RESEARCH ARTICLE

STUDY OF BODY MASS INDEX AND FREE RADICALS IN PULMONARY TUBERCULOSIS PATIENTS

Background: The burden of pulmonary tuberculosis is very high in India and developing countries.
Aims & Objective: The main objective of present study was to assess the changes in body mass index and oxidative stress in pulmonary tuberculosis patients with antitubercular treatment.
Materials and Methods: Fifty pulmonary tuberculosis patients and thirty healthy control were included in this study and they were matched for age, height and weight. The extent of oxidative stress was measured by blood level of malondialdehyde through spectrophotometer and changes in weight was measured by changes in body mass index.
Results: The study showed a statistically significant decrease in blood malondialdehyde level and there was significant increase in body mass index between control and cases but there was not significant increase in body mass index between new case and follow up cases.
Conclusion: Our study showed a negative correlation between oxidative stress and body mass index. It is suggested that antitubercular treatment increases body mass index and decreases oxidative stress even without antioxidant supplement and these physiological improvement is better if other factor of oxidative stress are avoided.
Key Words: Oxidative Stress; Free Radical; Lipid Peroxidation; Pulmonary Tuberculosis; Reactive Oxygen Species (ROS)

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INTRODUCTION

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 to low levels in developed countries, it still remains one of the most common causes of morbidity and mortality in developing countries. 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. These cells possess the capacity to 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. Inflammation-related oxidative stress has been implicated in the pathogenesis of lung fibrosis and dysfunction in patients with pulmonary tuberculosis.[1]

Lipid Peroxidation increases in a number of disease states.[2] Oxidative Stress induces Peroxidation of Membrane Lipids. This can be very damaging because it leads to alterations in the biological properties of the membrane such as the degree of fluidity. Thus it can lead to inactivation of membrane bound receptors or Enzymes which in turn may impair normal Cellular Function and increase in Tissue Permeability. Moreover, Lipid Peroxidation may contribute to and amplify cellular damage resulting from generation of oxidized products, some of which are chemically reactive and lead to covalently modifying critical Macromolecules. Products of Lipid Peroxidation, therefore, have been commonly used as biomarkers of Oxidative stress/Damage. It generates a variety of relatively stable decomposition of end-products. MDA (Malondialdehyde) is a physiological Ketoaldehyde, produced by Peroxidation of unsaturated Lipids as a byproduct of Arachidonate Metabolism. The excess MDA produced as a result of tissue injury can combine with the free Amino groups of Proteins producing MDA modified Protein products. Compared with Free Radicals the Aldehydes are relatively stable and can diffuse within or even escape from the cell. They attack targets far from the site of the original event. Some of these Aldehydes have been shown to exhibit facile reactivity with various Biomolecules including Proteins, DNA and Phospholipids, generating stable products at the end of the series of reactions that are thought to contribute to the pathogenesis of many diseases.[3]

Similarly, there is huge production of ROS and RNS in PTB patients which freely diffuses the cell membrane.[4] There is increased MDA level which is the indirect marker of oxidative stress in PTB patients.[5,6] The degree of oxidative stress and therefore decreased antioxidant enzymes are determined by radiology, sputum and cavity status of PTB patients.[7]

MATERIALS AND METHODS

The patients included in this study were selected from those attending the Out-Patients Department, Emergency Ward, and from Indoor Patients admitted in the Ward of Dept. of TB and Respiratory disease, Jawaharlal Nehru Medical College, Aligarh. Cases were taken which comprised of either sexes between 21 to 40 years and 50 number of cases were studied and were divided into two subgroup: (i) Newly diagnosed case, also designated as new case or pre follow up; (ii) Two month follow up case which were also designated as follow up case. Controls were taken from healthy population and institution and 30 number of either sexes and similar ages were selected for the study. The present study was a case control study.

Inclusion Criteria: Cases were comprised of both newly diagnosed and the follow-up cases of same patients after two months of Anti tubercular treatment (ATT). Diagnosis of new cases were done on the basis of sputum examination and chest X-ray.

Exclusion Criteria: Patients with previous history of TB and/ or treatment, any another respiratory diseases, smoking habits, patients who were not taking antitubercular drugs regularly i.e. defaulters and those patients who were taking antioxidants, pregnancy or currently breast feeding, diabetes mellitus, patients with known HIV positive status or AIDS, those with complications such as renal, endocrine or hepatic diseases and another infections etc. were excluded.

Blood samples were collected from all subjects and estimated for Malondialdehyde (MDA). Also Body mass index (BMI) of cases and control were studied and compared.

Estimation of Free Radicals: Free radicals were estimated by the method adopted by Philpot.[8] Its Principle is that Free Radicals, by their unstable and transient nature are difficult to measure directly. Their ability to cause Lipid Peroxidation has been used as an indirect measure. Hence estimation of MDA (Malondialdehde) which is a physiological Ketoaldehyde and relatively more stable byproduct of Lipid Peroxidation was done. One Molecule of MDA reacts with two Molecules of Thiobarbituric Acid (TBA) at pH 3.5. The pink color Chromagen measured spectophotometrically at 532 nm. For assay 1.0 ml of Serum was mixed with 2.5 ml of 20% Trichloroacetic Acid (TCA) and 1.0 ml of 0.67 per cent of Aqueous Solution of TBA. The mixture was heated in the boiling water bath for 30 minutes, the pink Pigment Formen was extracted with 2 ml of n-Butanol and its absorbance was read at 532 nm against m-Butanol as blank. The results were expressed as nmol/ml of the sample.

Anthropometric Measurement: BMI was calculated in SI units and expressed as, BMI = Weight (kg)/ [Height (m)]$^{2.}$ Height was measured in cm without shoes in standing position, with the heels put together and with calve buttocks, heels and back touching the Stadiometer and head of the patient tilted in such a manner that the lower Orbital Margin was at the level with the External Auditory Meatus (Frankfurt Plane). Weight was recorded in Kilogram without shoes and with minimal clothing. Room Temperature was recorded in Centigrade's. Grading of BMI done on the basis of WHO, 2004 classification as Underweight <18.50, Normal 18.50 – 24.99, pre obese 25.00 – 29.99 and obese ≥ 30.00.

Statistical analysis was done, using the statistical package for social science (SPSS 17.0) for Windows Software, Microsoft Excel 2007 and Scientific Calculator. The results were expressed as Mean ± Standard Deviation (S.D). The difference in the Serum levels of MDA, and changes in BMI were evaluated by means of t-test. Unpaired t test was applied to analyze the statistical significance of changes in MDA and BMI between control and newly diagnosed cases and also between control and 2 month follow up cases. Paired t test was applied to analyze statistical significance of change in MDA and BMI between newly diagnosed cases and 2 month follow up cases. P Value of less than 0.05 was taken as statistically significant.

RESULTS

Table 1 shows the comparison of the anthropometric parameters between new case and their healthy matched control and results were expressed as Mean ± SD. There was no significant difference in body mass index (BMI) and Malondialdehide (MDA). Table 2 shows the comparision of the anthropometric parameters between 2 month follow up cases and their healthy matched

control. There was very significant difference (p <0.001) in body mass index between controls (23.43 ± 2.86 kg/m 2) and follow up cases (16.78 ± 3.24 kg/m 2). Also, there was very significant difference (p <0.001) in Malondialdehide level between control (1.23 ± 0.06) and follow up cases (1.46 ± 0.14 nmol/ml).

Table 3 shows the comparison of the anthropometric parameters between new and 2 month follow up cases. In majority of follow up cases there is gain in BMI but that gain is not significant (p =0.155) but there is very significant decrease (p <0.001) in MDA level in follow up case (1.46 ± 0.14 nmol/ml) new cases (1.54 ± 0.14 nmol/ml).

Table-1: Control vs new cases (unpaired t test)

Parameter	Group	Number	Mean ± SD	P value
Age	Control	30	29.47 ± 5.37	0.468
	New cases	50	28.56 ± 5.40	
BMI	Control	30	23.43 ± 2.86	<0.001
	New cases	50	15.89± 3.10	
MDA	Control	30	1.23 ± 0.06	<0.001
	New cases	50	1.54 ± 0.14	

Table-2: Control vs 2 month follow-up cases (unpaired t test)

Parameter	Group	Number	Mean ± SD	P value
Age	Control	30	29.47 ± 5.37	0.468
	Follow up	50	28.56 ± 5.40	
BMI	Control	30	23.43 ± 2.86	<0.001
	Follow up	50	16.78 ± 3.24	
MDA	Control	30	1.23 ± 0.06	<0.001
	New cases	50	1.54 ± 0.14	

Table-3: New Case vs 2 months follow-up cases (paired t test)

Parameter	Group	Number	Mean ± SD	P value
BMI	New case	50	15.89± 3.10	0.155
	Follow up	50	16.78 ± 3.24	
MDA	New case	50	1.54 ± 0.14	<0.001
	Follow up	50	1.46 ± 0.14	

DISCUSSION

Oxygen is important to sustain life. It is relatively non-reactive in a ground state. The Cells use Oxygen to generate ATP, the energy currency of body. During the process, Free Radicals are generated. It may be stated here that the Theory of Free Radicals has been known over the years.[9] Free Radicals by virtue of their having one or more unpaired Electrons are highly unstable and reactive. They are continuously produced by a variety of mechanisms in the body.[10,11] At low concentrations they play their role in the physiological functions of body. But being unstable, they can lead to radical chain reactions, if unchecked. Oxidants consist mainly of Reactive Oxygen Species (Superoxide Anions, Hydroxyl, Alkyoxyl, Peroxyl Radicals) and Reactive Non-radical Oxygen Species (Hydrogen Peroxide and Singlet Oxygen) that are formed by Oxidation of partially reduced Oxygen Species and also by Radicals of Carbon, Sulphur and Nitrogen. The Nitric Oxide Radicals can be converted to a variety of Reactive Nitrogen Species, namely Nitrosonium Cation, Nitroxyl Anion or Peroxynitrite. Hydroxyl Radicals react with a variety of biomolecules, resulting in the formation of Unsaturated Bonds. They are formed in Fenton Reaction via decomposition of Hydrogen Peroxide or by reaction of Superoxide Anion with the Transition Metals like Copper or Iron.

Hydrogen Peroxide in itself is not reactive enough to oxidize Organic Molecules, though it has the ability to generate Reactive Radicals by interacting with transition Metals.[12] The radical chain reaction leads to Lipid Peroxidation of Membrane Phospholipids, resulting in alteration of Cellular Physiology. The Lipid Peroxides yield a variety of byproducts including Aldehydes.[13] ROS and RNS cause alteration of Proteins which leads to change in normal Protein function, Chemical fragmentation or increased susceptibility to proteolytic attack of Free Radicals, react with nucleic acid by addition to bases or abstractions of Hydrogen Atoms from the Sugar Moiety.[14] The tissues are protected against the Oxidants by the presence of Enzymatic and Non-enzymatic Antioxidant defense systems.[15]

In the present study there was significant difference in BMI between groups but there was no significant difference between subgroups. So, there is increased in weight and BMI in majority of follow up cases relative to newly diagnosed cases of pulmonary TB. It might be due to increased appetite, increased intake of food and nutritional supplement, decreased physical activity and decreased oxidative stress and increased antioxidant enzymes due to ATT during intensive phase. These findings are supportive of previous study that Weight gain and other improvements in nutritional indicators occur after effective chemotherapy for tuberculosis.[15.16] Also, there was lesser weight gain or no weight gain therefore BMI was not changed much in some of the follow up cases after 2 month of ATT completion in comparison to those majority of follow up cases which gained more weight. This less weight gain and few no weight gain in follow up cases might be due to decreased intake of food and nutritional supplement and increased physical activity.

However, there is significant decrease in oxidative stress in majority of weight gainer follow up cases which might be due to beneficial effect of ATT during intensive phase for two month. Also, majority of newly diagnosed cases of pulmonary tuberculosis in our study had BMI ≤18.50

which means majority were underweight or malnourished supporting the previous study of[17] that PTB is more common in underweight malnourished subjects which have increased risk of developing tuberculosis. Also very few number of cases of newly diagnosed pulmonary tuberculosis in this study had BMI ≥25.00 which means minor cases of PTB were overweight the earlier study[18] that PTB is less common in overweight or obese subjects which are more immunocompetent and less prone to be infected with PTB.

There was significant decrease in MDA level within sub group of follow up cases after 2 month of ATT. These findings are similar with many previous Studies. Also, there was a significantly low BMI in PTB patients than controls, which is was consistent with the significantly lower weights of the patients groups. There was a higher level of MDA in the pulmonary tuberculosis patients than the controls, which decreased significantly after two months of treatment with ATT. This result suggested that after two months of treatment there was a significant reduction in ROS generation and the extent of lipid peroxidation was diminished by chemotherapeutic destruction of mycobacteria. So, there was a significantly lower MDA concentration and higher antioxidant levels in patients with clinical improvement after chemotherapy.[19] Similarly, there are several circulating markers of free radical activity which were increased in PTB patients and in some of them it remained elevated even after completion of ATT, which may contribute to the development of lung functional abnormalities.[20] The lipid peroxides formed at the primary site may be transferred through the circulation to other organs and tissues and provoke damage by propagating lipid peroxidation.[2] Therefore, high MDA concentrations and low levels of enzymatic antioxidant might be due to excessive lipid peroxidation by ROS in PTB patients.

Some anthropometric parameters were measured in PTB patients before and after two months of chemotherapy. There were significantly lower BMI in PTB patients than in controls. The combination of undernourishment with decreased supplementation of antioxidants enhances ROS generation leading to increased utilization of these compounds. It represents a pathogenic loop that may result in markedly enhanced oxidative stress responsible for inflammation and subsequently weight loss, during pulmonary tuberculosis infection.[21] There were no significantly higher levels of BMI in most of post-treatment PTB patients than pre-treatment in our study. This shows that parameters eventually increased as there was clinical improvement in patients after two months of ATT. The elevated levels of cytokines and enhanced free radical production, although designed to combat the invader, has the potential to damage host tissue.[22] However, the host tissue damage is limited by concurrent enhancement of antioxidant defense in the host. The oxidative stress and host tissue damage is consistent with a number of other studies.[23-26] Oxidative stress increases highly significantly in cavitary pulmonary tuberculosis and the levels of the antioxidants decreased highly significantly with increase in lipid peroxidation.[27]

Serum reactive oxygen metabolite (ROM) levels of active pulmonary tuberculosis cases and follow up cases remains higher than those of the healthy population. The serum ROM levels of active cases also remains higher than those of follow up cases due to which serum total ROM values can be used as an activity criterion in the differentiation of active and follow up cases.[28] Also most of PTB cases were malnourished before beginning ATT supporting, undernutrition is common among tuberculosis patients.[29,30] Undernutrition is associated with severity of TB and may also be a determinant of treatment outcomes[31] and also survival[32]. TB can lead to malnutrition and malnutrition may predispose to TB.

Although, ATT generates some oxidative stress itself as shown in many studies but overall effect is beneficial to overcome respiratory burst and oxidative stress induced by fatal tubercle bacilli after two month treatment even without antioxidant supplement. So, PTB patients can recover faster provided they keep themselves free from other oxidative stress inducers and even without antioxidants supplement who cannot take them. However, the relation with oxidative stress and BMI should be studied in future prospective with interventional trials.

CONCLUSION

Our study showed a negative correlation between oxidative stress and body mass index. It is suggested that antitubercular treatment increases body mass index and decreases oxidative stress even without antioxidant supplement and these physiological improvement is better if other factor of oxidative stress are avoided.

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