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# Impact of HIV and Mycobacterium Tuberculosis Co-Infections on Antioxidant Status in Nigeria

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**Abstract:** Severe Oxidative stress has been reported in Tuberculosis infected individuals as a result of tissue inflammation, poor nutrition and poor immunity and this stress becomes more severe in those co-infected with HIV. Therefore the present study was designed to assess the antioxidant status of HIV infected participants with or without tuberculosis co-infections and in HIV seronegative participants infected with tuberculosis. 193 participants were randomly recruited for the study and grouped into: (i) Symptomatic HIV infected participants with tuberculosis co-infections (n = 67) (ii) symptomatic HIV infected participants without tuberculosis (n = 45) (iii) HIV seronegative participants with Tuberculosis (n = 52) and (IV) HIV seronegative control participants without tuberculosis (n = 29). Blood samples collected from the participants were used for HIV screening, CD4+T cell count, glutathione reductase activity, glutathione peroxidase activity, Total Antioxidant Status and albumin estimations. The results showed that glutathione reductase, glutathione peroxidase and Total Antioxidant Status were significantly lowered in both HIV infected participants with or without tuberculosis and HIV seronegative participants with tuberculosis ($P<0.01$), compared (in each case) with HIV seronegative participants without tuberculosis. The CD4+T cell count were significantly low in HIV infected participants with tuberculosis co-infections and HIV infected group without tuberculosis when compared with HIV seronegative participants with or without tuberculosis. However the CD4+T cell count in HIV infected participants with tuberculosis was not significantly different when compared with HIV infected participants without tuberculosis. The serum albumin were lowered in HIV infected participants with tuberculosis and tuberculosis infected participants ($P<0.01$ in each case). Correlation studies amongst groups showed significant correlation between CD4+T cell count and antioxidants in both HIV and tuberculosis co-infected participants and in HIV infected participants without tuberculosis ($P<0.01$ in each case). Serum albumin correlated positively with the antioxidants in both HIV infected participants and those co-infected with tuberculosis. There was no significant correlation between CD4+T cell count and the antioxidants in HIV seronegative participants with or without tuberculosis. The study observed alterations in the levels of glutathione reductase, glutathione peroxidase, total antioxidant status and albumin in tuberculosis infected participants and in HIV infected participants with and without tuberculosis. This could be as a result of greater utilization of antioxidants subsequent to increased oxidative stress. These findings also further support a link between oxidative stress, tuberculosis and HIV infection.

**Key words:** Oxidative stress, albumin, CD4+T cell, glutathione reductase

## INTRODUCTION

An alarming epidemic of tuberculosis has followed the rapid rise in HIV/AIDS cases particularly in developing countries such as Nigeria. Tuberculosis (TB) is the leading infectious killer of people living with HIV and accounted for an estimated 13% of Acquired Immune Deficiency Syndrome (AIDS) death worldwide (Sharma *et al*., 2005). HIV and TB are so often closely connected that they are often referred to as co-epidemics or dual epidemics. HIV activates dormant TB in a person who then becomes infectious and able to spread TB bacilli to others (UNAIDS, 2010). Th1 type immune response characterized by adequate cell mediated immunity is the crucial host defense against *Mycobacterium Tuberculosis* (Schluger and Rom, 1998). HIV infection primarily affects those components of host immune response responsible for cell mediated immunity. Thus in HIV infected individuals with latent tuberculosis, the

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fine balance between *Mycobacterium tuberculosis* and the host immunity is lost favouring reactivation of latent TB (FitzGerald and Houston, 1991).

Oxidative stress has emerged in recent years as a suspected component in the pathogenesis of HIV disease (Carole and Sterrit, 1994). Increasing number of reports have shown that even in the earliest stages of infection, a deleterious reductive-oxidative (redox) imbalance may occur, this means that increased reactive oxygen intermediates are generated at the same time that stores of naturally occurring reducing agents are depleted. The possible result is the uncontrolled presence of oxygen-containing molecules which may overwhelm the antioxidant capacity and cause damage to cell membranes, protein and nucleic acids and also cause alterations in the intra and inter-cellular environment (Carole and Sterrit, 1994).

Mycobacteria are intracellular pathogens that grow and replicate in the host macrophages. In an attempt to kill mycobacterium, host cells namely macrophages, neutrophils and monocytes generate huge amount of reactive oxygen species which contribute to inflammatory injury to host tissues and may further lead to immunosuppression particularly in those with impaired antioxidant capacity such as HIV infected individuals. Moreover the malnutrition which is commonly present in patients with tuberculosis can add to the impaired antioxidant capacity in these patients (Kauri *et al*., 2005). Therefore TB and HIV infections lead to continuous production of free radicals that may cause a resultant low level of antioxidants (Wiid *et al*., 2004).

Albumin is an important component of plasma with antioxidant activity that primarily binds free fatty acids, divalent cations and hydrogen peroxides. The Serum albumin is an important index of the nutritional status of an individual. In an advanced HIV/AIDS and TB, albumin concentration may be reduced due to reduced intake resulting from loss of appetite and reduced hepatic synthesis (Yamanaka *et al*., 2001).

Therefore, examination of antioxidants in participants with HIV and tuberculosis infections may identify deficiencies that predispose to severe oxidant injury and immunodeficiency. However our knowledge of the antioxidant profile in HIV patients with or without TB is scarce particularly in developing countries. Thus to further study the interaction between HIV, TB and antioxidants we investigated antioxidant status and its relationship to CD4+T cell count and albumin in HIV and TB co-infected participants.

## MATERIALS AND METHODS

**Subjects:** Participants were recruited at the Voluntary Counseling and Testing (VCT) unit of Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, South-Eastern Nigeria. At the VCT, participants were counseled on HIV infection, health implication and management of positive HIV test. Before recruiting of participants for this research at the VCT, they were screened for HIV infection and Mycobacterum tuberculosis infection. The participants who were HIV seropositive were further grouped into asymptomatic HIV stage 1 and symptomatic HIV stage 2, 3 and 4 using the World Health Organization (WHO) guidelines for HIV disease staging. Stages 1, 3 and 4 individuals were excluded from the present study. Control group consisted of participants who were HIV seronegative with or without tuberculosis infection. The recruited participants were not on Anti Retro viral Therapy (ART) and TB therapy. Based on the above criteria, the participants were grouped as follows:

- Symptomatic HIV stage 2 aged 39±18 (n = 112, M = 43, F = 69): Symptomatic HIV stage 2 with tuberculosis co-infections (n = 67). Symptomatic HIV stage 2 infected without tuberculosis (n = 45)
- Similarly the Control Subjects consisted of HIV seronegative participants aged 38±19 years (n = 81 M = 36, F = 45): HIV seronegative participants with Tuberculosis (n=52), HIV seronegative participants without Tuberculosis (n = 29)

**Blood sample collection:** 6ml of blood sample was collected from each participant and dispensed into lithium heparinized container for Glutathione peroxidase enzyme activity, plain tubes for total antioxidant status, glutathione reductase using spectrophotometric method respectively and serum albumin measurement by Bromo Cresol Green (BCG) method and the remaining into EDTA container for HIV screening using immunoassay and immunochromatography method and CD4+T-cell count by Cyflow.

Also three early morning sputum specimen were collected from each of the participants for three consecutive days for Zeihl-Neelson staining for acid fast bacilli.

Informed consent was obtained from those who participated in the study. The Nnamdi Azikiwe University Teaching Hospital Board of Ethical Committee approved the study design.

### HIV screening were by both immunoassay and immunochromatographic methods

**Immunoassay method:** HIV screening was performed using Abbot determine™ HIV-1 and 2 kit. (Abbot Japan Co ltd. Tokyo, Japan). The procedure was as described by the manufacturer. Briefly, 50µl of plasma sample from participants were applied to appropriately labeled sample pads. After 15 minutes of sample application, the results were read. The inherent quality control of the kit validates the results. Two visible red lines occurring in the region labeled control and test represents HIV seropositive reaction while a single red colour in the

region of control validates the test kit. Absence of red line in the test region represents HIV seronegative reaction.
Immunochromatographic method.
HIV screening by Immunochromatographic method using HIV 1 and 2 STAT-PAK assay kit. (Chembio diagnostic system, INC New York, USA). This method utilizes immobilized antigen for the detection of antibodies to HIV 1 and 2 in the human plasma. The procedure was as described by the manufacturer of the kit. In brief, 50µl of plasma sample was dispensed into appropriately labeled sample wells, then three drops of running buffer was added drop-wise into the appropriately labeled sample wells. The results of the test were read at 10 minutes after the addition of the running buffer. This method had inherent quality control that validates the results. The presence of two pink lines in the region of test sample and control indicates HIV seropositive reaction while a single pink line at the control region indicates HIV seronegative reaction. HIV seropositive results using these two methods were used to confirm participants presenting with HIV infection.
Determination of CD4+T cell count by Cyflow (Germany) 50µl of whole blood in EDTA anti-coagulant was dispensed into a partec test tube and 10 µl of CD4 PE antibody was added. The reaction mixture was incubated in the dark for 10-15 minutes. After incubation, 800 µl of the already prepared diluted buffer (Xn 0.09% $NaN_3$) was added to each reaction tube and vortexed. The partec tubes containing these reactions were plugged in position in the Cyflow SL Green (Partec Germany) which has already been connected to flow max software, CD4 count template data file and CD4 count instrument. The test was run on the Cyflow for 90 seconds. The results were displayed as histogram and printed. The CD4+T-Cell count was read off the histogram correcting for the dilution factor.

**Determination of Glutathione peroxidase enzyme:** The procedure was as described by the manufacturer of the kit (Randox Laboratories Ltd, UK). In brief, 0.05 ml of heparinized whole blood from each of the participant was put in appropriately labeled test tubes containing 1ml of diluting agent (0.18 mmol/L). This was incubated for 5 minutes. After the incubation, 1ml of haemoglobin reagent (containing I volume of haemoglobin reagent with 4 volumes redistilled water) was added. This was mixed very well and assayed within 20 minutes for Glutathione peroxidase as follows: 0.02 ml of the diluted sample was added in a labeled separate test tubes containing 1ml of Reagent 1 [Glutathione 4 mmol/l, Glutathione Reductase 0.5U/L and NADPH 0.34 mmol/L in phosphate buffer $P^H$ 7.2 (0.05mol/L and EDTA, 4.3 mmol/L)] and 0.04 ml of Cumene solution (Cumene Hydroxide, 0.18 mmol/L). The reagent blank was prepared similarly with 0.02 ml of distilled water in place of the sample. This was mixed gently and the absorbance of sample and reagent blank was read at 340nm against air after 1, 2 and 3 minutes. The change in absorbance of both sample and reagent blank was calculated and the concentration of Glutathione Peroxidase calculated using a factor of 8412 after subtracting the absorbance of blank from that of sample.

**Determination of glutathione reductase enzyme:** The procedure was as described by the manufacturer of the kit (Randox Laboratories Ltd, UK). 40 µl of serum from each of the participant was put in appropriately labeled test tube containing 1000 µl of substrate (2.2mmol/L Reduced Glutathione in 5ml of 250mmol/L Potassium Phosphate buffer, PH 7.3 and 5mmo/L EDTA) and 200µl NADPH (0.17mmol/L). This was mixed and the initial absorbance was read after 1 minute at 340nm against air. This was read again after 2, 3, 4 and 5 minutes. The change in absorbance was calculated and used to calculate the Glutathione Reductase activity in U/L by multiplying by a factor of 4983.

**Determination of total antioxidant status:** This was as described by the manufacturer of the kit (Randox Laboratories Ltd., UK). 20 µl of serum was added into a test tube containing 1000 µl of 2, 2'-Azino-di-[3-ethylbenzthiazoline sulphonate] and 1000 µl of chromogen (6.1 µmol/L of Metmyoglobin in 10 ml of 80 mmol/L phosphate buffered saline (PH 7.4). Standard tube and reagent blank was prepared in the same way using a standard solution containing (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) and double distilled water respectively. 25 µl hydrogen peroxide was added to all tubes and mixed. The absorbance was read after 3 minutes at 660nm against air at 37°C. The change in absorbance of sample and blank was calculated and used to determine the total antioxidant status.

**Determination of serum albumin by Bromocresol Green (BCG):** The procedure used was as described by the manufacturer of the kit (Randox Laboratories Limited UK). Briefly, 10 µl of serum from each participant was added to appropriately labeled test tubes containing 3mls of BCG. The reaction mixture was allowed to stand for 5 minutes at room temperature. For the standard albumin and blank reagent test reactions, similar procedure as explained for sample test was followed except addition of serum sample. The absorbance of samples and standard was read using spectrophotometer at 620 nm wavelength against reagent blank and the concentration of serum albumin in each sample was calculated.

**Statistical analysis:** Statistical analyses were performed by using SPSS for WINDOWS, version 16.0. Data were

presented as means with 95% CIs or as means±SDs unless stated otherwise. Statistical analyses within the groups were assessed by one-way analysis of variance (ANOVA), while post hoc was used to assess statistical comparisons between groups. Correlations between variables were calculated by Pearson's correlation test. P-values were considered statistically significant when $\alpha$ level was P<0.05.

## RESULTS

There was significant difference in serum glutathione reductase (U/L) activity amongst symptomatic HIV infected participants with tuberculosis (39.71±4.28), symptomatic HIV infected without tuberculosis (47.96±4.02), HIV seronegative control participants with tuberculosis (55.06±2.13) and HIV seronegative control participants without tuberculosis (57.16±2.92) (F = 25.75, P<0.01). Between group comparison showed a significantly lower glutathione reductase activity amongst symptomatic HIV infected participants with and without tuberculosis compared with the HIV seronegative control participants with or without tuberculosis (P<0.01 in each case). There was also significantly lower glutathione reductase activity in HIV seronegative control participants with tuberculosis compared with HIV seronegative control participants without tuberculosis (P<0.01). Others groups compared with respect to glutathione showed no differences in mean (P>0.05) see Table 1. There was significant difference in glutathione peroxidase activity (U/gHb) amongst the symptomatic HIV participants with tuberculosis (39.26±3.72) symptomatic HIV without tuberculosis (46.46±2.92), HIV seronegative control participants with tuberculosis (52.74±1.23) and HIV seronegative control participants without tuberculosis (55.47±3.08) (F = 29.85, p<0.01). Similarly between groups showed significant lower glutathione peroxidase activity amongst symptomatic HIV with tuberculosis and symptomatic HIV participants without tuberculosis compared with the HIV seronegative control participants with tuberculosis and without tuberculosis respectively (P<0.01) in each case. See Table 1.

Significant difference in total antioxidant status (mmol/L) was observed amongst symptomatic HIV with tuberculosis (1.31±0.04), symptomatic HIV without tuberculosis (1.40±0.02), HIV seronegative control participants with tuberculosis (1.45±0.05) and HIV seronegative control participants without tuberculosis (1.52±0.02) (F = 18.16 P<0.01). Between group comparison showed that Total antioxidant activity was significantly reduced in symptomatic HIV with tuberculosis compared with the control participants with and without tuberculosis (P<0.01 in each case). Similarly, total antioxidant activity was lowered in symptomatic HIV without tuberculosis when compared with HIV seronegative control participants without tuberculosis (P<0.01). There was also significantly reduced total antioxidant status in HIV seronegative control participants with tuberculosis compared with HIV seronegative control participants without tuberculosis

Table 1: Mean (±SD) concentration of the antioxidants, CD4+T Cells and albumin in Symptomatic HIV with or without tuberculosis and control participants

| Group | Glutathione reductase (U/l) | Glutathione peroxidase (U/gHb) | Total status Antioxidant (mmol/l | CD4+T cell count (/µl) | Albumin g/dl |
|---|---|---|---|---|---|
| Symp HIV with TB {a} n = 67 | 39.71±4.28 | 39.26±3.72 | 1.31±0.04 | 168.29±29.53 | 2.54±0.37 |
| Symp HIV without TB {b} n = 45 | 47.96±4.02 | 46.46±2.92 | 1.40±0.02 | 158.91±24.74 | 3.07±0.24 |
| Control with TB {c} n = 52 | 55.06±2.13 | 52.74±1.23 | 1.45±0.05 | 634.32±124.05 | 3.49±0.17 |
| Control without TB {d} n = 29 | 57.16±2.92 | 55.47±3.08 | 1.52±0.02 | 896.19±120.67 | 4.13±0.22 |
| F (P) Value | 27.75$^{c}$ | 29.85$^{c}$ | 18.16$^{c}$ | 49.38$^{c}$ | 22.94$^{c}$ |
| {a} Vs {b} | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.98$^{a}$ | 0.00$^{c}$ |
| {a} Vs {c} | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ |
| {a} Vs {d} | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ |
| {b} Vs {c} | 0.00$^{c}$ | 0.00$^{c}$ | 0.20$^{a}$ | 0.00$^{c}$ | 0.02$^{b}$ |
| {b} Vs {d} | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ |
| {c} Vs {d} | 0.77$^{a}$ | 0.02$^{b}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ |

Key: Symp = Symptomatic, n= No. of participants, SD = Standard deviation

F(P) = Symptomatic HIV with or without tuberculosis and HIV seronegative control with or without tuberculosis compared (using ANOVA)

{a} Vs {b} = Symptomatic HIV with tuberculosis compared with symptomatic HIV without tuberculosis (using t-test)

{a} Vs {c} = Symptomatic HIV with tuberculosis compared with HIV seronegative control with tuberculosis (using t-test)

{a} Vs {d} = Symptomatic HIV with tuberculosis compared with HIV seronegative control without tuberculosis (using t-test)

{b} Vs {c} = Symptomatic HIV without tuberculosis compared with HIV seronegative control with tuberculosis (using t-test)

{b} Vs {d} = Symptomatic HIV without tuberculosis compared with HIV seronegative control without tuberculosis (using t-test)

{c} Vs {d} = HIV seronegative control with tuberculosis compared with HIV seronegative control without tuberculosis (using t-test)

a = p value at P>0.05, b = P value at P<0.05, c = P value at P<0.01

(P<0.01). However, the symptomatic HIV without tuberculosis showed similar mean value with HIV seronegative control with tuberculosis (P>0.05) see Table 1.

A significant difference in mean (+SD) serum albumin (gldl) was seen amongst symptomatic HIV with tuberculosis (2.54±0.37), symptomatic HIV without tuberculosis (3.07±0.24), HIV seronegative control participants with tuberculosis (3.49±0.17) and HIV seronegative control participants without tuberculosis (4.13±0.22) (F = 22.94, P<0.01). A lower mean value was observed in both symptomatic HIV with tuberculosis and symptomatic HIV without tuberculosis compared with HIV seronegative control p±articipants without tuberculosis. (P<0.05 in each case) Table 1.

The mean (±SD) blood concentration of $CD4^+$T cells (/µl of blood) of symptomatic HIV with tuberculosis (168.29±29.53), symptomatic HIV without tuberculosis (158.91, 24.74), control participants with tuberculosis (634.32±124.45) and control participants without tuberculosis (896.19±120.67) were seen to be significantly different (F = 49.38, P<0.01). No significant difference in mean was observed between symptomatic HIV with tuberculosis compared with symptomatic HIV without tuberculosis (P>0.01). However, there was significant difference between symptomatic HIV with and without tuberculosis compared with HIV seronegative control with and without tuberculosis respectively in CD4+T cell count (P<0.05) in each case. Also mean±SD of CD4+T cell count of HIV seronegative control without tuberculosis show significantly high value compared with the HIV negative control with tuberculosis (P<0.05).

The data in Table 2 shows a significant correlation between CD4+T cell count and glutathione reductase (r = 0.702), glutathione peroxidase (r = 0.693) and TAS (r = 0.593) in symptomatic HIV infected participants with tuberculosis (P<0.01 in each case). A significant correlation was also seen between albumin and glutathione reductase (r = 0.656), glutathione peroxidase (r = 0.546) and TAS (r = 0.622) in symptomatic HIV infected participant with tuberculosis. These values were significant at P<0.01, Table 2 and Fig. 1, 4 and 5.

There was also a significant positive correlation between CD4+T cell count and glutathione reductase (r = 0.670), glutathione peroxidase (r = 0.553) and TAS (r = 0.655) in HIV infected participants without tuberculosis at P<0.01 in each case. Among the HIV seronegative control with tuberculosis infection, no significant correlation was found between serum albumin and glutathione reductase (r = 0.126), glutathione peroxidase (r = -0.102) and TAS (r = 0.179). (P>0.05) Table 2 and Fig. 2, 3, 5 and 7.

## DISCUSSION

The study documents alterations in the levels of glutathione reductase, glutathione peroxidase and total antioxidant status in HIV infected participants. The implication of this finding of lowered antioxidant status in HIV infected participants is that their ability to protect against free radical induced stress is compromised. This might predispose the HIV infected participants to internal tissue/cell damage which may also cause nuclear lesions considering the activities of free radicals. This study suggests evidence of oxidative stress and disturbed glutathione metabolism in HIV infected participants. HIV infected participants have been shown to have decreased antioxidant concentrations, disruption in glutathione metabolism and enhanced spontaneous generation of reactive oxygen species (Sundarama *et al*., 2008). Lower glutathione reductase activity and oxidative stress in general are known to up regulate inflammatory cytokine activity (Akpotuzor *et al*., 2007). Glutathione peroxidase plays an important role in the metabolism of reactive oxygen species as a defense mechanism against oxidative damage by catalyzing the reduction of a variety of hydroperoxidation via glutathione as a reducing substrate. The observations in this study therefore reveal that antioxidant supplementation might have an effect in decreasing oxidative stress in HIV infected individuals.

Our results also clearly show that severe antioxidant depletion occurred in HIV seropositive individuals in comparison with controls and this depletion becomes more severe as the disease progresses. This is

Table 2: Correlation between antioxidants, CD4+T cell counts and albumin

| | Glutathione Reductase (U/l) | Glutathione peroxidase (U/gHb) | Total antioxidant status (mmol/l) |
|---|---|---|---|
| **HIV infected participant With tuberculosis n = 67** | | | |
| CD4+T cell | r = 0.702[c] | r = 0.693[c] | r = 0.593[c] |
| Albumin | r = 0.656[c] | r = 0.546[c] | r = 0.622[c] |
| **HIV infected participants Without tuberculosis n = 45** | | | |
| CD4+T cell | r = 0.670[c] | r = 0.553[c] | r = 0.655[c] |
| Albumin | r = 0.822[c] | r = 0.483[c] | r= 0.630[c] |
| **Control participants with Tuberculosis n = 52** | | | |
| CD4+T cell | r = -0.127 | r = 0.154 | r = 0.138 |
| Albumin | r = 0.126 | r = -0.102 | r = 0.179 |
| **Control participants without Tuberculosis n = 29** | | | |
| CD4+T cell | r = 0.236 | r = 0.010 | r = 0.006 |
| Albumin | r = 0.104 | r = 0.156 | r = 0.142 |

Key: b = P<0.05, c = P<0.01, r = Pearson's correlation

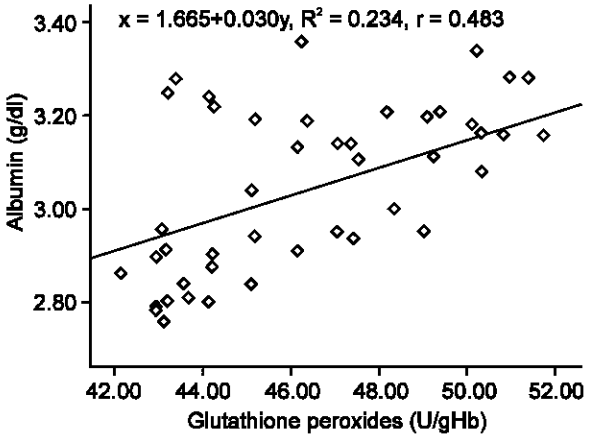


Fig. 1: Scatter plot between albumin and glutathione peroxidase in HIV infected participants without tuberculosis

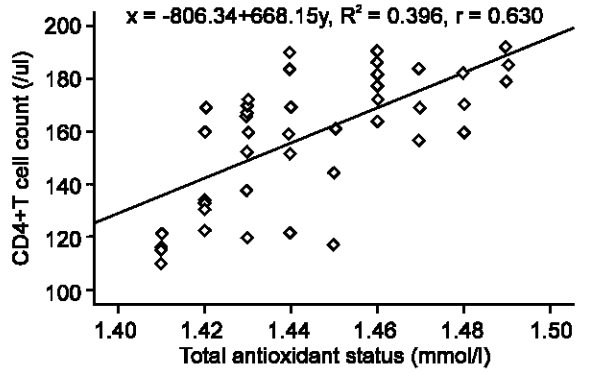


Fig. 2: Scatter plot between CD4+T cell counts and total antioxidant status in HIV infected participants without tuberculosis

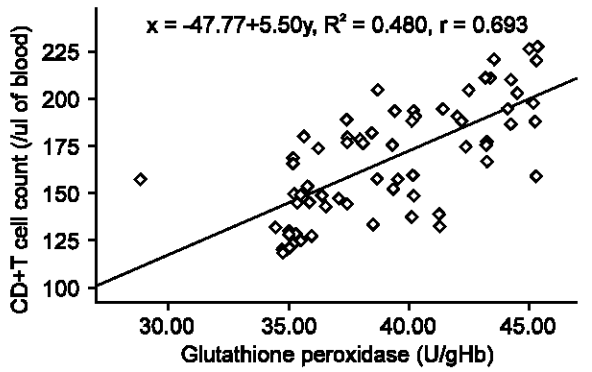


Fig. 3: Scatter plot between CD4+T cell count and glutathione peroxidase in HIV infected participant with tuberculosis

confirmed with the finding of a strong correlation between CD4+T cell count and the antioxidants. The lower the CD4+T cells count the lower the antioxidants.

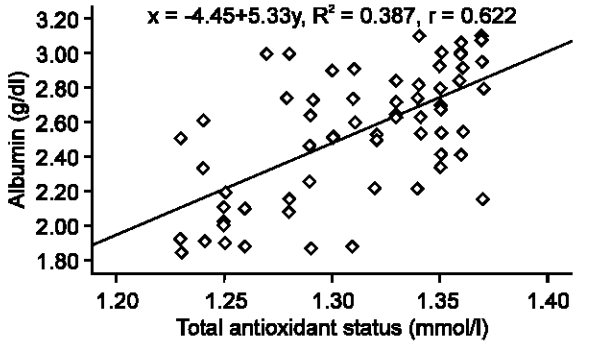


Fig. 4: Scatter plot between albumin and total antioxidant status in HIV infected participant with tuberculosis co-infection

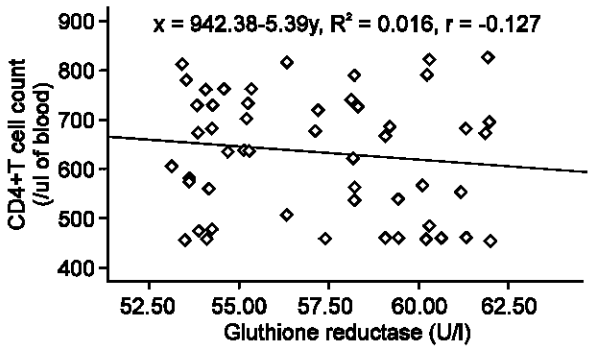


Fig. 5: Scatter plot between CD4+T cell counts and glutathione reductase in control participants with tuberculosis co-infection

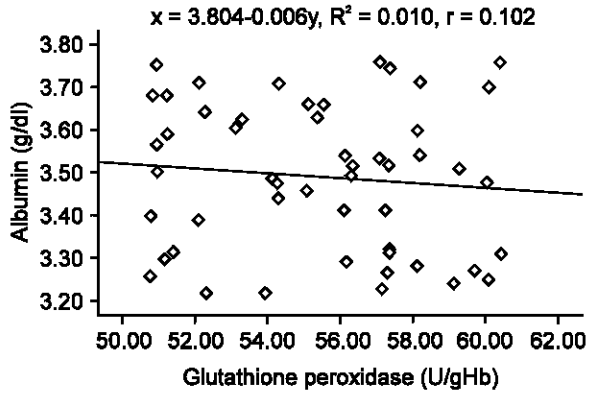


Fig. 6: Scatter plot between albumin and glutathione peroxidase in control participants with tuberculosis co-infection

This assertion is considered valid considering the role of antioxidants in normal immune function. Exposure to oxidants challenges cellular systems and their responses may create conditions that are favourable for the replication of viruses in HIV and decrease in CD4+T cells. As oxidative stress increases so does viral replication which increases the destruction of CD4+T

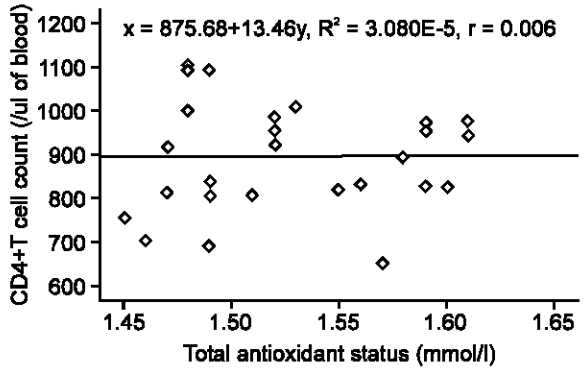


Fig. 7: Scatter plot between CD4+T cell counts and total antioxidant status in control participants without tuberculosis co-infection

cells and progression of disease (Cole *et al*., 2005). Since the immune system is constantly stimulated in HIV infection and antioxidant level is lower in HIV seropositive individuals than in control participants as observed from the study, adequate intake of antioxidants and phytochemicals is critical in minimizing oxidative stress.

The significantly lower glutathione reductase, glutathione peroxidase, total antioxidant status observed in participants presenting with tuberculosis without HIV infection and in symptomatic HIV participants with tuberculosis suggests that tuberculosis also exerts a negative impact on antioxidant enzyme activity/status. This has serious implication in HIV infected participants who are also very susceptible to tuberculosis infection. In tuberculosis infection, several factors may contribute to reduced antioxidant status such as tissue inflammation and malnutrition. The findings show that in participants with tuberculosis and HIV co-infection there is a severe depression in antioxidant enzymes which may lead to progression of disease since infection by HIV and/or TB is known to cause persistent chronic inflammation. There are evidences that individuals infected with HIV and/or TB are under chronic oxidative stress (Oguntibeju *et al*., 2010), with resultant decrease in endogenous and nutritional antioxidants as well as micronutrients. Oxidative stress due to over production of free radicals and antioxidant deficiency causes damage to vital biological macromolecules and organs and further contributes to disease complications, progression and morbidity. Nevertheless, this combination of enhanced oxidative stress and decreased concentrations of several antioxidants may have important pathogenic consequences in HIV and TB co-infected participants. Oxidative stress has been shown to enhance HIV replication, to induce the production of inflammatory cytokines (Israel and Gougerot-Pocidalo, 1997) and to promote lymphocyte apoptosis (Famularo *et al*., 1997) and T cell dysfunction (Aukrust *et al*., 1995) these effects could therefore contribute to increased viral replication and progression of immunodeficiency in patients dually infected with HIV and TB.

Another significant finding in this study was that participants with HIV infection had more depleted levels of CD4+T cells and the rate of CD4+T cell counts decreased more in tuberculosis infected symptomatic HIV participants. CD4+T cells are required for host's resistance to mycobacterium tuberculosis (Barber *et al*., 2011) and they regulate the protective immune response which follows exposure to mycobacterium tuberculosis by activating macrophages through the cytokines the CD4+T cells secrete (Boom *et al*., 1991). This implies that tuberculosis co-infection in HIV can lead to increased disease burden and severe immune derangement. Although this is in contrast to the report by Audu *et al*. (2005) who observed that co-infection with tuberculosis or malaria appeared not to have any impact on the degree of depletion of CD4+T cells in HIV infected subjects but Sharma *et al*. (2005) had different observation that HIV and tuberculosis have synergistic interaction, each accelerates progression of the other and tuberculosis co-infection in HIV leads to higher rate of CD4+T cell depletion.

TB is the first sign of immune dysfunction associated with HIV infection and active TB is an AIDS defining illness. TB infection is an ever increasing concern for people living with HIV. In some parts of the world, TB is the leading cause of death of people infected with HIV. Indeed the risk of developing active TB disease after TB infection or following an apparent cure for several years, increases considerably for people with deficient immune system. It was calculated that in case of HIV co-infection, this risk is multiplied 50-300 times (Sylvain *et al*., 2007). It has also been established that serum anti BCG is mostly impaired by HIV infection even in cases of malaria co-infection in endemic areas (Onyenekwe, 2008). T helper cells Type 1 (Th 1) immune response characterized by adequate cell-mediated immunity is the crucial host defense against *M. tuberculosis*. HIV infection primarily affects those components of host immune response responsible for cell-mediated immunity. Thus in HIV infected individuals with latent TB infection, the fine balance between *M. tuberculosis* and the host immunity is shifted favouring reactivation of *M. tuberculosis*. However the infection is poorly contained following reactivation, resulting in widespread dissemination causing extrapulmonary disease (Zang *et al*., 1994).

The study shows that albumin concentration was significantly low in symptomatic HIV infected subjects with or without tuberculosis co-infection and in tuberculosis infected control participants. Albumin is an important component of plasma with antioxidant activity that primarily binds free fatty acids, divalent cation and

hydrogen oxochloride (HOCl). These pro-oxidants attack the cell membranes thereby causing tissue damage and wasting disease. The low level of albumin may therefore contribute to the complications associated with TB and HIV (Oguntibeju *et al*., 2010). This reduction of albumin in HIV infection and in tuberculosis infection may be attributed to negative nitrogen balance, anorexia, malnutrition, malabsorption and increased catabolic activities associated with these diseases. Albumin has been used as a prognostic indicator in HIV disease. Onyenekwe *et al*. (2008) and Olawumi and Olatunji (2006) in their studies have documented a decrease in serum albumin in HIV infected individuals. Matos and Moreira Lemos (2006) documented that the presence of low albumin levels was strongly and independently associated with in-hospital death due to TB. Since HIV infected subjects with tuberculosis had significantly low albumin, this subgroup may potentially benefit from nutritional intervention. Several studies have revealed that micronutrients supplementation to patients with tuberculosis and HIV improves their health by increasing CD4+T cell count, increasing their weight and improving the efficacy of their drug treatment (Jon *et al*., 2006). This study highlights the importance of measuring serum albumin levels in people infected with TB and HIV as an indicator of the prognosis and disease progression.

From the study we conclude that the antioxidants glutathione reductase, glutathione peroxidase, total antioxidant status, albumin and CD4+T cell count were significantly reduced in symptomatic HIV infected participants with or without TB as well as in participants infected with TB. This finding suggests possible impairment of antioxidant function leading to oxidative stress in HIV and TB infection. Therefore the study suggests that prediction of severity and monitoring of disease could be performed by determining the CD4+T cell count, albumin and antioxidant capacity of HIV infected individuals especially those co-infected with TB. Thus Antioxidant supplementation could sufficiently scavenge free radicals, increase total antioxidant capacity with the potential to reduce disease progression and complications, increase survival and improve the general well being of people living with TB and HIV/AIDS.

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