



## VARIATION PROFILE OF SERUM CALCIUM IN PATIENTS SUFFERING FROM PULMONARY TUBERCULOSIS

Saad un Nisa Mahesar<sup>1</sup>, Mahvish jabeen Channa<sup>2\*</sup>, Beenish Khanzada<sup>3</sup>, Shagufta jabeen<sup>4</sup>, Jennica Rose<sup>5</sup>, Autif Hussain Mangi<sup>6</sup>, Shah Muhammad Mahesar<sup>7</sup>

<sup>1</sup>M.phil scholar, Institute of Biochemistry University of Sindh Jamshoro

<sup>2\*</sup>Asst. Prof. University of Sindh Jamshoro

<sup>3</sup>Asst. Prof. University of Sindh Jamshoro

<sup>4</sup>Institute of Microbiology University of Sindh Jamshoro

<sup>5</sup>Institute of Biochemistry, University of Sindh Jamshoro

<sup>6</sup>Asst. Prof. University of Sindh Jamshoro Sindh Pakistan

<sup>7</sup>Prof. Dean IBN-E-SINA university Mirpurkhas

**\*Corresponding author:** Mahvish jabeen Channa

\*Institute of Bio-Chemistry, University of Sindh Jamshoro, Pakistan,  
Email: Mahvishj.Channa@Usindh.Edu.Pk

### Abstract

**Background** Pulmonary Tuberculosis is a serious global health issue that has a significant influence on public health systems all over the world.

**Aims and Objectives:** This study investigates the variation profile in serum calcium alterations associated with pulmonary tuberculosis (TB) caused by Mycobacterium in a Pakistani population. Study carried in Institute of Biochemistry University of Sindh Jamshoro in collaboration with Chest Centre disease Kotri, Indus hospital and Civil Hospitals respectively, samples were collected from Pulmonology wards of respective hospitals, from December 2022 to June 2023.

**Methods** A retrospective case control study comprises total 200 participants, randomly selected 100 subjects of pulmonary tuberculosis and 100 age and gender matched healthy non T.B controls were analyzed for demographic characteristics of the patients and determination of calcium profile in PTB patients. Statistical analysis was done on SPSS software.

**Results** The detailed analysis of serum Calcium with Pulmonary Tuberculosis presents as calcium level mean  $\pm$  SD 8.95 + 0.77 than in controls mean  $\pm$  SD 9.49 + 0.81, with a p-value of 0.000, showing results are statically significant.

**Conclusion** we have observed altered biochemical abnormalities such as hypocalcaemia, serum calcium levels were observed significantly low in TB patients as compare to healthy controls. This study may contribute to the development of improved diagnostic and monitoring tools for TB patients, facilitating early detection and effective disease management.

**Key words:** Pulmonary Tuberculosis, Biochemical parameters, serum calcium

### Introduction

This study aims to investigate the effect of mycobacterium on serum calcium in pulmonary tuberculosis. Understanding the changes in biochemical parameter, such as serum calcium, in TB patients is crucial for several reasons. Firstly, it may provide valuable insights into the

pathophysiology of TB, shedding light on the host response to Mycobacterium infection [1]. Pulmonary Tuberculosis is a serious global health issue that has a significant influence on public health systems all over the world. This disease, which is primarily brought on by Mycobacterium tuberculosis [2]. One of the intriguing aspects of this interaction is the impact of Mycobacterium infection on serum calcium levels. Understanding these biochemical changes is essential for elucidating the pathogenesis of pulmonary TB and may have diagnostic and therapeutic implications [3].

### **1.1 Pulmonary Tuberculosis**

Pulmonary Tuberculosis caused by mycobacterium is an infectious disease. Pulmonary Tuberculosis commonly affects the lungs but it can also distresses other parts of the body [4]. Mycobacterium Tuberculosis mainly transmits by inhaling infectious respiratory droplets produced by an active TB patient while they cough, sneeze, or speak [5]. Active Tuberculosis infection usually affects the lungs (in 90% of cases [6]. Symptoms may include, fever, hemoptysis, chest pain, persistent cough producing sputum, dyspnea, weight loss, loss of appetite chills, night sweats, fatigue, nail clubbing and a prolonged cough producing sputum. About 25% remain asymptomatic those people may not have any signs. [7]. Mycobacterium is the causative agent of TB, has a remarkable ability to manipulate host physiological processes for its survival and replication within the host [8]. Mycobacterium commonly known as Koch's bacillus is a kind of pathogenic bacteria belonging the family of Mycobacteriaceae [9]. [10].

### **1.2 Prevalence**

Pulmonary Tuberculosis causes one death out of every ten deaths globally. More than 95% of Tuberculosis deaths take place in low- and middle-income nations. Pakistan, China, India, Nigeria, South Africa, the Philippines, and Indonesia have recorded 64% of the world's Tuberculosis cases [11]. In Pakistan, the TB pandemic is severe. The incidence, prevalence, and mortality rates of TB in Pakistan are now estimated by the WHO to be 230 per 100 000, 310 per 100 000, and 39 per 100 000, respectively. According to this, there are 69,000 TB deaths and 410 000 incident cases in Pakistan per year. Pakistan is ranked seventh worldwide in terms of the high burden of tuberculosis [12].

### **1.3 Diagnosis**

Tuberculosis is identified by finding the presence of Mycobacterium Tuberculosis bacteria in the clinical specimen taken from the patient. There are several other diagnostic techniques which are used to investigate the Mycobacterium tuberculosis but they cannot confirm its presence [13]. A medical history, physical examination, chest X-ray, microbiological examination, gene expert test, and other tests are required for a thorough evaluation for tuberculosis. Moreover, X-rays, surgical biopsies, other scans, and a tuberculin skin test may be performed [14].

### **1.4 Calcium**

Calcium is an essential mineral for various physiological processes in the body, including bone health of bones, contraction of muscles, nerve function, and blood clotting. It plays a crucial role in maintaining the integrity and function of cells and tissues [15]. There is strong relationship between calcium and tuberculosis (TB). Studies have reported that TB is a granulomatous disease caused by Mycobacterium tuberculosis can affect calcium metabolism, leading to changes in serum calcium levels. In pulmonary TB, both hypercalcemia (high levels of calcium) and hypocalcemia (low levels of calcium) may occur [16].

### **Methodology**

The present study was carried out in Institute of biochemistry university of Sindh jamshoro, Pakistan. This study was conducted involving tuberculosis (TB) diagnosed patients from three different Hospitals, namely Chest Diseases centre Kotri, Indus Hospital Tando Muhammad Khan,

and Civil Hospital Hyderabad. The study was carried out in seven months from December 2022 to June 2023. The population of the study comprised 200 participants, 100 T.B patients and 100 healthy Non T.B controls. All subjects provide written informed consent. The study is carried with the approval of Ethical committee of the Institute of Biochemistry, University of Sindh Jamshoro. Cases in this study were individuals diagnosed with Mycobacterium infections by gene expert test. A questionnaire is designed especially for this study documented demographic characteristics of the patients, patient's history and clinical analysis of all the participants. Cases and controls were matched based on relevant demographic variables such as age, gender, and, where appropriate, other variables to ensure that potential confounding variables were controlled for in the analysis. This matching process aimed to enhance the comparability of cases and controls and reduce the potential for bias. Study subjects includes male as well as female between the age group 20 to 80 years. Individuals with Mycobacterium infections having all symptoms of PTB were included in this study. Patients who experienced recent surgery or had any disorder other than T.B. were excluded in the study. Patients with established Kidney diseases, Liver diseases, HIV patients, Pregnant and lactating subjects were also excluded from this study. Patients who participated in this study signed written consent form.

## 2.1 Sample collection

Sputum samples were taken from each individual who had PTB symptoms, apparatus Cepheid Gene Expert System was used to examine the samples [17].

5 milliliters (ml) of venous blood samples were aseptically collected from the study subjects. Using sterile syringes with minimal consistency, blood was drawn by venipuncture and then placed into sample containers that had been chemically cleaned, dried, and labeled with the patient's name, age, and ward bed number. To assess biochemical parameter serum calcium samples were drawn into Vacutainer tubes with yellow top. The serum was separated out on centrifuge machine at about 3000 rpm and transferred to clean and well label vials for further analysis on visible spectrophotometer employing kit method.

## Data Analysis Procedures

The collected data were subjected to comprehensive statistical analysis on (SPSS) software, version 29.3.0 and excel version 2010.

## Determination of calcium

Patients was having 8 hours of fasting, for estimation of serum calcium quantitative methods were used, standard methods are used in this study, Colorimetric methods are commonly used for the quantitative measurement of serum calcium levels [18]. These methods typically involve the formation of a colored complex between calcium ions and a chromogenic reagent, which can be measured spectrophotometric methods. The amount of calcium ions present in the sample directly correlates with the color's intensity, we have used Arsenazo III method or the O-Cresolphthalein Complex one method, are commonly used for the colorimetric estimation of serum calcium levels on Diagnostest KIT method by Diag.M(private) [19].

## Calculation:

$$\frac{\text{Absorbance of sample} \times n}{\text{Absorbance standard}}$$

## Results

In evaluating the demographic profile of patients afflicted with pulmonary tuberculosis, our study encompassed an array of variables. The mean ages of patients and control (**Table.1**) were  $45.63 \pm$

9.87 vs.  $39.63 \pm 13.30$ . Indicating a middle-aged group with moderate age variability in patients group. While controls had a lower mean age with a larger standard deviation suggesting a wider age range among this group compared to the patient cohort. Concerning body weight, the mean of patients vs. control group was found to be  $43.13 \pm 15.05$ , vs.  $67.68 \pm 12.81$ , which points to a wide range of body weights within the subjects studied. The mean weight of the controls was substantially higher, indicating less variability in body weight than observed in the patient group. The height of patients averaged at  $1.65 \pm 0.65$ , reflecting a diverse stature distribution among the participants. The average height of control individuals was notably shorter at  $1.53 \pm 0.74$ , which could be indicative of a broader distribution in height. The Body Mass Index (BMI), an indicator of body fat based on height and weight, had a mean value of  $21.16 \text{ kg/m}^2$  and a standard deviation of 3.02, situating the average patient in the normal weight. The Body Mass Index (BMI) among the controls averaged at a higher  $26.6 \text{ kg/m}^2$  with a smaller standard deviation of 2.4  $\text{kg/m}^2$ , placing the average control individual in the overweight category according to BMI classifications category, according to the World Health Organization's BMI classifications. These demographic details provide a foundational understanding of the physical characteristics of the patients and control group under scrutiny.

**Table 1. Demographic characteristics of patients and control**

VARIABLE	DESCRIPTION	
AGE	CASES (N=100)	CONTROL (N=100)
Mean Age + SD (Years)	45.63 + 9.87	39.63 + 13.30
<b>WEIGHT</b>		
Mean Weight + SD (Kg)	43.13 + 15.05	67.68 + 12.81
<b>HEIGHT</b>		
Mean Height + SD (Meter)	1.65 + 0.65	1.53 + 0.74
<b>BMI</b>		
Mean BMI + SD (KG/m <sup>2</sup> )	21.16 + 3.02	26.6 + 2.4

The lifestyle-related factors among patients with pulmonary tuberculosis were investigated, focusing on their source of drinking water and smoking habits

For the source of drinking water, patients were nearly evenly split between those using surface water and those using underground sources. (Table 2) A slight majority of 56 patients relied on underground water, while 44 patients consumed surface water. This distinction could be indicative of different environmental exposures and socioeconomic conditions within the patient population. In terms of smoking habits, the study revealed a diverse range of tobacco-related behaviors. Out of the patients, only 15 were current smokers, which constitute a relatively small proportion. Notably, a significant number of the patients, 47 in total, were ex-smokers, indicating a past history of smoking that may have contributed to their current health status. Non-smokers amounted to 11 individuals, suggesting limited exposure to direct smoke inhalation. Additionally, 27 patients reported using edible nicotine products, which suggests an alternative mode of tobacco consumption that may also have implications for their health.

**Table 2. Factors related to lifestyle**

VARIABLES	DESCRIPTION
<b>Source Of Drinking Water</b>	
Surface	44
Underground	56
<b>SMOKING HABITS</b>	
Smoker	15

Non-Smoker	11
Ex-Smoker	47
Edible Nicotine Products	27

The clinical presentation of tuberculosis among the 100 patients was characterized by a pattern of signs and symptoms, many of which were experienced concurrently by the individuals. **(Table 3)** Almost all patients, 98 in total, presented with a cough, making it the most common symptom within the cohort. Fever was also a prevalent symptom, reported by 96 patients, suggesting systemic infection typically associated with tuberculosis. Chest pain and dyspnea, symptoms indicative of respiratory distress, were reported by 79 patients each. These symptoms point to the direct impact of the disease on pulmonary function. Weight loss, another common symptom associated with chronic infection and tuberculosis in particular, was observed in 78 patients. This symptom reflects the systemic effects of the disease, such as increased metabolic demand and decreased appetite. Production of sputum was noted in 67 patients, which is a key symptom in tuberculosis, often leading to the diagnosis through sputum analysis. A flu-like syndrome, consisting of symptoms such as fatigue, body aches, and malaise, was experienced by 52 patients. This set of symptoms overlaps with many other conditions, making the diagnosis of tuberculosis more challenging without specific tests. Hemoptysis, or coughing up blood, was present in 50 patients. This alarming symptom can be a sign of severe lung damage and is often associated with advanced stages of pulmonary tuberculosis.

**Table 3. Clinical manifestations among patients**

SIGNS & SYMPTOMS OF TB	FREQUENCY
Cough	98
Fever	96
Chest Pain	79
Dyspnea	79
Weight Loss	78
Sputum	67
Flu	52
Hemoptysis	50

Serum calcium was significantly lower in patients **(Table 3)** mean  $\pm$  SD 8.95 + 0.77 than in controls mean  $\pm$  SD 9.49 + 0.81, with a p-value of 0.000, indicating a meaningful difference that may reflect changes in calcium metabolism due to tuberculosis or its treatment. The T-test results clearly indicate significant differences between patients and controls across all measured biochemical parameters. These differences are likely reflective of the pathophysiological changes and the impact of tuberculosis on the overall health status of the patients.

**Table 3. Calcium profile comparison between cases and control**

VARIABLES	PATIENTS	CONTROLS	P-VALUE
Serum Calcium	8.95 + 0.77	9.49 + 0.81	0.000*

## Discussions

In this study the mean values  $\pm$  SD of serum calcium level in patients 8.95 + 0.77 than in controls mean  $\pm$  SD 9.49 + 0.81. Suggesting serum calcium levels in patients group is significantly low as compared to control group. This findings is related to the studies of Rohini et al. in India ,Serum calcium levels in TB patients were considerably lower than in the follow-up and non-TB control group; these results are consistent with those of Ali-Gombe et al. in Maiduguri and Rohini et al. in India [20]. The granulomatous disease tuberculosis is linked to variations in the metabolism of calcium, including hyper- or hypocalcemia. A lack of vitamin D, poor absorption, and malnutrition have all been linked to hypocalcaemia in pulmonary tuberculosis. Serum calcium levels were substantially higher in the treated follow-up TB group than in the newly diagnosed cases who were

not receiving therapy. Uchenna Chinyelu Ufoaroh et al., and colleagues PAMJ - 38(66). January 20, 2021 [21]. Sales, A.C.d.S., et al., This study relates with present study as Hypocalcemic T.B patients but study showing Narrow information on the prevalence and significance of hypocalcaemia in pulmonary tuberculosis (TB) patients: The article mentions that 88.9% of TB patients in their study had Hypocalcemia. However, there is a lack of comprehensive studies investigating the prevalence and clinical implications of Hypocalcemia in TB patients. Further research is needed to determine the frequency of Hypocalcemia in TB patients and its association with disease severity and treatment outcomes. Further studies are needed to address these gaps and provide a better understanding of the role of hypocalcemia in TB pathogenesis and treatment [22]. Dosumu, E. and J. Momoh et al., this study in contrast related to Hypercalcemia in pulmonary TB has been attributed to factors such as malnutrition, impaired absorption, and vitamin D deficiency. Research studies have found that TB patients, especially those who are newly diagnosed and not yet on treatment, it is rarely symptomatic, hypercalcemia is not unusual in Nigerian patients with recently diagnosed tuberculosis. [23].

### **Conclusion**

Evaluating the findings of current study, a significant decrease in serum calcium level in pulmonary tuberculosis patients have observed. Monitoring serum calcium levels, throughout PTB treatment not only helps to prevent therapy-related complications, but also improves patient adherence to the regimen.

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### **Authors Contribution**

SNM and MJC explored the literature and combined and edited the manuscript and BK, and SJ helped in literature search and review and edited the manuscript and contributes to the literature search. All authors read and approved the final version of the manuscript.

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