To Evaluate the Effect of Vitamin E Therapy on the Oxidative Stress Markers (Nitric Oxide, SOD, Glutathione Peroxidase) & Vitamin E Levels in Pulmonary Tuberculosis Patients

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ABSTRACT

BACKGROUND

Severe oxidative stress has been reported in TB patients because of infection associated with malnutrition and poor immunity. Mycobacteria can induce reactive oxygen species (ROS) production by activating phagocytes, and enhanced ROS production may promote tissue injury and inflammation. We wanted to compare the effect of antioxidant administration in the outcome of ATT treatment between the test and the control group.

METHODS

This perspective study was conducted in the Departments of Biochemistry and Chest Medicine, CMC & Hospital. Hundred patients (fifty controls and fifty tests) who were diagnosed as pulmonary tuberculosis and started on DOT therapy under RNTCP during this period were included in the study. Each participant in the study was subjected to the following test at the first visit, 2nd month and 6th month follow up (biochemical markers Nitric oxide, SOD, Glutathione Peroxidase and Vitamin E levels). Statistical analysis was done using SPSS version.

RESULTS

The results were based on four categories (male / female, alcoholic / non-alcoholic, smoker / non-smoker, and younger / older age group). Females had responded better with greater fall in percentage of nitric oxide values (69 %) than males (64.1 %). The mean of SOD activity (277.5 + / - 31.5) was more in smokers than non-smokers (261.3 + / - 36.0) & percentage fall of nitric oxide in smokers (65 %) & non-smokers (67 %). In alcoholics the percentage fall of nitric oxide (68.3 %) was higher with more SOD activity (Mean 278.7 + / - 27.6) than non-alcoholics (Mean 256 + / - 38.0) indicating a positive correlation of smoking & alcoholism with tuberculosis. Younger age group responded better with more fall in the percentage of nitric oxide (67 %) & mean SOD activity (265.8 + / - 30.1) than older age group.

CONCLUSIONS

Antioxidant supplementation reduces oxidative stress, improves the effectiveness of ATT therapy, and thus helps in improving the outcome in pulmonary tuberculosis.

KEY WORDS

Pulmonary TB, ATT (Anti-Tubercular Treatment), Antioxidants & Free Radicals

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BACKGROUND

Tuberculosis is a global public health problem & is responsible for more than 2 million deaths each year.[1] Pulmonary tuberculosis is an infectious and contagious disease which apparently develops under conditions of a deficient immunologic response. The immune system requires a wide variety of nutrients to function adequately, and some studies suggest that nutritional supplementation may represent a novel approach for a fast recovery.[2] Tuberculosis is also associated with various socio-economic factors and often occurs in populations suffering from poverty, poor housing, and economic deprivation. These are the major factors predisposing to poor nutritional status and impaired immune function.[3,4,5] Malnutrition and tuberculosis are both problems of considerable magnitude in most of the underdeveloped regions of the world. Malnutrition may predispose to the development of clinical disease or tuberculosis can contribute to malnutrition[6]. Before the advent of anti-tuberculosis chemotherapy, a diet rich in calories, proteins, fats, minerals and vitamins were generally considered to be an important, if not essential factor in the treatment of tuberculosis. Nutritional status determines normal health and functioning of the all the systems in the body, including immune system which is responsible for host resistance to various infectious diseases.[7] Furthermore, the reactivation of latent or previously sub-clinical TB infection is 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 nutritional status, since this can help prevent or modify many complications of diseases and also help in making projections of the interaction of nutritional status on the clinical course of the disease.[8] Three antioxidants that were significantly reduced in tuberculosis patients are glutathione, ascorbic acid and alpha tocopherol, which are important in regenerating a redox cycle.[9.10.11] Thus a combined decrease in these antioxidants may markedLy decrease antioxidant capacity in these patients. Accordingly, the deficiency of these antioxidants may markedLy increase oxidative stress, possibly adversely affecting the immune response and predisposing to drug toxicity.[12]

An alarming epidemic of tuberculosis has followed due to the rapid rise in HIV / AIDS cases, particularly in developing countries. *Mycobacterium* can induce Reactive oxygen species (ROS) by activating phagocytes[13.14,15] and although an important part of host defence mechanism against mycobacterium, enhanced ROS generation may promote tissue injury and inflammation. This further contributes to immunosupression[16,17,18], particularly in those with impaired antioxidant capacity, such as HIV infected individual.[19,20,21] Reactive oxygen species 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.[22] Free radicals are thought to play a major role in the aetiology of a wide variety of diseases including atherosclerosis, respiratory tract, and neurodegenerative disease, inflammatory bowel disease, cancer and in aging[23,24]. ROS (Reactive Oxygen Species) has been found to be an important contributing factor to lower the concentration of antioxidants in tuberculosis patients. In fact, the combination of malnutrition leading to decreased "supplementation" of antioxidants and enhanced ROS generation leading to increased utilization of these compounds may represent a pathologic loop, which results in markedly enhanced oxidative stress during tuberculosis infection.

Three of the antioxidants that were significantly found to be reduced in tuberculosis patients are Glutathione, ascorbic acid & α tocopherol, which are integral components of a regenerating redox cycle.[25] Accordingly the combined deficiency of these antioxidants markedly increases oxidative stress, possibly adversely affecting the immune response and predisposing to the drug toxicity. Moreover, water soluble antioxidants such as glutathione & vitamin C, and the lipid soluble vitamin E may act synergistically to protect cells from oxidative stress induced damage.[26] Also the dietary supplementation of vitamin E can significantly increase the LDL of vitamin E and in turn, confers significant protection against oxidative stress.[27] Human tissues are protected from the oxidative damage by a variety of mechanisms including small molecular weight antioxidants like vitamin C and E.[28] Vitamin E is one of the major, antioxidant found in the membranes and is found to improve the immune system. Short term vitamin E supplementation is known to improve immune responsiveness.[29] Reduced levels of vitamin C and E are associated with an impaired immune response.[30] 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 lung function abnormalities.[31] Therefore, the supplementation with vitamins may prove to be beneficial in oxidative stress conditions like tuberculosis, thereby improving the effect of ATT drugs.

METHODS

This is a one-year prospective interventional study conducted in the Departments of Biochemistry and Chest Medicine, CMC & Hospital, after obtaining ethical clearance & taking the consent of the patients. Hundred patients diagnosed of pulmonary tuberculosis were started on DOT's therapy under RNTCP regime in the institution were included in the study. Fifty odd numbers were designated as test group who received Vitamin E (200 mg OD) & other fifty even numbers were designated as control group (placebo). The patients were followed up at 2nd & 6th month after initial visit. The informed consent was taken for patients which were enrolled in the study and then estimated for the various biochemical parameters.

5 mL of venous blood was drawn & collected in EDTA and plain vials for further estimation. The collected samples were separated into plasma, serum and haemolysate for the nitric oxide, superoxide dismutase, glutathione peroxidase and vitamin C estimation. Nitric oxide was measured in plasma using the Griess reaction[32]. This system detects nitrite ion (NO^{2-}) in a variety of biological & experimental liquid matrices such as plasma, urine & tissue culture medium.

Reagents (Nitric Oxide)

1. 0.1 % NED Solution – (N-1-napthylenediamine dihydrochloride)
2. 1 % Sulphamide solution in 5 % Orthophosphoric acid

Working Griess Reagent

Griess reagent was prepared freshly by adding equal volumes of of reagent 1 and reagent 2.

Superoxide Dismutase

Superoxide Dismutase levels were determined in red blood cells (RBC) by colorimetric assay using Ransod kit from Randox which employs reaction between xanthine oxidase and INT to form coloured complex.

Reaction Principle-

Xanthine → XOD → Uric acid + 02-
INT → 0^{2-} → Formazan dye
OR
02 - + 02 - + 2H + → SOD → 02 + H_20

Sample Preparation - EDTA whole blood 0.5 mL was centrifuged for 10 mts. at 3000 rpm and plasma aspirated. The erythrocytes were washed for 4 times with 3 mL of 0.9 % NaCl solution, centrifuging for 10 mts. at 3000 rpm after each wash. The washed, centrifuged, erythrocytes were than made up to 2.0 mL with cold redistilled water, mixed and left to stand at 4 °C for 15 mts. The Lysate was diluted with 0.01 mol / L of phosphate buffer (PH 7.0), so that percentage inhibition falls between 30 % and 60 %. A 25-fold dilution of lysate was done (10 µL of sample dissolved in 240 µL of phosphate buffer).

Alpha Tocopherol (Vitamin E)

Alpha tocopherol was estimated by colorimetric method using Emmeric Engel reaction of Baker and Frank, 1968.[33]

Principle - Serum tocopherol was measured by its reduction of ferric to ferrous ions which then formed a red complex with α, α' dipyridyl. Tocopherols & carotenes were first extracted into xylene & than absorbance read at 460 nm to measure carotenes. A connection for carotenes made after adding ferric chloride & reading at 520 nm.

Reagents

1. Absolute Alcohol, aldehyde Free.
2. Xylene.
3. Alpha, alpha, dipyridyl, 1.2 q / L in n propanol.
4. Ferric Chloride solution, 1.20 g $FeCl_3.6H_2O$ / L in ethanol. Kept in a brown bottle.
5. Standard solution of DL – alpha tocopherol, 10 mg / L in ethanol.

Glutathione Peroxidase (GPx)

Glutathione peroxidase was estimated by Ransel kit of Randox Laboratories. Based on the method of Paglia & valentine, 1967.

Principle - Glutathione peroxidase (GPx) catalyses the oxidation of glutathione (GSH), by cumene hydro peroxidase. In the presence of glutathione reductase (GR) and NADH, the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance is measured at 340 nm.

Reaction –

$2GSH + ROOH \rightarrow GPx \rightarrow ROH + GSSG + H_2O$
$GSSG + NADPH + H^+ \rightarrow GR \rightarrow NADP^+ + 2\ GSH$

Statistical Analysis

All the collected Data was entered in the computer and analyzed using SPSS version 15. The statistical test used were Independent T test, repeated Anova analysis, Chi square test.

RESULTS

Hundred patients were enrolled in the study. All the odd numbers were grouped as "TEST" who were given vitamin E (200 mg OD) along with ATT & the even numbers were grouped as "CONTROL". The following were the significant outcomes of the study:

1. At the last evaluation (6 months) the percentage increase of SOD activity was more in the males (35 %) (mean 250 + / - 40.5) as compared to females (28 %) (Mean 247 + / - 46.1) in the test group, shows that males responded more with SOD activity in response to stress
2. The percentage decrease of nitric oxide value was more in the non-smoker (66.6 %) group than smokers (65.9 %). It showed that smoking had a positive correlation with Tuberculosis outcome (non-smokers respond better to treatment than smokers) as in (Table 2). This also corroborated with the higher fall in nitric oxide in females as most of the smokers were males.
3. The percentage activity of SOD was more in smoker (38 %) than non-smokers (35 %), indicating more inflammatory damage in smokers than non-smokers.
4. The mean of Glutathione peroxidase activity was more in the non-smoker group at the end of six months, as compared to the control in the test group. No positive correlation was found with smoking.
5. The percentage increase of vitamin E was more in Non-smokers (22 %) as compared to smokers (10 %), indicating a positive correlation between better responses in non-smoker than smokers.
6. The % decrease of nitric oxide value was more in the non-alcoholic group (14.7 %) than in alcoholic group (10.0 %) at the end of six-month evaluation, indicating a positive correlation of TB and alcohol.
7. The percentage increase of activity in SOD and glutathione peroxidase was more in the non-alcoholic group (10.5 % & 26.0 %) as compared to alcoholic group (10.0 & 21.0), indicating a positive correlation between alcohol and TB pathogenesis
8. In the age group wise comparison, the younger age group (18 - 39) yrs. showed a significant decrease in the nitric oxide value (% decrease 66.8) as compared to the older

group (% decrease is 63.9), indicating a better outcome in younger age group as compared to older age group

9. The % increase of activity of SOD and Glutathione peroxidase were found to be more in younger age (SOD 37.6 % & Glutathione Peroxidase 71.2 %) as compared to older age group (SOD 32.4 % & Glutathione peroxidase 69.8 %), indicating a better response in younger age group.

Parameters	Male (%) (n = 56)	Female (%) (n = 44)
Nitric Oxide	64.1	69.0
Superoxide Dismutase (SOD)	38	35
Vit. E Levels	45	43.5
Parameters	**Smoker (%)**	**Non-Smoker (%)**
Nitric Oxide	65	66.6
Superoxide Dismutase (SOD)	38.0 (Mean 277.5 + / - 31.5)	35.0 38.0 (Mean 261.3 + / - 36.0)
Vit. E Levels	45.1	43.8
Parameters	**Alcoholic (%)**	**Non-Alcoholic (%)**
Nitric Oxide	40.9	44.9
Superoxide Dismutase (SOD)	36.0 (Mean 278.7 + / - 27.6)	37.4 (Mean 256 + / - 38.0)
Vit. E Levels	45.6	43.1

Table 1. Outcome in Different Categories in Our Study

Study	Year	Controls	Test
Madhab et al[41]	2007	32.8 ± 11.9	47.1 ± 18.4
Mohod et al[38]	2011	0.19 ± 0.05	0.60 ± 0.10
Akiibinu et al[37]	2011	14.9 ± 9.6	8.4 ± 7.0
	Nitric	**Oxide (µM / L)**	
Reddy et al[42]	2004	74. 58 ± 6.1	56.4 ± 5.5
Kaur et al[43]	2005	24.04 ± 6.87	18.83 ± 5.1
Akiibinu et al[37]	2011	26.3 ± 10.0	18.0 ± 8.0
	Superoxide	**Dismutase (U / mL)**	
Madhvi et al[44]	2009	1.27 ± 0.10 (mg / dL)	0.83 ± 0.01 (mg / dL)
Madhab et al[41]	2007	32.8 ± 11.9 (mg / dL)	47.1 ± 18.4 (mg / dL)
Pawar et al[40]	2011	13.62 ± 7.91 (mg / L)	7.91 ± 1.4 (mg / L)
Johnkennedy et al[39]	2011	1.64 ± 0.41 (mg / dL)	0.84 ± 0.31 (mg / dL)
	Vitamin E (Alpha	**Tocopherol mg / dL)**	

Table 2. Comparable Results from Other Studies

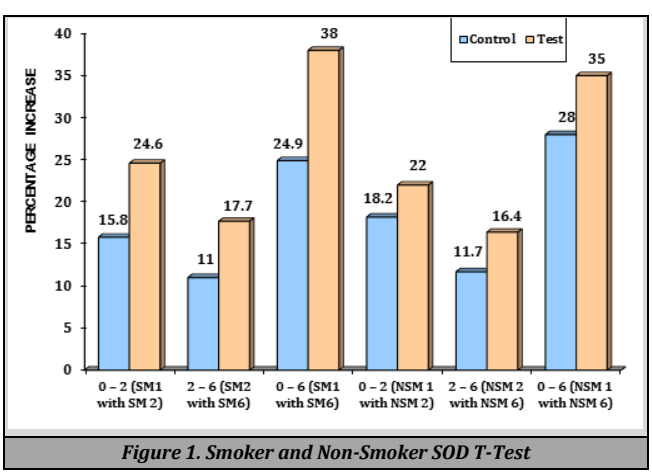

Figure 1. Smoker and Non-Smoker SOD T-Test

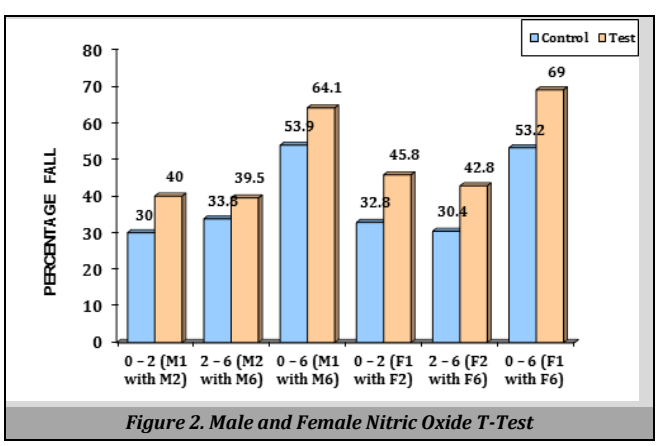

Figure 2. Male and Female Nitric Oxide T-Test

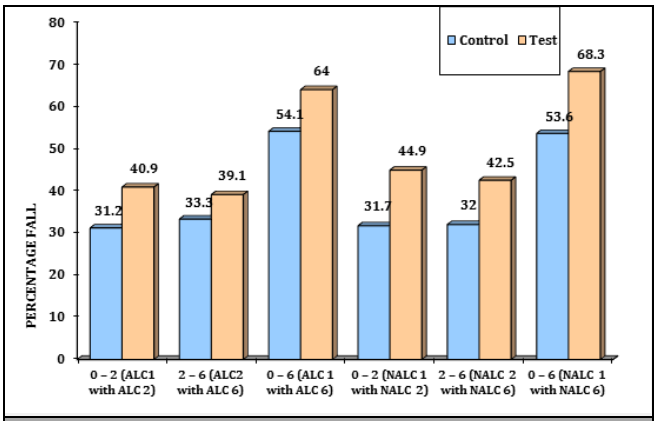

Figure 3. Alcoholic and Non-Alcoholic Nitric Oxide T-Test

DISCUSSION

At the end of last evaluation (6 months), the percentage increase of SOD activity was more in the males as compared to females in the test group. Males responded more with SOD activity in response to stress (Male 277.8 + / - 26.0) & (Female 252.9 + / - 40.9). The mean SOD activity was found to be higher in smokers (277.5 + / - 31.4) than non-smokers (261.3 + / - 36.0) indicating SOD as a marker of inflammation. The low SOD activity leads to both oxidative damage & activation of mediators of inflammation. The average increase in the mean of SOD in test group correlates well with the role of antioxidants in pulmonary inflammation which correlates well with Akiibinu et al.[34]

The statistically significant decrease of nitric oxide value was more in the non-smoker group than smokers indicating that smoking had a positive correlation with Tuberculosis outcome (non-smokers respond better to treatment than smokers) (p value 0.0). This also corroborated with the higher fall in nitric oxide in females as most of the smokers were males in accordance in accordance with Mohod et al[35] & Akiibinu et al.[34]

There is positive correlation of Tuberculosis & Alcohol, as the percentage fall of Nitric oxide value was more in non-alcoholics (68.3 %) rather than alcoholic (64.0 %) at the end of six-month evaluation, which are in agreement with Mohod et al.[35] The vitamin E levels were increased in the control group when compared to test, which shows a positive correlation between decrease antioxidant status in pulmonary tuberculosis & improved status after the treatment.

The percentage increase of vitamin E level was more in non-smokers (45.1) as compared to smokers (43.8), indicating a positive correlation between better responses in non-smoker than smokers. The younger group (0.7814 + / - 0.08) showed improved vitamin E levels in the test group than the older age group (0.7537 + / - 0.067) indicating a decrease antioxidant status in pulmonary Tuberculosis and improved status after treatment. The younger age group responded better to treatment which is in agreement with John Kennedy et al[36] & Pawar et al.[37]

CONCLUSIONS

Pulmonary tuberculosis patients exhibit oxidative stress which is depicted by the change in levels of various markers of oxidative stress (chemical and enzymatic). Along with this, there are decreased levels of non-enzymatic antioxidants (Vitamin E). However, antioxidant (Vitamin E) supplementation for two months as an adjuvant to DOT's therapy not only improves its level, but also the antioxidant status of the pulmonary tuberculosis patients. The results suggest that antioxidant supplementation reduces oxidative stress, improves the effectiveness of ATT therapy and thus helps in improving the outcome in pulmonary tuberculosis. Hence antioxidant supplementation may have a role in faster recovery. Based on the results of our study, large clinical trials are indicated and supplementation with antioxidant vitamins like vitamin E / vitamin C as an adjunct to anti-tuberculosis treatment is advisable.

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