Assessment of Serum Calcium, Phosphorus, C-reactive protein and Procalcitonin in Tuberculosis Patients

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Abstract

Introduction: Procalcitonin (PCT), a 116 amino acid is the prohormone precursor of calcitonin, is expressed primarily in C-cells of the thyroid gland and to a smaller extent in neuroendocrine tissue of other organs, such as lungs and intestines. PCT is a marker of inflammatory response to infection. C-reactive protein (CRP) is a widely used marker for diagnosis of infections.

Objective: The purpose of our study was to assess the levels of serum PCT, CRP, calcium and phosphorus in patients with pulmonary tuberculosis.

Method: Forty patients diagnosed with tuberculosis were recruited from The Institute of Thoracic Medicine, Chennai, India and compared with age- and sex-matched healthy volunteers (n=40). Serum procalcitonin concentration was analyzed using semi-quantitative PCT-Q kit (B.R.A.H.M.S Diagnostica GmBH, Germany). According to standard procedures, CRP level was determined by immunoturbidimetic assay, calcium and phosphorus by photometric Arsenazo III and phosphomolybdate methods respectively.

Results: Levels of PCT in 27 out of 40 healthy volunteers was <0.5 ng/dl, 3 showing >2 ng/dl. However, 15 out of 40 patients had PCT >2 ng/dl and 25 had PCT>10 ng/dl. CRP levels was significantly elevated in TB patients ($p<0.001$); Calcium and phosphorus were significantly decreased ($p<0.001$and $p<0.01$ respectively) when compared to healthy volunteers.

Conclusion: Several studies have reported that PCT values were not significantly elevated in TB patients, the contrary results in this study calls for further research on PCT in TB based on the differences in severity of the disease.

Key words: Procalcitonin, CRP, Tuberculosis, Calcium, Phosphorus, Infection

Introduction

Procalcitonin (PCT), a propeptide of the hormone calcitonin, is a novel marker of the inflammatory response to infection. It has been used to discriminate between infectious and non-infectious causes of inflammation, and as a marker of severe sepsis in the intensive care unit.[1] However, there have been contradictory reports by researchers where the utility of PCT in diagnosing pulmonary tuberculosis is concerned. While some studies implicate a poor diagnostic value to PCT in lower respiratory tract infections like pulmonary tuberculosis,[2] some others report that PCT could be a good indicator of inflammation in patients with chronic diseases and in persons exposed to long-lasting infections like tuberculosis.[3]

Researchers have also concluded that PCT is a useful biomarker for discriminating between pulmonary tuberculosis and community acquired pneumonia (CAP) because elevation of PCT in tuberculosis was not as significant as in CAP.[4] It is well known that during host defense, neutrophil-mediated killing of mycobacteria is a Ca2+-dependent process.[5] Malik ZA et al[6] have demonstrated that M. tuberculosis inhibits CR-mediated Ca (2+) signaling in a bid to inhibit phagosome-lysosome fusion and promote intracellular mycobacterial survival. Thus, the contradictory reports of diagnostic utility of PCT could be due to variations in the severity of the disease with respect to host immunity status. Also, measurement of alteration in serum PCT levels in conjunction with that in serum calcium levels could be useful in the diagnosis of pulmonary tuberculosis. This study is aimed to assess the combined diagnostic value of serum PCT and serum calcium in pulmonary tuberculosis patients, in the background of changes in serum CRP which is a well-known acute phase protein as well as a non-specific marker of systemic inflammation and serum phosphate which is known to be closely associated with serum calcium.

Subjects and Methods

The study subjects were recruited from The Institute of Thoracic Medicine, Chennai, India. The study group consisted of 40 patients (25–75 yrs.) diagnosed with pulmonary tuberculosis. The diagnosis of tuberculosis was performed using Ziehl-Neelsen staining

method for Acid-fast Microscopy (AFM)[7] and culture for growth of the organism on Lowensterin-Jensen (LJ) medium.[8] The patients were also tested for radiographic abnormalities. Age and sex matched healthy volunteers (n=40) were included in the study as normal controls for comparison of results. 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.

Blood samples were collected immediately following diagnosis using standard sampling tubes. Ten milliliter of venous blood was drawn and transferred to a centrifuged tube without any anticoagulant. The blood sample was allowed to clot for about one hour and subjected to centrifugation at 3000 × g for 15 min at room temperature and serum was separated. All the experiments were performed within 8 hours of sample collection.

PCT measurements were done using semi-quantitative PCT-Q kit (B.R.A.H.M.S. Diagnostica GmbH, Berlin, Germany) which employs a monoclonal mouse anti-catacalcin antibody conjugated with colloidal gold (tracer) and poly sheep anti-calcitonin antibody (solid phase) to form a sandwich complex with PCT in the test sample. The color intensity of the band thus formed is directly proportional to the PCT concentration in the sample and is expressed as four grades of > 0.5 ng/ml, 0.5-2 ng/ml, 2-10 ng/ml and > 10 ng/ml with the help of the reference card.

CRP level was determined by immunoturbidimetic assay.[9] Calcium was analyzed by photometric test using Arsenazo III end point.[10] and phosphorus was determined by phosphomolybdate method.[10]

Statistical analysis

Statistical analysis was made by the students' T-test. 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

Results of PCT estimation are shown in Figure 1. Serum PCT levels in thirty seven out of forty normal controls had were <0.5 ng/ml and three showed PCT values of > 2 ng/ml. However, PCT levels in fifteen out of forty TB patients were > 2ng/ml and the rest of the patients showed PCT levels > 10 ng/ml, thus indicating that PCT levels served as a useful marker of infection in TB patients.

As shown in Table 1, the increase in serum CRP levels and the decrease in serum calcium

levels was extremely significant ($p<0.001$) in TB patients when compared to normal controls. Serum phosphate levels were significantly lower in TB patients ($p<0.01$) when compared to normal controls. Serum CRP, calcium and phosphate levels of the three normal controls who recorded PCT values > 2 ng/ml, were in the range of values recorded by normal controls (Table 2). These results indicate that measurement of serum PCT in conjunction with serum calcium and phosphate levels could serve as a useful diagnostic tool in tuberculosis.

Discussion

Mycobacterium tuberculosis has been rated as the leading cause of mortality due to an infectious disease.[11] Despite aggressive research conducted on this disease and its mechanism, the question still remains," how to control the disease"? The presence of reliable diagnostic markers is an important factor contributing to the successful treatment of any disease. Serum PCT has been reported as a useful biomarker for diagnosis and prognosis of CAP by several researchers.[1,4,12,13] However, most studies [2,14,15] have reported that serum PCT was not elevated significantly in pulmonary tuberculosis. Baylan et al[16] has reported that PCT level was not a reliable indicator in the diagnosis of active PTB because of its low sensitivity (41.3%), and PCT test for the presumptive diagnosis of PTB cannot be a substitute for microbiological, epidemiological, clinical and radiological data. However, Ozlem K et al[3] report that PCT could be a good indicator of inflammation in tuberculosis. These contradictory reports have made it necessary to understand the biochemical mechanism of elevation of PCT in bacterial infections. Increased efforts in tuberculosis research are being aimed at better defining the interactions between the causative bacterium and the host immune system. Since PCT synthesis and release are determined by the inflammatory cytokine cascade during systemic infection, the intensity depends on the number of organisms entering the systemic circulation.[4] The number of organisms entering circulation in TB patients depends on the severity of the disease which in turn depends on the effectiveness of the host defense. *Mycobacterium tuberculosis* evades the innate antimicrobial defenses of macrophages by inhibiting the maturation of its phagosome to a bactericidal phagolysosome. Phagosome formation triggers a preprogrammed pathway of maturation into the phagolysosome, a process controlled by Ca2+.[17,18] In the present study, the decreased serum calcium levels in tuberculosis patients indicates a decreased availability of calcium for phagolysosome maturation, decreased efficiency of host antimicrobial activity and hence increased severity of the disease. Although there were three healthy controls with PCT > 2 ng/ml, serum calcium levels in them were well within normal range. This indicates that simultaneous measurement of serum calcium could increase the diagnostic utility of PCT in tuberculosis patients.

Conclusion

Although currently available research does not validate the diagnostic utility of PCT in tuberculosis patients, results of the present study indicate that measurement of serum PCT along with serum calcium could prove as a useful diagnostic marker for the disease. The findings imply that it is imperative to crack the underlying mechanism of increase in PCT during bacterial infections (namely- Why bacterial infections induce the PrePCT gene? Is PrePCT preferentially proteolysed to PCT over the proteolysis of PCT to calcitonin, to result in the increased serum PCT and so on) to understand and improve its diagnostic utility.

The present study encourages further research to validate the role of serum PCT-serum calcium combination in differential diagnosis of latent versus active tuberculosis and mild versus severe tuberculosis. It also calls for research at the molecular level on the relative rate of post translational modifications of PrePCT and PCT. This could help us understand the specific situations in which serum PCT is increased/not increased significantly in bacterial infections

Conflicts of interest: None declared

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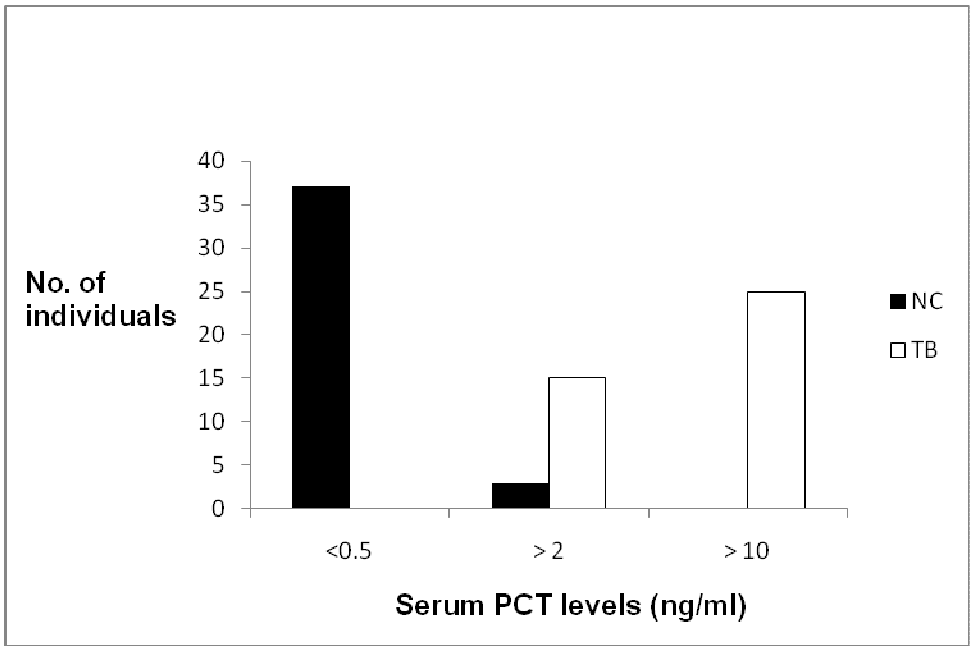

Figure 1: Serum PCT levels (ng/ml) in normal controls, NC and tuberculosis patients, TB (n=40 for both) according to Triage kit grading (PCT values <0.5 ng/ml, > 2 mg/ml and > 10 ng/ml).

Table 1: Levels of serum Procalcitonin, C-reactive protein, Calcium and Phosphorus in normal controls, NC and tuberculosis patients, TB. Values are mean ± SD

Parameters	NC (n = 40)	TB (n = 40)	p value
CRP (mg/dl)	1.33 ± 0.51	19.69 ± 3.93	<0.001
Calcium (mg/dl)	9.34 ± 0.46	7.72 ± 1.02	<0.001
Phosphorus (mg/dl)	3.45 ± 0.46	3.06 ± 0.8	<0.01

Table 2: Comparison of serum C-reactive protein, calcium and phosphorus in normal controls with PCT levels > 2 ng/ml, to the mean ± SD of normal control group (n = 40)

Parameters	Subject 1	Subject 2	Subject 3	NC group
CRP (mg/dl)	1.1	1.1	1.5	1.33 ± 0.51
Calcium (mg/dl)	9.6	9	9.2	9.34 ± 0.46
Phosphorus (mg/dl)	3.5	3.7	3.4	3.45 ± 0.46