

Effect of micronutrients supplementation on oxidative stress and antioxidant status in pulmonary tuberculosis.

Bhimrao D. Pawar*, ****Adinath N. Suryakar****, **Archana S. Khandelwal*****

*Department of Biochemistry, ***Department of Pathology, TPCT's Terna Medical College, Nerul, Navi Mumbai, Maharashtra, India.

**Registrar, Maharashtra University of Health Sciences, Nashik, Maharashtra, India

Abstract

During pulmonary inflammation increased amounts of reactive oxygen species (ROS) are produced as a consequence of a phagocytic respiratory burst. One of the manifestations of these free radical mediated processes is lipid peroxidation. Study was under taken to evaluate the level of lipid peroxidation product malondialdehyde (MDA) and levels of antioxidants like vitamin C, vitamin E with total antioxidant status in patients with active pulmonary tuberculosis (PTB). Fifty patients with positive sputum for acid fast bacilli (Ziehl Neelsen (ZN) staining) were divided into two group and 25 healthy controls without any disease were included for this study. 25 patients with active pulmonary TB were treated with Anti tuberculosis therapy (ATT) and remaining 25 cases were treated with antioxidants as an adjuvant therapy along with ATT. Serum levels of MDA, Vit.C, Vit.E and total antioxidant status were measured .Our results showed significantly decreased level of MDA and increased levels of Vit.C, Vit.E and total antioxidant status after six month follow up of antioxidants supplementation along with ATT. This study concludes that antioxidants (micronutrients) supplementation as an adjuvant therapy helps in reduction of oxidative stress and promotes recovery of patients.

Key Words: Pulmonary tuberculosis, oxidative stress, MDA, Vit.C, Vit.E, Total antioxidant status.

Accepted May 192011

Introduction

Tuberculosis (TB) is a global public health problem and it is second to HIV/AIDS as the leading infectious cause of death of adults worldwide [1]. Tuberculosis or TB is an infectious disease caused by acid fast bacillus, *Mycobacterium tuberculosis* (MTB) in humans [2]. Tuberculosis most commonly affects the lungs, where it is called pulmonary tuberculosis (PTB). In lungs, it forms a localized infection after inhalation of bacilli [3]. It can also affect extra pulmonary organs like lymph nodes, bones, joints, subcutaneous tissue, meninges, eyes, kidneys and gastrointestinal tract, where it causes an insidious disease that develops without striking clinical evidence [4].

World Health Organization declared TB to be a global emergency in the year 1993 [5]. India is the highest TB burden country accounting for one fifth of the global incidence. Global annual incidence estimate is 9.1 million cases out of which it is estimated that 1.9 million cases

are from India. India is 17th among 22 High Burden Countries (HBC) in terms of TB incidence rate [6].

MTB is a recognized facultative intracellular bacteria that replicates and persists within macrophages [7]. MTB can induce reactive oxygen species (ROS) production by activation of macrophages [8-10], which is an important part of host defense against mycobacterium. But generation of ROS may promote tissue injury and inflammation in affected individual. This further contributes to immunosuppression [11,12], particularly in those with impaired antioxidant capacity.

Due to poor dietary intake of micronutrients, malabsorption, poor immunity and altered metabolism, an increased free radical generation is observed in patients with TB. Combined effect of these causes oxidative stress which is also responsible for pathogenesis of tuberculosis. It is not known that antioxidants supplementation along with anti-tuberculosis therapy is useful to reduce oxidative stress in patients with tuberculosis. Hence, this study was under-

taken to evaluate the effect of antioxidants supplementation along with ATT on oxidative stress and antioxidants level in patients with PTB.

Material and Methods

Fifty outpatients with newly diagnosed active PTB in the age group of 15-60 years and 25 age and sex matched healthy control individuals were selected from Dr. V. M. Govt. Medical College and Chatrapati Shivaji Maharaj Sarvopchar Rughalaya, Solapur and Terna hospital and research center, Nerul, Navi Mumbai. Present study was approved by institutional ethical committee and selected subjects were informed about this research project and included in the study with their consent and without any economical burden to them.

All the patients were sputum smear positive for acid fast bacilli (AFB) by direct microscopy based on Ziehl Neelsen (ZN) staining, microbiological culture and radiology confirmed pulmonary infection.

Patients with renal diseases, endocrine dysfunction, hepatic diseases, diabetes mellitus, extra pulmonary TB, drug resistance at the baseline or during the follow up study, PTB with HIV/AIDS infection, non tuberculous pulmonary infection, pregnancy, previous history of treatment of tuberculosis, viral or bacterial infections, hypertension, chronic alcoholic etc were excluded from the study. Patients who did not take their medication regularly were also dropped from the study.

Subjects were grouped as follows:

Group I : control group included 25 healthy subjects

Group II : Included 25 pulmonary tuberculosis patients receiving ATT regimen only.

Group III : Included 25 Pulmonary tuberculosis patients receiving ATT regimen and one tablet of antioxidant A-Z (Alkem Lab Ltd) daily.

Antituberculous treatment was given as per the guideline of Revised National Tuberculosis Control Programme (RNTCP) and DOTS strategy and doses were adjusted as per body weight of patients.

Composition of A-Z antioxidant tablet:

Vitamin C-100mg, Niacinamide -50mg, Vitamin E acetate-25mg, Calcium pantothenate-12.5mg, Thiamine mononitrate-10mg, Riboflavin-10mg, Pyridoxine HCl-3mg, Vitamin A acetate-5000IU, Folic acid-1mg, Cyanocobalamin-5µg, Zinc oxide -15mg, Cupric oxide-2.5mg,

Magnesium chloride-1.4mg, Chromium chloride-65µg, Sodium selenate-60µg.

Under all aseptic precautions, 5 ml fasting venous blood sample was collected in plain bulb from all the control and PTB patients at baseline i.e. before starting anti tuberculosis treatment and after 7th day, 1st, 2nd, 3rd and 6th months of ATT. Blood samples were centrifuged at 5000 rpm for 10 min and serum was separated after clot retraction. The serum was analyzed at the earliest for levels of Malondialdehyde (MDA) [13], Vitamin C [14], Vitamin E [15] and Total Antioxidant Status (TAS) [16] on the same day.

The results were compiled and the statistical analysis was done by using 'T' test. Values were expressed as mean ± standard deviation (SD).

Results

As shown in table, serum level of MDA were significantly elevated ($p < 0.001$) in group II and group III in all newly diagnosed pulmonary tuberculosis patients as compared to control group. Level of MDA gradually decreased in group II after six months of ATT as compared to baseline level ($t = -21.05$, $p < 0.001$). Level of MDA also decreased in group III after six months of ATT and antioxidant supplementation as compared to baseline level ($t = -34.01$, $p < 0.001$). But better improvement was found in group III as compared group II ($t = -2.32$ and $p < 0.05$) after six months of treatment. Serum levels of vitamin C, vitamin E and TAS were significantly low ($P < 0.001$) before starting the treatment in group II and group III as compared to control group. Levels of vitamin C ($t = 19.91$, $p < 0.001$), vitamin E ($t = 21.65$, $p < 0.001$) and TAS ($t = 18.09$, $p < 0.001$) gradually increased in group II following ATT treatment after six months as compared to baseline levels. Also, Levels of vitamin C ($t = 28.38$, $p < 0.001$), vitamin E ($t = 20.58$, $p < 0.001$) and TAS ($t = 26.19$, $p < 0.001$) gradually increased in group III following ATT and antioxidant supplementation after six months as compared to baseline levels.

But levels of vitamin C ($t = 5.24$ and $p < 0.05$), vitamin E ($t = 3.29$ and $p < 0.05$) and total antioxidant status ($t = 11.28$ and $p < 0.05$) of group III were slightly higher than group II after six months of treatment.

Above results show better improvement in group III (ATT + antioxidant supplementation) as compared to group II (ATT only) after six months of treatment. This helps in outcome of patients and reduction in oxidative stress.

Table 1. Values of MDA, Vitamin C, vitamin E and TAS in serum of controls & Pulmonary tuberculosis patients during and after treatment.

Parameters	Control (Group I)	Group	Baseline	After treatment				
				7 th Day	1 st Month	2 nd Month	3 rd month	6 th Month
MDA (µmol/l)	3.76±0.79	II	8.39*±1.03	8.30±1.03	8.06±1.01	6.67±0.78	5.87±0.64	4.51 [□] ±0.66
		III	8.37*±1.02	8.26±0.99	7.82±1.07	6.35±0.95	5.03±0.92	4.09 ^{#□} ±0.66
Vitamin C (Mg/l)	14.54±0.77	II	7.83*±1.33	7.91±1.26	8.86±1.20	10.80±1.06	12.09±0.81	12.86 [□] ±0.67
		III	7.84*±1.13	7.97±1.20	9.12±1.19	11.32±1.19	12.80±0.70	14.02 ^{#□} ±0.89
Vitamin E (Mg/l)	13.62±0.74	II	7.71*±1.48	7.82±1.44	8.74±1.37	10.76±1.13	12.18±0.94	12.97 [□] ±0.77
		III	7.91*±1.4	8.18±1.04	9.30±0.88	11.84±0.84	13.01±0.48	13.53 ^{#□} ±0.33
TAS (µmol/l)	1719.76±99.62	II	1189.86*±119.88	1190.22±119.70	1192.55±119.68	1200.85±118.83	1212.78±101.90	1403.14 [□] ±99.65
		III	1187.19*±104.41	1190.68±104.40	1227.92±92.21	1261.79±82.08	1360.63±93.75	1666.62 ^{#□} ±60.90

Values are expressed as Mean ± SD,

*Indicates $p < 0.001$ as compared to control

□ Indicate $p < 0.001$ compared to baseline

Indicate $p < 0.05$ as compared to Gr.II after six month of treatment

Discussion

Tuberculous bacilli induce oxidative substances such as reactive oxygen species (ROS) derived from free radicals, which in turn can promote tissue injury and inflammation. 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 pulmonary TB [17]. Antioxidants scavenge free radicals and suppress the actions of ROS and thus protect the host from tissue inflammation. Ascorbic acid

and alpha tocopherol act as potent and probably the most important hydrophilic and lipophilic antioxidants respectively. Reduced levels of vitamin C and E are associated with an impaired immune response [18].

In many studies, concentration of MDA, product of lipid peroxidation was found to be significantly Higher [19, 20] and vitamin C [20-22], E [20-22] and TAS [23, 24] significantly lower in newly diagnosed tuberculosis patients than healthy controls and associated with clinical severity. There is inverse correlation between MDA and Vitamin C

and E levels. These results show an imbalance between free radicals and antioxidants in tuberculosis patients. Our findings are also same as above.

Vijayamalini M et al. [17] reported significantly increased level of thiobarbituric acid reactive substance (TBARS), a marker of lipid peroxidation in 30 newly diagnosed and untreated pulmonary tuberculosis patients as compared with healthy matched controls, while plasma levels of non enzymatic antioxidants vitamin C and E were significantly decreased. Higher level of malondialdehyde in tuberculosis patients indicates oxidative stress and more lipid peroxidation and lower levels of antioxidants. This is responsible for pathogenesis and severity of tuberculosis.

Janiszewska et al. showed that plasma MDA concentration in patients with pulmonary tuberculosis were significantly higher before and after 1 month and 2 months of treatment with antituberculosis drugs in comparison to the control group [25].

Lamsal et al. [26] observed serum MDA and nitrite levels were significantly higher ($p=0.0001$) and plasma levels of vitamin C and E were significantly lower ($p=0.001$) before commencement of ATT in sputum smear positive cases of pulmonary tuberculosis than controls. But serum MDA and nitrite levels decreased significantly and plasma level of vitamin C significantly increased after 2 months of ATT as compared to before ATT. No change was observed in plasma vitamin E before and after ATT. He suggested elevated levels of MDA and nitrite with concomitant depressed vitamin C and E levels before commencement of ATT indicative of lipid peroxidation and oxidative stress. But our results show serum level of Vitamin C as well as E significantly increased and level of MDA decreased significantly after six months of antioxidants supplementation. This helps in the outcome of tuberculosis patients. The response of immune system to tuberculosis infection depends critically on interaction between monocytes, macrophages, T cell lymphocytes and various cytokines released by them. Micronutrients (A-Z tablet) may improve T-cell mediated immune response in the body. This is accomplished by increased production of interleukin-2 (IL-2) leading to increased proliferation of T- cells. This also reduces production of Prostaglandin-2, T-cell suppressive factor. Micronutrients protect cells from deleterious effects of free radicals generated during disease conditions or natural body metabolism. In pulmonary TB patients micronutrients increase effectiveness of ATT which helps in reducing lesion area in lung and improves the outcome of patients.

Kowalski L et al. found decrease in plasma antioxidative activity in patients with tuberculosis as compared to the controls before as well as after therapy with tuberculostatic drugs. He observed patients taking vitamins C and

E (after 1-month tuberculostatic therapy) showed increase in plasma antioxidant activity [24].

Total antioxidant status (TAS) of an individual is a function of dietary, enzymatic and other systemic antioxidants and is therefore an indicator of the free radical load. Some researchers observed that total antioxidant status in tuberculosis patients was lowered significantly as compared to control and level rises during and after ATT [23, 24]. Reddy et al. [27] reported that the serum level of TAS was significantly low in untreated and under treatment patients of tuberculosis in comparison to normal healthy volunteers. Level of TAS gradually increases following ATT. However, our result shows better improvement after antioxidants supplementation as an adjuvant therapy.

This study showed increased level of MDA and decreased levels of non enzymatic antioxidants i.e. vitamin C (water soluble) and E (lipid soluble) and TAS in tuberculosis patients as compared with controls. This indicates oxidative stress in TB patients. However, antioxidants supplementation for six months as an adjuvant therapy increases the levels of vitamin C and E as well as TAS and lowers the MDA level. This suggests that antioxidants supplementation reduces oxidative stress, effectiveness of ATT and helps in outcome of tuberculosis patients.

Acknowledgement

I am thankful to Mr. Behera Abhiram for his help in statistical calculations. I also would like to acknowledge the kind cooperation extended by the staff of biochemistry, TB and Chest and PSM department of Dr. V. M. Govt. Medical College, Solapur and TPCT's Terna medical college, Nerul Navi Mumbai.

References

1. Global tuberculosis control: a short update to the 2009 report is a short update to the WHO report on global tuberculosis (TB) control that was published in March 2009 based on data collected from July to September 2009. The threat of TB.
2. Kumar V, Abbas AK, Fausto N, Richard N. (2007). Robbins basic pathology (8th ed.). Saunders Elsevier. pp. 516–522.
3. Moses AO, Emmanuel OO, Ganiyu AO, Fidelis AA, Dickson AO. Assessment of antioxidants and nutritional status of pulmonary tuberculosis patients in Nigeria. *Eur J Gen Med* 2008;5 (4): 208-211.
4. Hardy MA, Schumidek HH. Epidemiology of tuberculosis aboard a ship. *JAMA*. 1968;203 (3):175-179.
5. World health organization. TB-a global emergency. WHO Report on TB epidemic. WHO/TB/94.177. WHO Geneva, Switzerland; 1994.

6. TB India 2009, RNTCP status report, central TB division, DGHS, ministry of health and family welfare, Nirman Bhavan New Delhi. 2009. P-9.
7. Kaur K, Kishan J, Bedi GK, Ahiet RS. Oxidants stress and antioxidants in pulmonary tuberculosis. Chest Meeting Abstracts 2005;128:397S-b.
8. May ME, Spagnuolo PJ. Evidence for activation of a respiratory burst in the interaction of human neutrophils with Mycobacterium tuberculosis. Infect Immun 1987; 55:2304–2307.
9. Attwood EM, Weich DJ, Oosthuizen JM. The influence of carbon particles on the concentration of acid phosphatase and lysozyme enzymes within alveolar macrophages during the killing and degradation of Mycobacterium bovis. Tuber Lung Dis 1996; 77: 341–7.
10. Kuo HP, Ho TC, Wang CH, Yu CT, Lin HC. Increased production of hydrogen peroxide and expression of CD11b/CD18 on alveolar macrophages in patients with active pulmonary tuberculosis. Tuber Lung Dis 1996; 77:468–75.
11. Jack CI, Jackson MJ, Hind CR. Circulating markers of free radical activity in patients with pulmonary tuberculosis. Tuber Lung Dis 1994; 75:132–7.
12. Grimble RF. Malnutrition and the immune response. 2. Impact of nutrients on cytokine biology in infection. Trans R Soc Trop Med Hyg 1994; 88: 615–9.
13. Kei Satoh. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chemica Acta 1978; 90; 37 – 43.
14. Caraway WT. Carbohydrates in Tietz N. W. Ed., Fundamentals of clinical chemistry. W.B.Saunders Company 2000; 173-176.
15. Baker and Frank, Varleys Practical Biochemistry, 1988, 6th edition. Heinemann professional publishing P-902-903.
16. Iris F.F. Benzie and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of ‘antioxidant power’: the FRAP assay. Analytical Biochemistry; 1996; 239; 70- 76.
17. Vijayamalini M, Manoharan S. Lipid peroxidation, vitamins C, E and reduced glutathione levels in patients with pulmonary tuberculosis. Cell Biochem Funct. Jan-Feb 2004; 22(1):19-22.
18. Chandra RK. 1990 McCollum Award lecture. Nutrition and immunity: lessons from the past and new insights into the future. Am J Clin Nutr. May 1991; 53(5):1087-1101.
19. Deveci F, Ilhan N. Plasma malondialdehyde and serum trace element concentrations in patients with active pulmonary tuberculosis. Biological trace element research, 2003Oct; 95(1):29-38.
20. Madebo T, Lindtjorn B, Aukrust P, Berge RK. Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia. Am J Clin Nutr 2003; 78:117- 122.
21. Rwangabwoba JM, Fischman H, Semba RD. Serum vitamin A levels during tuberculosis and human immunodeficiency virus infection. Int J Tuberc Lung Dis, 1998; 2:771-3.
22. Panasiuk AV, Penenko OR, Kuz'menko IV, Suslov EI, Klimenko MT, Kuznitsa NI, et al. Vitamin E and its structural analogs in tuberculosis. Ukr Biokhim Zh 1991; 63:83-8.
23. Wiid I, Seaman T, Hoal EG, Benade AJ, Van Helden PD. Total Antioxidant Levels are Low During Active TB and Rise with Anti-tuberculosis Therapy. IUBMB Life.2004 Feb; 56(2): 101-106.
24. Kowalski J, Janiszewska-Drobińska B, Pawlicki L, Cegliński T, Irzmanski R. Plasma antioxidative activity in patients with pulmonary tuberculosis. Pol Merkur Lekarski. 2004 Feb; 16(92):119-22.
25. Janiszewska-Drobińska B, Kowalski J, Błaszczyk J, Kedziora J, Kaczmarek P, Ciećwierz J. Estimation of plasma malonyl dialdehyde concentration in patients with pulmonary tuberculosis. Pol Merkur Lekarski 2001 Oct; 11(64):310-3.
26. Lamsal M, Gautam N, Bhatta N, Toora BD, Bhattacharya 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 Jul; 38(4):695-703.
27. Reddy YN, Murthy SV, Krishna DR, Prabhakar MC. Role of free radicals and antioxidants in tuberculosis patients. Indian J Tuberc 2004; 51:213-218.

Correspondence:-

Bhimrao D. Pawar
Department of Biochemistry,
TPCT's Terna Medical College,
Nerul, Navi Mumbai, 400706
Maharashtra, India

