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Calcium Metabolic Profile in Various Categories of Pulmonary Tuberculosis Patients

BIOCHEMISTRY

KEYWORDS : Calcium, Vitamin D, Pulmonary Tuberculosis, Parathyroid Hormone.

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ABSTRACT

BACKGROUND: Pulmonary Tuberculosis is a deadly infectious disease caused by acid fast bacilli Mycobacterium Tuberculosis and is responsible for more than 1.5 million deaths every year, hence is a major global health problem specially in developing countries. Interrelationship between Tuberculosis and malnutrition is as old as the disease itself. Disease outcome and mortality in PTB depends on strength of immune system which in turn influenced by the nutritional status of an individual. Calcium, is an essential macromineral is altered in various infection including granulomatous diseases. Vitamin D not only maintains calcium homeostasis but also acts as immunomodulator in cell mediated immunity. Thus, calcium profile constitutes important part of nutritional monitoring in patients with PTB . AIM It deals with study of macronutrient Calcium, Phosphorus Vitamin D and Parathyroid Hormone (PTH) level in various categories of PTB patients . MATERIAL AND METHOD: The study was conducted on 100 adults (both males and females) from low socioeconomic status out of which 40 were newly diagnosed (Group A) , 30 were relapsed PTB cases (Group B) and 30 were healthy age and gender matched non family members of patients that served as controls (Group C) . After an informed consent, all were subjected to Anthropometric measurements and Biochemical investigations i.e Total protein (Biuret Method, Accurex), Albumin (BCG dye binding method, Accurex), Total Calcium (Arsenozo III Method, Accurex), Phosphorus (Phosphomolybdate UV Method, Accurex) and ALP (Kinetic Method), Vitamin D and PTH (Chemiluminescene). RESULT AND DISCUSSION: No significant difference was seen in ages among all the groups. BMI, Total protein and Albumin were significantly ($p<0.05$) low in Pulmonary Tuberculosis (PTB) than controls. Since TB is associated with severe chachexi ,wasting and low albumin, Ionic and corrected TCa was calculated and were significantly ($p<0.05$) higher in cases than control. Prevalence of Vitamin D deficiency (VDD) was maximum group A, followed by Group B and C . This was partly due to utilization of calcitriol in antimicrobial activity of macrophages in CMI response and partly due to TB associated undernutrition of selected population. CONCLUSION: The study concluded exsistence of hypercalcemia in PTB cases (both newly diagnosed and relapsed) causing clinical manifestation in some. High incidence of Vitamin D and PTH deficiency is found in PTB that compromised Cell mediated immunity and increases susceptibility to infection and possibility of relapse. This further support immunomodulator rple of Vitamin D.

INTRODUCTION

Pulmonary Tuberculosis (PTB) is a deadly infectious disease caused by different strains of Mycobacterium Tuberculosis (MTB) that usually affects apices of lungs. One-third of the world's population is thought to have been infected with MTB with new infection occurring in about 1 % of the population each year (1) ; making PTB a global health issue. The disease is called by some, "The Mother of Diseases" and is as much a social disease as an infectious disease. TB is associated with poverty, overcrowding, alcoholism, stress, drug addiction and malnutrition found commonly in developing countries like India (2). India is the highest TB burden country with WHO statistic for 2011 giving an estimated figure of 2.2 million cases in India out of a global incidence of 8.7 million cases (2). Although the causative agent is MTB, among various risk factors for TB, undernutrition is the most significant accounting for the highest population attributable risk (PAR) for TB in India (3). On one hand , malnourished are highly vulnerable to TB due to reduced immune strength, on the other hand, TB can lead to or worsen preexisting undernutrition by decreasing appetite and by increasing catabolism (3). It is a known that both TB and malnutrition are synergistically associated to each other . Among various methods of nutritional assessment, apart from anthropometric , macronutrient level in blood is important indicator of nutritional status of an individuals. Calcium and Phosphorus are essential macronutrients whose level is altered to a significant extent in various diseased states causing clinical manifestation.

Calcium is an essential macro mineral, the 5th most common metallic element found in three body compartments: The skeleton, soft tissue and the Extra Cellular fluid (ECF) and is altered in various granulomatous disease and simultaneously causing a spectrum of clinical features (4). Phosphorus is an important and widely distributed element of the body present both extracellularly and intracellularly. An acute decline in serum phosphate level may result in rapid complication and altered RBC function specially in immune compromised individuals (5). Since MTB infection is associated with compromised cell mediated immunity, assessment of Ca and Phosphorus (P) status will provide additional information for effective management of the disease. Calcium homeostasis is affected by ALP, vitamin D and PTH level. Serum Alkaline phosphatase (ALP) comprises of group of enzymes that catalyses hydrolysis of phosphate esters in alkaline medium generating organic radical and inorganic phosphate (6). It is a widely distributed membrane enzyme with highest concentration in liver and bones. Evaluation of serum ALP in degenerative disease like PTB will not only provide information on liver dissemination of Tuberculosis but also will help in understanding the systemic manifestation of the disease.

Vitamin D not only is a Calcium regulating hormone but also has profound effect on human immunity. It acts as an immunomodulator , prevents excessive expression of inflammatory cytokines and increases oxidative burst potential of macrophages thereby enhancing bacterial killing (7). Consumption of vitamin D during immune response can lead to Vitamin D deficiency (VDD). On the other hand, VDD can compromise cell mediated immunity and lowers antibacterial activity of macrophages

which reduces immune strength reflecting that VDD increases risk of TB. Such association between hypovitaminosis D and PTB varies widely among countries due to socio-economic status, demographic variations, vitamin D and Ca intake in diet, exposure to sunlight , extent of disease severity and criteria of Vitamin D deficiency (8) Serum 25(OH)D is used to assess vitamin D status as it is the main circulatory form. Level of 25(OH) D more than 30ng/ml is an ideal sufficient level. Level between 20-30 ng/ml is considered as insufficiency while level less than 20 ng/ml as deficient. The above criteria was used to define VDD in this study.

Parathyroid hormone (PTH) , secreted by two pairs of parathyroid gland closely associated with thyroid gland. is under the negative feedback regulation of serum calcium. Its prime function is to elevate serum calcium level by causing decalcification and demineralization of bones and by increasing Ca reabsorption by renal tubules. The latter is the most rapid action of PTH. Assessment of calcium and its metabolites will not only help in effective nutritional monitoring of PTB patients but will provide insight in understanding the role of Vitamin D and PTH in disease process.

AIM

The present study aims to determine the level of vitamin D in newly diagnosed and relapse cases of Pulmonary Tuberculosis. Another objective is to characterize changes in Calcium-PTH-Vitamin D axis in various categories of PTB patients and compare them with healthy controls.

MATERIAL AND METHOD:

The study was conducted in the Institute of Respiratory Diseases (IRD) in association with Department of Biochemistry, SMS Medical College, Jaipur. 100 Adult patients (both male and female) from low socio-economic status diagnosed with Pulmonary Tuberculosis (PTB) of which 40(M=30;F=10) were newly diagnosed (GroupA) and 30 (M=20;F=10) relapse cases (Group B) were recruited for the study. 30 (M=22; F=8) healthy, age matched individuals tested free of MTB without any previous or present symptoms of Tuberculosis or any other pulmonary disease and non family member of patients served as Controls (Group C). Following diagnostic criteria was used for PTB 1. Positive culture for MTB. 2. Positive (2 consecutive samples) smear for AFB. 3. Typical chest X-ray showing bilateral upper zone involvement with or without cavitations & with/without +ve sputum smear but with typical PT symptoms. 4. Patients with 2 or 3 criteria had to show clinical and radiological improvements with anti tuberculosis therapy. Relapse cases are patients who have completed treatment and declared cured, however they came up with typical PTB symptoms with +AFB smear or radiological detoriation. Patients suffering from drug resistant TB (MDR), extrapulmonary TB, those with significant renal, cardiac, neoplasm or respiratory disease (other than PTB like lung cancer) etc., diabetes, endocrine or genetic disorder were excluded from the study. HIV positive cases, Pregnant or lactating women and those on oral nutritional supplements were also excluded. None of the patients or control subjects were on calcium supplements. All subjects gave their written consent to participate in the study.

Sample collection and bacteriological examination

Two consecutive sputum sample of each patient were collected and subjected to Acid fast Staining. In order to determine Smear Positivity index, number of Acid Fast Bacilli (AFB) were counted and analysed as follows:1. No AFB in 100 fields-negative; 1-9 AFB in 100 fields-scanty ; 10-99 AFB in 100 fields- +1 ; 1-10 AFB per field-+2 and more than 10 AFB per field- +3.

Measurement of biochemical parameters

After an overnight fast (12 hrs), venous blood was drawn from anticubital vein of each subject by using aseptic technique in plain vial.Total protein (Biuret Method, Accurex), Albumin (BCG dye binding method, Accurex), Total Calcium (Arsenozo III Method, Accurex), Phosphorus (Phosphomolybdate UV Method, Accurex) and ALP (Kinetic Method) were estimated routinely on Clinical Chemistry RANDOX , IMOLA 3 Autoanalyzer. Vitamin D and PTH were estimated by Chemiluminescent method on Adviacentuar XP (Siemann, Germany) immunoassay system and on immulite respectively.

Statistical Analysis :

Quantitative data were expressed as mean ± SD. Comparison was made using student-t test (independent sample t-test). P value less than 0.05 was considered significant. Correlation between various parameters was studied by Pearson Correlation.

Results and Discussion

Tuberculosis is commomnly a disease of lungs where it forms localized infection after inhalation. Almost one third of the world's population is infected with *Mycobacterium tuberculosis* and the majority of these individuals live in less developed countries (Swaminathan et al., 2008).Therefore, despite recent progress, TB remains an important global public health problem (WHO, 2009). Nutrition and infection interact with each other synergistically . Recurrent infection leads to loss of body nitrogen and worsen nutritional status and malnutrition produces greater susceptibility to infection. The study deals with status of Calcium and its metabolites in Tuberculosis infection.

General characterstics of study populations is depicted in **Table 1.** Newly diagnosed PTB cases (Group A) studied were 40 out of which 75% were males and 25% were females and in Group B i.e. relapsed cases, 66.67% were males and 33.33% females. Patients group was compared with healthy controls that were 30 in number (22 males and 8 females) and constitute Group C. Difference was non significant with regard to male female ratio among all groups. Average age of Group A (Newly diagnosed) was 48.53± 19.58 years , Group B (relapse cases) was 43.07±18.7 and controls were of 45.13± 14.08 years. No significant difference was seen in the ages among all groups. Mean Body Mass Index (BMI) of Pulmonary Tuberculosis patients (both Group A and Group B) was 14.6± 2.2 and 17.1± 2.8 Kg/m^2 respectively and was significantly lower than controls i.e 26.8± 3.0 ($p<0.001$). Nutrition is the most important factor affecting susceptibility to any infection and disease outcome. BMI is much better indicator of nutritional status than weight alone because it takes height into account (9). It has been well documented that PTB is associated with severe chachexia, weight loss and generalized weakness. All these factors beside low economic status of our study population causing nutritional depletion are all responsible for low BMI in cases than in controls. Significant difference was observed in smoking and alcoholic habits of Group A and Group B patients when compared to controls ($p<0.001$). As seen in **table 1**, Nonsignificant difference was seen among all the Groups with regard to their smoking and alcoholic habits ($p<0.001$). Since smoking adversely affects lung function and reduces its vital capacity, cases of PTB disease and relapse are more common in smokers than non-smokers. Significant difference was seen with regard to the locality of study population. 87.5% of Group A and 76.67% of Group B patients belong to rural areas. Only 33.3% of control subjects belonged to rural areas.

Table 2 indicates the mean level of all biochemical parameters and Calcium metabolic profile of all Groups. The present study shows significantly low level of serum Proteins and Albumin (6.679± 0.53 g/l and 3.076±0.64 g/l respectively) in Newly Diagnosed PTB patients than that of controls (7.908± 0.75 g/l and 4.523± 0.569 g/l respectively) ($p<0.001$). Mean serum Protein and Albumin level in Relapse cases were 7.363 ± 0.89 and 4.020 ± 1.03 g/l respectively which were significantly less than controls.

Difference in Total protein and albumin level between Group A and Group B was not significant. Reduced Total Protein found in our study might be due to pre-exsisting undernutrition, malabsorption in TB infection, increased catabolism and anorexia in PTB cases. Further, low albumin and high globulin level are known complications of PTB observed in various studies (10). Albumin is an important component of plasma antioxidant activity and is a negative acute phase protein which decreases during any inflammatory condition, injury or in stress as a result of increased metabolic need for tissue repair and free radical utilization (11). Further leakage of albumin through vascular endothelium and reduced hepatic synthesis is are the factors combined with undernutrition and loss of appetite associated with PTB results in low albumin level in patients (both newly diagnosed and relapse) than in controls (12).

Mean Total Ca in Group A, Group B and Controls was 8.640±0.882 , 8.100±0.507 and 8.154 ± 0.54 mg/dl respectively. Regarding total Calcium, no significant difference was seen among all the groups. when adjusted for albumin concentration, significant hypercalcemia was present in PTB patients, Corrected Total Ca (TCa) for group A and Group B were 8.869 ± 0.988 mg/dl and 7.662 ± 0.938 mg/dl respectively which were significantly higher than controls (7.100± 0.457 mg/dl) ($p < 0.001$). Level of ionic calcium in group 1 and Group 2 were 4.729± 0.52 and 4.149± 0.441 mg/dl respectively. It is known that a component of TCa is complexed with either albumin (40%) or Globulins (8%); however, only the free or ionized form is physiologically active. If serum Ca is reduced as often occurs with anorexia and catabolic wasting that accompany TB, then TCa may be normal although ionized hypercalcemia and its resultant symptoms are present. It is therefore necessary to estimate Ca corrected according to serum albumin level. Studies from India and U S that did not use correction for Hypoalbuminia indicated that 16-28 % of TB patients may develop hypercalcemia. In contrast, studies using albumin adjusted Ca concentration have reported higher prevalence rates (13). PTB is a cell mediated immune response in which requirement of vitamin D is enhanced. The latter enhances synthesis of antimicrobial peptides and increases phagocytic activity of macrophages. Studies have shown elevated 1α-hydroxylase activity in monocytes from patients with active TB (14). This enzyme converts 25-OH Cholecalciferol into calcitriol by 1α- hydroxylation i.e the active form of Vitamin D. The activity of enzyme is enhanced during acute phase of the disease, however if produced in large quantity it spills into circulation causing hypercalcemia.

Present study showed nonsignificant difference in serum phosphorus in TB patients (both newly diagnosed and relapse) and controls ($p>0.05$). Mean serum Phosphate levels in all 3 groups were 4.632 ± 1.21, 4.560 ± 0.671 and 4.360 ± 0.542 mg/dl respectively. Phosphorus is present in all soft tissue and muscles specially at its membrane. Since PTB is associated with muscle wasting, there may be slightly high phosphorus level in patients group than controls (Table 2.) Further Phosphorus tends to be higher in those with elevated polymorphs suggesting its association with active phase of disease and tissue destruction.

Mean serum ALP level in newly diagnosed PTB patients was 261.4 ±105.5 U/L which was significantly higher than relapsed and control cases i.e 190.2 ± 57.8 and 185.61 ±31.8 U/L respectively. A significantly ($p<0.05$) high serum ALP is found in PTB patients (both newly diagnosed and relapse) suggest that though the selected population have PTB, some may have disease affecting liver causing disorganized liver function. ALP is non specific enzyme raised in critically ill and hospitalized patients. 52.5% of Group A individuals and 50% of Group B patients were alcoholic that may result in abnormal Liver Function Test. TB though confined to lung may have disseminated to other parts of the body particularly to liver causing misdiagnosis resulting in high ALP concentration in PTB patients. Further, drug induced hepatotoxicity during Anti-Tuberculosis Therapy (ATT) have been documented in various studies (15). No significant difference was seen with regard to Serum Glutamate Oxaloacetate transaminase and Serum Glutamate Pyruvate transaminase levels among the groups **(Table 2.)**. Non of the patient suffered from any serious liver disease. Probably, selection of patients from low socio economic population, poor nutrition, old age, alcohol intake, intestinal parasitism and ATT induced hepatotoxicity are factors that cause significantly high ALP in our PTB patients than controls. Thus, ALP should be employed as routine test for monitoring PTB patients to assess liver metastasis in TB and their drug regime can be properly designed.

The present study demonstrated high incidence of Vitamin D Deficiency (VDD) in PTB cases (both newly diagnosed and relapse) as compared to normal controls **(Table 2)**. Vitamin D level in group A and B was 16.17±7.2 and 23.83± 11.0 ng/ml respectively which was significantly less than controls i.e. 30.023 ± 6.70 ng/ml. **Table 3** demonstrated prevalence of VDD in all the three groups. 95% of group A patients suffered from severe deficiency with Vitamin D level less than 20 ng/ml, which was 66.6% in group B and non of controls had severe VDD. Vitamin D insuffiency was seen in 5% of group A patients, 6.67% of group B and 43.3% of groupC individuals. 26.67% of group B and 56.6% of group C cases had vitamin D suffiency ie > 30 ng/ml. Non of the group A patients had Vitamin D sufficiency. Non of the studied subjects had vitamin D toxicity. Various Studies have attributed low Vitamin D level in PTB cases, however variation occurs due to sun exposure, calcium and vitamin D intake in diet, socioeconomic factors, prevalence of PTB disease and difference in criteria for defining VDD among countries. For instance, in a study conducted in Greenland (16), a country with less sunlight, low vitamin D level increases susceptibility to TB. Once an individual develops active TB, impaired apettite and confinement to indoor life contribute to low 25(OH)D concentration through reduced Vitamin D synthesis in skin and reduced intake. Further supplementation with vitamin D to normalize serum 25(OH) D causes 29 % reduction in number of TB cases (16). A recent prospective study in Finnish men found that serum 25(OH)D was associated with increased risk of respiratory tract infection and that VDD in TB is a determinant of treatment outcome and co-morbidities (17). Low Vitamin D level causes 5 fold increase in risk for progression to TB and also increases probability of conversion from latent TB to active form by compromising cell mediated immunity (18,19). Antimicrobial role of Vitamin D was established long back when during the pre-antibiotic era cod liver oil (rich in Vitamin D) and sun exposure were the methods used to cure active TB . It improves immunological capacity of macrophages by inducing production of an endogenous antimicrobial peptide Cathelicidin (LL-37), effective against AFB mycobacteria . It involves enhanced consumption of 25(OH)D , the main circulatory form and is an indicator of body's vitamin D status. In addition, any infection may influence the time spend in sun that reduces vitamin D synthesis in the skin. VDD in our population is multifactorial in origin. Dietary deficiency due to low economic status of the cases selected, loss of apettite, malabsorption and reduced sunlight exposure are the factors that contribute to VDD. The most important explanation of VDD in MTB infection is increased utilization of body's Vitamin D stores for effective macrophage and neutrophil function as a part of cell mediated immune response.

Similar to Vitamin D, Parathyroid Hormone (PTH) level was significantly ($p<0.001$) low in both group A and B patients (27.8± 15.7, 38.6 ± 16.3 pg/ml respectively) than controls (42.0± 7.76 pg/ml). It has been found that both PTH and Vitamin D influence cellular immunity (20). Low level of PTH can be explained by poor nutrient status of study population and negative feebback effect of ionized calcium on PTH secretion. Further in-

creased active form of Vitamin i.e. calcitriol level in acute phase of the disease causes direct suppressive effect on PTH synthesis and secretion.

CONCLUSION

The present study shows significant hypercalcemia and slight hyperphosphatemia in both newly diagnosed and relapsed PTB cases that requires clinical attention. Serum ionized calcium i.e the physiologically active form of Calcium is increased in PTB. Higher incidence of Vitamin D and PTH deficiency is found in both Newly Diagnosed and Relapse PTB patients. Vitamin D deficiency occurs commonly in TB disease affecting immunological capacity of individuals, hence this parameter should be monitored during nutritional assessment of the patients. Improvement in Nutritional status of TB patients will help reduce overall mortality, improve survival rate and prevent cases of relapse.

TABLES TO BE INSERTED IN THE TEXT

Table 1. General characterstics of Study subjects:

Characte tics	Group A (Newly Diagnosed cases)	Group B (Relapsed cases)	Group C (Controls)	P - value
No. of Cases	40	30	30	
No. of males	30 (75)	20 (66.67)	22 (73.33)	0.063 NS
No. of females	10 (25)	10 (33.33)	8 (26.66)	
Average Age (mean±SD) in years	48.53 ± 19.58	43.07± 18.7	45.13 ± 14.08	o.441 NS
Average BMI (mean±SD) kg/m^2	14.6± 2.2	17.1± 2.8	26.8 ± 3.01	< 0.001 S
SPUTUM STATUS				
Negative	10 (25)	15 (50.0)	30 (100)	< 0.001 S
+1	10 (25)	11 (36.67)	0 (0.00)	
+2	13 (32.5)	3 (10.00)	0 (0.00)	
+3	7 (17.5)	1 (3.33)	0 (0.00)	
Smoking status				
No. smoking	32 (80.0)	20 (66.67)	8(26.66)	< 0.001 S
No. Non-smoking	8 (20.0)	10 (33.33)	22 (73.33)	
Alcohol Intake				
No. of Alcoholics	21 (52.57)	15 (50.00)	3 (10)	< 0.001 S
No. Non Alcoholics	19 (47.5)	15 (50.00)	27 (90)	
No. of Rural cases	35 (87.5)	23 (76.67)	10 (33.33)	< 0.001 S
No. of Urban cases	5 (12.5)	7 (23.33)	20 (66.67)	

Numbers in parenthesis are percentage

Table 2 : Biochemical parameters and Calcium metabolic profile of study population

S. No.	Biochemical Parameter	Group A (Newly diagnosed)	Group B (Relapsed)	Group C (Control)	Significance (P VALUE)		
					I vs III	II vs III	I vs II
1	Total Protein (g/l)	6.679± 0.53	7.363 ± 0.89	7.908 ± 0.75	S	NS	S
2	Albumin (g/l)	3.076 ± 0.64	4.020 ± 1.03	4.523 ± 0.56	S	NS	S
3.	SGOT (U/L)	38.76± 14.6	36.00± 18.7	28.31 ± 13.0	NS	NS	NS
4.	SGPT (U/L)	22.3 ± 10.1	27.14 ± 15.5	25.36 ± 14.4	NS	NS	NS
3	Serum Phosphate (mg/dl)	4.632 ± 1.21	4.560 ± 0.671	4.360 ± 0.54	NS	NS	NS
4	Total Calcium (mg/dl)	8.640± 0.882	8.100 ± 0.51	8.154 ± 0.54	NS	NS	S
5	Ionic Calcium (mg/dl)	4.729 ± 0.52	4.149 ± 0.44	4.028 ± 0.24	NS	NS	S
6	Corrected TCa (mg/dl)	8.869 ± 0.98	7.662 ± 0.93	7.100 ± 0.45	S	S	S
7	Alk. Phosphatase (U/L)	261.4 ±105.5	190.2 ± 57.0	185.6 ± 31.8	S	NS	S
8	Vitamin D (ng/ml)	16.1 ± 7.2	23.8 ± 11.0	30.02 ± 6.7	S	NS	S
9.	Parathyroid Hormone (pg/ml)	27.8 ± 15.7	38.6 ± 16.3	42.0 ±7.7	S	S	S

Values are mean±SD; S - Significant; NS - Non Significant

Table 3: Prevalance of Vitamin D deficiency (VDD) among all the groups:

S. No.	Vitamin D status	Group A (Newly diagnosed)	Group B (Relapsed)	Group C (Control)
	No. of subjects with	N = 40	N = 30	N = 30
1.	VDD (< 20 ng/ml)	38 (95)	20 (66.6)	0 (0.00)
2.	Vitamin D insufficiency (20-30 ng/ml)	2 (5)	2 (6.67)	17 (43.33)
3.	Vitamin D sufficiency (> 30 ng/ml)	0	8 (26.67)	13 (56.67)
4.	Vitamin D toxicity (> 100 ng/ml)	0	0 (0)	0 (0)

Values are numbers and values in parenthesis are percent.

Chi-square= 47.468 with 4 degree of freedom ; p < 0.001

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