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## Oxidative Imbalance and Non-Enzymic Antioxidant Status in Pulmonary Tuberculosis Infected Subjects: Carcinogenic Potential

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**Abstract:** Oxidative imbalance and non-enzymic antioxidant status in plasma of pulmonary tuberculosis patients in Nigeria were investigated. Forty HIV/AIDS seronegative pulmonary tuberculosis patients with the active infection (24 males, 16 females) aged 20-60 years diagnosed by Ziehl Neelson staining/demonstration of mycobacterium tuberculosis in sputum and sputum culture Lowenstein Jensen medium visiting Federal Medical Centre, Owerri were selected for the study. Sixty normal subjects free from pulmonary tuberculosis and HIV/AIDS (30 males and 30 females) ages 20-60 years were also used as control. Patients with complication such as renal diseases, viral and other bacterial infections, etc. were excluded from the study. In the analysis of the results using Duncan multiple range test, pulmonary tuberculosis infected subjects presented significantly higher mean values of plasma lipid peroxide ( $p < 0.05$ ) when compared with control. Also the levels of non-enzymic antioxidants such as Vitamin C, vitamin E and reduced glutathione in plasma were significantly depleted in the pulmonary tuberculosis infected subjects ( $p < 0.05$ ) when compared with control. This shows that pulmonary tuberculosis could probably be associated with excess ROS production.

**Key words:** Oxidative imbalance, non-enzymic antioxidant, pulmonary tuberculosis

### Introduction

Mycobacterium tuberculosis is considered as an etiologic agent of tuberculosis and the unifying feature of the genus is an acid-fast property (Bloom and Murray, 1992). Tuberculosis is a major cause of morbidity worldwide and is spread primarily through airborne transportation by aerosolized droplets during coughing, sneezing or talking especially in poorly ventilated areas and over 10 million cases of this disease are diagnosed annually with about 3 million deaths attributed to tuberculosis (WHO, 1997).

This disease is characterized by unexplained caught persisting for more than three weeks, haemoptasis, pleural pain not associated with an acute illness, loss of appetite, spontaneous pneumothorax, dehydration/vomiting, unexplained tiredness, loss of weight, high remittent or intermittent pyrexia, pleural effusion, severe and continuous diarrhoea in cases of tuberculosis enteritis, drenching sweats during sleep and anaemia (Macleod, 1981).

Factors contributing to the resurgence of tuberculosis in developing countries and problems of its control include co-infection with HIV, emergence of multi drug resistance tuberculosis, inadequate treatment, poverty, malnutrition, over crowding, armed conflict and increasing number of displaced persons (Cheesbrough, 2000).

The contribution of Reactive Oxygen Species (ROS) in the bacterial activity of phagocytes has been in the limelight ever since Klebanoff revealed the bactericidal potency of the myeloperoxidase- $H_2O_2$ -halide system of human neutrophil granules (Klebanoff, 1975). The

sensitivity of mycobacteria to the bactericidal potency of phagocytes cells *in vitro* differs from one Mycobacterium species to the other, (Orme and Collins, 1983).

A growing literature documents a possible interaction between oxidative stress and pathobiology of mycobacterial infections (Eze *et al.*, 1993). For example, local lung oxidant damage has been implicated to play a role in the pathogenesis of lung mycobacterium infections (Niwa *et al.*, 1984). This should not be surprising, given the combination of direct bacterial lung cell injury and the subsequent massive infiltration of "activated" inflammatory cells that occurs. The concept that oxidant stress may be present is buttressed by the mortality reduction produced by superoxide dismutase administration to mice challenged with an otherwise lethal influenza infection (Oda *et al.*, 1989).

Wu *et al.* (1988) have published epidemiological studies to the effect that either tuberculosis (TB) or pneumonia predisposes the individual to adenocarcinoma of the lungs. In these studies, increased intake of  $\beta$ -carotene decreased the risk. This salutary effect of  $\beta$ -carotene probably originates from its free radical scavenging ability.

In his present study, one of the indices of oxidative stress, malondialdehyde (MDA), a by product of lipid peroxidation and non-enzymic antioxidants were assayed with a view to provide information on oxidative imbalance in pulmonary tuberculosis infected subjects.

### Materials and Methods

**Subjects:** Forty HIV/AIDS seronegative pulmonary tuberculosis patients with the active infection (24 males,

## Nwanjo and Oze: Oxidative imbalance and non-enzymic antioxidant status

Table 1: Changes in the levels of plasma Lipid peroxide, vitamin C, Vitamin E and GSH in pulmonary tuberculosis infected patients and control groups

Parameters	Control	Hypertensive patients
Lipid peroxide (mmol/MDA/ml)	3.78±0.43	*8.32±0.82
Vitamin C (mg/dl)	1.05±0.41	*0.62±0.48
Vitamin E (mg/dl)	1.84±0.60	*0.69±0.28
GSH (mg/dl)	16.81±2.83	*9.45±1.51

Results are shown as mean±standard deviation, \*Significantly different from control (p<0.05) using Duncan multiple range test

16 females) aged 20-60 years diagnosed by Ziehl Neelson staining/demonstration of mycobacterium tuberculosis in sputum and sputum culture Lowenstein Jensen medium (Cheesbrough, 2000). The subjects were also screened for HIV/AIDS infection using genie II-HIV ½-9 a dual recognition enzyme immunoassay 92430 Marnesia Cogutte-France.

Patients with complications such as renal, endocrine or hepatic diseases, diabetes mellitus, obesity, viral and other bacterial infections etc were excluded from the study. Fresh patients yet to be placed on medication were used since most antibiotics used for treatment of pulmonary tuberculosis may increase oxidative stress.

Sixty normal subjects free from pulmonary tuberculosis and HIV/AIDS (30 males and 30 females) ages 20-60 years were also used as control. HIV/AIDS and mycobacterium tuberculosis screening was carried out on each of the subjects.

**Blood sample:** In all subjects 8 mL of venous blood was collected in Na-EDTA (1mg/ml) tubes after a fasting period of 10-12 hours. The plasma was extracted by centrifuging the whole blood in a Wisperfuge (model 684) centrifuge at 2500g for 5 min and used for the analysis of malondialdehyde (MDA), vitamin C, E and reduced glutathione (GSH) and HIV/AIDS screening. The sputum of each of the subjects was collected in a sterile universal bottle for Ziehl Neelson staining (3 consecutive times) and cultured, on Lowenstein Jensen medium for the identification and confirmation of the presence of mycobacterium tuberculosis.

**Estimation of lipid peroxidation:** Lipid peroxidation in plasma and liver was estimated colorimetrically by measuring malondialdehyde (MDA) as described earlier by us (Nwanjo, 2005; Nwanjo and Ojiako, 2005). In brief, 0.1 mL of plasma was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCL reagent (TBA 0.37%: 0.25N HCL: 15% TCA) and placed in water bath for 15min, cooled and centrifuged and then clear supernatant was measured at 535 nm against reference blank.

**Estimation of non-enzymic antioxidants:** Reduced glutathione (GSH) was determined by the method of Ellman (1959). 1 mL of supernatant (0.5 mL plasma/0.5

mL liver homogenate precipitated by 2 mL of 5% TCA) was taken and 0.5 mL of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 mL of phosphate buffer (pH 8.0) were added. The colour developed was read at 412nm.

Vitamin C (ascorbic acid) concentration was measured by Omaye *et al.* (1979) method. To 0.5 mL of plasma/0.5 mL liver homogenate, 1.5 mL of supernatant, 0.5 mL of DNPH reagent (2% DNPH) and 4% thiourea in 9N sulphuric acid) was added and incubated for 3 hours at room temperature. After incubation 2.5 mL of 8.5% sulphuric acid was added and colour developed was read at 530nm after 30 min.

Vitamin E ( $\alpha$ -tocopherol) was estimated by the method of Desai (1984). Vitamin E was extracted from plasma/liver homogenate by addition of 1.6 mL ethanol and 2.0 mL petroleum ether to 5.0 mL plasma and centrifuged. The supernatant was separated and evaporated. To the residue, 0.2 mL of 0.2% 2, 2, -dipyridyl, 0.2 mL of 0.5% ferric chloride was added and kept in dark for 5min, an intense red colour layer obtained on addition of 4 mL butanol was read at 520nm.

**Statistics:** Statistical evaluation of data was performed by using one-way analysis of variance ANOVA followed by Duncan's Multiple Range Test (DMRT) (Duncan, 1957).

### Results

Table 1 shows a significantly higher level of plasma lipid peroxides in pulmonary tuberculosis infected subjects (p<0.5) when compared with control groups. The levels of plasma vitamin C, Vitamin E and reduced glutathione in pulmonary tuberculosis infected subjects and control are also shown in Table 1. The levels of these non-enzymic antioxidants in plasma were significantly depleted in pulmonary tuberculosis infected subjects (p<0.05) when compared with the control.

### Discussion

The contribution of ROS in the bactericidal activity of phagocytes has been in the limelight ever since Klebanoff revealed the bactericidal potency of the myeloperoxidase-H<sub>2</sub>O<sub>2</sub>-halide system of human neutrophil granules (Klebanoff, 1975). The measurement of lipid peroxidation is a convenient method to monitor oxidative imbalance (Viani *et al.*, 1991). In this study the increase levels of plasma MDA in pulmonary tuberculosis infected patients reflected the lipid peroxidation as a consequence of oxidative imbalance. This may be due to the high endogenous ROS in phagocytes of patients with pulmonary tuberculosis, as well as, the high ROS in the mycobacterium tuberculosis (Niwa *et al.*, 1984). It is pertinent to comment here, that these ROS are

endogenous. Therefore, any ROS escaping from the phagocytes to the surroundings could damage tissue and cellular DNA. The most likely link between these bacteria diseases and cancer is the DNA damage cause by ROS, which are produced in excess in the lungs as the phagocytes fight the invading pathogens (Eze *et al.*, 1993).

As an example of the effects of diseases caused by these bacteria, Wu *et al.* (1988) have published epidemiological studies in the effect that either tuberculosis (TB), or pneumonia predisposes the individual to adenocarcinoma of the lungs. In their study, increased intake of  $\beta$ -carotene decreased the risk. The salutary effect of  $\beta$ -carotene probably originates from free radical scavenging ability. Non-enzymic antioxidants such as reduced glutathione, vitamin C, Vitamin E and  $\beta$ -carotene play an excellent role in protecting the cells from oxidative damage (Farombi *et al.*, 2000). It is well established that GSH in blood keeps up the cellular levels of the active forms of vitamin C and E by neutralizing the free radicals. When there is reduction in GSH the cellular levels of vitamins C and E are closely interlinked to each other. In agreement with this report, the decreased level of GSH, vitamin C and vitamin E in pulmonary tuberculosis infected patients were observed in this study when compared with control. This may be as a result of increase in free radical scavenging activities of these antioxidants or utilization of these antioxidants in quenching excess ROS production in pulmonary tuberculosis infected patients.

In conclusion, the increase in concentration of lipid peroxidation product (malondialdehyde) along with the decrease in concentration of non-enzymic antioxidant could probably be associated with excess ROS production in pulmonary tuberculosis infected patients. The most likely link between these bacterial disease and cancer may be the DNA damage caused by ROS, which are produced in excess in the lungs as the phagocytes fight the invading pathogens.

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