Original Article

Assessment of Oxidative Stress Biomarkers and Body Mass Index in Pulmonary Tuberculosis Patients: A Case-Control Study

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Abstract

Background: Pulmonary tuberculosis (PTB) is a major public health concern in most underdeveloped and developing countries. PTB affects the nutritional status of the patients and influences the body mass index (BMI). There is tissue inflammation and free radical burst from activated phagocytes resulting in oxidative stress. The present study was designed to assess the relationship between oxidative stress and body mass index in newly detected pulmonary tuberculosis patients.

Method: This was a case-control study designed to assess oxidative stress parameters such as nitric oxide (NO) and malondialdehyde (MDA) in 40 consecutive newly diagnosed PTB patients and compared with 40 age-matched healthy controls. The nutritional status of the study subjects was measured by calculating the BMI.

Results: The mean BMI was 21.61±3.52 Kg/m^2 in controls and 17.47±1.56 Kg/m^2 in PTB patients and the difference was statistically significant ($p < 0.0001$). The mean levels of MDA (7.65±0.65 nmol/ml) and NO (36.12±1.07 µmol/l) were significantly higher in PTB patients compared to controls (MDA 3.56±0.41 nmol/ml and NO 14.48±0.93 µmol/l).

Conclusions: Increased levels of malondialdehyde and nitric oxide were observed in newly diagnosed PTB patients when compared to controls indicating oxidative stress in PTB. The BMI of these patients was significantly lower than the controls. Thus it is concluded that there is an inverse relationship between oxidative stress and BMI in PTB patients and antioxidant supplementation in addition to nutritional intervention under the National Tuberculosis Elimination Program may help to improve the BMI and promote better recovery in these patients.

Keywords: Pulmonary tuberculosis, Body mass index, Malondialdehyde, Nitric oxide, reactive oxygen species.

Introduction

Pulmonary tuberculosis (PTB) is a chronic infectious disease caused by *Mycobacterium tuberculosis (M.tb)* bacillus, which most commonly affects the lungs but can also involve almost all organs of the body including the gastrointestinal tract.[1] It remains a major global public health problem with increased morbidity and mortality especially in developing and underdeveloped countries.[2]According to Global Tuberculosis Report 2019, 10.0 million new TB cases with an estimated 1.2million TB death among HIV-negative people have been reported worldwide. Estimate suggested that TB affects people of both

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sexes in all age groups but males (aged ⩾15 years)are affected most accounting for 57% of all TB cases.[3]

Mycobacterium tuberculosis bacilli after inhalation, reach lung alveoli where they are engulfed by host macrophages. These intracellular pathogens grow and replicate in host macrophages which later on undergo respiratory burst. During this process, there occurs the generation of huge amounts of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) as an important part of the host defence mechanism essential for the destruction of ingested microorganisms.[4]ROS and RNI are highly toxic molecules and include hydrogen peroxide, nitric oxide, and peroxynitrite.[5] During the normal metabolic process, these ROS and RNI are produced in the cell at basal levels, but they are efficiently neutralized by different antioxidant systems (enzymatic and non-enzymatic) existing in every cell. However, upon infection, ROS and RNI are produced at high concentrations beyond the neutralizing capacity of antioxidants, leading to increased oxidative stress.[6]

Oxidative stress causes peroxidation of membrane lipids leading to alterations in the biological properties of the membrane which in turn may cause impairment of normal cellular function. Malondialdehyde (MDA), a byproduct of lipid peroxidation, has been most commonly used as a biomarker to assess oxidative stress.[7] Nitric oxide is another important biomarker of lipid oxidation often used to study oxidative stress in bacterial infections.[8]

Association between malnutrition and Tuberculosis is well recognized. Malnutrition adversely affects both the innate and adaptive immunity of individuals making them susceptible to a variety of infections such as tuberculosis.[9]Tuberculosis itself can contribute to malnutrition through various mechanisms which include cytokine activation, abnormal protein metabolism and loss of lean tissue as well as body fat reserves. Thus there is a bi-directional relationship between the two. [10] The nutritional status of an individual is most reliably estimated by calculating the Body Mass Index (BMI). An inverse relationship has been observed between BMI and the incidence of TB burden. About 14% reduction has been reported in the incidence of TB per unit increase of BMI. [11]It has been suggested that lower levels of nutritional profiles in pulmonary tuberculosis patients might be associated with a heavy load of free radicals, oxidative stress and lipid peroxidation. [12]

Keeping all these facts in mind, the present study was designed to investigate the levels of oxidative stress biomarkers and BMI in newly detected pulmonary tuberculosis patients.

Material and Methods

Selection of Cases and Controls

Control Group: included 40 normal healthy, age-matched volunteers. Healthy volunteers with their consent were taken from our hospital.

Pulmonary tuberculosis patient Group: The study population consisted of 40 newly diagnosed PTB patients, who attended the National Tuberculosis Elimination Program (NTEP) OPD under the Department of Medicine. The subjects who were enrolled in the study were 18-50 years of age. The diagnosis of pulmonary tuberculosis was made based on clinical symptoms and sputum smear and/or culture positivity for acid-fast bacilli.[13]Written consent was taken from all consecutively enrolled study subjects after informing them about the study protocol. The study was approved by Institutional Ethics Committee(Reference no: IEC/09/2019). Subjects with the coexistence of other diseases such as other respiratory disorders, hypertension, diabetes mellitus, liver diseases, human immunodeficiency virus infection, and cancer were excluded. Other exclusion criteria were age <18 years, age >50 years and pregnancy.

Assessment of oxidative stress markers

Estimation of Nitric oxide (NO) was performed using Griess's reagent method. [14]Griess reagent composed of 1% sulfanilamide solution in 2.5% phosphoric acid and 0.1% Napthylethylene diamine dihydrochloride (NED) solution. It was allowed to equilibrate at room temperature. Thereafter, 50µl of plasma separated from the patient's blood sample was taken in a tube where 450 µl of distilled water and 500 µl of Griess reagent were added. The final reading was determined spectro-photometrically at 540nm.

Malondialdehyde (MDA) was measured by the modified TCA TBA method of Stater et al (1971).[15] To 0.5 ml of the patient's plasma, 3ml of 10% trichloroacetic acid (TCA) was added. Then 1.5 ml of 0.67% thiobarbituric acid (TBA) was added to it after following standard laboratory protocol. Colour intensity was measured spectro-photometrically at 530nm to get the final reading.

Assessment of nutritional status- Nutritional status of the study subjects was measured by calculating the body mass index (BMI) using the standard formula as BMI= Weight (Kg)/Height (m^2). BMI was categorized based on WHO, 2004 classification [16][Table 1].

Statistical analysis: The results of the study were expressed as Mean ± Standard Deviation (SD). The student's unpaired T-test was used to analyze the relationship between case and control groups. P-Value less than 0.0001 was taken as statistically significant. Statistical analysis was done using the statistical package for social science (SPSS) and Graphpad Prism 7.0 version.

Results

When enrolled the mean age of the study population was 28.14±11.23 yrs for control and 32.24±8.52 yrs for PTB patients. There were 40 healthy controls in the study group in which males comprised 60% (24/40) and females were 40% (16/40) whereas out of 40 pulmonary tuberculosis patients,75% (30/40) males and 25% (10/40) females. [Table 2]. The mean height was 155.85±6.36 cm in controls and 156±6.04 in PTB patients while mean weight was observed to be 52.21±6.82 kg and 42.54±4.64 kg in controls and PTB patients respectively showing a significant decrease in body weight in PTB patients as compared to controls [P < 0.0001]. There was highly significant difference (p <0.0001) in body mass index between controls (21.61±3.52 Kg/m^2) and PTB patients (17.47±1.56 Kg/m^2) [Table 2]. There were 24/40 (60%) PTB patients that were undernourished (BMI <18.50) while there was none in the control group.

The mean value of MDA was 3.56±0.41 nmol/ml and 7.65±0.65 nmol/ml in controls and PTB patients respectively. The mean NO level in the study population was 14.48±0.93 µmol/l and 36.12±1.07 µmol/l in controls and PTB patients respectively. The difference in these biochemical parameters compared to controls was found to be statistically highly significant (p <0.0001) [Table 3].

Table 1: Classification of Body mass index (BMI)

Category	Values of BMI (Kg/m^2)
Underweight	<18.50
Normal	18.50 – 24.99
Pre Obese	25.00 – 29.99
Obese	⩾ 30.00

Table 2: Demographic features of the study population

Demographic features	Control	Pulmonary Tuberculosis patients	t value	P-value
No. of participants	40	40	-	-
Age (Years)	28.14±11.23	32.24±8.52	1.83	P > 0.0001
Height (cm)	155.85±6.36	156±6.04	0.10	P > 0.0001
Weight (Kg)	52.21±6.82	42.54±4.64	7.40	P < 0.0001*
BMI (Kg/m^2)	21.61±3.52	17.47±1.56	6.80	P < 0.0001*
Male: Female	24(60%):16(40%)	30(75%):10(25%)	-	0.23 †

Values are expressed as Mean±SD; * =statistically highly significant; † =Not significant

Table 3: Biochemical parameters for estimation of oxidative stress in study population

Parameters	Control (n=40)	Pulmonary Tuberculosis patients (n=40)	t value	p value
MDA (nmol/ml)	3.56±0.41	7.65±0.65	33.63	p < 0.0001*
NO (µmol/l)	14.48±0.93	36.12±1.07	95.90	p < 0.0001*

Values are expressed as Mean±SD; n=number of study participants; * =statistically highly significant.

Discussion

Pulmonary tuberculosis is a multifaceted disease involving several host-mediated responses resulting in an array of oxidative stress. [17] The present case-control study was carried out to assess the association of oxidative stress and BMI in new PTB patients compared to healthy controls and the results showed that the mean Body mass index was decreased significantly in PTB cases (17.47±1.56) as compared to controls (21.61±3.52) indicating thereby that malnutrition is very closely associated with PTB, and probably plays a pivotal role in the pathogenesis of active PTB. Our finding of decreased BMI among PTB cases correlates with the studies conducted in Nigeria, Ethiopia and Zimbabwe.[18-20] One study in individuals with low BMI and latent TB has suggested that diminished circulating levels of protective proinflammatory cytokines (IFN-γ, TNF-α, IL-22, IL-1α, IL-1β, and IL-6) and raised levels of regulatory cytokines (IL-10, TGF-β, IL-5, IL-13) may be an important contributory factor for PTB in these patients.[21] However, the biological mechanism correlating BMI with the risk of developing TB is still not properly comprehended.

To assess the presence of oxidative stress in PTB, we estimated the levels of MDA and NO in our study groups. Our data suggested that there is a significantly

increased level of oxidative stress markers (MDA & NO) in newly diagnosed PTB patients in comparison with healthy controls. This is supported by the fact that infection with mycobacterium tuberculosis leads to increased production of ROS and RNI secondary to phagocyte respiratory burst, thereby creating oxidative stress. [22]Hashni et al (2012) observed 2.5 fold increases in MDA levels in TB patients which is in agreement with the present study.[23]Increased levels of MDA in PTB patients infected with the human immunodeficiency virus have also been noted by Nwanjo and Oze (2007). [24] Our finding of a significant increase in MDA also coincides with the findings of Reddy et al (2004),[25]Talhar et al (2019) [26] and Pawar et al (2011) [27] indicating that PTB patients present with enhanced lipid peroxidation.

We also observed significantly increased levels of NO in PTB patients in comparison to controls and this agrees with the findings of Lamsal et al (2007) who reported significantly raised levels of nitrite in sputum smear-positive PTB cases compared to controls before the commencement of ATT.[28] In another related study the concentration of NO, an important reactive nitrogen species, was higher in all PTB cases with varying bacillary load compared to controls.[29] One of the important mediators of immune homeostasis is NO[30] and significantly raised NO levels were demonstrated in newly diagnosed PTB patients [31] which coincided with the present study. Thus increased oxidative stress in PTB patients in the present study is consistent with the report of previous studies. [25-27, 29, 31] The limitations of this study include relatively smaller sample size and lack of follow-up of these PTB patients.

Conclusion

In conclusion, our study showed increased levels of MDA and NO in newly diagnosed pulmonary tuberculosis patients as compared to normal healthy controls thereby indicating the presence of oxidative stress in PTB. This may be a result of tissue inflammation, increased lipid peroxidation and a heavy load of free radicals. Antioxidant supplementation therefore might help to reduce oxidative stress burden.

In addition, in similarity with previous findings, a negative association between BMI and PTB infection was found in this study thereby supporting the concept that low body weight is associated with an increased risk of acquiring PTB infection. Thus it is suggested that along with better nutrition, supplementation with antioxidants may prove to be a useful adjuvant therapy to routine ATT drugs to reduce oxidative stress burden, improve BMI and promote better recovery of the patients.

Conflicts of interest: None

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References

1. Hardy MA, Schumidek HH. Epidemiology of tuberculosis aboarda ship. *JAMA* 1968; **203**:175.
2. Lyons JG, Stewart S. Commentary: tuberculosis, diabetes and smoking: a burden greater than the sum of its parts. *Int J Epidemiol* 2013; **42**:230–232.
3. World Health Organization. Global Tuberculosis Report 2019. Available at https://www.who.int/tb/publications/global_report/en/ (Accessed on 20 July 2020)
4. Kwiatkowska S, Piasecka G, Zieba M, Piotrowski D. Increased serum concentrations of conjugated diens and malondialdehyde in patients with pulmonary tuberculosis. *Respiratory Medicine* 1999; **93**:272- 6.
5. Gwinn MR, Vallyathan V. Respiratory burst: role in signal transduction in alveolar macrophages. *J Toxicol Environ Health.* 2006;**9**:27–39.
6. Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proceedings of the Proc Natl AcadSci US.* 2000; **97**:8841–8848.
7. Valenzuela A. The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. *Life Sci* 1991; **48**:301-9.
8. Pearl JE, Torrado E, Tighe M, Fountain JJ, Solache A, Strutt T, et al. Nitric oxide inhibits the accumulation of CD4+CD44hiTbet+CD69lo T cells in mycobacterial infection. *Eur J Immunol* 2012;**42**:3267-79.
9. Chandrasekaran P, Saravanan N, Bethunaickan R, Tripathy S. Malnutrition: modulator of immune responses in tuberculosis. *Frontiers in immunology*. 2017;**8**:1316.
10. Macallan DC. Malnutrition in tuberculosis. *DiagnMicrobiol Infect Dis* 1999;**34**:153-157.

11. Lonnroth K, Williams BG, Cegielski P, Dye C. A consistent log-linear relationship between tuberculosis incidence and body mass index. *Int J Epidemiol* 2010;**39**:149e55.
12. Moses AO, Emmanuel OO, Ganiyu AO, Fidelis AA, Dickson AO. Assessment of Antioxidants and Nutritional Status of Pulmonary Tuberculosis Patients in Nigeria. European Journal of General Medicine. 2008; **5**.
13. World Health Organisation Global Tuberculosis Programme, Treatment of Tuberculosis: Guidelines for National Programmes, WHO/CDS/TUBERCULOSIS, World Health Organization, Geneva, Switzerland, 3rd edition, 2003.
14. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR, et al. Analysis of nitrate, nitrite, and nitrate in biological fluids. *Anal Biochem*. 1982; **126**:131e138.
15. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used. *Biochem J*. 1971; **123**:805e814.
16. Who EC. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet (London, England)*. 2004; **363**:157.
17. Bhargava A, Khare NK, Bunkar N, Lenka RK, Mishra PK. Role of mitochondrial oxidative stress on lymphocyte homeostasis in patients diagnosed with extra-pulmonary tuberculosis. *Cell BiolInt* 2016; **40**:166-76.
18. Yeldu MH, Ibrahim Y, Akuyam SA, Danasabe IM, Shehu B, Danjuma M et al. Oxidative stress biomarkers in pulmonary tuberculosis patients in Gombe, North-eastern Nigeria. *Asian Journal of Medical Sciences* 2019;**10**:57-62.
19. Gebrehiot G, Daniel S, Getnet Y and Menon MKC. The non-enzymatic antioxidants and level of oxidative stress of tuberculosis patients in selected treatment centres in Addis Ababa Ethiopia. *J Tuberc Res* 2015; **3**: 63-71.
20. Benova L, Fielding K, Greig J, Nyang'wa BT, Casas EC, da Fonseca MS et al. Association of BMI category change with TB treatment mortality in HIV-positive smear-negative and extrapulmonary TB patients in Myanmar and Zimbabwe. *PLoS One* 2012; **7**: e35948 .
21. Anuradha R, Munisankar S, Bhootra Y, Kumar NP, Dolla C, Kumaran P, et al. Coexistent malnutrition is associated with perturbations in systemic and antigen-specific cytokine responses in latent tuberculosis infection. *Clinical and Vaccine Immunology*. 2016; **23**:339-45.
22. Sudha K, Amareshwara M, Rakesh M. Study of protein oxidation and antioxidants status in pulmonary tuberculosis patients. *International Journal of Pharma and Bio Sciences*. 2011; **2**:104-9.
23. Hashni MA, Ahsan b, Shah SIA, Khan MIU. Antioxidant Capacity and Lipid peroxidation product in Pulmonary Tuberculosis. *Al Amen J. Med. Sci*. 2012; **5**:313-319.
24. Nwanjo HU, Oze GO. Oxidative imbalance and non-enzymic antioxidant status in pulmonary tuberculosis-infected subjects: carcinogenic potential. *Pakistan Journal of Nutrition*. 2007; **6**:590-2.
25. Reddy YN, Murthy SV, Krishna DR, Prabhakar MC. Role of free radicals and antioxidants in tuberculosis patients. *Indian Journal of Tuberculosis*. 2004; **51**:213-8.
26. Talhar SS, Ambulkar PS, Sontakke BR, Waghmare PJ, Shende MR, Pal AK, et al. Oxidative stress and its impact on mitochondrial DNA in pulmonary tuberculosis patients-a pilot study. *Indian Journal of Tuberculosis*. 2019; **66**:227-33.
27. Pawar BD, Suryakar AN, Khandelwal AS. Effect of micronutrients supplementation on oxidative stress and antioxidant status in pulmonary tuberculosis. *Biomedical Research.* 2011; **22**:455-9.
28. Lamsal M, Gautam N, Bhatta N, Toora BD, Bhatta-charya SK, Baral N. Evaluation of lipid peroxidation product, nitrite and antioxidant levels in newly diagnosed and two months follow-up patients with pulmonary tuberculosis**.** *Southeast Asian J Trop Med Public Health.* 2007; **38**:695-703.
29. Mohod K, Dhok A, Kumar S. Status of oxidants and antioxidants in pulmonary tuberculosis with varying bacillary load. Journal of Experimental Sciences. 2011 May 23.
30. Pavanelli WR, Jerley J, Silva N. The Role of Nitric Oxide in Immune Response against Trypanosoma Cruzi Infection. *The Open Nitric Oxide Journal* 2010; **2**:1-6.
31. Wagh V, Rajopadhye S, Mukherjee S, Urhekar A, Modi D. Assessment of oxidative stress in serum of pulmonary tuberculosis patients. *International Journal of Research in Medical Sciences*. 2016; **4**:3328-32.