Evaluation of Some Antioxidants in tuberculosis Patients

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ABSTRACT

Objective: Serum levels of vitamins C, E, selenium, malondialdehyde (MDA), activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GRx) in individuals with Mycobacterium tuberculosis (MTB) infection was investigated.

Methods: 251 individuals consisting 120 treatment naïve individuals with active TB [26 (TB+HIV+) and 12 malaria parasite (MP) and TB (TB+MP+)) co-infection, 82 HIV negative (TB+HIV−)], 26 Latent TB (LTB) and 105 apparently healthy control (AHC). TB infection was determined by Ziehl-Neelsen sputum smeared microscopy and GeneXpert. MP was confirmed by Giemsa staining technique, HIV by immuno-chromatographic method. Analytes were evaluated spectrophotometrically, but selenium was by atomic absorption spectrometer. SPSS version 21 ANOVA was used for statistical analyses.

Results: The mean levels of vitamins E, C and selenium were significantly lower in individuals with TB infections compared with AHC (p<0.05). Significantly higher differences were observed in MDA levels in TB infections than in AHC (p<0.05). Significantly lower differences existed in the mean levels of vitamin C and selenium in LTBI than in control, MDA was significantly higher in the same group (p<0.05). The mean levels of vitamin E in TB+HIV+ (1.57±1.30) and TB+MP+ co-infections (2.23±1.70) were significantly lower compared with those with TB+(7.01±3.40)(p<0.05).The mean SOD, catalase and GPx activities in active tuberculosis, TB+HIV+ and TBMP co-infections were significantly lower than in the control (p<0.05). The mean GRx activity in AHC, active TB, TB+HIV+ and TB+MP+ co-infections were significantly lower than in LTBI(p<0.05).

Conclusions: Glutathione reductase may have prognostic potential in the differential screening for latent tuberculosis.

Keywords: Antioxidants; Tuberculosis; Malondialdehyde.

INTRODUCTION

The main cause of tuberculosis is Mycobacterium tuberculosis (MTB). The high lipid content of this pathogen is the reason for many of its unique clinical characteristics. One third of the world population has latent Mycobacterium tuberculosis. HIV/ AIDS epidemics have increased the burden of tuberculosis by enhancing the rate of tuberculosis acquisition and activation of latent MTB to active Mycobacterium tuberculosis. Screening for latent tuberculosis with Mantoux tests is rarely done in any of the major hospitals in Anambra state. Individuals identified as positive from the rare screening hardly get treated. These individuals and others who may have been missed to non screening have the potentials of progressing to active tuberculosis and
infect other people. A vicious cycle of tuberculosis (TB) spread is thus set in motion. Sputum smear AFB microscopy, culture and GeneXpert used for the diagnosis of TB currently relies mostly on sputum. However, there are some patients who may not be able to produce sputum and so cannot be screened with it. These problems could result to more deaths. Tuberculosis may infect any part of the body, but most commonly occurs in the lungs (pulmonary tuberculosis). Extrapulmonary TB occurs outside the lungs, may coexist with pulmonary TB. [6] The infection may extend into the pulmonary artery in rare cases resulting in massive bleeding. [1,7] A potentially more serious, widespread form of TB is called disseminated or miliary tuberculosis. [8] Free radicals and reactive oxygen species (ROS) are produced during tuberculosis infection. [9-10] Free radicals are cytotoxic and need to be removed by efficient antioxidant system. [11] Consequently, enhanced ROS and free radical production may lead to imbalance in the host antioxidant capacity. [12] This may cause oxidative stress and lipid peroxidation. [13] The effect of Mycobacterium tuberculosis infection on malondialdehyde and antioxidant status of individuals with Mycobacterium tuberculosis infection was investigated in this study.

2.0 MATERIALS AND METHODS

Study Area/ Design

This cross sectional research was conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State Nigeria. 251 study subjects composed of 120 treatment naïve active TB patients [26 HIV co infected (TB+HIV+) and 82 HIV negative (TB+HIV−), 12 malaria parasite co-infected (TB+MP+)], 26 latent TB infected (LTBI) and 105 healthy control (TB−HIV−TST−) were recruited. At recruitment, all study subjects were interviewed using a standard questionnaire and demographic data were collected. The weight and height of all the subjects were measured and used to determine their Body Mass index. TB infection was determined by Ziehl-Neelsen (ZN) sputum smeared microscopy and confirmed positive using gene Xpert.

Study population

The study population consisted of five thousand five hundred and eighteen (5518) suspected individuals with cardinal symptoms of tuberculosis who presented at TBDOT clinics of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi between May, 2015 and January, 2018.

Sampling Technique

A convenience and consecutive sampling techniques were used to select individuals from the TB DOT centres before the initiation of therapy. Participants were individuals who met the inclusion criteria and consented after the purpose was explained to them and were recruited as they became available until the sample size was attained.

Sample Size

Sample size was determined using the formula of. [14]

Inclusion criteria

Newly diagnosed TB positive individuals with or without mp, and or HIV co-inflections were recruited. The individuals above were Category one, first line TB positive individuals between 15-66years, attending the TB DOTS Clinic, NAUTH, Nnewi, State Anambra.

Exclusion criteria

Individuals infected with TB on antiretroviral therapy were excluded. Patients diagnosed with pulmonary tuberculosis but having diabetes mellitus were excluded from the study. Tobacco smokers, alcohol drinkers and participants who had other clinical problems such as diabetics and cardiovascular diseases were excluded from the study.

Ethical consideration

Ethical approval for the study was obtained from Nnamdi Azikiwe University Teaching Hospital Ethics Committee (NAUTHEC)
Informed consent
Participation was voluntary and informed consent was obtained from all the participants.

Sample collection
Sputum collection
Sputum samples were collected using the Directly Observed treatment short Course (DOTs) strategy specification. Sputum sample was collected twice (consisting of on the spot sample and early morning sample next day) into a wide mouth container from each of the individuals.

Sputum processing
Sputum samples were processed using the Ziehl Neelsen Staining Method and confirmed using the Gene Xpert by Cepheid, especially for HIV positive cases with cough.

Blood sample collection
Blood samples were collected once from individuals with active Mycobacterium tuberculosis infection. Firstly, immediately the individual(s) was confirmed to be positive for pulmonary tuberculosis, before the initiation of anti tuberculosis treatment (ATT). Blood samples were collected from individuals with latent TB and apparently healthy individuals (control) once. Eight milliliters (8mls) of blood was collected from each individual at each period of blood collection, thick and thin blood films were made for microscopic detection of P. falciparum on recruitment and malaria plasmodium falciparum rapid test device (CARESTARTTM Malaria HRP2 (Pf) by ACCESS BIO,INC. USA).

Mantoux test
Tuberculin purified protein derivative (PPD) was utilized for the mantoux test. The PPD used was obtained from BB – NCIPD Ltd, sofia, Bulgaria. Each vial contained 1ml (10 doses) containing 50TU of PPD = STU/0.1ml per dose.

Determination of enzymic antioxidants
i. Superoxide dismutase activity was evaluated by the Method of [16] as described by [17,18]  
ii. Catalase activity was estimated by the method of [19]  
iii. The activity of glutathione peroxidase was determined by the method of [20]  
iv. Glutathione Reductase activity was evaluated by colorimetric method as described by [21,22]

Determination of non-enzymic antioxidants.

i. Vitamin C was estimated by [22] method  
ii. Serum vitamin E (α-tocopherol) was estimated by the method of Desai [23]  

Determination of Malondialdehyde (MDA)
Malondialdehyde was estimated by the method of. [25]

**Statistical analysis**

The IBM Statistical Package for Social Sciences (SPSS) version 21 ANOVA and LSD’s post hoc were used for statistical analyses. The results were presented as mean ± standard deviation. Significant levels were considered at p< 0.05

**RESULTS**

Table 1. Serum baseline levels of non enzyme antioxidants in individuals with latent tuberculosis (LTB) and Tuberculosis (TB) infections, individuals with human immunodeficiency virus and tuberculosis co-infection (HIV&TB) , individuals with tuberculosis and malaria parasite co-infection (TB&MP) and apparently healthy control (mean±SD).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>VIT E (mg/dl)</th>
<th>VIT C (mg/dl)</th>
<th>Selenium (mg/l)</th>
<th>MDA (vol.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.AHC (n=105)</td>
<td>14.21±3.96</td>
<td>2.18±0.69</td>
<td>20.57±12.53</td>
<td>0.55±3.30</td>
</tr>
<tr>
<td>2.LTBI (n=26)</td>
<td>13.75±4.55</td>
<td>1.95±0.75</td>
<td>18.21±14.17</td>
<td>1.25±0.39</td>
</tr>
<tr>
<td>3.TB (n=82)</td>
<td>7.01±3.40</td>
<td>0.37±0.20</td>
<td>0.42±0.22</td>
<td>2.55±1.49</td>
</tr>
<tr>
<td>4.TB &amp;HIV (n=26)</td>
<td>1.57±1.30</td>
<td>0.51±0.22</td>
<td>0.62±0.18</td>
<td>2.35±1.64</td>
</tr>
<tr>
<td>5.TB&amp;MP (n=12)</td>
<td>2.23±1.70</td>
<td>0.32±0.17</td>
<td>0.57±0.16</td>
<td>3.00±1.84</td>
</tr>
<tr>
<td>F-value</td>
<td>83.55</td>
<td>172.11</td>
<td>27.79</td>
<td>47.52</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 VS 2</td>
<td>0.480</td>
<td>0.040 *</td>
<td>0.040 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 VS 3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 VS 4</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 VS 5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 VS 3</td>
<td>0.000</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2 VS 4</td>
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<td>0.00</td>
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<td>2 VS 5</td>
<td>0.000</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>3 VS 4</td>
<td>0.007</td>
<td>0.23</td>
<td>0.96</td>
<td>0.42</td>
</tr>
<tr>
<td>3 VS 5</td>
<td>0.008</td>
<td>0.76</td>
<td>0.98</td>
<td>0.19</td>
</tr>
<tr>
<td>4 VS 5</td>
<td>0.873</td>
<td>0.297</td>
<td>0.993</td>
<td>0.095</td>
</tr>
</tbody>
</table>

*AHC: apparently healthy control, TBHIV: tuberculosis and HIV co-infection, TBMP: tuberculosis and malaria parasite co-infection.

LTBI: Latent tuberculosis infection, TB: active tuberculosis infection. Results are expressed as Mean±SD and are statistically significant at p<0.05 (Confidence Interval (CI) is set at 95%).

The mean serum level of Vitamin E was significantly lower in individuals with active tuberculosis (TB+), individuals with human immunodeficiency virus and tuberculosis co-infection (HIV&TB), individuals with tuberculosis and malaria parasite co-infection (TB&MP) (7.01±3.40, 1.57±1.30, 2.23±1.70) respectively compared with AHC (14.21±3.96) (p<0.05). Likewise, there was significantly lower mean serum level of Vitamin E in individuals with active TB (7.01±3.4) compared with LTBI (13.75±4.55) (p<0.05) .Further, there was significantly lower mean serum level of Vitamin E in individuals with HIV&TB (1.57±1.30) and in individuals with TB&MP (2.23±1.70) than those with TB+ (7.01±3.4) (p<0.05). No significant difference exists in the mean serum level of Vitamin E in those with HIV&TB (1.57±1.30) compared with individuals with TB&MP (2.23±1.70) (p>0.05). Furthermore, the mean serum level of Vitamin C was significantly lower in individuals with LTBI, active TB+, individuals with HIV&TB and those with TB&MP (1.95±0.75, 0.37±0.20, 0.51±0.22 and 0.32±0.17) respectively compared with AHC (2.18±0.69) (p<0.05). Likewise, the mean serum level of Vitamin C was significantly lower in individuals with TB+, HIV&TB and those with TB&MP (0.37±0.20, 0.51±0.22 and 0.32±0.17) respectively compared with LTB (1.95±0.75) (p<0.05). There was significantly lower mean serum level of Vitamin C in individuals with active TB (0.37±0.20) and LTBI (1.95±0.75) compared with AHC (2.18±0.69) (p<0.05). There was significantly lower mean serum level of selenium in individuals with TB+, in individuals with HIV&TB and those with TB&MP.
Table 2. Serum baseline activities of enzyme antioxidants in individuals with latent tuberculosis (LTB) and Tuberculosis (TB) infections, individuals with human immunodeficiency virus and tuberculosis co-infection (HIV&TB), individuals with tuberculosis and malaria parasite co-infection (TB&MP) and apparently healthy control (mean±SD).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SOD (U/ml)</th>
<th>GPX (umol/l)</th>
<th>GRX (mu/ml)</th>
<th>Catalase(U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.AHC (n=105)</td>
<td>1.58±0.51</td>
<td>0.46±0.20</td>
<td>49320.11±10.30</td>
<td>50.80±24.55</td>
</tr>
<tr>
<td>2.LTBI (n=26)</td>
<td>1.61±0.23</td>
<td>0.58±0.25</td>
<td>87388.42±58.17</td>
<td>52.46±54.46</td>
</tr>
<tr>
<td>3.TB (n=82)</td>
<td>1.13±0.46</td>
<td>0.34±0.22</td>
<td>26452.31±16.23</td>
<td>31.96±13.69</td>
</tr>
<tr>
<td>4.TB&amp;HIV(n=26)</td>
<td>0.48±0.40</td>
<td>1.36±1.12</td>
<td>1212.70±1.33</td>
<td>1.53±1.14</td>
</tr>
<tr>
<td>5.TB&amp;MP (n=12)</td>
<td>0.65±0.63</td>
<td>2.00±1.32</td>
<td>1111.40±1.30</td>
<td>11.27±0.60</td>
</tr>
<tr>
<td>t-value</td>
<td>98.85</td>
<td>23.78</td>
<td>23.78</td>
<td>105.78</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*AHC: apparently healthy control, TBHIV: tuberculosis and HIV co-infection. TBMP: tuberculosis and malaria parasite co-infection. LTB: Latent tuberculosis infection, TB: active tuberculosis infection. Results are expressed as Mean±SD and are statistically significant at p<0.05 (Confidence Interval (CI) is set at 95%).

The mean serum activity of superoxide dismutase was significantly lower in active TB, TBHIV and TBMP co-infections (1.13±0.46, 0.48±0.40, 0.65±0.63) respectively compared with those with LTBI (1.61±0.23) and AHC (1.58±0.32) (p<0.05). Moreover, the mean serum activity of superoxide dismutase in individuals with TBHIV co-infection (0.48±0.40) and TBMP co-infection (0.65±0.63) were significantly lower than in individuals with TB+ (1.13±0.46) (p<0.05). There existed a significantly higher mean serum activity of glutathione peroxidase in individuals with TBHIV (1.36±1.12) and TBMP co-infections (2.00±1.32) compared with those with active TB, LTBI and the control (0.54±0.22, 0.58±0.25 and 0.46±0.20) (p<0.05) respectively. Also, the mean serum activity of glutathione peroxidase was significantly higher in LTBI (0.58±0.25) and active TB (0.54±0.22) compared with AHC (0.46±0.20) (p<0.05). Moreover, the mean serum activity of glutathione peroxidase in individuals with LTBI (0.58±0.25) was significantly higher than with AHC (0.46±0.20) (p<0.05). However, the mean serum activity of glutathione reductase in LTBI was significantly higher (87388±58.17) compared with those with TB+, TBHIV, TBMP co-infection and AHC (26452.31±16.25, 1212.70±1.33, 1111.40±1.30 and 49320.11±10.30) (p<0.05) respectively, while no significant difference was observed in the mean serum activity of glutathione reductase in individuals with TBHIV co-infection (1212.70±1.33) compared with individuals with TBMP co-infection (1111.40±1.30) (p>0.05). But serum activity of glutathione reductase (mu/ml) was significantly lower in
individuals with active TB (26452.31±16.25) than those with LTBI (87388.42±58.17) and AHC (49320.11±10.30) (p<0.05). Serum activity of catalase was significantly lower in active TB (31.96±13.69) compared with individuals with latent tuberculosis (52.46±34.46) and AHC (50.80±24.55) (p<0.05). However, the mean serum activity of catalase in individuals with TBHIV co-infection (11.53±1.14) and individuals with TBMP co-infection (11.27±0.60) was significantly lower than in individuals with active TB, LTBI and AHC (31.96±13.69, 52.46±34.46 and 50.80±24.55)(p<0.05).

DISCUSSION

The degree of oxidative stress is not only evaluated by the generation of free radicals and reactive oxygen species (ROS) but also by antioxidants. [12] The mean levels of antioxidants were significantly reduced in the test groups while that of malondialdehyde were significantly higher in the test groups. The mean serum levels of baseline non enzyme antioxidants (vitamin E, vitamin C and selenium were significantly lower in individuals with TBHIV and TBMP co-infections compared with the control group. This could be as a result of oxidative damage that occurs during tuberculosis disease. Hence, malondialdehyde a product of lipid peroxidation was significantly raised in Mycobacterium tuberculosis co-morbidity in this study. The significantly lower mean serum levels of vitamins C and E observed in this study have been previously reported by some authors. [26,15,29,13] The reported no significant difference in the mean serum level of selenium in individuals with LTBI and significant difference observed in individuals with active tuberculosis corroborated the work of. [13] Further, the significantly higher difference observed in the mean serum level of MDA (nmol/ml) in individuals with active tuberculosis compared with AHC, and a significantly lower difference in the mean serum level of MDA in LTBI compared with active TB in this study was consistent with the findings of. [26,15,13] It could be due to high oxidative stress in TB infected individuals. Vitamin-E has been described as the major chain breaking antioxidant in humans because of its lipid solubility. Vitamin E is located in cell membranes, where it interrupts lipid peroxidation and probably plays a major role in modulating intracellular signaling pathway that relay on reactive oxygen species. Vitamin E can also directly stop ROS, including superoxide radical, OH, and O2.Vitamin E plays a primary defense against lipid peroxidation and oxidation. [27] Vitamin C is the most abundant water soluble antioxidant in the body. It directly removes oxygen radical, hydroxyl radical and hydrogen peroxide. Vitamin C renders inactive oxidants from stimulated neutrophils. Vitamin-C contributes to the reformation of vitamin E to its active form. Vitamin C is the most important compound implicated in recycling of alpha-tocopherol radicals. [28] However there is paucity of information on the effect of Mycobacterium tuberculosis - Plasmodium falciparum (Pf)(TBMP) and Mycobacterium tuberculosis- Human Immunodeficiency virus (HIV)co -infection (TBHIV) on non enzyme antioxidants(vitamins E and C, selenium )and MDA. Most studies compared active TB individuals and control only. [26,10,13] All the studies observed reduced levels of non enzyme antioxidants and raised levels of MDA in individuals with tuberculosis. Unlike, in this study, individuals with tuberculosis with either HIV or MP co-infection were compared with the control group. They suffered enhanced lipid peroxidation due to tuberculosis and this probably impaired their antioxidant capacity. One of the manifestations of these free radical mediated processes are lipid peroxidation. [3] This finding corroborates with the study of. [13] The observed no statistically significant difference in individuals with TBHIV and TBMP comorbidity, could be because they had in common active TB infection. Similar result was obtained by, [29] in their study. Likewise, [30] remarked that co-infection
with tuberculosis or malaria appeared not to have any impact on the degree of depletion of CD4+ T cell counts in individuals living with HIV. See table 1.

The significantly lower mean serum activities of superoxide dismutase and catalase in individuals with *Mycobacterium tuberculosis* infection in this study could be attributed to the development of TB disease. [31] There was also significantly higher mean serum activity of catalase in individuals with LTBI. Similar result was also obtained by. [26] Consequently, [27] and [31] in their two months follow-up study had the same report. MDA levels gradually decreased with clinical improvement following treatment. Erythrocyte SOD, serum catalase, plasma glutathione levels and serum total antioxidant status were decreased significantly in TB1 and TB2, TB3 patients than the control, these levels gradually increased with clinical improvement with ATT [26] Oxidative stress was observed in all the TB patients (TB1, TB2, TB3), irrespective of treatment status, however, none of the researchers recruited individuals with latent tuberculosis. Further, a significantly higher mean serum activity of GPX (μm/ml) in individuals with LTBI and active TB was seen. This observation supported the work of. [27] Besides, there was a significantly lower mean serum activity of GRX in individuals with active TB infection with or without comorbidity compared with LTBI and AHC in this research; these findings substantiated the study by, [32] in their work. It confirmed the role of oxidative stress in the pathogenesis of TB, reduced antioxidant levels and free radical generation in the TB patients than in control group. Individuals with LTBI are immune competent, which explains the no significant differences observed in Vitamin E levels, SOD, and catalase activities in individuals with LTBI. Unexpectly, an outstanding significantly higher activity of GRx was seen in individuals with LTBI compared to other groups may suggest the prognostic potential of the enzyme in the differential screening for latent tuberculosis.

Also, the observed significantly reduced mean serum activities of baseline enzyme antioxidants (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) in individuals with TBHIV and TBMP co-infection could be attributed to tuberculosis disease. This is consistent with the work of, [29] which reported lower serum activities of glutathione peroxidase, glutathione reductase and total antioxidant capacity in both HIV infected participants with or without tuberculosis and HIV seronegative participants with tuberculosis, (in each case) compared with apparently healthy control and no significant difference was also observed compared with comorbidity or TB participants as in this study. Table 2

**CONCLUSIONS**

The findings of this study suggest reduced levels of antioxidants in individuals with active *Mycobacterium tuberculosis* infection with increased levels of malondialdehyde. However, an outstanding significantly higher activity of GRx in individuals with LTBI may suggest the prognostic potential of the enzyme in the differential screening for latent tuberculosis.

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**Authors’ Contributions**

ACI, SCM, CCO and CEO conceived and designed the research proposal. ACI, CCO and CEO performed sample collection, and experiments and ACI,CEO and SCM analyzed the data. Discussion was done by SCM while ACI, CCO and SCM contributed to the final version of the manuscript.SCM and CCO also supervised the work.
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