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Original Article

TOTAL ANTIOXIDANT STATUS AND LIPID PEROXIDES IN PATIENTS WITH PULMONARY TUBERCULOSIS

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ABSTRACT

Free radicals are potentially damaging to tissues, producing irreversible damage to biomolecules such as enzymes, proteins and membrane lipids. The current study was conducted to assess the concentration of malionaldehyde(MDA) as a marker of lipid peroxidation and total antioxidant status(TAS) in patients with active pulmonary tuberculosis and to examine the influence of chemotherapy on these parameters. 45 patients were enrolled into the study. 20(Group I) had only small radiographical changes and were sputum negative. The remainder 25(Group II) had advanced pulmonary tuberculosis and were sputum smear positive. The control group consisted of 20 clinically healthy individuals. In group I & II blood samples were collected for examination before and after 6 months of treatment period with tuberculostatic drugs. Data was analyzed by SPSS software. In patients with pulmonary tuberculosis, both before and after chemotherapy , serum MDA concentration was significantly higher and the content of TAS was significantly lowered in comparison to the control group, though in the course of treatment MDA concentration decreased significantly while the concentration of TAS were stable. These results suggest that increased circulating levels of free radical activity along with decreased TAS are found in active pulmonary tuberculosis and hence may play a role in the resultant fibrosis. It also reinforces the belief that inclusion of antioxidants to the therapy may prove to be useful.

KEY WORDS: Antioxidant, Lipid Peroxides, Pulmonary Tuberculosis

INTRODUCTION

Free radicals are responsible for widespread and indiscriminate oxidation and peroxidation of lipids causing cell death or organ damage. Free radicals oxidative stress has been implicated in the pathogenesis of a variety of human diseases.[1] When a host tissue is challenged by a pathologic insult of either an immunologic or non-immunological nature, an inflammatory reaction may occur, with subsequent clearance of the pathologic stimulus by phagocytic cell. Tissue injury may result from either the direct effects of the pathologic agent or as a consequence of an inflammatory cell influx.[2] Upon recognition of a pathocytic or soluble stimulus, both neutrophils and macrophages experience a "respiratory burst" which is characterized by an increase in oxygen consumption and increase glucose metabolism via hexose monophosphate shunt. In conjunction with an increase in oxygen consumption, neutrophils and macrophages secrete both superoxide(O_2^-) and hydrogen peroxide(H_2O_2) as a defense mechanism.[2]

The biological effects of these highly reactive compounds are controlled in vivo by a whole spectrum of antioxidative defense mechanisms: vitamin E and C, carotenoids, metabolites such as glutathione and uric acid , and antioxidant enzymes(superoxide dismutase, glutathione peroxidase and catalase).[3]During pulmonary inflammation increased amounts of reactive oxygen species and reactive nitrogen intermediates are produced as a consequence of phagocytic respiratory burst. One of the manifestation of these free radical mediated processes is lipid peroxidation.[4] In some recent clinical studies, one or several of these antioxidant enzymes were measured in blood as possible biological indicators especially concerning hyperlipidemia, alcoholism, diabetes mellitus and cancer. Though pulmonary tuberculosis is a disease of most common occurrence and widely studied, many questions in this field still remain unanswered, such as role of lipid peroxidation process. Therefore in the present study an attempt has been made to define more precisely the circulating indicators of lipid peroxidation(measured as malionaldehyde,MDA) and total antioxidant status(TAS) in patients with pulmonary tuberculosis and to determine the influence of healing with tuberculostatic drugs on these biochemical parameters.

MATERIAL AND METHOD

A total of 45 patients with active pulmonary tuberculosis were studied at the outset and after 6 months of antimicrobial chemotherapy. Disease classification was based on patients self presented report of prior physician diagnosis. Patients were divided into two groups:

Group I: Comprised 20 patients (12 Males and 08 Females), aged 26 to 61 years (mean 48.3 years) had only small radiographical changes and were sputum smear negative.

Group II: Comprised 25 patients (14 Males and 11 Females), aged 27 to 65 years (mean 45.1 years) had only small radiographical changes and were sputum smear positive.

The control group consisted of 20 clinically healthy individuals (10 Males and 10 Females) aged 22 to 59 years (Mean 46.3 years). Mean values of age, blood pressure, BMI and lipid parameters did not significantly differ between the groups studied.

Fasting blood samples were collected from all the subjects. The blood was drawn into plain tubes and subjected to estimation of serum malionaldehyde concentration by the method of Satoh,[5] and serum total antioxidant status was measured by the method of Meller et al.[6](kit manufactured by Randox lab, U.K.)

Data analysis was performed with the SPSS statistical software. The results for continuous variables are mean $\pm$ S.D. Student t test for independent samples, analysis of variance (ANOVA) were used in the assessment of the significance of difference between group means.

RESULTS

Table I shows the mean values of age , serum MDA and TAS in different groups of pulmonary tuberculosis patients and in control subjects. In the healthy control group mean serum concentration of MDA was 4.06 $\pm$ 0.94 nmol/ml and mean TAS was 1.46 $\pm$ 0.12 mmol/L , these values were within the accepted normal range. There was no statistically significant sex related difference. The mean serum MDA level, in Group I and Group II at baseline were 6.36 $\pm$ 1.47 nmol/ml and 7.98 $\pm$ 1.91 nmol/ml respectively while total antioxidant status in respective group were 1.09 $\pm$ 0.16 mmol/L and 0.84 $\pm$ 0.07 mmol/L respectively. Taken together, data indicate a higher free radical activity in group II patients. The trend was similar when the sexes were examined separately. In both types no relation of disease prevalence with gender,, age at onset of DM, socio-economic status and other lifestyle variations was found.

DISCUSSION

In pulmonary tuberculosis patients a significant increase in MDA concentration ($p<0.001$) represent an increased lipid peroxidation, a general mechanism of tissue damage by free radicals. Free radicals and peroxides are clearly involved in physiological phenomenon such as synthesis of prostaglandins , thromoxanes and in the pathogenesis of various disease.[7]During pulmonary inflammation increased amounts of reactive oxygen species and reactive oxygen nitrogen intermediates are involved as a consequence of phagocyte respiratory burst.[4] Thus, toxic free radical are implicated in the development of lung fibrosis , which may be a

long term sequel of pulmonary tuberculosis.[8] Results of this study clearly shows a definite relation between the levels of lipid peroxides (measured as MDA) and severity of disease. Increase was seen in ascending order; levels were higher in subjects with advanced disease than in those with only small radiographical changes. These results are in accordance with Janiszewska et al[9] and Kominskaia et al.[10]

Table 1: Serum MDA (maliondialdehyde) and TAS (Total Antioxidant Status) in normal subjects and in patients with pulmonary tuberculosis before and after 6 months of treatment (Mean ± SD)

	Control Group	**Group I**	**Group II**
N	20 (10 M; 10 F)	20 (12 M; 8 F)	25 (14 M; 11 F)
Age(years)	46.3 ± 6.4	48.3 ± 8.2	45.1 ± 7.1
MDA (nmol/ml)	4.06 ± 0.94	**(i) Before therapy**	**(i) Before therapy**
	M: 4.18 ± 0.99	6.36 ± 1.47*,||	7.98 ± 1.91*,||
	F: 3.99 ± 0.84	M: 6.22 ± 1.94	M: 8.04 ± 1.23
		F: 6.45 ± 1.01	F: 7.91 ± 1.33
		(ii) After therapy	**(ii) After therapy**
		5.27 ± 1.04||	6.55 ± 1.39||
		M: 5.21 ± 1.21	M: 6.64 ± 1.07
		F: 5.46 ± 0.92	F: 6.47 ± 1.21
TAS (mmol/L)	1.46 ± 0.12	**(i) Before therapy**	**(i) Before therapy**
	M: 1.39 ± 0.17	1.09 ± 0.16†,§	0.84 ± 0.07†,‡,§
	F: 1.54 ± 0.14	M: 1.01 ± 0.11	M: 0.82 ± 0.09
		F: 1.14 ± 0.13	F: 0.89 ± 0.08
		(ii) After therapy	**(ii) After therapy**
		1.12 ± 0.11§,¶	0.97 ± 0.08§,¶
		M: 1.09 ± 0.16	M: 0.94 ± 0.07
		F: 1.17 ± 0.14	F: 0.97 ± 0.06

*p<0.001 (Group I Vs control group; Group II Vs control group)
†p<0.01 (Group I Vs control group; Group II Vs control group)
‡p<0.05 (Group II Vs Group I)
§ No significant difference before and after therapy in same group
|| p<0.05 (Before and after therapy in same group)
¶p<0.05(Group I Vs control group; Group II Vs control group)

There was a very significant decrease (p<0.01) in the level of total antioxidant status in both of the patient groups (Group I: 1.09 ± 0.16 mmol/L; Group II: 0.84 ± 0.07 mmol/L) when compared with control subjects (1.46 ± 0.12 mmol/L). Despite an great increase in MDA values in group II subjects , activity of various scavengers of oxygen free radicals (measured as TAS) further decreased (p<0.05) compared to group I subjects, representing a relative defect or deficient total antioxidant status – that is altered scavenger system in the pulmonary tuberculosis patients. Decreased TAS might be responsible for decreased host resistance. This may also be responsible for increased production/ accumulation of superoxides. Excess production of superoxides leads to accelerated lipid peroxidation in biomembranes, causing overall tissue damage. This explains the increased lipid peroxide levels in patients with pulmonary tuberculosis. On comparing the laboratory data before and after 6 months of chemotherapy, MDA concentration decreased significantly (p<0.05) in both the groups, yet remains elevated than the control group, whereas TAS does not show any significant difference before and after the treatment. As decreased antioxidants lead to increased lipid peroxidation , inclusion of antioxidants in the treatment may prove to be useful.

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