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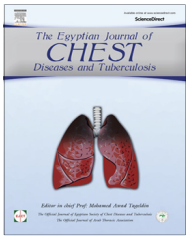

ORIGINAL ARTICLE

Plasma vitamins and essential trace elements in multi-drug resistant tuberculosis patients before and during chemotherapy

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Abstract Multi-drug-resistant tuberculosis (MDR-TB) is caused by *Mycobacterium tuberculosis* (Mtb) strain resistant to both rifampicin and isoniazid. Nigeria has an estimated MDR-TB rate of 2.9% and 14.3% among new and relapse cases respectively and is ranked among 4 high burden African countries for MDR-TB. Malnutrition has been implicated in the progression from dormant to active disease.

This study determined the plasma level of micronutrients (Fe, Zn, Cu, vitamins A, C, D and E) in MDR-TB patients before and throughout anti-TB chemotherapy. Plasma iron, zinc, copper, vitamins A, C, D and E were determined in twenty-four (24) MDR-TB patients before the commencement of anti-TB chemotherapy, 2 months, 4 months and 6 months post-commencement of anti-TB chemotherapy, as well as in twenty (20) healthy controls. Plasma vitamin A level was significantly decreased before chemotherapy compared with controls. At 2 months of anti-TB treatment there were significant decreases in plasma levels of iron, vitamins A, C and E compared to controls whereas plasma zinc level was significantly increased compared with levels before treatment. At 4 months of treatment, plasma levels of copper and vitamin D were significantly increased while plasma vitamin E level was reduced significantly compared with controls. There were significant increases in iron, zinc, copper, vitamin A and vitamin D levels with decreased plasma levels of vitamins C and E at 4 months of treatment compared with their levels before chemotherapy. At 6 months of treatment, plasma levels of iron, zinc, copper and vitamin D were significantly increased while vitamin E was significantly decreased compared with controls. Plasma levels of iron, zinc, copper, vitamins A and D were significantly increased whereas the levels of vitamins C and E were significantly reduced at 6 months of treatment compared with levels before chemotherapy.

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Plasma levels of iron, zinc, copper, vitamins A and D were raised while plasma levels of vitamins E and C were reduced in MDR-TB patients from 4 months post commencement of anti-TB chemotherapy. Thus, there is need to monitor micronutrient supplementation and plasma levels of these micronutrients to avoid complications associated with overload or deficiency.

Background

Tuberculosis (TB) continues to be a major public health challenge globally and now ranks alongside HIV as a leading cause of death worldwide [1]. Recent statistics show a rise in the number of new TB cases from 9 million in 2013 to 9.6 million in 2014 with the number of TB associated deaths in 2013 and 2014 remaining constant at 1.5 million [1,2]. In recent years, progress in TB control and eradication has been threatened by the emergence of drug resistant strains of *Mycobacterium tuberculosis* (Mtb). Multi-drug-resistant tuberculosis (MDR-TB) is defined as a disease caused by Mtb strain resistant to both rifampicin and isoniazid, which are frontline anti-tuberculosis drugs presently used in chemotherapy. Nigeria is reported to have an estimated MDR-TB rate of 2.9% and 14.3% among new and relapse cases respectively and is ranked among 4 high burden African countries for MDR-TB [3]. Among other factors, malnutrition has been implicated in the progression from dormant to active disease [4].

The innate immune cells; macrophages, neutrophils and dendritic cells, play an important role in immunological response, as first responder, activator of adaptive immunity and effector cell in Mtb infection [5]. Mtb bacilli upon entry into the lungs are engulfed by resident innate immune cells (macrophages and dendritic cells). This leads to transformation of macrophages from a resting state to an activated state with characteristic feature of increased oxygen uptake, enlargement and increased protein synthesis [6]. Also there is increased influx of neutrophils to the lungs, and a report demonstrated that neutrophils are the most commonly infected phagocytes in human TB [7]. Macrophage and neutrophil apoptosis have also been reported to be a potent mechanism for control of inflammation and removal of infected cells in Mtb infection [8]. These lead to a high rate of cellular turnover during infection and increased demand for essential nutrients by the immune cells [9].

A close relationship was suggested between micronutrients and modulation of neutrophil and macrophage functions [10]. Micronutrient malnutrition has been described in pulmonary TB patients [11–14] and several studies have suggested that patients with TB are at high risk of deficiency of vitamins A, C, D and E as well as zinc [13,14]. Our previous study [15] reported micronutrient malnutrition in patients with drug-sensitive TB at diagnosis and throughout the period of anti-TB chemotherapy. Because micronutrient deficiency has been reported to impair resistance to infection, lead to active TB disease and poor outcome of anti-TB chemotherapy, this present study therefore determined the plasma level of micronutrients (Fe, Zn, Cu, vitamins A, C, D and E) in MDR-TB patients before and throughout anti-TB chemotherapy.

Materials and method

Study participants

Twenty-four (24) patients admitted into the MDR TB center, University College Hospital (UCH) Ibadan, Nigeria for anti-TB treatment were recruited for the study after obtaining written informed consent. Patients had been previously diagnosed as being infected with isoniazid and rifampicin resistant strains of Mtb using clinical history, chest X-ray and GENE Xpert test. Twenty (20) apparently healthy participants were recruited as controls. Five (5) milliliters of blood was drawn from the anti cubital fossa vien into lithium heparin tubes before the commencement of chemotherapy, and 2 months, 4 months and 6 months of anti-TB therapy. Blood samples were centrifuged and plasma obtained was analyzed.

MDR-TB treatment protocol

All bacteriologically confirmed MDR-TB patients received intensive phase for 6–8 months in the hospital followed by 12 months of continuation phase in the community based on WHO updated guidelines in 2011 [16]. Standardized treatment regimen was used including five drugs: kanamycin/Amikacin, Levofloxacin, Prothionamide, Cycloserine, Pyrazinamide (with Pyridoxine). This present study was conducted during the intensive phase of treatment.

While patients were admitted for MDR-TB treatment, they were supported by non-governmental organizations which provided nutritional support in the form of meals and micronutrient supplements. Vitamin C (Spartan C), folic acid (Vitabiotics), vitamin B complex (Vitabiotics), vitamin B6 (Pauco) and multivit (Pauco) supplements were administered daily with anti-TB drugs as part of the treatment regimen at the center where this study was conducted.

Micronutrient analysis

Plasma levels of micronutrient vitamins (A, C, D and E) were determined by high performance liquid chromatography method using WATERS 616/626 (USA) machine as previously carried out [17].

Plasma concentrations of trace metals (Fe, Zn and Cu) were determined using atomic absorption spectrophotometry (Buck Scientific, 210, Atomic Absorption Spectrophotometer, Connecticut, USA) as previously described [15].

Statistical analysis

Data obtained were analyzed using statistical package for social sciences (SPSS) version 17.0. Independent Student

t-test was used to compare the mean values of MDR TB patients and controls while paired *t*-test was used to compare the mean values of MDR TB patients before the commencement of chemotherapy, and 2 months, 4 months and 6 months of anti-TB chemotherapy. Values were considered significant at $p < 0.05$.

Results

Plasma vitamin A level was significantly decreased before the commencement of chemotherapy compared with controls. At 2 months of anti-TB treatment regimen, there were significant decreases in plasma levels of iron, and vitamins A, C and E when compared to controls whereas plasma zinc level was significantly increased when compared with levels before treatment. At 4 months of treatment the plasma levels of copper and vitamin D were significantly increased while plasma vitamin E level was reduced significantly when compared with controls. There were also significant increases in iron, zinc, copper, vitamin A and vitamin D levels with decreased plasma levels of vitamins C and E at 4 months of treatment when compared with their levels before the commencement of chemotherapy.

At 6 months of treatment, the plasma levels of iron, zinc, copper and vitamin D were significantly increased while vitamin E was significantly decreased when compared with controls. Plasma levels of iron, zinc, copper, and vitamins A and D were significantly increased whereas the levels of vitamins C and E were significantly reduced at 6 months of treatment compared with levels before the commencement of chemotherapy (see Table 1).

Discussion

Most previous micronutrient supplementation studies in TB patients concentrated on drug sensitive TB patients and assessed effects of micronutrient supplementation on mortality, sputum culture conversion, chest X-ray resolution, weight gain and bacterial clearance [18]. These studies produced inconsistent results. In this present study plasma vitamin A concentration was significantly reduced in patients before the commencement of chemotherapy compared with control. This supports previous findings in drug-sensitive TB patients [14,15]. However, significantly reduced levels of micronutrients (Fe, Zn, Cu, vitamins C, D and E) observed in drug-sensitive TB patients before chemotherapy compared with controls in our previous study [15] was not observed in this present study. This might be due to differing severity or class of TB. The present patients were MDR-TB patients while the previous were drug-sensitive TB patients. MDR-TB patients might have been using conventional or herbal-traditional medicines which may be rich in micronutrients [19–21] during the waiting period before hospital admission in a bid to better their health and resolve TB symptoms. Vitamin A has been shown to inhibit multiplication of Mtb in macrophages *in vitro* [22,23] and plays a vital role in lymphocyte proliferation and maintaining the function of epithelial tissues [24]. However, vitamin A deficiency is a common feature of pulmonary tuberculosis. Reduced plasma vitamin A concentration observed before chemotherapy in this present study might be due to reduced intake, reduced absorption, increased utilization, increased urinary excretion of vitamin A or a combination of these factors as demonstrated in other infections.

Following the commencement of anti-TB treatment combined with micronutrient supplementation, this present study observed reduced plasma concentrations of iron and vitamins A, C and E in MDR-TB patients at two months of chemotherapy compared with controls. This finding is similar to our previous study [15] on drug-sensitive TB patients on standard anti-TB treatment without micronutrient supplementation. To explain this observation, we suggested possible drug–micronutrient interaction or drug induced nutrient depletion [15] within the first two months post commencement of anti-TB chemotherapy. Though a study [25] reported a modulating effect of rifampicin on nutritional supplements, the nature and mechanism of interaction between the anti-TB drugs and micronutrients are still largely unclear. Plasma levels of copper and vitamin D increased at 4 months post commencement of treatment with increased concentrations of iron, zinc, copper and vitamin D at 6 months post commencement of treatment when compared with controls. Also, from 4 months post commencement of treatment the levels of iron, zinc, copper, and vitamins A and D concentrations increased when compared to controls or before chemotherapy. This observation is similar to our previous report in drug-sensitive TB patients [15]. This might be due to adaptation to continuous drug use. This present study also supports the findings of Kassu et al. [26] that serum levels of trace elements in TB patients increased after the treatment when compared to their levels before treatment.

The pathogenesis of lung fibrosis and dysfunction in TB has been associated with induced production of oxidative substances such as reactive oxygen species (ROS) derived from

Table 1 Plasma concentrations of micronutrients in MDR-TB patients before chemotherapy, during treatment, and healthy controls.

Parameters	Control	Before chemotherapy	2 months PC	4 months PC	6 months PC
Iron (μg/dl)	98.76 ± 3.64	92.17 ± 10.47	89.12 ± 8.02*	106.12 ± 11.70°	131.44 ± 11.68*,°
Zinc (μg/dl)	66.27 ± 9.85	62.57 ± 4.63	68.36 ± 4.49°	79.81 ± 25.59°	114.73 ± 17.72*,°
Copper(μg/dl)	97.18 ± 7.38	96.33 ± 9.59	100.35 ± 4.87	110.09 ± 14.74*,°	132.33 ± 14.30*,°
Vitamin A (μg/dl)	71.78 ± 20.28	49.81 ± 11.43*	54.79 ± 9.55*	73.67 ± 11.50°	80.91 ± 5.63°
Vitamin C (mg/dl)	1.82 ± 1.07	2.40 ± 0.52	1.04 ± 0.83*,°	1.62 ± 1.11°	2.03 ± 0.53°
Vitamin D (pg/ml)	45.80 ± 13.30	44.08 ± 9.58	45.02 ± 11.84	64.04 ± 13.85*,°	62.73 ± 9.24*,°
Vitamin E (mg/dl)	2.04 ± 0.52	2.16 ± 0.59	1.03 ± 0.53*,°	0.94 ± 0.48*,°	0.83 ± 0.18*,°

PC – post commencement of chemotherapy.
* Significantly different from control ($p < 0.05$).
° Significantly different from before commencement of chemotherapy ($p < 0.05$).

free radicals, which in turn can promote tissue injury and inflammation. ROS are highly toxic to all types of cells, but especially to lipids causing peroxidation which results in damage to cell membranes [27,28]. Antioxidants in different forms scavenge free radicals and suppress the actions of ROS, protecting the host from tissue inflammation. Vitamins C (ascorbic acid) and E (alpha tocopherol) act as potent and probably the most important hydrophilic and lipophilic antioxidants respectively. Vitamin C scavenges superoxide radical, hydrogen peroxide and thiol radicals and is a potent quencher of singlet oxygen while vitamin E converts superoxide radical, hydroxyl and lipid peroxyl radicals to less reactive forms [27]. It has been demonstrated that vitamin E acts as a mobilizable antioxidant being released from tissue stores and diverted to lungs of TB patients during oxidative stress and radical mediated pulmonary fibrosis [29]. This leads to the formation of tocopherol radical which is converted to tocopherol by ascorbic acid (vitamin C), thereby conferring vitamin E sparing ability on vitamin C [30]. In this present study, plasma concentrations of vitamin E were reduced at 2 months, 4 months and 6 months of anti-TB treatment when compared to controls or before the commencement of anti-TB chemotherapy while plasma vitamin C concentrations were reduced at 2 months when compared to controls or at 2 months, 4 months and 6 months when compared to before the commencement of chemotherapy. Despite supplementation of TB chemotherapy with vitamins, the levels of vitamins C and E were not raised as expected, therefore, there is need to determine effective dosage of vitamins for supplementation in MDR-TB patients.

Active TB disease has been associated with a low iron status [14,15,26] which has been attributed to anemia resulting from chronic inflammation or a shift of iron from transferrin-bound available state to a ferritin-incorporated storage state [26]. This shift may have evolved as a cytokine-mediated defense against microbial pathogen to effectively withholding iron from microbes [31]. In our previous study, we suggested that caution be exercised in the supplementation of iron in TB patients [15]. Increased levels of iron and copper from 4 months after the commencement of treatment observed in this present study underscores a need for reduction in the dosage of iron and copper supplements administered to MDR-TB patients. Iron and copper are pro-oxidants which produce toxic and indiscriminately reactive hydroxyl radical from hydrogen peroxide by Fenton chemistry [32]. This may also explain the low levels of vitamins C and E observed in these patients due to increased scavenging of resulting ROS. In a previous prospective study of the relationship between TB and increased dietary iron, increased dietary iron was associated with a 3.5-fold increase in estimated odds of developing active TB and a trend toward higher mortality among TB patients [33]. Also, free iron is used by microbes to thrive [34], excessive iron in MDR-TB patients might explain susceptibility to continuous TB and other infectious agents. Moreover, Mtb uses iron as prosthetic group for superoxide dismutase which transfers free electron from superoxide to form hydrogen peroxide [35]. Superoxide is a more potent antibacterial agent than hydrogen peroxide. Moreover, hydrogen peroxide is easily converted to water by catalase in Mtb [35]. Based on this, excess iron in MDR-TB patients may be one of the factors used by resistant Mtb to evade respiratory burst mechanism employed by phagocytes to kill ingested Mtb.

In conclusion, multi-micronutrient supplementation of MDR-TB patients on anti-TB chemotherapy raised plasma levels of iron, zinc, copper, and vitamins A and D from 4 months post commencement of anti-TB chemotherapy. Thus, there is need to monitor micronutrient supplementation and plasma levels of these micronutrients to avoid complications associated with overload or deficiency.

Conflict of interest

None declared.

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