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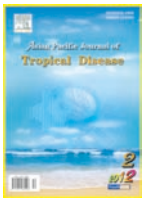

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Hypercalcemia in patients with tuberculosis and HIV infections in Northwest Ethiopia

Bemnet Amare[1*], Solomon Meseret[2], Tomoki Yabutani[3], Beyene Moges[4], Afework Kassu[4]

[1] *Department of Medical Biochemistry, College of Medicine and Health Sciences, University of Gondar, P.O. Box 196, Gondar, Ethiopia.*

[2] *Department of Biostatistics, School of Public Health, College of Medicine and Health Sciences, University of Gondar, P.O. Box 196, Gondar, Ethiopia.*

[3] *The Department of Chemical Engineering, University of Tokushima, Tokushima, Japan*

[4] *Department of Microbiology, Immunology and Parasitology, College of Medicine and Health Sciences, University of Gondar, P.O. Box 196, Gondar, Ethiopia.*

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ABSTRACT

Objective: To determine incidence of hypercalcemia among TB patients with and without HIV infection before and after anti-TB chemotherapy in tropical settings of Northwest Ethiopia. **Method:** Serum levels of calcium were determined using an inductively coupled plasma mass spectrometer from all subjects at baseline and from 60 TB patients (34 with HIV co-infection) at the end of an intensive phase of anti-TB chemotherapy. **Results:** At baseline, significant difference was found between the mean albumin-adjusted calcium levels in TB patients with HIV (11.95±3.72) and controls (9.81±1.59) (P=0.03). Hypercalcemia (serum calcium of > 10.5 mg/dL) was observed in 62.2% and 43.2% of TB patients with and without HIV co-infection, respectively. The serum levels of calcium did not significantly change after anti-TB chemotherapy both in patients with (n = 34) and without (n = 26) HIV co-infection. **Conclusions:** The present study suggests that TB itself appears not to be responsible for hypercalcemia; however, rates of hypercalcemia are high in TB and HIV co-infected patients in the tropical settings of Northwest Ethiopia, of rarely symptomatic. Anti-TB chemotherapy does not improve the incidence of hypercalcemia in both groups with HIV co-infection and without.

1. Introduction

Estimates by the World Health Organization indicate that there are more than 9 million new active cases of TB and close to 2 million deaths per year[1], and that 2.6 million new cases of HIV infection and 1.8 million AIDS-related deaths occur per year[2]. Literatures show that TB is the largest single cause of death in the setting of AIDS, accounting for about 26% of AIDS-related deaths[3]

Both TB and HIV have profound effects on the immune system, as they are capable of disarming the host's immune responses through mechanisms that are not fully understood[4]. HIV coinfection is the most powerful known risk factor for progression of M. tuberculosis infection to active disease, increasing the risk of latent TB reactivation[3]. Likewise, TB has been reported to exacerbate HIV infection[5]. Multiple disturbances of electrolyte metabolism and endocrine regulation have been observed in patients infected with the HIV[6]. However, its effect on the development of hypercalcemia in patients with and without TB has not been established.

Calcium is the most abundant mineral in the body and is used for strong bones and teeth. It is also required for muscle contraction, to transmit pulses throughout our nervous system, blood vessel expansion and contraction and the secretion of hormones and enzymes. The association between TB and hypercalcemia is well recognized[7-10]. The reported incidence of sever hypercalcemia in TB varies widely between countries, probably because of differences in the vitamin D and calcium intake, the amount of sun exposure, the extent of disease and the criteria for hypercalcemia[11-13]. Longitudinal studies from India[14], in Malaysia[11] and the United States[9] reported

*Corresponding author: Bemnet Amare, Department of Medical Biochemistry, University of Gondar, P.O. Box 196, Gondar, Ethiopia.
Tel: +251 918 71 1800
Fax: +251 581 11 14 79
E-mail: amarebem6@gmail.com

hypercalcemia in 19%, 27.5% and 16% – 28% of TB patients. Although a single study has documented the status of three zinc, iron, copper and selenium among TB patients[15], no information is available on the calcium levels of TB patients in Ethiopia. This study was, therefore, aimed at determining the incidence of hypercalcemia among TB patients with and without HIV infection before and after anti-TB chemotherapy in tropical settings of Northwest Ethiopia.

2. Material and methods

Consecutive TB patients attending hospital of the University of Gondar in Ethiopia with a diagnosis of TB were enrolled. As controls, apparently healthy volunteers with no previous history of TB, and who were living in the same geographic locale with TB patients, were recruited. The University teaching hospital is a major tertiary levels referral hospital rendering health services for over 5 million inhabitants in the Northwest Ethiopia. The study proposal was approved by the research ethics committees of the University of Gondar, Gondar, Ethiopia and the University of Tokushima, Tokushima, Japan. Informed consent was obtained from all patients. TB was diagnosed by medical doctors as described previously[15].

To be considered as a case of TB, an individual had to have two or more of the following: (1) positive sputum smear, (2) histopathological evidence, (3) radiographic examination consistent with TB and (4) clinical response to anti-TB chemotherapy. After TB diagnosis, all patients received directly observed therapy short course (DOTS) as per the protocol of the National Tuberculosis and Leprosy Prevention and Control Programme. All HIV-infected TB patients were naive to antiretroviral drugs as antiretroviral therapy was not in use in the country during the study period. Patients with hyperparathyroidism and other known calcium metabolism disorders were excluded from the analysis.

2.1. Nutritional assessment

Body weight was determined to the nearest 0.1 kg on an electronic digital scale and height was measured to the nearest 0.1 cm. Body mass index (BMI), defined as the weight in kilogram of the individual divided by the square of the height in meter, was used to determine the nutritional status of the patients into severe malnutrition (BMI<15.9 kg/m^2), moderate malnutrition (BMI1/416-16.9 kg/m^2), mild malnutrition (BMI=17-18.4 kg/m^2) and normal (BMI=8.5-25 kg/m^2) as recommended by WHO[16].

2.2. Blood collection, Clinical chemistry and HIV serology

Blood specimens were taken with minimal venostasis after overnight fasting for the measurement of serum calcium from TB patients prior to initiation of anti-TB treatment and healthy controls. Sub samples of the TB patients were followed up during therapy and blood samples were collected again at the end of intensive phase of anti-TB chemotherapy. The presence of HIV antibodies was determined by an enzyme linked immunosorbent assay following the manufacturer's instruction (Vironostica HIV Uni-Form II plus O, Organon Teknika, Boxtel, the Netherlands). The concentration of albumin and was determined photometrically (AUTOLAB PM 4000/3, Analyser Medical System, Italy). Hypoalbuminemia was defined as serum albumin level below 3.5 gm/dl[17].

2.3. Determination of calcium in serum

The frozen serum samples were kept on dry ice and air freighted to Japan. Concentration of calcium in serum was determined using an inductively coupled plasma mass spectrometer (ICP-MS) (model 8500, Schimadzu, Tokyo, Japan), at Department of Analytical Chemistry, the University of Tokushima, Japan[18]. In brief, serum sample (200 μl) was aliquoted in to teflon tube and covered with teflon ball. After adding 1ml of concentrated HNO_3 (Wako Pure Chemicals, Japan), the tube was heated on an aluminum heating block (IWAKI, Asahi Techno Glass, Japan) at 120 ^{0}C for 5 h. The sample was further heated almost to dryness at 200 ^{0}C after removing the teflon ball. Finally, the residue was dissolved with 2ml of 0.1M HNO3 which contained 10 ng/ml internal standard elements (In, Re and Tl). The diluted serum solution was used for analysis of the calcium in ICP-MS. Commercially available singleelement standard solutions (1000 mg/ml) were purchased from Wako Pure Chemicals (Osaka, Japan) and used for standardization of calibration curves. To allow for protein binding of calcium, measured serum total calcium concentrations were corrected for hypoalbuminemia using the following equation as published by[19]

Corrected Ca = serum Ca + 0.8 (4 – serum albumin)

2.4.Statistical analysis

Data were analyzed using SPSS version 13 statistical package. A one-sample Kolmogorov- Smirnov test was used to assess whether the data were normally distributed. Serum calcium values were log transformed for analysis. Comparisons of serum values of calcium among TB patients with and without HIV co-infection and control groups were made using a one-way ANOVA. Post-hoc Tukey test was used to determine which pairs of means differ significantly. The paired T-test was used to compare pretreatment and post-treatment data. Hypercalcemia was defined at its serum levels greater than 10.5 mg/dl[20]. P-values less than 0.05 were considered statistically significant.

3. Results

A total of 155 TB patients (81 HIV seronegative and 74 HIV seropositive), 32 asymptomatic HIV-positive blood donors and 31 healthy controls were included in the study. Table 1 shows demographic, nutritional and clinical status of the patients and controls. Malnutrition (BMI<18.5 kg/m^2) was detected in 71.6% of HIV-positive and 65.4% of HIV-negative patients with TB, respectively, whereas it was seen in 50% of asymptomatic HIV-positive blood donors and in 16.1% of healthy controls. Severe malnutrition (BMI<15.9 kg/m^2) was more pronounced in TB patients with HIV co-infection. Majority of the patients manifested cough, weight loss, fever and night sweats irrespective of HIV serostatus. Extra pulmonary TB was diagnosed in greater proportion of TB patients with HIV co-infection (Table 1).

Table 2 shows the concentrations of albumin adjusted serum calcium in TB patients and healthy controls. The mean concentration of albumin adjusted serum calcium in sera of TB patients co-infected with HIV was significantly higher than that in TB patients without HIV co-infection (P<0.05) and healthy controls (P=0.03). Although the albumin adjusted serum calcium concentration was remarkably higher in HIV patients compared to its level in healthy controls, the difference was not statistically significant (P=0.07). In this study, the mean serum albumin level at the time of diagnosis was (3.07±0.33 g/dl) (range 1.2-4.6 g/dl). Hypoalbuminaemia (serum albumin level <3.5 g/dl) was present in 79 TB patients (51%) at diagnosis before commencement of anti-tuberculosis treatment. No significant improvement was observed after anti-tuberculosis treatment.

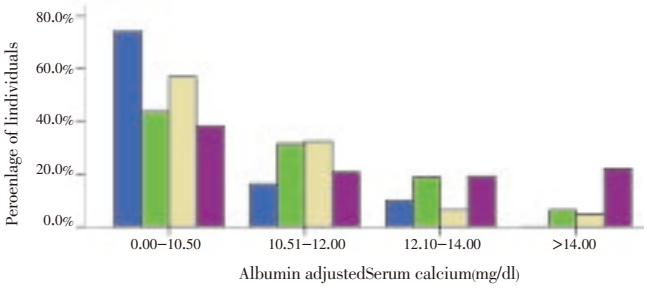

Figure 1. Frequency distribution of albumin adjusted serum calcium concentrations in TB patients with or without HIV co-infection in comparison with apparently healthy controls and asymptomatic HIV-infected blood donors in Gondar, Ethiopia. HIV indicates asymptomatic HIV-infected subjects; TB, patients with TB; TB&HIV, TB patients with HIV co-infection.

Table 1.
Demographic, clinical and calcium of TB patients by HIV serostatus and healthy controls

Parameter	TB +and HIV+N=74	TB+N=81	HIV+TB-N=32	Controls N=31
Age	31.72+8.72a	33.59+14.30	29.8+7.24	31.02+6.54
Sex				
Male	26 (35.1)b	34 (42.0)	13(40.6)	13 (41.9)
Female	48 (64.9)	47 (58.0)	19(59.4)	18 (58.1)
BMI	17.57+2.5a	18.0+2.4	18.7+11.1	21.0+2.9
Nutritional status				
Severe malnutrition	18 (24.3)	14 (17.3)	10(31.3)	-
Moderate malnutrition	11 (14.9)	17 (21.0)	2(6.3)	1 (3.2)
Mild malnutrition	24 (32.4)	22 (27.2)	4(12.5)	4 (12.9)
Normal	21 (28.4)	28 (34.6)	16(50)	26 (83.9)
Clinical signs				
Cough	65 (87.8)	65 (80.2)	-	-
Fever	70 (94.6)	73 (90.1)	-	-
Wt. loss	70 (94.6)	72 (88.9)	-	-
Night sweats	66 (89.2)	67 (82.7)	-	-
TB type				
Smear negative	14 (18.9)	24 (29.6)	-	-
Smear positive	18 (24.3)	19 (23.5)	-	-
Extra pulmonary	42 (56.8)	38 (46.9)	-	-

TB: tuberculosis; TB and HIV: tuberculosis and HIV co-infection.
a Mean±s.d. bNumber (%).

Table 2.
Serum levels of calcium (mg/dL) in TB patients with and without HIV co-infection before and after completion of intensive phase of anti-TB chemotherapy compared with those in apparently healthy controls in Gondar, Ethiopia. Calcium was adjusted for albumin, if it was below the lower reference of 8.4 mg/dl using the equation (see methods)

	Control(n = 31)	HIV(n = 32)	TB(n = 81)	TB and HIV(n = 74)	TB (n = 26)		TB and HIV (n = 34)	
					Before Treatment	AfterTreatment	Before Treatment	After Treatment
Mean ±SD	9.81±1.59	11.11±1.46	10.32±2.21	11.95±3.72*,†	10.98±2.05	10.82±2.75	12.21±2.95*	12.20±2.38*
Median (range)	9.98(8.81-10.98)	10.77(8.83- 15.35)	10.18(2.24-19.62)	11.31(4.90-23.02)	10.90(6.39-16.98)	10.65(2.01-17.54)	11.35(6.83-22.11)	11.65(9.44-19.23)
>10.5mg/dLa n (%)	8(25.8)	18(56.3)	35(43.2)	46(62.2)	16(61.5)	15(57.7)	24(70.6)	25(73.5)

aCutoffs according to (Diana M.et al) (2007).
*P<0.05 versus control †P<0.05 versus TB

Hypercalcemia was found in 62.2% and in 43.2% of TB patients with and without HIV co-infection, respectively. Twenty-six percent of healthy controls and 56.3% of asymptomatic HIV-infected blood donors were found to have serum calcium levels above 10.5mg/dl (Table 2). Fig. 1 shows the frequency distribution of albumin adjusted serum calcium levels in TB patients with and without HIV co-infection, asymptomatic HIV-infected blood donors, and healthy controls. Although hypercalcemia was observed in all groups, the proportion of TB-HIV patients with moderate hypercalcemia (12-14 mg/dL) and severe hypercalcemic crisis (>14.1 mg/dL) was significantly higher (p=0.03).

As summarized in Table 2, in 60 TB patients (26 HIV seronegative and 34 HIV seropositive), effect of anti-TB chemotherapy on the serum calcium concentration was studied by comparing their level at baseline and at the end of intensive phase of anti-TB chemotherapy. The group with follow-up did not differ from the whole group at baseline in clinical features of TB, age, and sex distributions. The mean levels of serum albumin adjusted calcium levels were not significantly changed in patients with and without HIV co-infection at the end of the intensive phase of anti-TB chemotherapy compared with the corresponding baseline levels. The proportion of TB patients with and without HIV co-infection with calcium levels of greater than 10.5mg/dL (hypercalcemia) were not significantly changed at the end of the intensive phase of anti-TB chemotherapy.

Regression analyses of age, sex, body weight, BMI, clinical signs of TB, and TB type as independent variables and serum level of albumin adjusted calcium as dependent variables did not show any significant association between the parameters and hypercalcemia.

In the present study, the incidence of hypercalcemia was significantly different in TB/HIV co-infected patients and control; however, the incidence was not significantly different in Ethiopian patients with TB and controls.

The results of this study show that hypercalcemia is extremely high, occurring in about 52% of patients with TB in Gondar, Northwest Ethiopia. This is higher than that reported in most previous studies, ranging from nearly 16% to 28%[9, 11, 13-14, 21-23]. A possible explanation for the difference in frequency may lie in regional differences in vitamin D status and calcium intake[24].

The high rates of hypercalcemia among patients may be related to the extent of disease, the intake of vitamin D and calcium, and the amount of sun exposure[25-27]. The staple dish of many people in the study area and its environs is a pancake named enjera made from a cereal called Tef (Eragrostis teff) which has higher calcium than those of wheat, barley, or sorghum[28]. It could also be due to tropical climates, Ethiopia is located in the tropics in the horn of Africa between 3 ° and 15 ° N, 33 ° and 48 ° E. The country is blessed with thirteen months of sunshine, and this may explain the relatively high incidence of hypercalcemia in our TB patients with or with out HIV. When sunlight is plentiful, relatively high serum 25(OH) D3 may give rise to hypercalcemia in the presence of TB[29]. Ultraviolet (UV) light is essential in this reaction. In tropical areas where there is a large amount of sun exposure, the concentration of 25(OH) D3 increases with an increase in active vitamin D synthesis. This theory is supported by the fact that incidence of tuberculous hypercalcemia is high in areas with large amount of sun exposure such as Australia and Malaysia[11].

On the other hand, in spite of abundant availability of UV radiation, it has been reported that the population from Addis Ababa situated at latitude 10 8N, 2700m above sea level had a high rate of biochemical vitamin D deficiency compared with the Norwegian group at latitude 60 8N at sea-level[30], In addition; it was also reported that increased risk of vitamin D deficiency in darker skinned individuals is due in the main part to decreased dermal synthesis of vitamin D as a result of the absorption of UV radiation by the increased melanin pigmentation[31]

It is well-established that activated immune cells can produce the hormonally active metabolite of vitamin D. Macrophages and other immune cells can express 1 α-hydoxylase, the enzyme that converts circulating 25-hydroxyvitaminD3 into 1, 25-dihydroxyvitamin D3, the active form of vitamin D[32]. Moreover, M. tuberculosis infection activates Toll-like receptors (TLR1/2) that mediate the activation of different cells in the innate immune system and their expression of cytokines and antimicrobial peptides[33-34]. However, in the case of tuberculosis infection, increased 1,25-dihydroxyvitaminD3 synthesis may further contribute to unwanted hypercalcemia. Most cases of hypercalcemia with tuberculosis are asymptomatic or mild. Active tuberculosis is more likely to be associated with severe hypercalcemia[21]. In the present study, none of the hypercalcemic patients with pulmonary TB had symptoms related to hypercalcemia before anti-TB therapy; however, a few weeks after starting anti-TB therapy, clinical evidence related to hypercalcemia was present in one of our TB and HIV co-infected patient.

Tuberculosis has been reported to enhance HIV replication and progression to AIDS in dually infected patients, possibly involving enhancement of inflammatory cytokines[35]. In turn, AIDS-related infections may lead to hypercalcemia, infection with Pneumocystis carinii[36], Mycobacterium avium[37-38], Cryptococcus neoformans and Coccidioides immitis[39-40], Candidiasis and paracoccidioidomycosis[41] and concurrent Epstein-Barr virus infection[42] have all been reported to be associated with hypercalcemia in HIV patients. As a result, the pathogenesis of hypercalcemia in pulmonary tuberculosis with and without HIV co-infection may involve more than one simple factor. Whatever the mechanism, there is the possibility that a modest increase in vitamin D in the daily diet and adequate sunlight may cause hypercalcemia in active pulmonary TB patients with HIV.

It is interesting to note that antiretroviral treatment (ART)-induced immune reconstitution may lead to the

possibility of hypercalcemia[43-44]. The roles of cytokines, macrophage activation and TB-associated immune reconstitution inflammatory syndrome in the pathogenesis of hypercalcemia among HIV/AIDS patients attending antiretroviral therapy (ART) require further investigation.

In addition to being hypoalbuminemic[24], patients with active TB commonly have protein and caloric deficiencies[45]. Hypoalbuminemia was reported in 69% of patients with TB at admission in a study from Nigeria[13] and in approximately half of patients in the present study at the time of diagnosis. The relatively high frequency of hypoalbuminemia among the series in the present study may have been related to malnutrition, which was mainly due to the low socioeconomic status of these patients plus chronic ill-health as a result of TB. If the serum calcium concentration is not corrected for a low serum albumin concentration, then hypercalcemia may not be recognized.

It has been suggested that anti-TB therapy may prevent the development of hypercalcemia[46]. However, the findings of the current study found no significant change in rates of hypercalcemia was observed after intensive phase of anti-TB treatment in both HIV positive and HIV negative TB patients from the baseline result obtained before initiation of therapy. No patient received vitamin D supplements before or during the study. In agreement with our observation, Kitrou et al[47] reported maximal increase in serum calcium occurred three weeks after initiation of treatment. The mechanism(s) by which anti-tuberculosis treatment affects calcium metabolism remains uncertain.

Our study has some potential limitations. We did not score subjects' recent sun exposure nor measure their degree of skin pigmentation. However, the cultural habit of this population included regular daily sun exposure. The etiology of the hypercalcemia was unclear, as vitamin D metabolites were not measured. Further studies are therefore warranted to evaluate the effect of vitamin D on health outcomes in TB patients with and without HIV infection.

Although, the present study demonstrated that rate hypercalcemia and hypercalcemic crisis is high in Ethiopian patients with TB and HIV co-infection, it is usually asymptomatic. The present study also suggests that TB itself appears not to be responsible for hypercalcemia. Anti-TB chemotherapy does not avert the incidence of hypercalcemia; however, further in-depth studies are needed to substantiate these findings.

Conflict of interest statement

We declare that we have no conflict of interest.

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