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## ORIGINAL ARTICLE

## Oxidative Stress And Ascorbic Acid Levels In Cavitory Pulmonary Tuberculosis

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**Abstract:** Oxidative stress is implicated in the pathogenesis of many diseases. It has been evidenced by various studies that free radicals are involved in the progression of pulmonary tuberculosis and also, in the damage caused to the lung tissue.

**Aim:** The present study was conducted to assess the severity of oxidative stress (MDA) and the levels of antioxidants (vitamin C) in advanced (cavitory) pulmonary tuberculosis by comparing it with the non cavitory cases. **Methodology:** 50 cases and 30 controls were included in this study. The cases were further divided into cavitory and non-cavitory on the basis of the chest X-ray reports. The levels of lipid peroxidation (MDA) were estimated by the method of Stocks and Dormandy and those of vitamin C were estimated by the method of Varley. **Result:** The levels of lipid peroxidation increased significantly in the cavitory cases and also, the levels of the antioxidants (vitamin C) were found to be decreased significantly in the cavitory cases as compared to the non-cavitory cases. **Conclusion:** Oxidative stress was found to be increased highly significantly in cavitory pulmonary tuberculosis. The levels of the antioxidants (vitamin C) decreased highly significantly with an increase in lipid peroxidation levels.

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### Introduction

M. tuberculosis is most commonly transmitted from a patient with infectious pulmonary tuberculosis to other persons by droplet nuclei, which are aerosolized by coughing, sneezing or speaking. The disease primarily affects lungs and causes pulmonary tuberculosis and is the most important form of tuberculosis which affects humans [1]. Pulmonary tuberculosis is India's biggest public health problem. Every year, approximately 1.8 million people develop tuberculosis, of which about 0.8 million are new smear positive highly infectious cases. The annual risk of becoming infected with Tuberculosis is 1.5% and once infected, there is a 10% life time risk of developing tubercular disease. Two out of every five Indians are infected with the TB bacillus [2]. A free radical can be defined as a

chemical species, because it has an unpaired electron. Free radicals are generally produced in the cells by electron transfer reactions. These are extremely reactive species and are thus, short lived. All the major classes of biomolecules may be attacked by free radicals. Lipids, perhaps, are the most susceptible ones [3]. Their toxic effects are limited by an antioxidant defence system, which prevents cell damage by these free radicals. Antioxidative defence mechanisms include certain enzymes like superoxide dismutase, catalase and glutathione peroxidase and nonenzymatic biomolecules like ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, glutathione, albumin, ceruloplasmin, transferrin, etc [3]. Lipid peroxidation is a free radical mediated process. In lipid peroxidation, a primary reactive free radical interacts with

polyunsaturated fatty acids to initiate a complex series of reactions that result in a variety of degradation products. The uncontrolled peroxidation of biomembranes can thus lead to profound effects on the membrane structure and function and may be sufficient to cause cell death [4].

Malonyldialdehyde (MDA) is a decomposition product of oxidized polyunsaturated fatty acids. This three- carbon dialdehyde has been proposed to arise from fatty acid hydroperoxides via several mechanisms. The most frequent precursors of MDA are five-membered hydroperoxy epidioxides (endoperoxides) and 1,3-dihydroperoxides [5]. Most lipid hydroperoxides are unstable and undergo decomposition to secondary lipid peroxidation products such as MDA [6]. Cell injury associated with free radicals occurs either in situations in which the scavenging system for free radicals is overwhelmed by the excessive production of free radicals (“hyperoxidants stress”) or in the diseased state in which this protective antioxidant system is impaired [7]. Vitamin C or L-Ascorbic acid is water soluble and is present in its deprotonated state under most physiological conditions. It is considered to be the most important antioxidant in extracellular fluids [8] and has many cellular activities of an antioxidant nature as well [9]. Oxidative stress reflects a shift towards the pro-oxidants in the prooxidant-antioxidant balance that characterises the normal aerobic state, thus leading to the production of damaged products [10]. Lipid peroxidation products (LPPs) diffuse from the site of inflammation and can be measured in blood [11]. The granulomatous destruction of the lung tissue itself may cause the liberation of toxic radicals, or indeed, it may be that the activated macrophages release highly reactive radicals which may then cause the local disruption of the essential structure including membrane lipids, deoxyribonucleic acid and proteins and hence, cause tissue destruction [12]. Kwiatkowska et al [13] observed increased levels of lipid peroxidation products (LPP), conjugated dienes and malonyldialdehyde (MDA) in patients with active TB. The highest level of conjugated dienes (CD) and malonyldialdehyde (MDA) were found in patients with advanced TB. Jack et al [14] concludes that increased circulating levels of

free radical activity are found in active pulmonary tuberculosis and that they also play a role in resultant fibrosis. It also reinforces the belief that a range of free radical activity (FRA) indicators are produced in any inflammatory process with fibrinogenic potential and that these indicators may measure different stages of the disease process.

The objectives of the present study were to assess the severity of oxidative stress in cavitory tuberculosis and also to assess the level of antioxidants and ascorbic acid and to compare these levels with the levels which were observed in non-cavitory tuberculosis.

### Material and Methods

The present study was conducted on fifty patients of pulmonary tuberculosis who attended the Adesh Institute of Medical Sciences and Research, Bathinda. In all cases, a detailed clinical history was taken and their physical examination was recorded on the proforma which was prepared for the present study. Thirty healthy individuals who were symptomless, free from any clinical abnormality and those who were not taking any drug, constituted the control group [Table/Fig 1]. The cases and controls were further divided according to age and sex [Table/Fig 2] and [Table/Fig 3].

**[Table/Fig 1]: Distribution Of Cases**

Group	No. of Cases
Control	30
Study	50
Total	80

**Total number of cases involved in the study were 80 out of which 30 healthy individuals served as control group and 50 diagnosed cases of sputum positive pulmonary tuberculosis served as study group.**

**[Table/Fig 2]: Showing Distribution Of Cases According To Age**

Age Groups (Years)	Control		Study	
	No.	%age	No.	%age
15-30	8	26.67%	15	30%
31-40	9	30%	11	22%
41-50	7	23.33%	13	26%
51-60	6	20%	5	10%
61-70	-	-	4	8%
≥70	-	-	2	4%
<b>Total</b>	<b>30</b>	<b>100%</b>	<b>50</b>	<b>100%</b>
Range (yrs.)	18-55		15-75	
Mean±SD(yrs.)	38.40 ± 11.01		40.58 ± 14.77	
t & p value	0.69 ; p > 0.05			
Significance	NS			

Table-2 shows the distribution of age in study group. The maximum number of patients in study group were in the range of 15-30 years of age while maximum number of patients in control group were in the range of 31-40 years of age with the range of 15-75 years in study group and 18-55 years in control group. The data when compared statistically was non significant (p>0.05).

[Table/Fig 3]: Distribution Of Cases According To Sex

Sex	Control		Study	
	No.	%age	No.	%age
Male	26	86.67%	40	80%
Female	4	13.33%	10	20%
<b>TOTAL</b>	<b>30</b>	<b>100%</b>	<b>50</b>	<b>100%</b>

[Table/Fig 3] shows that the number of male cases were more than female cases both in study group and control group. In study group 80% were males and 20% were females while in control group 86.67% were males and 13.33% were females. The data when compared statistically, was found to be non significant.

The following investigations were taken into consideration.

1. X-ray chest
2. Sputum examination
3. Estimation of Lipid peroxidation (MDA) levels in red blood cells [15].
4. Estimation of Vitamin C levels in plasma [16].

#### Collection and Treatment of the Samples:

Under aseptic conditions, 5ml of venous blood from human subjects was taken in a vial containing 3.8% sodium citrate.

**Washing of Erythrocytes:** Citrated blood was centrifuged at 3000rpm for 5 minutes. The buffy coats were separated by aspiration. The packed cell volume (PCV) was washed thrice with phosphate buffer (pH 7.4). The PCV was

used for lipid peroxidation (LPO) and haemoglobin estimation.

**Estimation of Lipid Peroxidation in Red Blood Cells:** Lipid peroxidation in red blood cells was estimated according to the method described by Stocks and Dormandy [15]. LPO was estimated in erythrocytes as malonyldialdehyde (MDA) which was formed by the thiobarbituric acid (TBA) reaction.

**Haemoglobin Estimation:** It was done by the method described by Dacie and Lewis [17].

**Ascorbic Acid (Vitamin C):** Vitamin C levels in plasma were estimated by a titration method by using the 2-6 dichlorophenolindophenol dye. It was based on the titration of 2-6 dichlorophenolindophenol in an acid solution. This blue compound, on titration with a solution of ascorbic acid, was reduced to a colourless leucobase, the vitamin C being oxidised to dehydroascorbic acid [16].

The statistical analysis was carried out by the Students t- test. The data was expressed as mean ± standard deviation with the level of significance set at P<0.01.

## Results

[Table/Fig 5] shows the comparison of the parameters between the cavitory and non-cavitory cases.

1. The range of MDA in the cavitory cases was 570-1026 nmol MDA/hr/gm Hb, with a mean ± SD of 785.70 ± 132.70 nmol MDA/hr/gm Hb, while in the non cavitory cases, it was 470-988 nmol MDA/hr/gm Hb, with a mean ± SD of 638.55 ± 149.05. The data when compared statistically, was found to be HS (P<0.01).
2. The range of vitamin C in the cavitory cases was 0.48-0.90mg% with a mean±SD of 0.64 ± 0.10mg% and that in the non cavitory cases was 0.52-1.0mg%, with a mean ± SD of 0.72 ± 0.11mg%. The data when compared statistically, was found to be HS (p<0.01).

## Discussion

Sputum examination was done for the presence of AFB (Acid Fast Bacilli). On the basis of the chest X-ray findings, the cases in the study group were divided into cavitory and non-cavitory cases [Table/Fig 4]. According to this finding, 46% of the cases in study group were found to be cavitory and the rest of the 54% were found to be non-cavitory.

**[Table/Fig 4]: Distribution Of Cases According To The Presence/Absence Of Cavity In Chest X-Ray**

S.No.	X-Ray finding	No. of Cases	Percentage
1.	Cavitory	23	46
2.	Non-Cavitory	27	54
TOTAL		50	100

The cases and controls were also divided on the basis of demographic data like age and sex, but however, on statistical analysis, these data when compared, were found to be non-significant [Table/Fig 2] and [Table/Fig 4]. By statistical analysis, oxidative stress (MDA) was found to be highly significant in the cavitory cases as compared to that in the non-cavitory cases [Table/Fig 5].

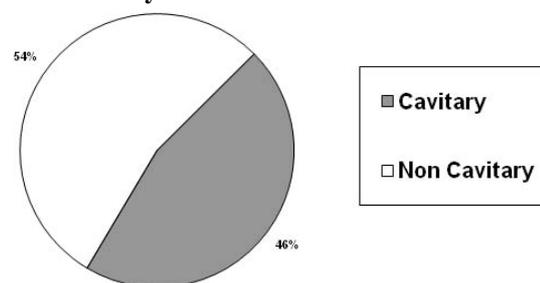
**[Table/Fig 5]: Showing Comparison Of Different Parameters Between The Cavitory And Non Cavitory Cases**

Parameter	Cavity	No. of Cases	Range	Mean $\pm$ SD	t	P	S
MDA (nmolMDA/hr/gm.Hb)	Yes	23	570-1026	785.70 $\pm$ 132.70	3.65	<0.01	HS
	No	27	470-988.1	638.55 $\pm$ 149.05			
Vit.C (mg%)	Yes	23	0.48-0.90	0.64 $\pm$ 0.10	2.80	<0.01	HS
	No	27	0.52-1.0	0.72 $\pm$ 0.11			

The levels of vitamin C were reduced highly significantly in the cavitory cases as compared to those in the non-cavitory cases. It was evident from the present study, that LPO in the cavitory tuberculosis of lung was increased as compared to that in the non-cavitory cases. Our work confirms the presence of increased oxidative stress in patients with cavitory pulmonary tuberculosis. It was measured as increased MDA levels. In pulmonary tuberculosis, the imbalance between the production of oxygen free radicals and antioxidant defences was found to be more in the cavitory cases as compared to the non-cavitory cases. This reflects increased oxidative challenge to the patients with

cavitory pulmonary tuberculosis. Our findings correlated well with the data which was published in the past. Increase in MDA levels is an indicator of increased LPO, thus reflecting that the imbalance between oxidative stress and the antioxidants is in favour of the former. The cavitory cases in the study group exhibited higher levels of oxidative stress and lower antioxidant (Vitamin C) levels as compared to those in the non-cavitory cases. In a study conducted in Ethiopian subjects, the concentrations of antioxidants like vitamin C, vitamin E and vitamin A were found to be significantly lower in tuberculosis patients and high malondialdehyde concentrations were associated with clinical severity [18]. High MDA concentrations and low levels of non-enzymatic antioxidants like vitamins C and E may indicate the depletion of antioxidants due to excessive lipid peroxidation by ROS in PTB patients. Ascorbate is the first antioxidant to be depleted upon exposure to both environmental and inflammatory oxidants, thus suggesting that it is the ultimate antioxidant either by directly scavenging these oxidants or trapping their intermediates [19]. Reduced levels of serum vitamin C have been reported in patients with pulmonary tuberculosis [20]. Ascorbic acid plays a major role in pulmonary antioxidant defense. Sufficient amounts of ascorbic acid are necessary to maintain normal metabolic processes in the lung [21].

**[Table/Fig 5]: Pie Chart showing distribution of cases according to presence/absence of cavity in chest x-ray**



According to the presence/absence of cavity in chest X-ray, the cases in study group were divided into cavitory and non cavitory group. The number of cases were 23 and 27 respectively.

The administration of nutrients such as ascorbic acid and alpha tocopherol have been shown to accelerate tuberculosis healing, based on decay cavity closure and negative sputum [22]. Furthermore, a clinical trial

cohort study of 26,975 Finnish men during a median follow-up of 6-7 years found a high inverse association between calculated vitamin C intake and the incidence of tuberculosis. Subjects with an intake of >90mg of vitamin C and with an increased consumption of fruits, vegetables and berries were found to have a significantly lower risk of tuberculosis [23]. It was evident from the above studies, that the supplementation of antioxidant vitamins had a positive bearing on the overall health and recovery of the patient. **The control and case groups varied in size i.e the number of cases and controls were not same in this study. This can be one of the limitations of this study.** Also, as the present study involved a small sample size, it is warranted to conduct studies with a larger sample size to find similar encouraging results and to include vitamin supplementation in the regimen of ATT. However, larger cross sectional and longitudinal studies are warranted to confirm our observations.

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