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Original Article

Immunological correlation of oxidative stress markers in tuberculosis patients

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ABSTRACT

Severe oxidative stress has been reported in tuberculosis patients because of malnutrition and poor immunity. Knowledge of the antioxidant status and its relation to lipid peroxidation in tuberculosis patients is scarce, particularly in developing countries. The purpose of this study was to investigate the serum lipid peroxidation products and total antioxidant capacity and to correlate with C-reactive protein (CRP) levels in tuberculosis patients. Methodology: The subjects for this study comprised of normal human volunteers (n=50) and tuberculosis patients (n=50) untreated with anti-tuberculosis therapy. In a cross-sectional study, we evaluated serum malondialdehyde (marker of lipid peroxidation), ferric reducing antioxidant power assay (marker of total antioxidant capacity) and CRP in tuberculosis patients and in healthy control subjects. Results: Total antioxidant capacity was significantly lower in tuberculosis patients than in healthy controls. Tuberculosis patients had higher malondialdehyde concentrations and high CRP than did control subjects. High malondialdehyde concentrations positively correlated with clinical severity as measured by the anthropometric scores and CRP. Conclusions: Our findings further support a link between oxidative stress and tuberculosis. However, whether antioxidant supplementation will improve tuberculosis outcome or not is of importance for its prevention should be further examined in future prospective studies.

1. Introduction

Several studies have reported decreased antioxidant concentrations, disturbed glutathione metabolism, and enhanced spontaneous generation of reactive oxygen species (ROS) in tuberculosis(TB) patients [1]. Mycobacterium can induce ROS production by activating phagocytes, and although an important part of the host defense against mycobacteria, enhanced ROS generation may promote tissue injury and inflammation. This further contributes to immunosuppression. Moreover, the malnutrition that commonly occurs in patients with TB may further contribute to the impaired antioxidant capacity in these patients [2].

Lipid peroxidation, a general mechanism of tissue damage by free radicals is known to be responsible for cell damage and may induce many pathological events. During pulmonary inflammation, increased amounts of ROS and reactive nitrogen intermediates are produced as a consequence of phagocytic respiratory burst. Examination of antioxidants in patients with TB may identify deficiencies that predispose to severe oxidant injury and immunodeficiency [3]. C-reactive protein is a proper test for evaluation and prognosis of pulmonary tuberculosis. It can reflect the course of disease and also the effectiveness of the drugs [4].

However, our knowledge of the antioxidant profile and its relation to lipid peroxidation in tuberculosis patients is scarce, particularly in developing countries. Thus, to further study the interaction between tuberculosis and antioxidants, we investigated antioxidant status and its relation to lipid peroxidation products in TB patients.

2. Materials and Methods

2.1. Patient selection

This cross-sectional study was conducted at Sri Siddhartha Medical Teaching Hospital & Research Centre and at Department of Biochemistry, Tumkur. The study included 50 cases aged >18 years, of either sex (31 men and 19 women), diagnosed as pulmonary TB patients (diagnosed by signs of clinical and radiologic pulmonary tuberculosis, including positive Ziehl Neelsen staining of sputum showing acid-fast bacilli) and 50 age and sex matched healthy blood donors as controls (38 men and 12 women). Selection of patients was by purposive sampling method. After a routine interview to exclude individuals with all types of acute or chronic disease, the blood donors underwent structured

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clinical and some basic laboratory examination to exclude those with HIV infection or diseases that can be transmitted with blood transfusion, such as hepatitis.

None of the TB patients or the control subjects were using any kind of treatment or prophylaxis for chronic disease, such as hypertension, diabetes mellitus, coronary artery disease, HIV infection, or other diseases that could affect the results of our analysis. In the patient group, all blood samples were taken before the start of anti-tuberculosis treatment.

Informed consent for participating in the study was obtained from all patients and control subjects. The study was approved by the ethical committee of Sri Siddhartha Medical College, Tumkur.

2.2. Investigation of patients

Standardized procedures were used to measure body weight, height, and midupper arm circumference. All subjects were weighed while wearing minimal clothing. Body mass index (BMI; in kg/m2) values of 18.5, 17.0, and 16.0 were used as the thresholds below which patients were classified as having mild, moderate, or severe malnutrition. Patients with a midupper arm circumference < 24 cm for men and < 23 cm for women were considered malnourished. In addition, dietary intakes were estimated by a dietary and history interview procedure with the use of a modified food-frequency questionnaire that included a food list adapted to include foods commonly consumed in South India [5].

Under aseptic precautions about 6 ml of a venous blood sample was collected from TB patients and from healthy controls. Blood was collected in appropriate tubes and centrifuged at 3000 g for 15 min and the separated serum was stored at 40 C until analysis was carried out. Ferric reducing antioxidant power (FRAP) assay, estimation of malondialdehyde (MDA), albumin and CRP were carried out on serum sample. All the chemicals used were of highest analytical grade available in India. CRP levels in serum were measured according to standard turbidometry procedures [6].

Total antioxidant capacity was measured by FRAP assay according to the method of Benzie.F.F. and J.J.Strain [7]. At low pH, when a ferric tripyridyltriazine (Fe III-TPTZ) complex is reduced to the ferrous (Fe II) form, an intense blue colour with an absorption maximum at 593 nm develops.

Lipid peroxidation was measured by serum MDA estimation according to the colorimetric method of Satoh.k [8]. Lipoproteins are precipitated from the specimen by adding trichloroacetic acid. 0.05M sulphuric acid and 0.67% thiobarbituric acid (TBA) in 2M sodium sulphate are added to this precipitate and the coupling of lipid peroxide with TBA is carried out by heating in a boiling waterbath for 30 minutes. The resulting chromogen is extracted in n-butanol, which is measured colorimetrically at 530 nm.

2.3.Statistical Analysis

Each result was expressed as mean ± standard deviation. The statistical significance of the data were determined by Student's t-test and one way ANOVA test at 5% level of significance. Pearson's correlation coefficient was determined at 5% level of significance. Statistical analysis was done using SPSS software version 16.0.

3.Results

Values for the clinical, hematologic, and biochemical indexes measured in the study group are shown in Table 1. Tuberculosis patients had significantly lower values for anthropometric (eg, weight, BMI, and midupper arm circumference) and some biochemical (eg, albumin, CRP) indexes than did healthy control subjects.

Table 1 . Clinical and biochemical indexes in the study group

	TB patients (n = 31 M, 19 F)	Healthy control subjects (n = 38 M, 12 F)	p value
Weight (kg)	46.9 ± 8.9	60.2 ± 6.2	<0.05
Height (cm)	165.1 ± 9.4	168.4 ± 7.4	>0.05
BMI (kg/ m2)	16.9 ± 2.4	21.8 ± 2.3	>0.05
MUAC (cm)	19.2 ± 2.7	25.0 ± 2.1	>0.05
Albumin (g/L)	28.7 ± 7.7	43.1 ± 2.9	>0.05
CRP (mg/dl)	3.93 ± 4.4	0.9 ± 0.1	>0.05
MDA (n mol/ml)	2.24 ± 0.7	0.95 ± 0.2	>0.05
TAC (μ mol/L)	764.6±133.6	1028.7±124.6	>0.05

3.1.Total Antioxidant capacity

As shown in Table 1, serum concentrations of the total antioxidant capacity, were markedly lower in patients with tuberculosis than in healthy control subjects.

3.2.Concentrations of MDA and its association with TAC

As shown in Table 1, serum MDA concentrations, as a measure of lipid peroxidation reflecting the degree of oxidative stress, were significantly higher in patients with tuberculosis than in healthy control subjects.

3.3.Oxidative stress and antioxidants in relation to nutritional state

Malnutrition may influence antioxidant concentrations and oxidative stress. As shown in Table 2, in patients with tuberculosis, but not in healthy control subjects, there was a weak but significant inverse association between low BMI and low weight and high MDA concentrations. Moreover, MDA was positively correlated with CRP.

Table 2: Correlations between oxidants and antioxidants in TB patients.

	r value	p value
MDA & TAC	- 0.56	< 0.01
MDA & BMI	- 0.168	< 0.05
MDA & CRP	0.01	< 0.05

4.Discussion

The present study is a comprehensive evaluation of markers of oxidative stress in TB patients. ROS acts as an important contributing factor to the lower concentrations of antioxidants in tuberculosis patients. Under normal conditions, the ROS produced in the course of metabolism are contained by the natural antioxidant system which consists of a series of antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase as well as numerous endogenous and dietary antioxidant compounds that are capable of reacting with and inactivating ROS, thereby protects the functional and structural molecules against ROS-mediated tissue damage. 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 pathogenic loop that results in markedly enhanced oxidative stress during TB infection [9, 10].

MDA is a three carbon, low molecular weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids of biological membranes. The determination of MDA is used for monitoring lipid peroxidation in biological samples. Although the concentration of plasma antioxidant components can be measured individually, these measurements may be time- and cost-consuming and labour intensive. In addition, it may not accurately reflect the total antioxidant status [11]. Thus, the accurate antioxidant capacity of the organism can only be determined by the measurement of total antioxidant capacity. FRAP assay is presented as a novel method of assessing total antioxidant capacity and is considered as a useful indicator of the body's antioxidant status to counteract the oxidative damage due to ROS. The advantage of the FRAP assay is in being fast, easy to handle, with highly reproducible results [12].

There are conflicting reports about lipid peroxidation and antioxidant status in patients with TB [13]. In the present study, higher concentrations of MDA and decreased TAC in tuberculosis patients compared to healthy controls, possibly reflects increased oxidative stress in the former group. Several factors such as low food intake, nutrient malabsorption, and inadequate nutrient release from the liver; acute-phase response and infection; and an inadequate availability of carrier molecules may influence circulating antioxidant concentrations [14]. Our findings of a correlation between several indices of malnutrition and low TAC may suggest the involvement of low food intake and nutrient malabsorption in the generation of oxidative stress in TB patients.

CRP is an acute-phase protein and a non-specific marker of systemic inflammation. The utility of the CRP level as a marker of bacterial infection of the lower respiratory tract has been studied in a number of populations. C-reactive protein seems to be reactive in those patients with pulmonary TB in whom the disease is in the active stage regardless of extension and site of involvement [15]. In this study, CRP level is increased in TB patients and significantly correlates with oxidative stress.

Significant correlations between high MDA concentration with slow TAC and between high MDA with high CRP suggests increased utilization by persistent immune activation and enhancement of oxidative stress may reflect interacting phenomena possibly secondary to an increased infectious load in TB.

5. Conclusion

The possibility of counteracting oxidative stress by a pool of proper antioxidants plus an appropriate diet, mainly in patients whose blood antioxidant deficiencies can be easily rebalanced, may have real health benefit and represent a promising way of inhibiting the progression of disease. Thus, based on our study results, elevated MDA and low TAC may be useful as novel markers of oxidative stress to monitor and optimize antioxidant therapy which might probably help as an adjunct in the management of TB patients. Nevertheless, larger clinical studies in this area are needed to establish the relationships between oxidative stress and TB progression to establish the prognosis and appropriate antioxidant intervention strategies in TB.

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