis Patients before and after taking anti tuberculosis treatment. Materials and Methods: In this comparative cross sectional study, a total of 210 individuals including: newly diagnosed TB patients as group-I (n = 70), TB patients who completed treatment as group-II (n = 70), and healthy volunteers as group-III (n = 70) were enrolled. Different methods were used to determine the parameters; vit-C (HPLC method), lipid peroxidation (thiobarbuituric acid method), and bilirubin (Colorimetric assay). Results: Vitamin-C (Vit-C) and of group-I showed a significant reduction ($p < 0.001$) as compared with both group-II and group-III whereas Malondialdehyde (MDA) level was increased. However, the total and direct bilirubin was not different among the groups. In group-III, there was a positive correlation between BMI and serum Vit-C ($r = -0.305$, $p = 0.010$). Vit-C showed a negative correlation with serum MDA in all the groups with values ($r = -0.265$, $p = 0.027$), ($r = -0.389$, $p = 0.001$) and ($r = -0.375$, $p = 0.001$) for group-I, group-II and group-III respectively. In addition to this Vit-C was negatively correlated with serum UA ($r = -0.285$, $p = 0.017$) in group-I. Conclusion: The findings of the current study suggest that the amount of Vit-C in the newly diagnosed TB patients and those who finished their treatment is much lower than the healthy

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volunteers. In contrast to this, the MDA value was significantly higher both in the newly diagnosed TB patients and TB patients who completed treatment than in healthy volunteers suggesting higher degree of oxidative stress.

Keywords

Tuberculosis, Non-Enzymatic Antioxidants, Oxidative Stress

1. Introduction

The term tuberculosis (TB) came from the Latin word "*tuberculum*" which meant small swelling, Pimple. "*Tuber*" means lump and "osis", a suffix of Greek origin. It is so called in reference to the tubercles which form in the lungs. It is caused by a bacterium, it is also known as Koch's Disease [1]-[3].

Mycobacterium tuberculosis infects one in three worldwide and kills more people each year than any other bacterial pathogen. Routine treatment of tuberculosis requires combination antibiotic therapy for a minimum of six months, and places a substantial burden on health care systems. Tuberculosis remains a major global health problem. It causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). The latest estimates included in the report indicated that there were almost 9 million new cases in 2011 and 1.4 million TB deaths (990,000 among HIV negative people and 430,000 HIV-associated TB deaths) [4].

According to WHO report on Tuberculosis profile of the countries, Ethiopia is among the 22 High-burden countries in the world. There were about 159,017 total notified TB cases by the year 2011. Out of this figure about 49,594 were smear-positive, 52,967 were smear-negative, 2530 Smear-unknown (not done) and 49,305 were extra pulmonary [4].

Several studies were done and still are going on the change in Non-Enzymatic Antioxidants and level of oxidative stress of tuberculosis Patients all over the world. However, still as no data exists in Ethiopia, this study will contribute a lot to evaluating the level of anti oxidants and change in oxidative stress of Ethiopian tuberculosis Patients and healthy volunteers.

1.1. *Mycobacteria tuberculosis* and the Antioxidant Defense Mechanisms

Mycobacteria are capable of inducing reactive oxygen species production by activating both mononuclear and polymorphonuclear phagocytes that may possess antimicrobial activity. The enhanced level of free radical production, although designed to combat the invader, has the potential to damage the host; however, host tissue damage is limited by the concurrent enhancement of the antioxidant defenses of the host [5]. In tuberculosis patients, there are also some reports of poor antioxidants defence that may expose to oxidative host tissue damage [6] [7].

Oxidant-antioxidant balance is essential for the normal lung function. Both, an increased oxidants and/or decreased antioxidant may reverse the physiologic oxidants—antioxidant balance in favor of oxidants, leading to lung injury. Recent research suggests that oxygen and its relative species (oxidants) may contribute to the pathogenesis of a number of important lung diseases. The lung exists in a high-oxygen environment and together with its large surface area and blood supply, is susceptible to injury mediated by these oxidants. Increased production of reactive oxygen species and reactive nitrogen intermediates secondary to phagocyte respiratory burst occur in pulmonary TB. Evidence suggests that increased circulating levels of free radical activity are found in pathogenesis of active pulmonary TB and hence play a role in resultant fibrosis [8].

Nutritional status determines normal health and functioning of all systems in the body, including the immune system which is responsible for host resistance to various infectious diseases. Because cell-mediated immunity is the key host defense against TB, malnutrition is therefore an important risk factor for the development of TB [9]. Furthermore, the reactivation of latent or previously sub-clinical TB infection is also often related to deteriorating nutritional status and this explains the observed increase in the prevalence of TB in association with HIV infection. Thus, the effective management of diseases, including TB, therefore requires detailed evaluation of the nutritional status since this can help prevent or modify many complications of the disease and also help in

making projection of the interaction of nutritional status on the clinical course of the disease.

Non-Enzymatic Antioxidants

Antioxidants are substances, which inhibit or delay oxidation of a substrate while present in small amounts. Endogenous antioxidant defences are both non-enzymatic (uric acid, glutathione, bilirubin, thiols, albumin, and nutritional factors, including vitamins and phenols). In the normal subject the endogenous antioxidant defences balance the reactive oxygen species production, but for the above-mentioned there is 1% daily leak [10].

Vitamin-C is the major water-soluble antioxidant and acts as first defence against free radicals in whole blood and plasma. It is a powerful inhibitor of lipid peroxidation and regenerates vitamin E in lipoproteins and membranes. A strong inverse association has been shown between plasma ascorbic acid and isoprostanes [11]. Isoprostanes represent a family of prostaglandin isomers which, in contrast to classic prostaglandins formed through an enzymatic action of the prostaglandin-H-synthase from arachidonic acid, result from a free radical-catalyzed mechanism [12]. For this reason, isoprostanes provide an optimal estimate of oxidative damage to cellular lipids [13] and represent an excellent biomarker of lipid peroxidation for aging studies [14].

Chronic vitamin-C treatment is able to decrease high levels of isoprostanes in animal models [15]. Ascorbic acid combined to α-tocopherol is particularly effective in inhibiting oxidation [16]. Vitamin-C reduces α-tocopheroxyl radicals rapidly in membranes andloe density lipoprotein to regenerate α-tocopherol and possibly inhibits α-tocopheroxyl radical-mediated propagation.

The non-enzymatic antioxidants consist of dietary supplements and synthetic antioxidants such as vitamin-C, GSH, taurine, hypotaurine, vitamin E, Zinc, selenium, betacarotene, uric acid, bilirubin and carotene [17].

Uric acid is by-far the highest concentration antioxidant in human blood. Uric acid is an antioxidant oxypurine produced from xanthine by the enzyme xanthine oxidase, and is an intermediate product of purine metabolism [18]. In animal studies that investigate diseases facilitated by oxidative stress, introduction of uric acid both prevents the disease or reduces it, leading researchers to propose this is due to uric acid antioxidant properties [19].

Decreasing uric acid production in a broiler using the xanthine oxidase inhibitor, allopurinol, results in increased oxidative stress accompanied by the accumulation of markers of free radical damage [20] [21].

Bilirubin is an endogenous compound that can be toxic [22], especially in neonates. However, it has recently been recognized that unconjugated bilirubin exerts a strong anti-oxidant activity, and that mild hyperbilirubinaemia might have positive health effects. Bilirubin is the ultimate breakdown product of hemoglobin and serves as a diagnostic marker of liver and blood disorders.

1.2. Oxidative Stress and Tuberculosis

Mycobacterium tuberculosis is intracellular pathogens, which grow and replicate in the host macrophages. It is well known that macrophages undergo respiratory burst after contact with this microorganism. These cells posses the capacity to generate huge amounts of reactive oxygen species (ROS) and ROS induce lipid peroxidation (LP), a chain process which affects unsaturated fatty acids mainly localized in cell membranes, in which end product as Malondialdehyde (MDA) is generated. MDA, which itself is responsible for some of the damaging effects of free radicals on DNA and on cell membranes [23]. LP products diffuse from the site of inflammation and can be measured in the blood.

MDA is a three carbon oxidative stress marker, low molecular weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids of biological membranes. The determination of MDA is used for monitoring lipid peroxidation in biological samples. Although the concentration of plasma antioxidant components can be measured individually, these measurements may be time and cost consuming and labour intensive. In addition, it may not accurately reflect the total antioxidant status [24]. Thus, the accurate antioxidant capacity of the organism can only be determined by the measurement of total antioxidant capacity.

Recent research suggest that in pulmonary tuberculosis there is increase in several circulating markers of free radical activity, indicating ongoing oxidative stress and decrease in the antioxidant activity which may contribute to development of lung function abnormalities [25].

2. Material and Methods

General objective: To determine changes in Non-Enzymatic Antioxidants, level of oxidative stress and Nutri-

tional Profiles of tuberculosis Patients before and after taking anti tuberculosis treatment (ATT).

Specific objectives:

- ✓ To compare the level of vitamin C, uric acid and bilirubin in newly diagnosed TB patients with TB patients who completed treatment and with healthy volunteers.
- ✓ To estimate the level of oxidative stress of TB patients by measuring the lipid peroxidation product Malondialdehyde (MDA).

Study design: Comparative cross-sectional study.

Study area and period: This study was carried out at Saint Peter TB specialized hospital (SPTBSH) and Tekle Haimanot health center from March 2012 to May 2013. SPTBSH is one of the largest hospitals in Ethiopia. It was established in 1953E.C. It is found in Zone five, Addis Ketema Kifle ketema Woreda, Kebele17, Addis Ababa, Ethiopia. Tekle Haimanot health center is also one of the oldest Governmental health centers in Addis Ababa city with high flow rate of TB patients. It is found in Lideta Kifle ketema Woreda, Kebele10, Addis Ababa, Ethiopia.

Ethical considerations: The study proposal was reviewed and approved by the ethical review committee of the department biochemistry, Addis Ababa University (protocol number 011). In addition, the ethical committee of SPTBSH and Tekle Haimanot health center also has given an approval and legal permission to conduct the research.

Study population: all TB patients at Saint Peter's TB Specialized Hospital (SPTBSH) and Tekle Haimanot health center, Addis Ababa, Ethiopia.

Sample size: In calculating sample size since no study has been done previously in this area the value of proportion (P) was taken from a study conducted in Ghana as 51%.

Inclusion criteria: Those who gave written consent, TB patients who are non-smokers, between age of 18 to 60 years and TB patients without any history of recent drug usage were recruited for the study.

Exclusion criteria: pregnant women, HIV patients, Hepatitis B patients, Hepatitis C patients and Patients with a history of previous treatment for tuberculosis were excluded from the study.

Experimental arrangement: **Group I:** Newly Diagnosed Pulmonary and extra pulmonary tuberculosis patients which are sputum smear-positive and smear negative who did not start anti TB drug treatment was included. **Group II:** Pulmonary and extra pulmonary tuberculosis patients who had finished anti TB drug treatment and checked they are cured were included. **Group III:** healthy volunteers: individuals who are under diagnosis for other medical checkup cases other than the cases listed under the exclusion criteria will be included in this group. In addition some volunteer nurses and laboratory technologists were also included in this study.

Sampling technique: The study participants who meet the inclusion criteria were selected. The selected study participants who comply with the inclusion criteria were examined and interviewed by a nurse, and latter on gave blood sample.

Sample and data Collection procedure: A questionnaire was administered for all eligible study participants to collect demographic, clinical and socioeconomical data (age, sex, nutritional states, smoking habit, drug use, etc) and anthropometric measurements (height, weight) were collected from each study participant.

A volume of 8 ml of venous Blood samples were collected by Nurses from antecubital vein of the arm from each eligible consenting study participant after an overnight fast on the morning. Sample tubes were properly labeled with participant identification code. Blood was collected using a disposable plastic syringe and it was dispensed into gel coated tubes. After the blood clot by keeping for about 15 minutes, the serum samples were immediately separated by centrifugation at 4000 rpm for 10 min at 4˚C. Then serum samples were separated immediately into two aliquots in sterile Eppindorffs tubes (Delta Lab, Barcelona, Spain) aseptically by using sterile Pasteur pipettes. The separated serum was stored frozen at −70˚C until analysis. Samples were processed and analyzed at different laboratories. MDA was analysed using the ELISA machine which is available in Biochemistry department laboratory, Addis Ababa University. Vitamin C was analysed in Ethiopian Health and Nutrition Research Institute (EHNRI).

Statistical analysis: Epi Info™ was used for data entry. SPSS version 16.0 for windows software was used for data analysis. Statistical analysis was carried out by two ways Analysis of Variance (ANOVA). Data was expressed as mean ± SD and the p-values ≤ 0.05 was considered as significant. Dunnet t-test was used to compare variables between control and study groups. Association variable were determined using spearman correlation coefficient.

3. Result

A total of 210 randomly selected (newly diagnosed TB patients, n = 70; TB patients that have completed their treatment and checked they were TB negative after treatment, n = 70 and healthy volunteers, n = 70) were recruited to participate in this study, and all of them completed the study giving 100% response rate. Among the participants 53.8% (113) were male and 46.2% (97) were female. In both group-I and group-II, the higher percentages of TB patients are illiterate, private employed and single.

Table 1 indicated that, there was no marked difference in the mean age of the three groups. The mean weight of the treated cases was significantly lower ($p < 0.001$) compared to the healthy controls and it was higher ($p < 0.05$) compared to the new cases.

As indicated in **Table 2**, 21 (10%) of the total participants were with BMI < 17.9 (chronic energy malnutrition). Out of this 19 of them were from the newly diagnosed TB patients and 2 from patients that have completed their treatment. Most of the study participants, 171 (81.4%) were in the range of 18 - 24.9 (normal). Out of this, the highest number was in the healthy volunteers group (69). Among the study participants no one was in range of BMI > 30 (obese).

3.1. Biochemical Variables

Non-Enzymatic Antioxidants

The biochemical testes performed on the study participants showed both significant and non-significant differences among the three groups as shown in **Table 3**. Most of the non-enzymatic antioxidants show significant differences except the total and direct bilirubin. Especially vitamin-c and MDA showed a significant difference ($p < 0.001$) both with healthy controls and between new cases and treated cases.

3.2. Correlation Results among Variables

3.2.1. Correlation Results among Variables Investigated in Group One

As indicated in **Figure 1**, Vitamin-C showed a negative correlation with serum MDA concentration ($r = -0.265$, $p = 0.027$) and with serum UA ($r = -0.285$, $p = 0.017$). Other correlations such as direct bilirubin and total bilirubin were not significant.

3.2.2. Correlation Results among Variables Investigated in Group Two

In group-II also there was a positive correlation between serum Vitamin-C and UA ($r = 0.114$, $p = 0.0347$). As indicated in **Figure 2**, Vitamin-C showed a negative correlation with serum MDA concentration ($r = -0.389$, $p = 0.001$). Other correlations such as direct bilirubin and total bilirubin were not significant.

4. Discussion

The current study indicated that the mean body mass index (BMI) of the new case was 19.6 ± 2.64. About19 (27.15%) of them had chronic malnutrition (BMI < 17.9). our finding was in agreement with a study conducted in Myanmar and Zimbabwe**,** which indicated that half of newly diagnosed adult TB patients were malnourished at the time of starting treatment, with more than a quarter having moderate to severe malnutrition [26]. The observed malnutrition among TB patients at the time of registration has been reported in other studies in both

Table 1. Anthropometric characteristics of the study participants*.

Anthropometric parameters	Group-I (n = 70)	Group-II (n = 70)	Group-III (n = 70)
Age(years)	34.47 ± 13.35	36.46 ± 11.52	34.3 ± 9.74
Weight (kgs)	53.43 ± 8.28	$57.67 \pm 10.62^{2,8}$	60.47 ± 7.95
BMI(kg/m^2)	19.6 ± 2.64	$22.1 \pm 2.35^{2,6}$	21.6 ± 1.499
Male/Female	1.59	1.33	0.75

*Data presented as mean ± SD, [2]significantly different from healthy control subjects (Dennett's test): [2]$p < 0.001$. [6,8]significantly different from new case TB patients (Dennett's test): [6]$p < 0.001$, [8]$p < 0.05$.

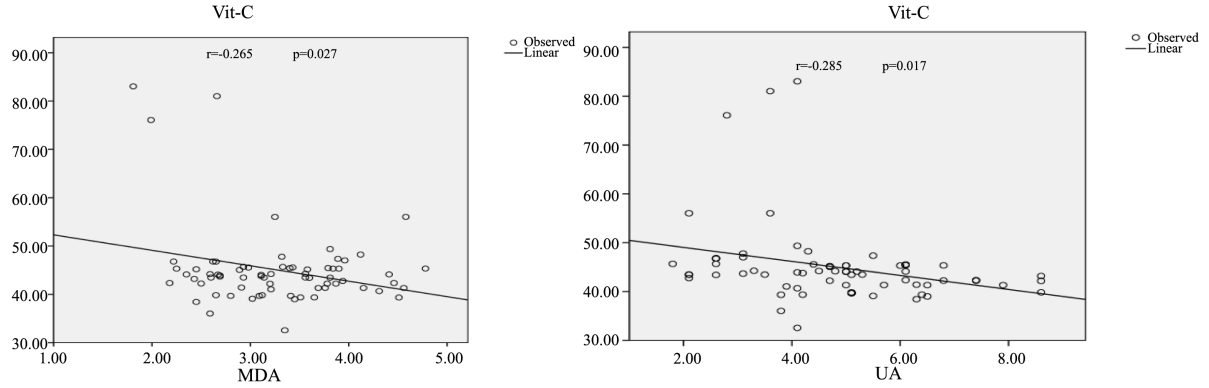

Figure 1. Regression fit of (a) Vit-C Vs MDA and (b) Vit-C Vs UA.

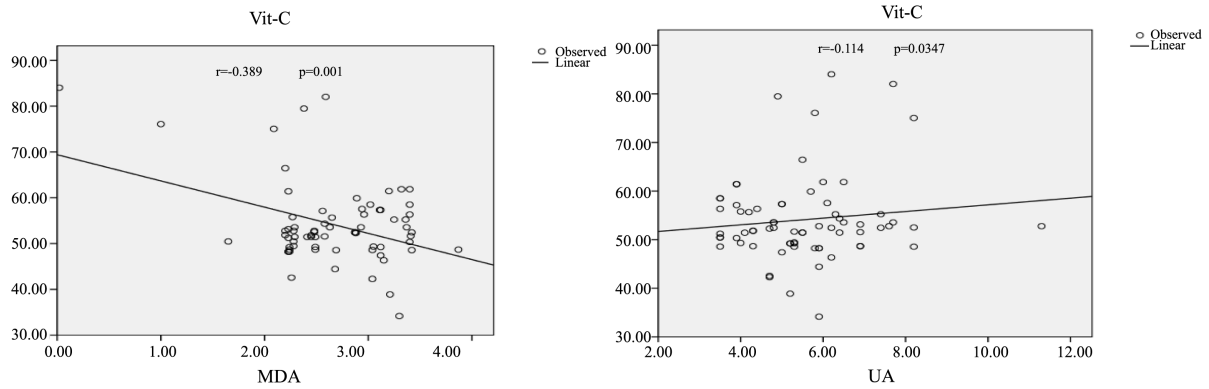

Figure 2. Regression fit of (a) Vit-C Vs MDA and (b) Vit-C Vs UA.

Table 2. Body dimension as measured by BMI.

BMI	Group-I n (%)	Group-II n (%)	Group-III n (%)	Total n (%)
<17.9 (chronically malnourished) (%)	19 (27.15)	2 (2.8)	0.0 (0)	21 (10)
18 - 24.9 (normal) (%)	47 (67.15)	55 (78.6)	69 (98.6)	171 (81.4)
25 - 29.9 (over wt) (%)	4 (5.7)	13 (18.6)	1 (1.4)	18 (8.6)
>30 (obese) (%)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Total (%)	70 (100)	70 (100)	70 (100)	210 (100)

Table 3. Serum concentrations of non-enzymatic antioxidants and (MDA)*.

Parameter	Group-I (n = 70)	Group-II (n = 70)	Group-III (n = 70)
Vit-C (µmol/L)	45.05 ± 8.32[2]	54.10 ± 8.83[2,6]	60.19 ± 9.52
UA (mg/dL)	4.81 ± 1.65[4]	5.52 ± 1.47[8]	4.85 ± 2.01
BulT (mg/dL)	0.532 ± 0.19	0.54 ± 0.15	0.55 ± 0.35
MDA (µmol/L)	3.23 ± 0.69[2]	2.65 ± 0.60[2,6]	1.35 ± 0.61
BulD (mg/dL)	0.0795 ± 0.0766	0.0813 ± 0.0895	0.0801 ± 0.0215

*Data presented as mean ± SD, [2,4]significantly different from healthy control subjects (Dennett's test): [2]$p < 0.001$, [4]$p < 0.05$. [6,8]significantly different from new case TB patients (Dennett's test): [6]$p < 0.001$, [8]$p < 0.05$.

developing and developed countries [27]-[29]. The degree of nutritional impairment found in this study is the same as in other developing countries, but what was different from this study was the percentage BMI of participants was somewhat higher. This could be because of the inclusion of extra pulmonary TB patients which was also observed during the time of sample collection their body weight was higher than the pulmonary TB patients. This needs further investigation with an increased sample size for each group.

This study also showed lower vitamin-C and enhanced lipid peroxidation products (MDA) in newly diagnosed TB patients and TB patients who completed treatment than in healthy volunteers. Our findings further support a role for oxidative stress in the pathogenesis of tuberculosis and suggest that lower anti-oxidant capacity and higher oxidative stress in the TB patients than the healthy human controls. The result of the present study was in agreement with the study by [30] which showed the elevation of free radicals activity was increased and total antioxidant status was low in all TB cases, irrespective of treatment status, indicating that there is an oxidative stress. The decrease in vitamin-C and increase in MDA was more pronounced in the newly diagnosed TB patients indicating that the anti-oxidants were nearly completely utilized to scavenge the superoxide free radicals. Different studies concluded similar findings regarding the non-enzymatic antioxidants and MDA in TB patients [5] [31]. Another study [32] conducted in Pakistan showed decreased levels of total antioxidant capacity and increases MDA levels. Another study has shown that lower levels of antioxidant were observed in pulmonary tuberculosis patients [4] [28]. This might be due to malnutrition and exhaustion in attempt to neutralize heavy load of free radicals in these patients.

Our finding of a significant negative correlation between high MDA concentrations and low concentrations of vitamin C, suggests increased utilization by ROS as an important contributing factor to the lower concentrations of anti-oxidants in TB patients. In fact, the effect of malnutrition leading to decreased level of anti-oxidants and enhanced ROS generation leading to increased utilization of these compounds may represent a pathogenic loop that results in markedly enhanced oxidative stress during tuberculosis infection. A study conducted in Nigeria has show the same result which indicated that the levels of vitamin-C in tuberculosis patients is significantly lower than the healthy controls, while the MDA level is higher than the healthy. After 6 weeks follow-up treatment with ATT in the tuberculosis patients the level of vitamin-C was improved and the MDA level decreased markedly [33].

The present study showed that a slight increase in uric acid level was observed in TB patients who completed treatment when compared to the two groups (newly diagnosed TB patients and healthy volunteers), though it was in the normal range. This increase may be due to Pyrazinamide whose one of the side effects is hyperuricemia. The result of the present study was in agreement with the study of [34] which showed the elevation of serum uric acid on Pyrazinamide therapy. In contrast with this, a study [35] conducted in Nigeria indicated that there was an increases in uric acid level of newly diagnosed pulmonary tuberculoses patients than normal control. Many studies [36] [37] also mentioned that there is an increase in uric acid during the course of anti TB therapy no signs of clinical gout or arthralgias. But fortunately it was also shown that the results returned back to normal once the drug was stopped. Another study reported that uric acid was endogenously produced as a compensatory mechanism for the neutralization of free radicals in tuberculosis patients [38].

In this study significant difference were not observed among the three groups in parameters including total protein, total bilirubin, direct bilirubin and total triglyceride. In contrast with this a study conducted in Mumbai have shown that total protein in non-treated TB patients was elevated marginally as compared to control group (6% high) and 1 month post-treatment periods gradually (7% and 14%). However it start slow decline from 2 month through 3, 4 and up to 6 month post treatment period. The value comes altogether equal to control on 6 month post treatment period [39].

5. Conclusion

In general, the current study showed that the amount of vitamin-C in the newly diagnosed TB patients and those of who completed treatment is much lower than the healthy volunteers. Although the value in the treated group was somewhat higher than the untreated TB patients, the value was significantly different from the healthy controls. In contrast to this, the MDA value was significantly higher in both the new TB cases and treated TB patients than that in the normal healthy controls. This indicated that TB patients did not have enough antioxidants to fight the free radical generation that aggravated oxidation of membrane lipids leading to the production of high MDA as marker of oxidative stress.

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