

A correlation of plasma malondialdehyde, whole blood glutathione peroxidase and lipid profile in pulmonary tuberculosis

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Abstract

Background: Tuberculosis is one of the commonest chronic infectious diseases; highly endemic in India, kills five lakh patients every year. Oxidative Stress plays important role in inflammatory & degenerative diseases including pulmonary tuberculosis. There is hardly any one study available in literature correlating oxidative stress, lipid profile values and antioxidant status together with the pulmonary tuberculosis; so we decided to conduct this study.

Methods: Study group included newly diagnosed 100 cases of pulmonary tuberculosis and control group included 100, age and sex matched healthy volunteers and employees. All the subjects were subjected to complete physical and systemic examinations, routine investigations including Sputum for AFB by Ziehl-Neelsen staining, AFB culture and Chest x-ray and special tests like fasting lipid profile, plasma malondialdehyde, whole blood glutathione peroxidase and findings recorded and statistically analysed.

Results: In the study group with 66 males and 34 females, we found total cholesterol mean \pm SD 149.16 \pm 19.02; Triglycerides 114.36 \pm 13.38; HDL-C 36.61 \pm 4.17; LDL-C 92.78 \pm 13.72; VLDL-C 20.04 \pm 2.97; MDA 6.03 \pm 1.62 and GPx 6192.9 \pm 790.75 while in controls with 61 males and 39 females, these values were 183.51 \pm 14.63; 134.52 \pm 8.48; 43.46 \pm 4.65; 111.98 \pm 12.62; 28.95 \pm 4.95; 1.79 \pm 0.50 and 7739.27 \pm 429.80 respectively.

Conclusion: Tuberculosis effect more males (66%) than females (34%). TC, TG, HDL-C, LDL-C, VLDL-C and GPx were found statistically significantly lower in study group when compared with control, (p <0.001) while MDA was found significantly higher in study group when compared with control, (p <0.001).

Key Words: Plasma Malondialdehyde, Whole blood Glutathione Peroxidase, Lipid profile, Pulmonary Tuberculosis, Antioxidants

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Introduction

Tuberculosis is one of the commonest chronic infectious diseases and highly endemic in India and five lakh patients die every year^[1,2] It usually affects lungs but cases of extra-pulmonary tuberculosis are not rare. Delay in diagnosis and in initiating treatment results in poor prognosis and sequelae in upto 25% of cases.^[3,4] Pulmonary Tuberculosis (PTB) can be confirmed by sputum examination and diagnosed easily but diagnosing extra-Pulmonary TB becomes frequently difficult, since the specificity and sensitivity of non-invasive methods is very low. Several workers have estimated the specificity and sensitivity of Adenosine Deaminase (ADA) and found out its reliability.^[5,6]

Several biochemical reactions occur in human body during health and disease; as a result of these essential reactions, there is formation of highly reactive oxygen species (ROS) which consist of free radicals

(FR). In reactions with FR, bio-molecules undergo oxidation and through donation of their own electrons, they themselves become new secondary radicals that continue radical chain reactions and support spatial and time-dependent oxidative stress (OS) propagation and consequently lead to the cell/ tissue damage.^[7]

In healthy conditions at the cellular level, there is a critical balance that exists between the FR generation and the various antioxidant defense mechanisms. But during certain disease processes there is a huge imbalance between these two mechanisms resulting in OS, hence this condition is characterized by disturbance in the pro-oxidant – antioxidant balance in favor of the former, which leads to a potential harm to the cell.^[8]

ROS can damage proteins, lipids, nucleic acids and other cellular components under oxidative stress conditions.^[9] OS plays an important role in inflammatory & degenerative diseases like pulmonary tuberculosis.^[10]

Mycobacterium tuberculosis (M. tuberculosis) is an intracellular pathogen which grows and replicates in the host macrophages. The pathogen activates the invaded macrophages and results in free radical burst.^[11,12] These FR induce lipid peroxidation (LP), a chain process which affects polyunsaturated fatty acids (PUFA) mainly localized in cell membranes, in which end products such as malondialdehyde (MDA) is

generated.^[13] MDA is itself responsible for some of the damaging effects of free radicals on Deoxyribonucleic acid (DNA) and on cell membranes.^[14] High levels of lipid peroxidation products like MDA is seen in advanced tuberculosis and can be measured in the blood as a parameter of oxidative stress.^[15]

There are number of studies available in the literature where different researchers have tried to find out the level of oxidative stress, lipid profile values and antioxidant status separately in pulmonary tuberculosis (PTB) patients, there is hardly any one study available in literature correlating these three parameters together with the disease; so we decided to conduct this study.

Objectives

1. To estimate plasma Malondialdehyde (MDA), whole blood Glutathione Peroxidase (GPx) levels and Lipid profile in patients of Pulmonary Tuberculosis (PTB).
2. To find plasma MDA, GPx levels and Lipid profile in age & sex matched healthy controls.
3. To compare the findings so obtained in control and study group patients and find out if there exist any significant difference between them.

Patients and Methods

The present study was conducted in the department of Biochemistry, Subharti Medical College, Meerut. Ethical clearance was obtained by the Institutional Ethical Committee. Patients attending OPD and IPD of Respiratory Medicine, Chhatrapati Shivaji Subharti Hospital (C.S.S.H.) were enrolled for the present study. Informed consent was taken from each individual subject.

Study group included newly diagnosed (fresh) 100 cases of pulmonary tuberculosis. Patients with previous diagnosis of PTB with or without treatment, Diabetes Mellitus, Hypertension, Coronary artery disease, Hepatitis B infections, Human immunodeficiency virus (HIV) infection, Chronic obstructive pulmonary disease (COPD) and Suppurative lung disease (SLD) were excluded from the study by undertaking fasting and 2 hours Blood Sugar, BP recording on two consecutive days, ECG, Stress testing, PFT, HBsAg and HIV tests in selective cases. Control Group included 100, age and sex matched healthy volunteers and employees.

General information and detailed medical history was recorded from each individual subject, all individuals were subjected to complete physical and systemic examinations, routine investigations including Sputum for AFB by Ziehl-Neelsen (ZN) staining, AFB culture and Chest x-ray (CXR) and special tests. Fasting Lipid profile, plasma MDA and whole blood GPx were performed in each case and findings recorded.

Criteria for newly diagnosed cases:

Patients with cough for 2 weeks or more, with or without other symptoms, suggestive of TB and patients with clinico-radiological findings suggestive of PTB were enrolled. Diagnosis was confirmed on the evidence of the presence of AFB in:

1. Sputum expectorated after deep inspiration or
2. Broncho-alveolar Lavage (BAL) by bronchoscopy by direct smear examination after Ziehl -Neelsen staining and/ or Culture on Lowenstein- Jensen (LJ) medium or by BACTEC methods.

Diagnostic algorithms for inclusion of cases is detailed in figure below:

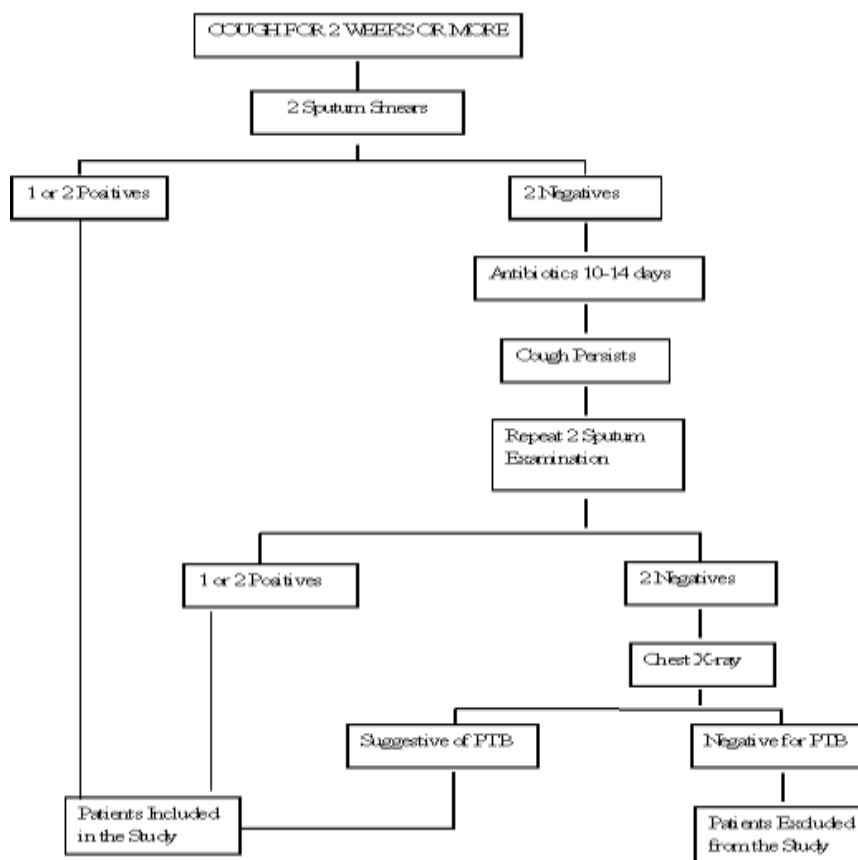


Fig 1: Diagnostic Algorithms for Inclusion of Cases

Sample collection:

Sputum: Two samples were collected as per the criteria laid down by Revised National Tuberculosis Control Program (RNTCP)^[16].

Blood: Blood sample was drawn from the antecubital vein aseptically through venipuncture after overnight fasting (>12 hrs) and collected in;

1. EDTA vacutainer for estimation of routine hematology,
2. Citrate vacutainer for estimation of ESR and
3. Plain vacutainer for estimation of lipid profile {Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C)}.

The blood sample in plain vial was left undisturbed for 30 minutes at room temperature. It was then subjected to centrifugation at 1500 g for 10 minutes. Serum was then carefully transferred to disposable cups for investigations. Vitros-250 auto analyzer of Ortho- Clinical Diagnostics, Johnson & Johnson, USA was used for processing for the estimation of lipid profile.

Heparinized vacutainers were used for estimation of plasma MDA and whole blood GPx. The sample for MDA was subjected to centrifugation at 2500 g for 15 minutes and plasma separated while for GPx, sample was stored as such; all samples were stored at -80°C in deep freezer. MDA was estimated by Derivative Spectrophotometry using thiobarbituric agent while GPx was estimated using the method described by Paglia and Valentine^[17] by using Shimadzu spectrophotometer model no. UV-1800 ENG, 240 Wt, Serial no. A11635304025-CD.

Statistical Analysis

The data collected was subjected to statistical analysis. Mean, Standard deviation and Students (t) test were applied between the values of the test and control group.

Results

In study group, we found TC mean \pm SD 149.16 \pm 19.02 mg/dl; Triglycerides 114.36 \pm 13.38 mg/dl; HDL-C 36.61 \pm 4.17 mg/dl; LDL-C 92.78 \pm 13.72 mg/dl; VLDL-C 20.04 \pm 2.97 mg/dl; MDA 6.03 \pm 1.62 nmol/ml and GPx

6192.9±790.75 U/L, while these values in controls were 183.51±14.63; 134.52±8.48; 43.46±4.65; 111.98±12.62; 28.95±4.95; 1.79±0.50 and 7739.27±429.80 respectively (Table 2). $p < 0.001$ was considered to be statistically significant.

TC, LDL-C, HDL-C, Triglyceride, VLDL-C and GPx was found significantly lower in study group when compared with control ($p < 0.001$); while MDA was found significantly higher in study group when compared with control ($p < 0.001$);

Table 1: Mean age and sex distribution of all subjects according to study and control group

	Control (N=100)	Study Group (N=100)	p-Value
Age (years) Mean± SD	37.96±13.39	37.13±15.49	0.686 (NS)
Sex Distribution			
Male	61%	66%	0.463 (NS)
Female	39%	34%	

Table 2: Comparison between study and control group of biochemical parameters

Parameters	Control (N=100)	Study Group (N=100)	p-Value
Total cholesterol (mg/dl)	183.51±14.63	149.16±19.02	<0.001 (Sig)
Triglyceride (mg/dl)	134.52±8.48	114.36±13.38	<0.001 (Sig)
HDL-C (mg/dl)	43.46±4.65	36.61±4.17	<0.001 (Sig)
LDL-C (mg/dl)	111.98±12.62	92.78±13.72	<0.001 (Sig)
VLDL-C (mg/dl)	28.95±4.95	20.04±2.97	<0.001 (Sig)
MDA (nmol/ml)	1.79±0.50	6.03±1.62	<0.001 (Sig)
GPx (U/L)	7739.27±429.80	6192.90±790.75	<0.001 (Sig)

Discussion

Pulmonary tuberculosis, a disease associated with a wide range of respiratory symptoms, is socially and economically very cumbersome to control. Although tuberculosis morbidity and mortality has decreased in developed countries, but owing to limited resources it still remains one of the most common causes of morbidity and mortality in developing countries due to delay in its diagnosis.

Mycobacteria are intracellular pathogens which grow and replicate in host macrophages. After phagocytosis survival of mycobacteria depends on their ability to avoid destruction by macrophages. Phagocytes (neutrophils, macrophages and monocytes) undergo respiratory burst after contact with microorganisms. Here, they generate huge amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which are essential for the destruction of ingested microorganisms and also contribute to inflammatory injury to host tissue. ROS are highly toxic to all types of cells, but especially to lipids (fat cells) causing peroxidation. This results in damage to cell membrane which promotes lung fibrosis and dysfunction in PTB.^[18] Antioxidants scavenge free radicals and suppress the actions of ROS and thus protect the host from tissue inflammation. But as malnutrition is commonly seen in patients of PTB, it can add to the impaired antioxidant capacity in these patients resulting in OS.^[19]

Association of oxidative stress, antioxidants status and lipid profile with PTB has long been the interest of

researchers. Several studies are available in the literature in which researchers have assessed the individual parameters like OS, antioxidant status and lipid profile in PTB patients but hardly any available study includes these three parameters and their correlation with the disease. This generated our interest in this direction and so this study was planned and conducted on the population of District Meerut and 50 Km area around; findings of which are discussed below:

Demographic

We studied 100 patients of PTB, all newly diagnosed; out of which 66 were males and 34 were females; indicating that the incidence of disease in the present study is 66% in males & 34% in females; males being involved more as compared to females (Table 1). Our study does not include adolescent patients. Some worldwide studies have shown that males are exposed to higher risk factors like smoking, alcoholism and drug addiction and their more outdoor involvement for work contribute to higher incidence in males. The global data on tuberculosis prevalence also shows that the prevalence of M. tuberculosis infection is similar in males and females until adolescence; but after that, it appears higher in males.^[20,21,22]

Comparison of different parameters between control and study group

Total cholesterol: In our study the TC values were found to be significantly lower in study group when

compared with healthy control (Table-2). This finding is consistent with several studies.^[23,24] Rao, Sukhesh et al. found that levels of serum cholesterol were found to be significantly lower in smear-positive group when compared with the smear-negative group.^[25] The reason postulated for this observation by Cole et al. and Van der Geize et al. was the presence of a complex repertoire of lipid metabolising genes in the genome of *M. tuberculosis* while Sasseti et al. reported upregulation of cholesterol uptake and catabolism genes^[26-28]. These workers suggested that cholesterol is utilized by the bacteria and so the TC levels falls in these patients.

Triglycerides: We found triglyceride values to be significantly lower in study group when compared with healthy control (Table 2). Similar findings were also observed by Cole et al. and Van der Geize et al. who further postulated that this was due to the presence of a complex repertoire of lipid metabolising genes in the genome of *M. tuberculosis*:^[26] indicating that lipids are being utilized by mycobacteria and hence causes decrease in the levels of lipids including triglycerides.

Moreover Virendra singh et al. observed raised levels of serum free fatty acids in patients with PTB and related it to the extent of the lesion and the bacillary status of the patients.^[35] They concluded that breakdown of adipose tissue with conversion of triglycerides into fatty acids and further decreased peripheral utilization and blocked entry of fatty acids into mitochondria may have caused raised levels of serum free fatty acids. So the finding of elevated free fatty acid is also an indirect evidence for decreased levels of triglyceride.

HDL- Cholesterol: We found HDL-C values to be significantly lower in study group when compared with healthy control (Table 2). Similar findings were observed by Akiibinu O Moses et al. in their study.^[84] The reason for the decreased serum HDL-C concentration in PTB patients might be due to acute phase response during inflammatory process that could be induced/ caused by the mediators of inflammation.^[29,30] Acute phase proteins such as secretory phospholipase A2 (sPLA2) and serum amyloid A (SAA) might be principally responsible for decreased concentration of serum HDL-C concentrations. In addition, ATP-binding cassette transporter (ABC) 1 has been described to play an obligatory step in HDL-C metabolism that may cause low HDL-C concentrations. Moreover Nakagawa H et al. in their study hypothesized that the decrease in serum HDL-C during inflammation may also be attributed to a decreased lecithin cholesterol acyltransferase activity.^[31]

LDL Cholesterol: We found LDL-C values to be significantly lower in study group when compared with

healthy control (Table 2). This lowering of LDL-C in PTB patients was noticed by Akiibinu O Moses et al. also.^[32] Generally, plasma LDL-C concentrations are reduced during infection and inflammation due to a host response which might induce LDL-C oxidation.^[33] Similar findings were observed by Memon RA et al. though this study was conducted on animals, they observed the infection is associated with increased oxidation of LDL-C.^[34]

VLDL-cholesterol: In our study VLDL-C values were found to be significantly lower in study group when compared with healthy control (Table 2). Significantly lower values of VLDL-C were observed in all groups of PTB patients when compared with healthy controls. Findings of Sasaki Y et al. and Akiibinu O Moses et al. are also similar.^[23,32]

Oxidative stress marker: Wiid IS Edmen T et al. hypothesized that in tuberculosis, oxidative stress is a result of tissue inflammation, poor dietary intake of micronutrients due to illness, free radical burst and anti-tuberculosis drugs.^[11] The formation of free radicals is although a normal consequence of a variety of essential biochemical reactions but can occur at elevated rates under pathophysiological conditions.^[36] Inflammation-related oxidative stress has been implicated in the pathogenesis of lung fibrosis and dysfunction in patients with pulmonary tuberculosis.^[15,37] Activated macrophages are capable of releasing a variety of chemicals including oxygen free radicals which degrade polyunsaturated lipids, forming MDA suggesting the involvement of lipid peroxidation in pulmonary damage.^[38] The production of MDA (an aldehyde) is therefore used as a biomarker to measure the level of oxidative stress in an infection.^[39]

MDA: We found MDA values to be significantly higher in study group when compared with healthy control (Table 2). R. Premanand et al. also observed similar finding of elevated lipid peroxide levels with concomitant reduction of tocopherol and glutathione peroxidase activity in their study on the patients of respiratory diseases.^[40] Devenci F et al. also found concentration of MDA to be significantly higher in PTB patients as seen in our study.^[41] In an another study, Kwiatkowska et al. reported high levels of lipid peroxidation in all categories of PTB patients, irrespective of treatment status and postulated that this may be the cause of reduction in the concentration of serum lipids.^[15] Reddy YN et al. showed greater lipid peroxidation in TB patients as compared to normal human volunteers supporting a link between oxidative stress and tuberculosis infection.^[42] Modebo T et al. also found concentration of MDA to be significantly higher in PTB patients and concluded that high MDA concentrations was associated with clinical severity and anthropometric scores.^[43] All these findings contribute

to the belief that inflammation-related oxidative stress with production of free radicals, damages host tissue including cell membranes and thus generates MDA which can be used to assess different stages of the disease process.^[44-45]

GPx: Glutathione directly reacts with ROS, and GPx catalyzes the removal of hydrogen peroxide. Decrease in GPx activity indicates impairment of hydrogen peroxide-neutralizing mechanisms. Here, we observed GPx values to be significantly lower in study group when compared with healthy control (Table 2). This correlates with the earlier observation of Islam et al. that substantial amounts of ROS are generated in cells infected with PTB due to cellular activation.^[46] Many similar studies have clearly exhibited an imbalance between oxidant and antioxidant defensive systems in the human body under such pathological situations.^[47] Rukmini, M.S. et al. also showed decrease in GPx activity as the disease progresses and explained that there is impairment of hydrogen peroxide- neutralizing mechanisms.^[48] In a similar study by R. Premanand et al. the patients of respiratory diseases showed reduction of tocopherol and glutathione peroxidase activity and elevated levels of lipid peroxides. Also a significant inverse relation was found between lipid peroxides versus tocopherol and glutathione peroxidase activity in these patients.^[49] Akiibinu O Moses *et al.* in their study observed lower levels of antioxidants and nutritional profiles in PTB patients and found it to be associated with heavy load of free radicals, oxidative stress and lipid peroxidation.^[32] Sies et al. in their study showed several factors—such as low food intake, nutrient malabsorption, inadequate nutrient release from the liver and decreased availability of carrier molecule to influence circulating antioxidant concentrations.^[50] They concluded that, improved nutrition and supplementation with antioxidant therapy in the treatment of pulmonary tuberculosis may prevent the oxidative stress and further complications.

Our finding of significant correlation between high MDA and low antioxidant suggests increased utilization by ROS as an important contributing factor to the lower concentration of antioxidants in tuberculosis. The present study is a comprehensive evaluation of circulating antioxidants and oxidative markers in tuberculosis patients. Our results show lower antioxidant potential and increased lipid peroxidation products (MDA) in tuberculosis. These findings support the role of oxidative stress in the pathogenesis of this disease.

We showed lower antioxidant potential and greater lipid peroxidation in TB patients as compared to normal healthy volunteers, these findings further support a link between oxidative stress and tuberculosis infection.

Conclusion

Tuberculosis effect more males (66%) than females (34%). TC, TG, HDL-C, LDL-C, VLDL-C and GPx were found significantly lower in study group when compared with control, ($p < 0.001$) while MDA was found significantly higher in study group when compared with control, ($p < 0.001$).

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