International Journal of Health Sciences and Research
Vol.10; Issue: 7; July 2020
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Evaluation of Some Antioxidants in tuberculosis Patients

Ihim Augustine Chinedu[1], Meludu Samuel Chukwuemeka[1,2], Onyenekwe Charles Chinedum[1], Onah Christian Ejike[1]

[1]Chemical Pathology Unit, Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State , Nigeria.
[2]Department of Human Biochemistry, Faculty of Basic Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra state, Nigeria.
[3]Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus

Corresponding Author: Ihim Augustine Chinedu

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).* [1] The high lipid content of this pathogen is the reason for many of its unique clinical characteristics. [2,3] One third of the world population has latent *Mycobacterium tuberculosis.* [4] 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.* [5,6] 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−), 12malaria 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 - infections 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)

NAUTH/CS/66/VOL.7/79 Nnewi, Anambra State Nigeria.

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 individuals 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/*pan rapid test device (Startcare ^{TM} Accessio USA) for the qualitative detection of circulating *P. falciparum* antigen in whole blood was also used. Two milliliters (2ml) of blood was dispensed in ethylene diamine tetracetic acid (EDTA) bottle and six milliliters (6ml) of blood was dispensed in plain tube to separate serum for various biochemical assays. [15] The blood in the plain tube was allowed to stand for 30 minutes to clot and further centrifuged at 3500 rpm for five minutes using Wisperfuge model 1384 centrifuge (Samson, Holland). Serum was separated from clot with micropipette into sterile serum sample bottle for the measurement of biochemical parameters. Each individual's blood sample was stored frozen at -20^{0}C in aliquot, in three vials to avoid repeated thawing and storing that would affect the result of the analysis.

Diagnostic Assessments

The HIV status of study subjects was determined using the Determine HIV-1/2 (Abbott laboratories, Japan) as the screening test, the Capillus HIV-1/2 (Trinity Biotech, Ireland) as the confirmatory test and Uni-Gold HIV-1/2 recombinant (Trinity Biotech, Ireland) as a tie breaker test.

Diagnosis of Malaria

P. falciparum malaria was determined employing Giemsa staining technique and malaria plasmodium falciparum rapid test device (CARESTARTTM Malaria HRP2 (Pf) by ACCESS BIO,INC. USA).

Mantoux test [16]

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 = 5TU/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]

iii. Selenium analysis was conducted using Varian AA240 Atomic Absorption Spectrophometer according to the method of APHA [24])

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 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).

GROUP	VIT E (mg/dl)	VIT C (mg/dl)	Selenium(mg/l)	MDA
1.AHC (n=105)	14.21±3.96	2.18±0.69	20.57±12.53	0.55±.30
2.LTBI (n=26)	13.75±4.55	1.95±0.75	18.21±14.17	1.25±0.39
3.TB (n=82)	7.01±3.40	0.37±0.20	0.42±0.22	2.55±1.49
4.TB &HIV(n=26)	1.57±1.30	0.51±0.22	0.62±0.18	2.35±1.64
5.TB&MP (n=12)	2.23±1.70	0.32±0.17	0.57±0.16	3.00±1.84
f-value	83.55	172.11	27.79	47.52
p-value	<0.001	<0.001	<0.001	<0.001
1 VS 2	0.480	0.040 *	0.040 *	<0.001
1 VS 3	<0.001	<0.001	<0.001	<0.001
1 VS 4	<0.001	<0.001	<0.001	<0.001
1 VS 5	<0.001	<0.001	<0.001	<0.001
2 VS 3	0.000	0.00	0.00	0.00
2 VS 4	0.000	0.00	0.00	0.00
2 VS 5	0.000	0.00	0.00	0.00
3 VS 4	0.007	0.23	0.96	0.42
3VS 5	0.008	0.76	0.98	0.19
4 VS 5	0.873	0.297	0.993	0.095

***AHC**: apparently healthy control, **TBHIV:** tuberculosis and HIV co-infection,: **TBMP:** tuberculosis and malaria pararsite 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 (0.42±0.22, 0.62±0.18 and 0.57±0.16) respectively compared with LTBI (18.21±14.17) and AHC (20.57±12.53) ($p<0.05$).Further, the mean serum level of MDA was significantly higher in individuals with LTBI, TB+, HIV&TB and those with TB&MP (1.25±0.39, 2.55±1.49, 2.35±1.64 and 3.00±1.84) respectively compared with AHC (0.55±.30) ($p<0.05$).

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 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).

GROUP	SOD (U/ml)	GPX (umol/l)	GRX (mu/ml)	Ctalase(U/l)
1.AHC (n=105)	1.58±0.31	0.46±0.20	49320.11±10.30	50.80±24.55
2.LTBI (n=26)	1.61±0.23	0.58±0.25	87388.42±58.17	52.46±34.46
3.TB (n=82)	1.13±0.46	0.54±0.22	26452.31±16.25	31.96±13.69
4.TB &HIV(n=26)	0.48±0.40	1.36±1.12	1212.70±1.33	11.53±1.14
5.TB&MP (n=12)	0.65±0.63	2.00±1.32	1111.40±1.30	11.27±0.60
f-value	98.85	23.78	9.18	105.78
p-value	<0.001	<0.001	<0.001	<0.001
1 VS 2	0.746	0.040	0.028	0.425
1 VS 3	<0.001	<0.001	<0.001	<0.001
1 VS 4	<0.001	<0.001	<0.001	<0.001
1 VS 5	<0.001	<0.001	<0.001	<0.001
2 VS 3	<0.001	<0.001	0.040	<0.001
2 VS 4	<0.001	<0.001	<0.001	<0.001
2 VS 5	<0.001	<0.001	<0.001	<0.001
3 VS 4	<0.001	<0.001	<0.001	0.004
3VS 5	<0.001	<0.001	<0.001	0.003
4 VS 5	0.248	0.059	1.000	0.936

*__AHC__: apparently healthy control, **TBHIV:** tuberculosis and HIV co-infection,: **TBMP:** tuberculosis and malaria pararsite 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 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 in 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 (mu/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. Unexpectedly, 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.

ACKNOWLEDGEMENTS

The authors would like to pay their most profound gratitude to the Tertiary Education Trust Fund (TETFUND), Nigeria for the research grants extended for the accomplishment of this research activity. The management and staff of Nnamdi Azikiwe University Teaching Hspital Nnew and Reene Medical Diagnostic Laboratory, Awada, Anambra State for the laboratory analyses of all biochemical parameters, Staff of TBDOT laboratory NAUTH Nnewi, Anambra State.

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.

REFERENCES

1. Gerald L, Mandell JE, Bennett R. Mandell D and Bennett's principles and practice of infectious diseases 7th ed. Philadelphia: Churchill Livingstone/Elsevier; 2010.
2. Jindal SK. Textbook of pulmonary and critical care medicine. 1st ed. New Delhi: Jaypee Brothers Medical Publishers 2011; p.549-550.
3. Niederweis M, Danilchanka O, Huff J, Hoffmann C, Engelhardt H. Mycobacterial outer membranes in: search of proteins. *Trends in Microbiol* 2010;18 (3): 109–116.
4. World Health Organisation. Latent TB Infection, Updated and consolidated guidelines for programmatic management.https://www.who.int/tb/publications/2018/latent-tuberculosis-infection/en/(retrieved October 10, 2019):1-78.
5. World Health Organization.https://www.who.int>tb>publications>tb control. Geneva: World Health Organization.(retrieved November 2019,):179.
6. Behera D. Textbook of Pulmonary Medicine 2nd ed. New Delhi: Jaypee Brothers Medical Publishers 2010; p. 457.
7. Halezeroğlu S, Okur E. Thoracic surgery for haemoptysis in the context of tuberculosis: what is the best management approach?. *J. Thorac. Dis.* 2014; 6 (3): 182–185.
8. Core Curriculum on Tuberculosis. What the clinician should know. 6th ed. Centers for Disease Control and Prevention: Division of Tuberculosis Elimination; 2013, p. 24.
9. Deveci F, Ilhan N. Plasma malondialdehyde and serum trace element concentrations in patients with active pulmonary tuberculosis. Biol *Trace Elem Res* 2003; 95(1): 29-38.
10. Nnodim J, Anyadoh S, Nwosu NE. The antioxidant status and lipid peroxidation product of newly diagnosed and six weeks follow up patients with pulmonary tuberculosis in Owerri, Imo State, Nigeria. *Asian Pac. J. Trop. Dis.2011; 1: 292-294*.
11. Akiibinu MO, Arinola OG, Ogunyemi EO. Plasma neopterin and peroxide levels in pulmonary tuberculosis patients on chemotherapy with or without micronutrient supplementation. *Pak J med Sci.2009; 25(3):380-385*.
12. Ihim AC, Nwosu DC, Nwanjo HU, Ozuruoke DF, Nwobodo EI, Obi PC et al.. Anti oxidative status of *Nauclea latifolia* leaves extract on ciprofloxacin induced oxidative stress in rats. *Int. J. Inf. Res. Rev.*2017; 04(04):33-37.
13. Oyedeji SO, Oguntuase N R, Esan A, Adesina AA, Oke OT.Oxidative stress and lipid profile status in pulmonary tuberculosis patients in South Western Nigeria. *Greener J Med Sci.*2013;3(6): 228–232.
14. Araoye MO. Sample size determination in research methodology with statistics for health and social sciences. 1st ed. Nathadex Publishers Ilorin 2004; p.115-121.
15. Nwanjo HU, Oze GO. Oxidative imbalance and non-enzymic antioxidant status in pulmonary tuberculosis infected subjects. Carcinogenic potential. Pak J Nutr 2007; 590-592.
16. Cheesbrough M. Protein. In: District laboratory practice in tropical countries 1st ed.Part 1 United Kingdom: Cambridge University Press p. 434-436.
17. Misra HP, Fridovich I. The Role of Superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; 247: 3170.
18. Akinduko DS, Temitope MO, Chukwuemeka ZU.Antioxidant capacity of *Moringa Oleifera* seed oil against CCL_4 induced hepatocellular lipid peroxidation in wistar albino rats. *Eur.J Exp. Biol. 2014; 4(1):514-518*.
19. Sinha AK.Colorimetric assay of catalase. *Analytical Biochemistry*.1972; 47:389-394.
20. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG.Selenium: Biochemical role as a component of glutathione peroxidase 1973;179 (4073):588-590.
21. Anderson JV, Davis DG. Abiotic stress alters transcript profiles and activity of glutathione *S*-transferase, glutathione peroxidase, and glutathione reductase in *Euphorbia esula. Int. J. Plant Sci.* 2004; 120 (3):421-433.
22. Omaye ST, Turbull TP, Sauberchich H.C. Selected Methods for determination of ascorbic acid in cells, tissues and fluids. *Method Enzymol*1979; 6: 3-11
23. Desai. Vitamin E analysis methods for animal tissues. *Method Enzymol*1984; 105:138-147.
24. APHA, (1995). Standard methods for the examination of water and wastewater. 19th

edn, American Public Health Association Inclination, New York 1995;p.213-223.
25. Gutteridge, JM., Wilkins S. Copper dependant hydroxyl radical damage to ascorbic acid; formation of thiobarbituric acid reactive products. *FEBS Letters* 1982; 137:327-330.
26. Reddy YN, Murthy SV, Krishna D R, Prabhakar MC. Role of free radicals and antioxidants in tuberculosis patients. *Indian Journal of Tuberculosis* 2004;213-218.
27. Pugalendhi V, Prema G, Panicker TMR, Paul KM, Jagadeesan K, TracyTina AJ. Lipid peroxidation and antioxidant status in patients of end stage renal disease with and without pulmonary tuberculosis. *J Int Med Sci Acad* 2012, 25(4):225-227.
28. Cooper DA, Eldridge AL, Peters JC. Dietary carotenoids and certain cancers, heart disease, and age-related macular degeneration. A review of recent research. *Nutr. Rev.* 1999; 57:201–214.
29. Osuji FN, Onyenekwe CC, Ifeanyichukwu MO, Ahaneku JE, Ezeani M, Ezeugwunne IP. Impact of HIV and *Mycobacterium tuberculosis* co-infections on antioxidant status in Nigeria. *Pak J Nutr* 2013; 12(5):496-504.
30. Audu DC, Onwujekwe J, Onubuogu N, Adedoyin A, Oyeyejepu J, Aje C. et al. Possible impact of co-infections of tuberculosis and malaria on the CD4+ cell counts in HIV infected subjects. *Ann Afr Med2005;* 4:10-13.
31. Lamsal M, Gautam N, Bhatta N,Toora BD, Bhattacharya SK, Baral N. Evaluation of lipid peroxidation product, nitrite and antioxidant levels in newly diagnosed and two months follow-up patients with pulmonary tuberculosis, *Southeast Asian J. Trop. Med. Public Health* 2007; 22(8):25-28.
32. Shubhangi MD,Vinayak WP, Nagsen NR. The Roles of glutathione, glutathione peroxidase, glutathione reductase and the carbonyl protein in pulmonary and extra pulmonary tuberculosis. *J. Clin. Diagn. Res* 2012; 6(9):1462-1465.

How to cite this article: Chinedu IA, Chukwuemeka MS, Chinedum OC et.al. Evaluation of some antioxidants in tuberculosis patients. Int J Health Sci Res. 2020; 10(7):238-246.

******