STUDY OF CALCIUM IN ACTIVE PULMONARY TUBERCULOSIS PATIENTS BY FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

Zainab Manzoor Memon¹*, Afsheen Mushtaque Shah¹ and Tasneem Gul Kazi²

¹Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan.
²National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan.

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ABSTRACT

Pulmonary tuberculosis (PTB) arising by mycobacterium tuberculosis (tubercle bacilli). It leads to morbidity and humanity worldwide comprising Pakistan. The aim of study was to assess the serum calcium level in patients with active pulmonary tuberculosis and compared with healthy volunteer. Blood sample was assembled from 100 active PTB patients and from 50 normal control subjects. Study was conducted at chest ward of Liaquat University of Medical & Health Sciences Jamshoro/Hyderabad, Rajputana Hospital Hyderabad and TB Sanatorium Hospital Kotri, Sindh, Pakistan. All patients and controls were selected from both genders having same age group (20-70 of years). Serum calcium was analyzed by using analytical technique named Flame Atomic Absorption Spectrophotometer (FAAS). In our study we found elevated serum calcium concentration in patients with mean ± SD 16.6 ± 6.7 mg/dl that is more than normal range (>10.5 mg/dl). While in normal control the mean ± SD was 8.8 ± 4.3 mg/dl that is under the normal range (8-10 mg/dl). So, it is conclude that hypercalcemia is linked with active pulmonary tuberculosis and also caused other different disorders.

Corresponding author
Zainab Manzoor Memon
Research Scholar, Institute of Biochemistry,
University of Sindh, Jamshoro, Pakistan
Zainabmemon81@hotmail.com

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INTRODUCTION

Tuberculosis (TB) is an olden disease from the past of human life [3]. It is a disease of developed nations; approximately 3 million fatalities reported in 2014 occur yearly and approximately five deaths happen in each minute. TB leftovers the solitary infectious virus and is the source of the uppermost death in person [37]. It is second foremost source of fatality following human immunodeficiency virus [6, 4, 5]. TB is bacterial infections illness caused by mycobacterium tuberculosis (tubercle bacillus or acid fast bacilli) [2]. Initially bacilli enter in the lungs and causes disease termed as pulmonary tuberculosis (PTB) [1].

It extends from infected person via air by sneezing, coughing, laughing, talking, sneezing, spitting, discharging mucus and kissing [28]. Abnormal radiographic findings, positive phlegm, prolonged cough, dyspnea, haemoptysis, weakness, fatigue, weight loss, fever, night sweating and anorexia are symptoms of active pulmonary TB [22, 27]. Large families, low monthly income, living in urban areas, poor shelter setting, breathe in gather together foundations, live in detention center, hostels, shelters for old group, public refuge, garden center and schools, scarcity, deprived cleanliness, low education, drug confrontation, reduced conformity with medications, diabetes and HIV/AIDS are most imperative risk factors [7]. Growing frequency of TB is an alarming situation for the community wellbeing policy creators of underdeveloped and industrial nations. Research stated that globally about 1/3 of the inhabitants are contaminated and three million individuals pass away in each year due to bacteria known as mycobacterium tuberculosis. Approximately 342 losses occur in a single hour because of this infected disease. To tackle the disease, precise diagnosis and quick opinion is required. In the existing conditions the regular investigations for diagnosis of TB have confines. Such assessment includes chest x-ray, culture, tuberculin skin test and acid-fast staining examination. Culture acquire much time in outcome while serological examinations are rapid but not too sensitive. Single chest x-ray is not a key in the diagnosis of TB and tuberculin skin test hasn’t explicit and consistency [36].

Several hematological and biochemical abnormalities are universal in pulmonary tuberculosis and are helpful in the diagnosis of TB. Hypercalcemia acknowledged in granulomatous disease and is sound documented obstacle of active pulmonary tuberculosis. In many published research the prevalence of hypercalcemia contrast broadly in different nations. These variations are due to intake of vitamin D, calcium and sun exposure [36].

TB and malnutrition relationship is sound documented [8]. Calcium is the abundant mineral and 5th most vital micronutrient of body [12]. Calcium is crucial for the growth of body including bones and teeth and located in the skeleton, soft tissues and extracellular fluid [13]. During pathogenesis, neutrophil mediated slaughter of mycobacteria is a Ca²⁺ needy process [10]. This granulomatous disease alters the concentration of serum calcium [9].

The aim of study was to assess the levels serum calcium in patients with active pulmonary tuberculosis at the main TB hospitals of Jamshoro and Hyderabad, Sindh, Pakistan and associated it with nutrition and related disorder of calcium.

MATERIALS & METHODS

Study was conducted among 100 active pulmonary tuberculosis subjects and 50 healthy volunteers of both genders with same age group 20 - 70 years respectively. The patients were selected from Liaquat University Hospital Hyderabad/Jamshoro, Rajputana Hospital and TB Sanatorium Hospital Kotri, Sindh, Pakistan. This study was approved by the members of Ethical Committee, Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan. Researcher was used face mask and surgical gloves during collection of data. The patients who had clinical sign and symptoms of active TB including anomalous chest radiography with cavity formation and shadow, lymph nodes deformity, cough more than three weeks, shortness of breath, chest tenderness, blood in sputum, weakness, tiredness, weight loss, fever, night sweating, loss of appetite and sputum positive test result were incorporated in this study. All subjects and healthy controls were examined, physically including blood pressure, pulse and temperature. All patients and volunteers were interviewed through questionnaire. Serum calcium and sputum smear microscopy were done of all patients and control groups. Calcium was detected by using Flame Atomic Absorption Spectrophotometer.

Experimental/ Methodology

A flame atomic absorption spectrophotometer equipped with photomultiplier detector. The Ca hollow cathode lamps were used and run according to conditions suggested by the company (Table 1). Biological samples were digested at 120-130 W for 5 minutes using thermostatic dry oven ranged from 20–250°C and the timer scope is from 1-9999 minutes respectively. For preparation and storage of solutions, acid wash poly tetrafluoroethylene (PTFE) vessels and flasks were used [29].

Chemicals and Reagents

Concentrated nitric acid (HNO₃) 65% and hydrogen peroxide (H₂O₂) 30% were purchased from Merck (Darmstadt, Germany) for the preparation of stock standards. All standards and chemicals were of analytical grade [29].

Stock solutions and metal standard

Calcium chloride (CaCl₂) was purchased from Merck Germany. 1000 ppm solutions were prepared from stocks by diluting with 1000 ml deionized water and obtained working standard solutions [29].

Microwave assisted acid digestion method (MDM)

Duplicate blood samples (0.5 mL) of each tuberculosis patient and control subject were placed into crucibles (Teflon PFA flasks). About 2 milliliter freshly prepared mixture of concentrated HNO₃:H₂O₂ with a ratio of (2:1, v/v) was added into flask and were left for 10 minutes. After completion of 10 minutes each flasks were placed in a preheated thermostatic oven at 120-130°C for 5-
6 minutes until clear solution obtained. Removed flask with the help of holder, after that the flasks were left to cool. To take away surplus acid, the solution was then evaporated to dryness; add 10 milliliter diluted 0.1 M nitric acid in each flask. Experiment was carried out at room temperature (30°C) by using Flame Atomic Absorption Spectrophotometer. The calibration graphs were obtained against reagent blank [29, 32].

Table 1: Measurement conditions for flame Atomic Absorption Spectrophotometer.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Slit width (nm)</th>
<th>Lamp current (mA)</th>
<th>Burner height (mm)</th>
<th>Oxidant (Air) L/minute</th>
<th>Fuel (Acetylene) L/minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>422.7</td>
<td>0.7</td>
<td>7.5</td>
<td>12.5</td>
<td>17.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Statistics
The data were analyzed using Statistical Package for Social Sciences (Ver. 6.0).

Table 2: Mean, SD and RSD of calcium in active pulmonary tuberculosis patients and control.

<table>
<thead>
<tr>
<th>Calcium</th>
<th>Mean</th>
<th>SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>16.60</td>
<td>6.74</td>
<td>4.10</td>
</tr>
<tr>
<td>Control</td>
<td>8.86</td>
<td>4.31</td>
<td>0.60</td>
</tr>
</tbody>
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RESULTS & DISCUSSION
Table: 2 highlight a mean, standard deviation and relative standard deviation of calcium in active PTB patients and normal control subjects. Figure: 1 shows a linear relationship between concentration of standard of calcium and absorbance on Atomic Absorption Spectrophotometer. Figure: 2 graphically indicate a difference in mean, in active pulmonary tuberculosis patients and control group.

Figure: 1 Calibration Cure of Calcium standard by Atomic Absorption Spectrophotometer

Figure: 2 Graphical representation of calcium in patients and control.
Calcium act in body in the form of free ion or bound complexes contribute in variety of functions of body. As bound calcium the most imperative functions is mineralization of skeleton. In skeleton 99% of total body calcium is present in form of calcium-phosphate complexes while 1% of total body calcium present in rest parts of body known as nonbone calcium. Nonbone calcium plays a role in extra- and intracellular signaling, nerve impulse transmission, and muscular contraction. The normal range of serum calcium is 8.8 to 10.4 mg/dl (2.2 to 2.6 mM) in which 51% free ions present, 40% are protein-bound complexes and ionic complexes are 9%. Nonionized calcium is bound to a proteins comprise serum albumin and globulin and calmodulin and cellular calcium-binding proteins. Serum calcium phosphate, calcium carbonate, and calcium oxalate are the main ionic complexes [12].

In this study, we observed a significant increase in serum calcium in pulmonary tuberculosis when compared to normal subjects. The same findings was reported by Okgun Godwin A in (2010) and also Burtis and Ashwood in (2001) [15]. This amplification may happen by more entry of calcium within extracellular fluid partition such as from skeleton and from the intestine and kidney. This arises mostly in tuberculosis, dehydration and hyper albuminaemia. The reason of hypercalcemia is hyperparathyroidism, vitamin D- intoxication, malignancy, medications, thiazide diuretics, lithium, milk alkali syndrome, immobilization infection, inflammation, inheritance, granulomatous disease, endocrine disorders and genetic disorders. In pulmonary tuberculosis unbalanced performance of parathyroid gland resulting surplus production of parathyroid hormone [12, 22]. Hypercalcemia in pulmonary tuberculosis patients is generally mild and asymptomatic. Though, extreme levels have been found in disseminated tuberculosis patients and pulmonary tuberculosis patients with pleural effusion. Protein energy malnutrition and hypoalbuminemia are general findings in patients with hypercalcemia in active tuberculosis. Vitamin D (25 hydroxycholcalciferol and 1, 25 dihydroxycholecalciferol) act in pathogenesis by activation of cell mediated immunity in pulmonary tuberculosis, thus increasing the number of macrophages. 

Mean and standard deviation of calcium level in active TB patients were 16.6 ± 6.7 mg/dl (>10.5 mg/dl) while in control the mean and standard deviation of calcium were 8.8 ± 4.3 mg/dl (8.8-10 mg/dl). Bemnet Amare et al. supported our finding which was conducted in (2012) and conclude that hypercalcemia is really found in PTB [17]. Insufficiency of vitamin D linked with progress of tuberculosis disease [19, 35]. Vitamin D enhances the manufacture of antimicrobial peptide cathelicidin which help in assassination of M. tuberculosis [19] and calcium equilibrium is controlled by parathyroid hormone (PTH), 1,25-dihydroxyvitamin D3 (calcitriol), and calcitonin in human body [20, 34]. Excess resorption of calcium in bones, higher gastrointestinal absorption of calcium, and lower renal excretion of calcium all are the basis of hypercalcemia [34].

CONCLUSION
It is accomplished that calcium abnormalities mainly hypercalcemia is quite common in our patients of pulmonary TB and physicians must maintain a high index of suspicion for diagnosis and correction of these abnormalities which are causing different other disorders.

Authors’ Statements
Competing Interests
The authors declare no conflict of interest.

REFERENCES
34. Moe M. S., Disorders Involving Calcium, Phosphorus, and Magnesium., Prim Care, 2008; 35:2: 215–VI.