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EC MICROBIOLOGY
Research Article

Cellular Activation and Antioxidants Depletion in Pulmonary Tuberculosis Patients

Moses Akiibinu[1]*, Oladele Mabekoje[2], Franklin Akinola[3], Adekunle Adesiyan[3], Olaniyi Duduyemi[4], Ebenezer Ademola[2], Maria Adeyemi[5] and Titiloye Oyewumi[6]

[1]Department of Biochemistry (Immunology Unit), Caleb University Lagos, Nigeria

[2]Department of Microbiology, Caleb University Lagos, Nigeria

[3]Department of Biomedical Science, Ladoke Akintola University, Ogbomoso, Osun state, Nigeria

[4]Department of Chemical Pathology and Immunology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria

[5]Department of Biochemistry (Nutrition Unit), Caleb University Lagos, Nigeria

[6]Department of Chemical Pathology, University of Ibadan, Ibadan, Nigeria

***Corresponding Author**: Moses Akiibinu, Department of Biochemistry (Immunology Unit), Caleb University Lagos, Nigeria.

Received: January 19, 2019; **Published:** February 27, 2019

Abstract

Background: *Tubercle bacilli* infection has the potential to modulate metabolic pathways and change the functional abilities of some affected organs in susceptible individuals. This study assessed the ability of the pulmonary tuberculosis to initiate immunological responses including inflammation and free radical generation.

Methods: One hundred and twenty-five newly diagnosed pulmonary tuberculosis (PTB) patients participated in this study. Diagnostic criteria for recruiting PTB patients included chest X-ray, sputum examination using Ziehl Neelson (ZN) staining technique and Mantoux test. Another one hundred and twenty apparently healthy individuals who were negative to Mantoux test and HIV-antibodies served as controls. Plasma levels of vitamin C (vit. C), vitamin E (vit. E), total antioxidant potential (TAP), uric acid, albumin, alpha-2-macroglobulin (AMG), transferrin (TRF), ceruloplasmin (CLP), C-reactive protein (CRP) and neopterin were measured using spectrophotometric methods, single radial immuno-diffusion (Maccini) and enzyme linked immunosorbent assay (ELISA) methods respectively.

Results: The result shows that PTB patients had significantly ($p < 0.05$) lower body weight, BMI, TAP, TRF, vit. C, vit. E and albumin compared with the controls. The plasma levels of neopterin, AMG, CLP, CRP and uric acid increased significantly ($p < 0.05$) in PTB patients compared with the controls.

Conclusion: Antioxidant depletion and chronic inflammatory responses are possible consequences of excessive macrophage activation in pulmonary tuberculosis. Since nutritional antioxidants are significantly lower in pulmonary tuberculosis patients, adjuvant micronutrient supplementation may be beneficial in the management of the disease to avert the risks of oxidative stress.

Keywords: *Tuberculosis; Cellular Activation; Inflammation; Antioxidants*

Abbreviations

PTB: Pulmonary Tuberculosis; vit. C: Vitamin C; vit. E: Vitamin E; TAP: Total Antioxidant Potential; U/A: Uric Acid; ALB: Albumin; AMG: Alpha-2-Macroglobulin; TRF: Transferrin; CLP: Ceruloplasmin; CRP: C-Reactive Protein.

Introduction

Mycobacterium tuberculosis is an intracellular pathogen that survives and replicates in the host macrophages [1,2]. Activation of the invaded macrophages subsequently leads to production of neopterin [3], cytokines and granuloma. While an effective mature granuloma renders the bacteria latent, inefficient granuloma due to malnutrition or immunodeficiency results to active tuberculosis. Common char-

acteristics of the activated macrophages include increased free radical generation [4]. But, the *M. tuberculosis* evade the host immune response in the macrophages by triggering anti-inflammatory responses, producing antioxidant enzymes that block the effects of reactive oxygen and nitrogen intermediates, and reducing the acidification of the *M. tuberculosis*-containing phagosome [5,6]. Excess free radical load beyond the detoxification capability of the antioxidant defenses results to oxidative stress [7]. The pathological effects of excess free radical load include fragmentation of proteins and peroxidation of lipids, dysfunction of cell membranes and enzymes, impairment of cell membrane function, decreased fluidity, inactivation of membrane-bound receptors, increased permeability of ions and gene mutation [8]. Despite the roles of free radicals in the pathophysiology of tuberculosis, little attention has been paid to the status of antioxidants in Nigerian tuberculosis patients.

Evidences show that inflammatory cytokines (IL-1 and IL-6) released by infected/activated cells initiate the production of spectrum of acute phase proteins (such as C-reactive protein, haptoglobin, transferrin etc.) by hepatocytes [9,10]. Studies have shown that epithelial cells of both respiratory tract and renal epithelium can also produce CRP under certain circumstances [11,12]. Recent studies have demonstrated that human coronary artery smooth muscle cells could also synthesize CRP upon stimulation by inflammatory cytokines. Cogent data have indicated that the CRP is also produced by the atherosclerotic lesions (especially by smooth muscle cells and macrophages), kidneys, neurons, and alveolar macrophages [13,14]. The acute phase proteins play significant physiological roles in tissue repair or inflammation and in several host immune-defense mechanisms. For example, C-reactive protein has been shown to cause bacterial capsular swelling, promotion of agglutination, complement fixation, enhancement of phagocytosis, and initiates opsonization, phagocytosis and lysis of invading organism such as bacteria and viruses [15]. The C-reactive protein activates macrophages, possesses anti-proteolytic activity and presumably blocks the migration of cells into the lumen of blood vessels thus helping to prevent the establishment of a generalized systemic inflammation [16]. The C-reactive protein has been used to assess the severity of several acute bacterial diseases, myocardial infarction and rheumatoid arthritis, and also to monitor the progress of these patients during treatment [17,18]. Also, serum CRP levels have been used in monitoring the efficacy of chemotherapy in patients with only radiological suspicion of tuberculosis [19] and are significantly correlated with disease severity [20]. Previous studies have not reported the status of alpha-2-macroglobulin, transferrin and ceruloplasmin in Nigerian pulmonary tuberculosis patients. The present study was therefore designed to bridge this gap in knowledge by determining the plasma levels of alpha-2-macroglobulin (AMG), transferrin (TRF), ceruloplasmin (CLP), vitamin C (vit. C), vitamin E (vit. E), total antioxidant potential (TAP), uric acid, albumin, C-reactive protein (CRP) and neopterin in newly diagnosed pulmonary tuberculosis patients.

Materials and Methods

One hundred and twenty-five (125) newly diagnosed PTB patients who were sputum smear- positive for acid fast bacilli (AFB), and one hundred and twenty (120) apparently healthy, sputum AFB-negative as controls were selected for this study. None of the patients and controls was positive to HIV-antibodies at the time of this study. Diagnostic criteria for the establishment of pulmonary tuberculosis status included clinical symptoms, chest X-ray, Mantoux test and Ziehl Nelson (ZN) staining for AFB. Those selected as controls were students and staff of University College Hospital, Ibadan, Nigeria and staff of Ultimate Medical Diagnostic Laboratory, Apata, Ibadan, Nigeria who were negative to Mantoux test and HIV-antibodies.

Methods

Estimation of albumin (ALB)

The albumin concentration was determined by using a commercially prepared reagent (brilliant cresol green solution) purchased from Dialab Production and Vertrieb vonchemich-technischen, Wien- Panikengasse. Albumin is a marker of secretory function of the liver and was used to assess the secretory activities of the liver in PTB patients before and after treatment.

Estimation of vitamin E

Vitamin E (α-tocopherol) was estimated by the method of Desai [21]. The principle was based on the fact that vitamin E complex with α-α-dipyridyl, ferric chloride and butanol, a colour complex is produced which is read at 520 nm.

Estimation of total antioxidant potential (TAP) and ascorbic acid

TAP was estimated using the ferric reducing/antioxidant power (FRAP) assay [22,23]. The vitamin C concentration was determined by using the method of Briggs [24].

Determination of AMG, TRF, CLP, CRP and neopterin

AMG, TRF, CLP and CRP were quantified by single radial immunodiffusion method as described by Salimonu*., et al* [25]. Neopterin was determined in the plasma using a commercially prepared reagent, Neopterin ELISA kit (RE59321) by IBL Hambunrg, as described by Smith., *et al* [26].

Statistical analysis of data

All statistical analysis were performed using Statistical Package for Social Sciences SPSS) for windows, version 21. The data were expressed as mean \pm SD. Student (t) test was used for the comparison of tuberculosis patients and controls. The changes were considered significant, when p-values were less than 0.05.

Results

The result shows that PTB patients had significantly ($p < 0.001$) lower body weight and BMI (Table 1). The plasma levels of TAP, vit. C, vit. E, albumin (Table 2) and TRF (Table 3) were significantly ($p < 0.05$) lower when compared with the controls. The plasma levels of uric acid (Table 2), neopterin, AMG, CLP and CRP (Table 3) increased significantly ($p < 0.05$) in PTB patients when compared with the controls.

	N	Age (years)	Height (M)	Weight (Kg)	BMI (Kg/M^2)
Controls	120	35.3 ± 12.5	1.64 ± 0.06	63.9 ± 11.2	23.7 ± 2.6
PTB patients	125	33.5 ± 12.0	1.60 ± 0.06	48.0 ± 10.0	18.6 ± 3.4
p -values		0.2	0.1	< 0.001(s)	< 0.001(s)

Table 1: *Physical parameters in PTB-patients and non-PTB controls.*

N: Number of Subjects; BMI: Body Mass Index; S: Significantly Different from Controls.

	N	Vit. C (mg/L)	Vit. E (mg/L)	TAP (mmol/L)	U/A (mg/dl)	ALB (g/dl)
Controls	120	22.3 ± 2.6	12.6 ± 1.7	1160 ± 280	4.3 ± 3.2	4.7 ± 0.4
PTB	125	17.8 ± 1.8	7.07 ± 1.3	582 ± 295	5.9 ± 3.8	2.9 ± 0.5
P- values		< 0.001(s)	< 0.001(s)	< 0.001(s)	0.02(s)	< 0.01(s)

Table 2: *Levels of antioxidants in tuberculosis and controls.*

N: Number of Subjects; vit. C: Vitamin C; vit. E: Vitamin E; TAP: Total Antioxidant Potential; U/A: Uric Acid; ALB: Albumin; S: Significantly Different from Controls.

	N	CRP (Mg/L)	AMG (g/l)	TRF (g/l)	CLP (g/l)	NEOP(nM/ml)
Control	120	2.46 ± 1.3	3.44 ± 2.03	3.43 ± 2.92	0.59 ± 0.21	10.4 ± 3.7
PTB	125	7.84 ± 2.6	4.0 ± 1.66	1.33 ± 0.64	1.04 ± 0.49	27.5 ± 6.8
p -values		< 0.001(s)	0.013(s)	< 0.001(s)	< 0.001(s)	0.0001(s)

Table 3: *Levels of AMG, TRF and CLP in tuberculosis and controls.*

N: Number of Subjects; CRP: C-Reactive Protein; AMG: Alpha-2-Macroglobulin; TRF: Transferrin; CLP: Ceruloplasmin; NEOP: Neopterin; S: Significantly Different from Controls.

Discussion

A number of clinical and immunologic changes in tuberculosis patients have been associated with the ability of the *Tubercle bacilli* to modulate the physiology of susceptible hosts. This modulation may enhance significant changes in the weight and body mass index as observed in our pulmonary tuberculosis patients. The cause of tuberculosis-associated wasting is incompletely understood. But available studies link the loss of weight to chronic anorexia, increased pro-inflammatory cytokines and malnutrition [6,9,10,27]. Poor nutritional status and inflammatory responses could contribute to the lower levels of BMI and weight of the tuberculosis patients. Karyadi., *et al.* [28] hypothesized that inflammatory mediators such as tumor necrosis factor-α (TNFα) and leptin may play significant role in the wasting symptom.

Previous reports reveal that *Mycobacterium tuberculosis*-invaded macrophages produce oxygen-derived free radicals and hydrogen peroxide. High serum levels of these free radicals and lipid peroxidation products are characterized by patients with advanced tuberculosis [27]. Imbalance in the free radical/antioxidants in favor of the free radical generation results in oxidative stress [29]. In health, the free radicals generated from normal metabolic activities are controlled at physiological levels by the antioxidant system. In this study, excessive production of free radicals and continuous neutralization by the antioxidant system could contribute to the significantly lower levels of vit. C and vit. E. Also, significantly lower level of albumin in this study could be due to its usurpation during neutralization of free radicals. It may also be due to the fact that protein synthesis in infection or injury is diverted to protective (e.g. immunoglobulins) rather than transport protein. Since TAP is an index of all antioxidants, deficiency or exhaustion of certain antioxidants molecules (vit. E, vit. C, albumin) during continuous neutralization of free radicals could contribute to the lower level in our tuberculosis patients. The significantly lower activity of antioxidant system in our PTB patients is consistent with the report of previous workers **[30,31]** who reported significantly lower levels of classes of antioxidants in tuberculosis. Sasaki., *et al.* [32] stated that albumin and total protein were significantly lower in pulmonary tuberculosis. Aily., *et al.* [33] also observed lower levels of albumin and hematocrit in tuberculosis. Akiibinu., *et al.* [29] have earlier reported decreased antioxidant activity in patients with pulmonary tuberculosis. Meanwhile, elevated level of uric acid in these tuberculosis patients could be a physiologic way of controlling excess free radical load generated due to chronic tissue damage.

Neopterin is a metabolic product of macrophage activation, an indicator of pro-inflammatory immune status and a marker of cellular activation that is released into the circulation [3]. Significantly higher level of neopterin was observed in our pulmonary tuberculosis patients. This is consistent with a previous study [27]. This report corroborates several other studies that show higher levels of neopterin in many diseases including tuberculosis and cancer [3,34]. Since high level of neopterin production has been associated with increased production of reactive oxygen species, the significantly higher level of neopterin in this study may be a consequence of excessive macrophage activation and excessive free radical generation in the PTB patients.

Inflammatory cytokines (e.g. IL-1 and IL-6) released by infected cells initiate the production of spectrum of acute phase proteins (such as C-reactive protein, haptoglobin, transferrin etc.) by hepatocytes [9,10,35]. The significantly higher level of CRP, AMG and CLP in this study could be due to the chronic inflammatory responses in the tuberculosis patients. Previous studies by Márton., *et al.* [36]; Immanuel., *et al.* [37]; Trajcevska., *et al.* [38] show that levels of alpha-1-antitrypsin, alpha-2-macroglobulin, C-reactive protein, haptoglobin, complement component C3 concentrations and oxidase activity of ceruloplasmin increased significantly in tuberculosis patients. In a study by Grange., *et al* [39], marked increases in serum ceruloplasmin, C-reactive protein and serum amyloid-A-protein were observed with significantly lower levels of albumin and transferrin. Since CRP binds to pathogens and activates the complement to enhance opsonisation and clearance, even before the production of specific IgM or IgG, the increased level might be beneficial to the tuberculosis patients in the clearance of secondary infections. Ceruloplasmin, known to possess significant oxidase activity and capable of scavenging oxygen-derived free radicals [40], are probably responsible for limiting the damage caused by these radicals. Haptoglobin and transferrin have been shown to play important roles in restricting the availability of iron, an essential nutrient for the survival and proliferation of microorganisms within the host. Haptoglobin binds hemoglobin which is known to support bacterial growth [41] and transferrin binds free iron available within the cell [42] to prevent increased microbial load. In the present study, plasma levels of albumin and TRF were significantly lower in the newly diagnosed pulmonary tuberculosis patients. This corroborates the report of Hernández-Pando., *et al.* [43] which states that liver mRNA concentrations of the negative APP (albumin) decrease significantly in tuberculosis patients. The decrease in transferrin concentrations during the acute phase reaction is attributed to an excess of catabolism over synthesis [41].

Conclusion

In conclusion, antioxidant depletion and chronic inflammatory responses are possible consequences of excessive macrophage activation in pulmonary tuberculosis. Since nutritional antioxidants are significantly lower in pulmonary tuberculosis patients, adjuvant micronutrient supplementation may be beneficial in the management of the disease.

Authors Contributions

MA, OM, AA, EA and TO designed the study, MA, OM, FA, AA, MA and OD did the analysis and all authors approved the write-up.

Acknowledgements

Appreciations to the Damien Foundation, Belgium, Nigeria Chapter; and Tuberculosis and Leprosy Unit, Oniyanrin Health Center, Ibadan, Oyo state, Nigeria for allowing us to use their tuberculosis patients for this study.

Conflict of Interest

Authors declare that they do not have conflict of interest.

Bibliography

1. Wiid I Seaman., *et al*. "Total antioxidant levels are low during active TB and rise with anti-tuberculosis therapy". *IUBMB Life* 56.2 (2004): 101-106.

2. McGarvey JA., *et al*. "Differential gene expression mono-nuclear phagocytes infected with pathogenic and non-pathogenic mycobacteria". *Clinical and Experimental Immunology* 136.3 (2004): 490-500.

3. Chandara I., *et al*. "Serum neopterin levels in HIV-infected patients with and without tuberculosis". *Indian Journal of Medical Research* 121.4 (2005): 220-225.

4. Vignais PV. "The superoxide generating NADPH oxidase: structural aspect and activation mechanism". *Cellular and Molecular Life Sciences* 59.9 (2003): 1428-1459.

5. Fenton MJ., *et al*. "Receptor-mediated recognition of Mycobacterium tuberculosis by host cells". In Tuberculosis and the Tubercle Bacillus, eds Cole ST, Eisenach KD, McMurray DN, Jacobs WR Jr., editors. (New York: ASM Press), (2005): 405-426.

6. Cooper AM. "Cell-mediated immune responses in tuberculosis". *Annual Review of Immunology* 27 (2009): 393-422.

7. Kwiatsowska S., *et al*. "Increased serum concentrations of conjugated diens and malondialdehyde in patients with pulmonary tuberculosis". *Respiratory Medicine* 93.4 (1999): 272-276.

8. Ramanujam MD. "Free radicals and antioxidants - current status" (2004).

9. Tillet WS., *et al*. "Chemical and Immunological Properties of a Species Specific Carbohydrate of Pneumococci". *The Journal of Experimental Medicine* 52.6 (1930): 895-900.

10. Peltola H., *et al*. "Quantitative C-Reactive Protein (CRP): Determined by an Immunoturbidimetric Method in Rapid Differential Diagnosis of Acute Bacterial and Viral Diseases of Children". *Acta Paediatrica Scandinavica* 73.2 (1984): 273-274.

11. Logering BA., *et al*. "The kidney as a second site of human C-reactive protein formation in vivo". *European Journal of Immunology* 33.1 (2003): 152-161.

12. Yeh ETH. "A new perspective on the biology of C-reactive protein". *Circulation Research* 97.7 (2005): 609-611.

13. Calabró P., *et al*. "Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells". *Circulation* 108.16 (2003): 1930-1932.

14. Venugopal SK., *et al*. "Macrophage conditioned medium induces the expression of C-reactive protein in human aortic endothelial cells. Potential for paracrine/autocrine effects". *American Journal of Pathology* 166.4 (2005): 1265-1271.

15. Pepys MB and Baltz ML. "Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins): and serum amyloid A protein". *Advances in Immunology* 34 (1983): 141-212.

16. Rienhoff HY Jr. "Molecular and cellular biology of serum amyloid A". *Molecular Biology and Medicine* 7.3 (1990): 287-298.

17. Morley JJ and Kushner I. "Serum C-reactive protein levels in diseases". *Annals of the New York Academy of Sciences* 389 (1982): 406-418.

18. Whither JT and Dieppe PA. "Acute phase proteins". *Clinical Immunology and Allergy* 5 (1985): 425-446.

19. de Beer FC., *et al*. "Serum Amyloid A Protein and C-Reactive Protein Levels in Pulmonary Tuberculosis: Relationship to Amyloidosis". *Thorax* 39.3 (1984): 196-200.

20. Shameem M., *et al*. "Association between serum C reactive protein levels and other important predictive markers of outcome in Central Out Patient Department". *Acta Medicine of Iran* 49 (2011): 18-20.

21. Desai ID. "Vitamin E methods for animal tissues". *Methods in Enzymology* 105 (1984): 138-143.

22. Harma M., *et al*. "Oxidative stress in women with pre-eclampsia". *American Journal of Obstetrics and Gynecology* 192.2 (2005): 656-657.

23. Benzie IE and Strain JJ. "The ferric reducing ability of plasma (FRAP): as a measure of antioxidant power (the FRAP assay)". *Annals of Biochemistry* 239.1 (1996): 70-76.

24. Briggs ME. "Vitamin in human biology and medicine". Boca Raton, Fla. CRC Press inc (1981).

25. Salimonu LS., *et al*. "Serum immunoglobulin levels in normal premature and postmature newborns and their mothers". *International Journal of Gynecology and Obstetrics* 16.2 (1978): 119-123.

26. Smith D., *et al*. "Neopterin levels in patients with coronary artery disease are independent of Chlamydia pneumoniae seropositivity". *American Heart Journal* 146.1 (2003): 69-74.

27. Akiibinu MO., *et al*. "Plasma Neopterin and Peroxide Levels in Pulmonary Tuberculosis Patients on Chemotherapy with or Without Micronutrient Supplementation". *Pakistan Journal of Medical Sciences* 25.3 (2009): 380-385.

28. Karyadi E., *et al*. "Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia". *Journal of Nutrition* 130.12 (2000): 2953-2958.

29. Akiibinu MO., *et al*. "Non-enzymatic Antioxidant Status and Nutritional Profiles in Newly Diagnosed Pulmonary Tuberculosis Patients in Nigeria". *African Journal of Biomedical Research* 10 (2007): 223-228.

30. Madebo T., *et al*. "Circulating antioxidants and lipid peroxidation products in untreated tuberculosis patients in Ethiopia". *American Journal of Clinical Nutrition* 78.1 (2003): 117-122.

31. Arinola OG and Akiibinu MO. "Influence of Mycobacterium tuberculosis infection on the serum levels of antioxidant vitamins and trace elements". *The Tropical Journal of Health Sciences* 15.2 (2008): 1-4.

32. Sasaki Y., *et al*. "A case of pulmonary tuberculosis with pan-cytopenia accompanied to bone marrow gelatinous transformation". *Kekkaku* 74.4 (1999): 361-364.

33. Aily DC., *et al*. "Systematic mycobacteriosis in AIDS patients as determined by blood cultures on biphasic medium". *Revista Argentina de Microbiología* 31.2 (1999): 53-57.

34. Turgut T., *et al*. "Serum interleukin-2- and neopterin levels as useful markers for treatment of active pulmonary tuberculosis". *Tohoku Journal of Experimental Medicine* 209.4 (2006): 321-328.

35. Kushner I., *et al*. "Control of acute phase response. Serum C-reactive protein kinetics after acute myocardial infarction". *Journal of Clinical Investigation* 61.2 (1978): 235-242.

36. Márton I., *et al*. "The role of acute phase proteins in the pathogenesis of chronic periapical granuloma". *Fogorvosi szemle* 83.8 (1990): 235-239.

37. Immanuel C., *et al*. "Acute phase proteins in tuberculous patients". *Indian Journal of Chest Diseases and Allied Sciences* 32.1 (1990): 15-23.

38. Trajcevska M., *et al*. "Acute phase reaction in pulmonary tuberculosis during treatment". *European Respiratory Journal* 46.59 (2015): PA4529.

39. Grange JM., *et al*. "A study of acute phase reactant proteins in Indonesian patients with pulmonary tuberculosis". *Tubercle* 65.1 (1984): 23-29.

40. Goldstein IM., *et al*. "Ceruloplasmin: An acute phase reactant that scavenges oxygen-derived free radicals". *Annals of the New York Academy of Sciences* 389 (1982): 368-379.

41. Ogata RT. "Factors determining bacterial pathogenicity". *Clinical Physiology and Biochemistry* 1 (1983): 145-159.

42. Gordon AH. "The acute phase plasma proteins". In: Plasma Protein Turnover. R Bianchi, G Mariani and AS McFarlane (Eds). Baltimore, University Park-Press (1976): 381.

43. Hernández-Pando R., *et al*. "The response of hepatic acute phase proteins during experimental pulmonary tuberculosis". *Experimental and Molecular Pathology* 65.1 (1998): 25-36.

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