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Short Communication

Total Serum Calcium and Inorganic Phosphate Levels in Tuberculosis Patients in Benin City, Nigeria

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ABSTRACT

Pulmonary tuberculosis (PTB) is still a very common cause of morbidity and mortality around the globe and the disorder of calcium and inorganic phosphate metabolism has been poorly associated with the infection. This study was aimed at assessing the total serum calcium and inorganic phosphate levels in PTB patients in Benin City, Edo State, Nigeria. Blood samples from seventy (70) PTB patients and twenty five (25) apparently healthy individuals were analysed for total serum calcium and inorganic phosphate using conventional colorimetric techniques. The result showed that there was a significant increase (P<0.05) in the mean serum calcium levels of tuberculosis patients (9.4±0.9mg/dl) when compared to control group (8.6±5.8mg/dl). Similarly, there was a decreased mean level of serum calcium (9.06±0.96mg/dl) among newly diagnosed tuberculosis patients when compared to tuberculosis patients on treatment (10.0±0.8mg/dl). However, there was no significant statistical difference (P >0.05) in the inorganic phosphate level between tuberculosis patients and control group as well as in newly diagnosed tuberculosis patients, when compared to tuberculosis patients on treatment. Thus, the study confirms that hypercalcemia is associated with tuberculosis infection.

Keywords: Inorganic phosphate, Serum calcium, Nigeria, Tuberculosis

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INTRODUCTION

Tuberculosis is a chronic or acute bacterial infection that primarily attacks the lungs, but which may also affect the kidneys, bones, lymph nodes and brain (Daniel, 2006). Symptoms of tuberculosis include coughing, chest pain, shortness of breath, loss of appetite, weight loss, fever, chills and fatigue (Moreira *et al.,* 1993). It is a disease with deep social and economical roots (Dye *et al.,* 1999) and has been attributed to poverty, malnutrition and poor environment (Jemikalajah and Okogun, 2009). Low-income people with large families, living in dense urban communities and deficient housing conditions have a high probability of becoming infected, developing active disease and dying from it (American Thoracic Society, 2000). Also, the risk of becoming infected and ill with TB is higher among people that live in congregated institutions such as prisons, youth correctional facilities, nursing homes for elderly people, social shelters, day nurseries and schools (Castelo-Filho *et al.,* 2004). Today, PTB is adjudged as one of the most important diseases in the world with an estimated global prevalence rate of 14.4 million cases and 9.2 million new cases (139 per 100,000 population). Indian, China, Indonesia, South Africa and Nigeria ranked first to fifth respectively (WHO, 2008).

Calcium, an important constituent of bones and teeth, is the fifth most common metallic element in the body and highly essential for normal body development. It is found in three body compartments: the skeleton, soft tissues and extracellular fluid.

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While soft tissues and extracellular fluid contain about 1% of the body's calcium, the skeleton contains 99% of the body's calcium (Lippinocott and Bilezikian, 2004). Tuberculosis is one of the granulomatous diseases that change the status of plasma calcium concentration (Burtis and Ashwood, 2001). In pulmonary tuberculosis, there is abnormal functioning of parathyroid gland to produce more PTH, leading to hypercalcaemia of free calcium. Very low albumin in the system may also results in hypocalcaemia of plasma-bound calcium. However, the increase in free calcium in tuberculosis is not as marked as other disease; it disappears as the tuberculosis is arrested (Abassi *et al.,* 1979). This has also been correlated with vitamin D intake (Anon Zoonotic Tuberculosis and Food Safety, 2003).

Inorganic phosphate is an important and widely distributed element in the human body. The skeleton is the major reservoir, providing phosphate for both extracellular and intracellular pools. Extracellular phosphate maintains the critical intracellular concentration and provides substrates for bone mineralisation (Burtis and Ashwood, 2001). An acute decline in the serum phosphate concentration may result in rapid complications and altered red blood cell function especially in the immune-compromised. In spite of the fact that PTB is a very common disease in Nigeria, there has not been any documented report on the level of calcium and inorganic phosphate in the serum of tuberculosis patients particularly in Edo state. Since the majority of the PTB patients generally present with muscle wasting and weakness, this present study was designed to access the changes in total serum calcium and inorganic phosphate levels in clinically ill PTB patients in Edo State, Nigeria. The data generated is hoped to provide additional information towards effective management of the disease.

MATERIALS AND METHODS

Study Population

A total of ninety-five (95) individuals selected by random sampling, comprising of 3 groups were studied. Group 1 comprises of 30 newly diagnosed subjects who tested positive for the presence of acid fast bacilli (AFB) in their sputum samples and had not commence any form of chemotherapy (anti-TB drugs). Group 2 comprises of 40 subjects who had been diagnosed of tuberculosis and were currently on anti-TB drugs such as rifampicin, isonizid and pyrazinamide for up to three months after the commencement of the therapy. Group 3 served as control group and composed of 25 subjects who had no form of tuberculosis or had received any previous anti-TB chemotherapy and were classified as apparently healthy individuals.

Collection of Specimen

Five milliliters of venous blood was collected from all subjects into plain (non-anticoagulant) container. The blood samples were allowed to clot. The clotted samples were then centrifuged at 2,500g for 10mins and the serum was immediately separated and transfer into another clean plain tube. The samples were stored at refrigerated temperature of 2-8^{o}C prior to analysis.

Determination of Serum Calcium

Serum calcium was determined as described by Appleton *et al.* (1959) using randox diagnostic laboratory kits. Calcium in the sample reacts with α-cresophthalein at alkaline pH. The coloured complex formed is proportional to the amount of calcium present in the sample.

Determination of Serum Inorganic Phosphate

Following the method described by Fraser *et al.* (1987), serum inorganic phosphate was determined using randox diagnostic laboratory kits. The phosphate ions present in the sample reacts with ammonium molybdate in the presence of sulphuric acid to form phosphomolybdate complex. The complex formed was measured at 340nm wavelength.

Statistical Analysis

Data generated from this study were analysed using statistical formula means, standard deviation, *t*-distribution at 95% confident limits using Graph Pad Prism version 5.

RESULTS

Table 1: Mean ± SD of Serum Calcium and Inorganic Phosphate in the Study Population

Parameters	Test (n=70)	Control (n=25)	*t*-value	*p*-value
Total calcium (mg/dl)	9.4 ± 0.9	8.61 ± 0.62	5.0	*P*<0.05
Inorganic phosphate (mg/dl)	3.0 ± 0.3	3.4 ± 0.42	4.4	*P*<0.05

The result shows that the mean+SD of total serum calcium (9.4 ± 0.9mg/dl) for tuberculosis patients was significantly higher (*P*<0.05) when compared to control groups (8.61 ± 0.62mg/dl). There was significantly lower level (*P*<0.05) in the mean+SD of inorganic phosphate (3.0 ± 0.3mg/dl) for tuberculosis subjects when compared to control groups (3.4 ± 0.42mg/dl) (Table 1).

There was significantly higher level (*P*<0.05) in the mean+SD of total serum calcium(10.0 + 0.8mg/dl) for tuberculosis subjects on treatment when compared to newly diagnosed patients (9.06 ± 0.96mg/dl). The mean+SD of inorganic phosphate (3.0 + 0.3mg/dl) for tuberculosis subjects on treatment was significantly lower (*P*>0.05) when compared to the newly diagnosed cases (3.03 ± 0.27mg/dl).

Table 2: Mean ± SD of Newly Diagnosed TB Patients and those on Treatment

Parameters	Newly diagnosed (n=30)	TB on treatment (n=40)	t-value	*p*-value
Total calcium (mg/dl)	9.06 ± 0.96	10.0 ± 0.8	4.3	*P*<0.05
Inorganic phosphate (mg/dl)	3.03 ± 0.27	3.0 ± 0.3	1.5	*P*>0.05

DISCUSSION

In this study, a significant increase in total serum calcium in PTB when compared to control subjects was observed. This may be due to increased influx of calcium into the extracellular fluid compartment from the skeleton, intestine or kidney which occurs in tuberculosis following dehydration and hyper-albuminaemia as earlier reported by Burtis and Ashwood (2001). In addition, abnormal functioning of parathyroid gland is known to produce more parathyroid hormone. This results in increased concentration of calcitonin leading to hypercalcaemia of free calcium. This might have resulted in very low albumin (hypoalbuminaemia) in the system resulting in hypocalcaemia and substantiating the findings of some investigators that in PTB, there is low plasma albumin; low binding capacity to calcium, and increased ionised concentration of calcium which in turn lower the serum total calcium level (Abassi *et al*., 1979; Fraser *et al*., 1987; Rich *et al*., 2005). The total serum calcium of patients on treatment increased significantly compared to the newly diagnosed cases. This corroborates the earlier reports of Abbasi *et al*. (1979) and Anon (2003) that marked increase in free calcium in PTB disappears as the tuberculosis is arrested during treatment.

Previously, Jemikalajah and Okogun (2009) suggested that these conditions may have resulted from malnutrition, the socio-economic status, and polluted environment which predispose the inhabitants to PTB infection. Although factors such as vitamin D and protein deficiencies were not assessed in this study group, the participants were of poor socio-economic status and feasibly malnourished. However, there was significantly low level of inorganic phosphate in the PTB patients when compared to control subjects. This finding agrees with Burtis and Ashwood (2001) that in rapid complications such as in PTB, rhybdomylysis and other diseases, there is acute decline in the serum phosphate level. This may be due to the fact that the extracellular phosphate that maintains the critical intracellular concentration and provides substrates for bone mineralisation were lost as a result of muscle wasting in PTB infection.

The inorganic phosphate level of patients that are newly diagnosed was observed to be higher compared to control level. This may be attributed to the presence of inorganic phosphate in virtually all soft tissue and muscle especially at the membrane since pulmonary tuberculosis is clinically associated with muscle wasting (Burtis and Ashwood, 2001). There was significantly higher inorganic phosphate level in the newly diagnosed PTB patients than in those on treatment. This agrees with the findings of Knochei (1986) that hyperphosphataemia occurs in acute and chronic diseases including PTB. It also proves that PTB patients on treatment in this study were responding to therapeutics.

The main limitation of this study however is that serum albumin level of the patients was not documented. Since, in PTB patients, there is malnutrition and hypoalbuminaemia which tends to decrease the levels of calcium despite "increase" in its value, further studies with a higher number of participants is being designed to evaluate the concentration of total calcium in patients with PTB in order to allow meaningful interpretation of the results and correct the level of albumin in the blood. In conclusion, this study has

shown that there was a significant change in total serum calcium and inorganic phosphate levels of subjects studied especially hypocalcaemia and hypophosphotaemia which is common in PTB. We therefore recommend that physicians must maintain a high index of suspicion for correction of these abnormalities.

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