Assessment of Serum Calcium and Phosphorus in Pulmonary Tuberculosis Patients Before, During and After Chemotherapy

K. Rohini · Surekha Bhat · P. S. Srikumar ·
A. Mahesh Kumar

Received: 27 May 2013 / Accepted: 10 September 2013 / Published online: 11 October 2013
© Association of Clinical Biochemists of India 2013

Abstract Our study was aimed to assess the levels of serum calcium and phosphorus in pulmonary tuberculosis patients. Blood samples were collected from 40 patients with pulmonary tuberculosis before treatment (PTB-0), at the end of 2 months of intensive phase of treatment (PTB-2) and after 6 months of treatment (PTB-6). Age and weight matched normal healthy volunteers ($n = 37$) served as normal controls. Serum was analyzed for calcium and phosphorus. Serum calcium significantly decreased to hypocalcemic levels and serum phosphorus significantly decreased but was within normophosphatemic limits in pulmonary tuberculosis. Chemotherapy for tuberculosis managed to raise serum levels of both the ions, with hypocalcemia still persisting in majority of patients during treatment but getting resolved in a significant percentage of patients at the end of 6 months of treatment. Results indicate the need for calcium and phosphorus supplements in tuberculosis patients during chemotherapy. This study also warrants the need for regular monitoring of serum calcium and phosphorus in patients undergoing anti-tuberculosis treatment.

Keywords Hypocalcemia · Normophosphatemia · Anti-tuberculosis treatment · Chemotherapy

Introduction

Pulmonary tuberculosis (PTB) is still a very common cause of morbidity and mortality around the world. Calcium abnormalities have been variedly reported in studies related to tuberculosis, with some studies reporting hypercalcemia and a few others reporting hypocalcemia as a major biochemical finding. Lind and Ljunghall [1] found hypercalcaemia in 25 % of the pulmonary TB patients in their study. Similar findings have been reported by other authors in their respective research studies on TB [2, 3]. Some workers have detected hypercalcemia in 25 % or more of their study population [4, 5] with pulmonary tuberculosis, with symptoms of hypercalcaemia present in only 5–12 % of these patients. Hypercalcaemia and hyperphosphataemia in pulmonary TB has also been reported by Meuthen et al. [6]. Chan et al. [7] have reported that albumin corrected calcium (ACC) was found significantly higher in pulmonary TB patients despite a lower calcium intake but comparatively low percentage of hypercalcaemia was found in another related study. Contrary to all the above reports, pulmonary TB has been found to be associated with hypocalcaemia in some studies [8–11].

Similarly, alterations in serum phosphorus levels have been variedly reported in tuberculosis. Wells et al. [12] referred to a few early reports of phosphorus retention and reduced excretion and also to a few reports of increased urinary levels of lipid-bound phosphorus in patients with tuberculosis. Sweany et al. [13] found that phosphorus levels varied considerably from patient to patient and they suggested that some of the phosphorus might be associated

K. Rohini (✉)
Unit of Biochemistry, Faculty of Medicine, AIMST University,
08100 Bedong, Kedah, Malaysia
e-mail: rohinik23@gmail.com

S. Bhat
Melaka Manipal Medical College, Melaka, Malaysia

P. S. Srikumar
Unit of Psychiatry, Faculty of Medicine, AIMST University,
Bedong, Kedah, Malaysia

A. Mahesh Kumar
Madras Medical Mission Hospital, Chennai, India

with lipoproteins liberated by the destruction of cell membranes. Sharma [14] observed elevated phosphorus levels in Indian patients who were hypercalcaemic but not in those who were normocalcaemic. Studies have shown that there was a significant change in total serum calcium and inorganic phosphate levels of subjects studied especially hypocalcaemia and hypophosphatemia which is common in PTB [15].

A thorough reading through all the above mentioned articles drove our attention to the fact that some studies related to alterations in calcium and phosphorus metabolism in tuberculosis per se while others assessed the serum ionic levels during chemotherapy. The present study was carried out to study the changes in serum calcium and phosphorus levels in tuberculosis before, during and at the end of chemotherapy in pulmonary tuberculosis.

Materials and Methods

Subjects

The study subjects were recruited from The Institute of Thoracic Medicine, Chennai, India. The study group consisted of 40 patients (25–75 years) diagnosed with PTB. The diagnosis of tuberculosis was performed using Ziehl–Neelsen staining method for Acid-fast Microscopy (AFM) and culture for growth of the organism on Lowenstein–Jensen (LJ) medium. The patients were also tested for radiographic abnormalities. Thirty seven age and sex matched healthy volunteers (*Normal controls; NC*) were included in the study for comparison of results.

Sampling and Analysis

Blood samples were collected from both groups and those collected from PTB were sub-grouped as PTB-0 (PTB at diagnosis), PTB-2 (PTB during treatment or PTB at 2 months post treatment) and PTB-6 (PTB at the end of treatment or PTB at 6 months post treatment). Blood samples were collected in PTB-0 immediately after diagnosis and these patients were put under intensive phase of chemotherapy with the four-drug regimen (Rifampicin, Isoniazid, Pyrazinamide and Streptomycin) for 2 months. At the end of 2 months, blood samples were collected again for the estimation of the ionic levels and these samples were labeled as PTB-2. All the PTB subjects were continued into the next 4 months of continuation phase with the 2 drug regimen (Rifampicin and Isoniazid). At the end of 6 months, all subjects were declared as clinically cured. Blood samples were collected yet again and labeled as PTB-6.

Blood samples thus collected using standard sampling techniques were centrifuged to obtain serum that was analyzed for calcium by photometric test using Arsenazo III end point [16] and phosphorus levels by phosphomolybdate method [16]. The study protocol was approved by the Institutional ethics committee and was carried out in accordance with the principle of declaration of Helsinki. Informed consent was obtained from all the subjects.

Statistical Analysis

Statistical analysis of the results was carried out using students' unpaired *t* test for comparison of groups PTB-0, PTB-2 and PTB-6 with NC, paired *t* test and repeated measures ANOVA for comparison between the subgroups. Following ANOVA for correlated samples, the Tukey HSD posttest was applied to check for significance. A *p* value of less than 0.05 was considered significant, less than 0.01 very significant and less than 0.001 as extremely significant.

Results

The clinical characteristics of the patients are shown in Table 1. As shown in Table 2, both serum calcium and serum phosphorus levels in PTB-0 group was less than that of normal controls (nearly 1.2-fold decrease in serum calcium and nearly 1.1-fold decrease in serum phosphorus) and this was statistically extremely significant ($p < 0.001$ for serum calcium, <0.01 for serum phosphorus). The mean serum calcium in PTB-2 group was less than that of normal controls and this was statistically extremely significant ($p < 0.001$), however, serum calcium had begun to rise during the intensive phase of treatment as indicated by the very significant *p* value (<0.01) in the paired *t* test of PTB-0 versus PTB-2, with PTB-2 showing higher serum calcium levels. The mean value for serum phosphorus levels in PTB-2 group was more than that of normal controls and this was statistically not significant. However, serum levels of the anion had significantly increased in comparison to PTB-0 in the paired *t* test (<0.01) indicating that serum phosphorus levels had managed to reach the normal range at the end of the intensive phase of treatment. The mean serum calcium in PTB-6 group was less than that of normal controls and this was statistically extremely significant ($p < 0.001$) as seen in Table 2. Repeated measures ANOVA (Table 3) in the table of the three test groups (PTB-0, PTB-2 and PTB-6) showed statistical significance ($p < 0.001$). Tukey HSD post-test between the three groups showed that the means of the three groups differed significantly from each other ($p < 0.01$ for PTB-0 vs PTB-2, PTB-2 vs PTB-6 and PTB-0 versus PTB-6).

Table 1 Clinical characteristics of tuberculosis patients

Clinical characteristics	No. of subjects
Total number	40
Age	25 ± 75 years
Body mass index	
Severe malnutrition (<15.9 kg/m ²)	30
Moderately (16–16.9 kg/m ²)	7
Mild (17–18.4 kg/m ²)	2
Normal (>18.5 kg/m ²)	1
Cough	40
Fever	25–30
Weight loss	40
Night sweats	25–30

Thus, although the decreased mean plasma calcium at diagnosis had not reached the normal range at the end of 6 months of treatment, there was a significant progressive increase in the cationic levels during the course of treatment. Serum phosphate levels had further increased during the continuation phase. The mean value for serum phosphorus levels in PTB-6 group was more than that of normal controls and this was statistically extremely significant ($p < 0.001$) as seen in Table 3. Repeated measures ANOVA (Table 4) of the three test groups (PTB-0, PTB-2 and PTB-6) showed statistical significance ($p < 0.001$). Tukey HSD posttest between the three groups showed that the means of the three groups differed significantly from each other ($p < 0.05$ for PTB-0 vs PTB-2, $p < 0.01$ PTB-2 vs PTB-6 and $p < 0.01$ for PTB-0 vs PTB-6). Although the mean serum phosphorus of PTB-6 was significantly more than that of NC, it was within the normal range of serum phosphate in humans.

Discussion

Tuberculosis is one of the granulomatous diseases that change the status of plasma calcium concentration [17]. While some studies report hypercalcemia associated with tuberculosis [1–7], some others report hypocalcemia as a finding in the disease [8, 9, 11]. Low serum calcium was not considered the predisposing cause of tuberculosis until the recent past, where different workers from different centers of the world were able to show that tuberculous patients were often associated with hypocalcaemia [10, 18]. In our study, the mean serum calcium in PTB-0 group was less (by nearly 1.2-fold) than that of normal controls and this was statistically extremely significant ($p < 0.001$). Ijaz et al. [11] have attributed their finding of hypocalcemia to malnutrition and malabsorption associated with tuberculosis. Hafiez et al. [9], in their studies attribute the

Table 2 Levels of serum calcium and phosphorus in normal controls, NC and pulmonary tuberculosis patients at diagnosis, PTB-0

Parameters	NC* (<i>n</i> = 37)	PTB-0 (<i>n</i> = 40)	<i>p</i> value	Magnitude of change
Calcium (mg/dl)	9.34 ± 0.46	7.72 ± 1.02	<0.001	~1.2-fold ↓
Phosphorus (mg/dl)	3.45 ± 0.46	3.06 ± 0.8	<0.01	~1.1-fold ↓

Values are mean ± SD

hypocalcemia to impaired intestinal absorption of calcium or deficient intake as a result of anorexia, decreased plasma albumin or decreased active metabolites of vitamin D. In the present study also, as can be seen in Table 1, 39 out of 40 patients were malnourished (30 severe, 7 moderate, 2 mild). This could be a reason for the observed hypocalcaemia in PTB-0 patients. According to Islam et al., tuberculosis is the disease of people who live in under-privileged conditions like dark homes and with poor nutritional intake. It is a well-known fact that poor exposure to sunlight results in vitamin D deficiency that along with low intake of milk leads to hypocalcaemia. We presume that the decreased physical activity experienced by the patients due to the rest advised in the event of the disease could be a reason for less exposure to sunlight. This could lead to vitamin D deficiency and hence hypocalcemia. Simultaneous with these findings, we have also observed significant increase in serum PCT in the same tuberculosis patients at diagnosis [19] and a decrease in the prohormone levels at the end of 6 months of treatment but not at the end of 2 months of intensive phase of chemotherapy [20]. However, measurement of PCT was semi quantitative and hence we could not correlate the changes in serum PCT with the changes in serum calcium. Although studies report that there are no enzymes in plasma to convert PCT to calcitonin (CT) nor does the PCT bind to calcitonin receptors [21]. Hafiez et al. have reported increased plasma calcitonin with concomitant decrease in plasma calcium in tuberculosis patients. These researchers also explain that one of the reasons of hypocalcemia in their study could be due to the increased plasma calcitonin, which is increased due to increased calcitonin secretion by bronchial K-cells. In view of our previous findings and the above mentioned reports by previous authors, we suggest that further research be conducted to determine the correlation between changes in serum PCT, calcitonin and calcium. A quantitative assay of serum PCT would be more preferable in these future studies. The suggested research becomes essential because with the available literature, the question remains unanswered as to why serum PCT as well as CT increase in tuberculosis although there are no enzymes in plasma to convert PCT to CT.

Table 3 Levels of serum calcium and phosphorus in normal controls, NC, pulmonary tuberculosis patients at diagnosis (PTB-0), pulmonary tuberculosis patients at 2 months (PTB-2) and post treatment (PTB-6)

Parameters	NC (<i>n</i> = 37)	PTB-0 (<i>n</i> = 40)	PTB-2 (<i>n</i> = 40)	PTB-6 (<i>n</i> = 40)
Calcium (mg/dl)	9.34 ± 0.46	7.72 ± 1.02	8.25 ± 0.27	8.71 ± 0.44***,###
Phosphorus (mg/dl)	3.45 ± 0.46	3.06 ± 0.8	3.67 ± 1.17	4.65 ± 0.96***,###

Values are mean ± SD

*** $p < 0.001$ for PTB-6 vs NC; ### $p < 0.001$, ## $p < 0.01$ for correlated samples ANOVA of PTB-0, 2 and 6

Table 4 Results of Tukey HSD post hoc test between the three subgroups (PTB-0; PTB-2; PTB-6) for the calcium and phosphorus levels

Parameters	<i>p</i> -values after Tukey HSD post hoc test		
	PTB-0 vs PTB-2	PTB-2 vs PTB-6	PTB-0 vs PTB-6
Calcium (mg/dl)	<0.01	<0.01	<0.01
Phosphorus (mg/dl)	<0.05	<0.01	<0.01

It has been reported that hypercalcemia is more often encountered during the first weeks of antituberculous treatment, when hypercalcemia may be found in approximately 30–50 % of the patients [22, 23]. According to Kitrou et al. [24] hypercalcaemia in patients with PTB seems to be triggered by chemotherapy although the mechanism(s) by which anti-tuberculosis treatment affects calcium metabolism remains uncertain. An increase in the calcium content of the body tissue and blood apparently tends to lessen capillary permeability, thereby lessening the amount of exudate, preventing an extension of the infecting organisms and tending to limit the destruction by their toxins [25]. In the present study, the four drug regimen resulted in an increase in serum calcium ($p < 0.01$ for paired t test of PTB-0 vs. PTB-2) although the mean was still within hypocalcemic range for human serum and significantly lower than that of normal controls ($p < 0.001$ for unpaired t test of PTB-2 vs NC). We also wish to add here that we have reported a body weight gain of less than the desirable 5 % in PTB-2 patients [26]. According to Khan et al. [27], a weight gain of 5 % or less during the first 2 months of therapy is associated with an increased risk of relapse, even after controlling for other risk factors for relapse. The lower weight gain observed in PTB-2 patients could be an additional factor, a nutritional factor responsible for the persisting hypocalcemic milieu. At this juncture, we agree with Islam et al. that calcium supplements must be added along with vitamin D to get excellent clinical response to antituberculous therapy. This would compensate for the slow reversion of calcium to normal levels by chemotherapy. Additionally we would like to suggest that in an already altered calcium homeostasis in

pulmonary tuberculosis, serum calcium needs to be closely monitored before deciding on adding calcium supplements to the treatment protocol. With the two drug continuation regimen, there was a further increase in serum calcium ($p < 0.01$ for PTB-0 vs PTB-2, PTB-2 vs PTB-6 and PTB-0 versus PTB-6 in Tukey HSD post test for repeated measures ANOVA). Unlike with the 4 drug regimen in PTB-2, 20 patients had become normocalcaemic whereas the rest 20 were still hypocalcemic in PTB-6. Hence, the mean was at the upper end of hypocalcemic range for human serum and significantly lower than that of normal controls ($p < 0.001$ for unpaired t test of PTB-6 vs NC). Noteworthy is the fact that chemotherapy did not induce hypercalcemia in any of our patients, contrary to what was observed by some earlier researchers [21, 22]. These findings warrant the need for calcium supplements not only during intensive phase but also during the continuation phase of treatment and perhaps for some time beyond cessation of treatment for those patients who fail to become normocalcaemic even after 6 months of treatment.

A lot of previous studies have reported an increase in serum phosphate in tuberculosis patients. Although more attention has previously focused on calcium than on phosphorus in tuberculosis, studies by Kardjito et al. suggests that the latter is much more affected than the former in this disease [27]. Unlike these previous reports, serum phosphorus levels in PTB-0 group in our study was less (by nearly 1.1-fold) than that of normal controls and this was statistically very significant ($p < 0.01$). However, the mean value was still within the accepted normal range of serum phosphate in humans (2.4–4.7 mg/dl) and hence our finding is one of normophosphatemia rather than hypophosphatemia, although the decrease in PTB-0 is statistically significant. This is similar to the findings of Hafiez et al. who have reported normophosphatemia in tuberculosis patients who presented with hypocalcemia and hypercalcitoninemia.

The mean value for serum phosphate levels in PTB-2 group was more than that of normal controls but this was statistically not significant. However, serum levels of the anion had significantly increased in comparison to PTB-0 in the paired t test (<0.01). Thus, consequent to chemotherapy, the phosphorus levels reached closer to those of normal controls. The mean value for serum phosphate

levels in PTB-6 group was more than that of normal controls and this was statistically extremely significant ($p < 0.001$). Surprisingly, the mean value of PTB-6 showed a 1.5-fold increase over that of normal controls. However, normophosphatemic status was still maintained, although at the higher end of the range. It is to be remembered that the intensive phase of treatment is marked by active phagocytosis of the bacilli resulting in cell lysis and increased liberation of lipoproteins and hence phosphate. This together with the finding that there was a progressive increase of phosphorus from PTB-0 through PTB-2 to PTB-6 in the present study, draws one's attention towards a closer monitoring of the anion during treatment to avoid situations of hyperphosphataemia.

Conclusion

In conclusion, serum calcium and phosphorus significantly decreased in pulmonary tuberculosis. While the cationic levels decreased to hypocalcemic range, the decrease in phosphorus was within normophosphatemic range. Chemotherapy for tuberculosis managed to raise serum calcium levels, with hypocalcemia still persisting in majority of patients in the intensive phase but getting resolved in a significant percentage of patients at the end of 6 months of treatment. Chemotherapy also increased serum phosphate levels and results indicate that there is a likelihood of development of hyperphosphataemia during tuberculosis treatment. These findings indicate the need for calcium and phosphorus supplements in tuberculosis patients during chemotherapy. This study also warrants the need for regular monitoring of serum calcium and phosphorus in patients undergoing anti-tuberculosis treatment.

References

- Lind L, Ljunghall S. Hypercalcemia in pulmonary tuberculosis. *Ups J Med Sci.* 1990;95(2):157–60.
- Pruitt B, Onarecker C, Coniglione T. Hypercalcemic crisis in a patient with pulmonary tuberculosis. *J Okla State Med Assoc.* 1995;88(12):518–20.
- Hournay J, Mehta JB, Hournay V, Byrd RP Jr, Roy TM. Hypercalcaemia and pulmonary tuberculosis in east Tennessee. *Tenn Med.* 1997;90(12):493–5.
- Roussos A, Lagogianni I, Gonis A, Ilias I, Kazi D, Patsopoulos D, Philippou N. Hypercalcaemia in Greek patients with tuberculosis before the initiation of anti-tuberculosis treatment. *Respir Med.* 2001;95(3):187–90.
- Liam CK, Lim KH, Srinivas P, Poi PJ. Hypercalcaemia in patients with newly diagnosed tuberculosis in Malaysia. *Int J Tuberc Lung Dis.* 1998;2(10):818–23.
- Meuthen I, Kirsch L, Saborowski F. Hypercalcemia in florid pulmonary and cervical lymph node tuberculosis. *Dtsch Med Wochenschr.* 1991;116(23):899–902.
- Chan TY, Poon P, Pang J, Swaminathan R, Chan CH, Nisar M, Williams CS, Davies PD. A study of calcium and vitamin D metabolism in Chinese patients with pulmonary tuberculosis. *J Trop Med Hyg.* 1994;97(1):26–30.
- Shirai M, Sato A, Suda T, Shichi I, Yasuda K, Iwata M, Okano A, Genma H. Chida Calcium metabolism in tuberculosis. *Kekkaku.* 1990;65(6):415–20.
- Hafiez AA, Abdel-Hafez MA, Salem D, Abdou MA, Helaly AA, Aarag AH. Calcium homeostasis in untreated pulmonary tuberculosis. I-Basic study. *Kekkaku.* 1990;65(5):309–16.
- Ali-Gombe A, Onadeko BO. Serum calcium levels in patients with active pulmonary tuberculosis. *Afr J Med Med Sci.* 1997;26(1–2):67–8.
- Ijaz A, Mehmood T, Saeed W, Qureshi AH, Dilawar M, Anwar M, Hussain S, Khan FA, Khan IA, Khan DA. Calcium abnormalities in pulmonary tuberculosis. *Pak J Med Res.* 2004;43:4.
- Wells HG, Dewitt LM, Long ER. The chemistry of tuberculosis. London: Bailliere Tindall and Cox; 1923.
- Sweany HC, Weathers AT, McCluskey KL. The chemistry of blood in tuberculosis. *Am Rev Tuberc.* 1923;8:405.
- Sharma SC. Serum calcium in pulmonary tuberculosis. *Postgrad Med J.* 1981;57:694.
- Godwin AO, Johnson DJ, Otimenbhor JO. Total serum calcium and inorganic phosphate levels in tuberculosis patients in benin city Nigeria sierra leone. *J Biomed Res.* 2010;2(2):87–90.
- Endres DB, Rude RK. Mineral and bone metabolism. In: Burtis CA, Ashwood ER, editors. *Tietz Fundamentals of Clinical chemistry.* Philadelphia: W.B. Saunders; 2001. p. 795.
- Baig MA, Ghulam Mustafa KK, Lutufullah B, Qureshi MA. Low serum calcium associated with tuberculosis. *Prof Med J.* 2006;13(4):583–6.
- Rohini K, Srikumar PS, Jyoti S, Mahesh Kumar A, Bhat S. Assessment of serum calcium, phosphorus, c-reactive protein and procalcitonin in tuberculosis patients. *Int J Collab Res Intern Med Pub Health.* 2012;4(12):1868–75.
- Rohini K. Diagnostic and prognostic value of procalcitonin in tuberculosis patients, Ph.D thesis, Banasthali University ('A' Accredited Institution), Rajasthan; 2013.
- Maruna P, Nedelnikova K, Gurlich R. Physiology and genetics of procalcitonin. *Physiol Res.* 2000;49(Suppl 1):S57–61.
- Abbasi AA, Chemplavil JK, Farah S, Muller BF, Arnstein AR. Hypercalcemia in active pulmonary tuberculosis. *Ann Intern Med.* 1979;90(3):324–8.
- Kitrou MP, Phytou-Pallikari A, Tzannes SE, Virvidakis K, Mountokalakis TD. Hypercalcemia in active pulmonary tuberculosis. *Ann Intern Med.* 1982;96(2):255.
- Kitrou MP, Phytou-Pallikari A, Tzannes SE, Virvidakis K, Mountokalakis TD. Serum calcium during chemotherapy for active pulmonary tuberculosis. *Eur J Resp Dis.* 1983;64(5):347–54.
- Goldberg B. Calcium therapy in tuberculosis. (1935). In: *Diseases of the chest*: <http://173.193.11.217/>. Accessed Dec 13 2012.
- Rohini K, Bhat S, Srikumar PS, Jyoti S, Mahesh Kumar A. Diagnostic and prognostic value of procalcitonin in tuberculosis patients. *Br J Med Med Res.* 2013;3(4):2189–96.
- Khan A, Timothy Sterling R, Reves R, Vernon A, Robert Horsburgh C, Tuberculosis Trials Consortium. Lack of weight gain and relapse risk in a large tuberculosis treatment trial. *Am J Resp Crit Care Med.* 2006;174:344–8.
- Kardjito T, Ediyanto SP, Grange JM. Serum phosphorus levels in pulmonary tuberculosis. *Postgrad Med J.* 1984;60:394–6.