

Study of serum TRACP 5b as a sensitive and specific Bone Resorption Marker of Bone Metastases in Prostate Cancer patients in comparison with Bone Scintigraphy

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Abstract— Skeletal metastases are a most common event in prostate cancer patients with advanced cancer disease. Early detection of bone metastases is crucial to initiate successful therapy. Now a days imaging technique such as bone scintigraphy is a frequently used method for detection of bone metastases. Without radiological tools it is difficult to diagnose, treat or follow clinically, bone metastases patients. This study was designed to evaluate the utility of bone resorption markers - serum tartrate-resistant acid phosphatase 5b (TRACP 5b), serum calcium and commonly used bone formation marker such as serum total alkaline phosphatase (ALP), in comparison with whole body skeletal scintigraphy with Technetium99^m MDP, for the diagnosis of bone metastases(BM) in prostate cancer(PC) patients.

Four groups of samples were analysed for this study. Group 1(GP 1) consists normal male (cancer free men), Group 2(GP 2) consists PC patients without BM, Group 3(GP 3) consists PC patients with limited BM and Group 4(GP 4) consists PC patients with extensive BM, conformed by whole body skeletal scintigraphy with Technetium99^m MDP.

One way ANOVA was used to compare serum TRACP 5b, serum ALP and serum calcium among these groups. Serum TRACP 5b is not markedly elevated in limited bone metastases ($p = 0.034$) but is strongly elevated in extensive bone metastases ($P < 0.0001$). Serum ALP is markedly elevated in both limited and extensive bone metastases ($P < 0.0001$). Serum calcium also shows significant additional background values in bone metastases ($P < 0.0001$).

Index Terms—Prostate Cancer (PC), Bone Metastasis (BM), limited bone metastases (Lim.BM), extensive bone metastases (Ext.BM), Scintigraphy, Technetium99^m MDP (methylene diphosphonate), Tartrate-resistant acid phosphatase 5b (TRACP5b), total alkaline phosphatase (ALP).

1. INTRODUCTION

ONE of the most common types of cancer is prostate cancer representing 19% of all diagnosed cancers in the western world in the year 2002 with 679000 new cases [1]. Bone metastases is common in prostate cancer patients, occurring in more than 50-60% of patients with advanced cancer disease [2]. Almost all patients who die of prostate cancer have skeletal involvement [3]. When the primary tumor metastasizes to the bone causing a lesion of high bone remodelling, destroying the bone structure that results in severe bone pain, pathological bone fractures, spinal cord compression, hypocalcaemia and increased mortality [4], [5]. The growth factors and cytokine mediated interactions lead to stimulation of osteoclastic bone resorption and results in both uncoupled and unbalanced bone remodelling [6]. Due to this invasive tumor cells in the bone micro environment, which affect the osteoblasts and osteoclasts by increasing their number, activity and survival of these bone remodelling cells by a phenomenon known as the vicious cycle of metastases [7]. The characteristic feature of prostate cancer is mainly by sclerotic bone lesions especially due to the failure of androgen therapy in men [8]. Early detection of bone metastases is crucial to initiate successful therapy with bisphosphonates, targeting the skeleton in prostate cancer with bone metastases.

Bone scintigraphy is traditionally used method for initial evaluation to detect bone metastases and also to monitor the same. It is considered as gold standard but there are some limitations as it is expensive and the fear of radiation due to repeated use on the same set of patients having bone metastases, for continuous evaluation. Hence there is a need for development of new biochemical markers which are useful to follow-up the patients with bone metastases. Serum tartrate-resistant acid phosphatase 5b (TRACP 5b) is such a novel bone resorption marker for bone metastases. Recent studies show that serum TRACP 5b is identified as a marker of osteoclasts and bone resorption [9], [10]. In human serum, type 5 TRACP is present in two isoforms, band 5a and band 5b [11]. TRACP 5a originating from macrophages and dendritic cells is a marker of inflammatory condition and TRACP 5b originating from osteoclasts is a marker of bone resorption. Band 5a consists of a single polypeptide chain with a molecular weight of 35 kD, band 5b is a proteolytically cleaved form with disulphide linked polypeptide subunits of 16 and 23 kD. One important difference between these two isoforms is the presence of sialic acid in 5a, and its absence in 5b. Osteoclasts synthesize and secrete tartrate resistant acid phosphatase 5b normally during the course of bone resorption

[12], [13]. This circulating serum TRACP 5b can be used as a marker of bone resorption. TRACP 5b has an added advantage than other bone markers as it does not show much dependence either on nutritional status or diurnal rhythm [14]. Its activity is not affected by liver and renal disorders. This is an important point in the case of cancer patients having additional liver metastases [15], [16]. Both physiological and pathological changes of bone turnover show influence on Serum TRACP 5b levels. TRACP 5b can be used as a sensitive and specific marker for bone metastases in prostate cancer patients as an osteoclast specific marker.

2. MATERIALS AND METHODS

The present study was carried out in the Department of Biochemistry, Gandhi Hospital, Secunderabad and in the Department of Nuclear Medicine, MNJ Institute of Oncology and Regional Cancer Centre, Red Hills, Hyderabad. The study population comprised of 152 males and they are classified into four groups.

Group 1 comprises 50 normal; cancer free males aged 20- 80 years.

Groups 2, 3 and 4 comprise male patients with confirmed prostate cancer, diagnosed earlier by histo-pathological and radiological studies. They were categorised on the basis of absence or presence of skeletal lesions, and their number detected through their whole body skeletal scintigraphy with Technetium 99m MDP.

Group 2 consists of 38 prostate cancer patients without bone metastases.

Group 3 consists of 27 prostate cancer patients with limited bone metastases (3 or less than 3 skeletal lesions).

Group 4 consists of 35 prostate cancer patients with extensive bone metastasis (4 or more than 4 skeletal lesions).

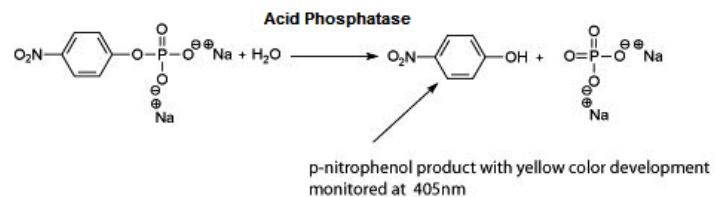
Patients and control group were recruited after informed consent was obtained. Venous blood samples were drawn before conducting bone scintigraphy (for the patients) and allowed to clot at room temperature for 30 to 60 minutes. They were centrifuged for 20 minutes in a refrigerated centrifuge at 10000g at -4° C and the serum stored at -70° C in aliquots, where TRACP 5b activity is stable for years. (The TRACP 5b activity was measured within one month of blood collection.) Samples were thawed to room temperature immediately before estimating TRACP 5b activity. In this study serum tartrate-resistant acid phosphatase 5b activity, serum alkaline phosphatase and serum calcium were estimated.

A dose of 20 mCi Technetium 99m MDP (methylene diphosphonate) is administered intravenously to these patients. After 2 - 4 hours, whole body bone scintigraphy was conducted on these patients using Dual Headed Gama Camera.

Subjects with fractures, primary and secondary hyperparathyroidism, and all bone related problems are considered as exclusion criteria for normal group. Patients treated with aromatase inhibitors and bisphosphonates and patients with extensive bone metastases for various times prior to enrolment are the exclusion criteria for carcinoma prostate samples in this study.

2.1 Serum TRACP 5b activity Assay [17], [18].

Tartrate-resistant acid phosphatase 5b activity was estimated spectrophotometrically as described by Lau et al. (1987) with modifications by Yamagishi et al. (2009) according to the following equation:



Reagents:

- 1) Substrate solution contains 100 mM pNPP (sigma chemical) in 200 mM sodium acetate buffer containing 80 mM sodium tartrate, 400 mM potassium chloride and 42U/ml heparin (sigma chemical) adjusted to pH 5.6 by addition of conc. HCl.
- 2) 1 M NaOH

The clear serum samples were obtained by centrifugation at 10,000g for 20 min at -4° C and were stored at -70° C. Serum Samples were diluted ten-fold with distilled water before analysis. Diluted samples were incubated for 1 hr. at 37° C. Then, 50 μ l of the diluted sample was added to 50 μ l of substrate solution in a micro plate. The reaction was carried out for 1 hr. at 37° C and quenched by adding 50 μ l of 1 M NaOH. (All the other chemicals used in this assay are analytical grade chemicals).

A standard calibration curve was constructed using p-nitrophenol (pNP) (sigma chemical) solution of known concentrations (5-25 μ g/ml in 0.05 M NaOH) and the absorbance was measured at 405 nm in a micro plate reader. The amount of p-nitrophenol production was calculated through a comparison with standard curve obtained with p-nitrophenol solution. The results were expressed as units per litre.

One unit (IU) of TRACP 5b activity was defined as the amount of enzyme required to hydrolyse 1 micro mole (μ mol) of p-nitrophenyl phosphate (pNPP) per minute at 37° C. The samples were diluted further and reanalysed if the activity of TRACP 5b exceeded the range of the standard curve.

2.2 Serum Alkaline phosphatase is estimated by pNPP-AMP (IFCC) Kinetic Assay method.

2.3 Serum Calcium is estimated by O-Cresolphthalein Complexone, End point Assay method.

3. RESULTS: One way ANOVA was used to compare serum TRACP 5b, serum ALP and serum calcium among groups. (Table1).

Table 1. Comparison of Mean and SD values of serum TRACP 5b, serum ALP and serum calcium in normal and prostate cancer patients

	TRACP5b		ALP		Calcium	
	mean	SD	mean	SD	mean	SD
GP 1 normal N=50	2.85	0.86	78.60	30.83	9.80	0.42
GP 2 PC without t BM N=38	2.63	0.90	81.89	67.22	9.62	0.48
GP 3 PC with limited BM N=27	3.26	0.99	148.51	87.86	9.2	0.81
GP 4 PC with Extensive BM N=32	9.04	2.99	731.28	290.63	7.82	2.26
F	165.3		168.1		29.6	
P value	0.0001		0.0001		0.0001	

3.1 Sensitivity and Specificity of TRACP 5b:

The sensitivity and specificity were estimated by Receiver Operating Characteristic (ROC) Curves plotted for serum TRACP 5b in 38 PC patients without BM vs. 50 control subjects. The area under the curve (AUC) generated from the serum TRACP 5b was 0.5679 (95%CI, 0.4463 to 0.6895) and the p value is 0.2772. The ROC curves plotted for serum TRACP 5b in 27 PC patients with Lim BM vs. 50 control subjects. The

area under the curve (AUC) generated from the serum TRACP 5b was 0.6474 (95%CI, 0.5165 to 0.7784) and the p value is 0.03369(Fig.1). The area under the curve increased to 1.000 (95% CI, 1.000 to 1.000) for the 32 patients with extensive metastasis vs. 50 control subjects, and the p value is < 0.0001 (Fig. 2).

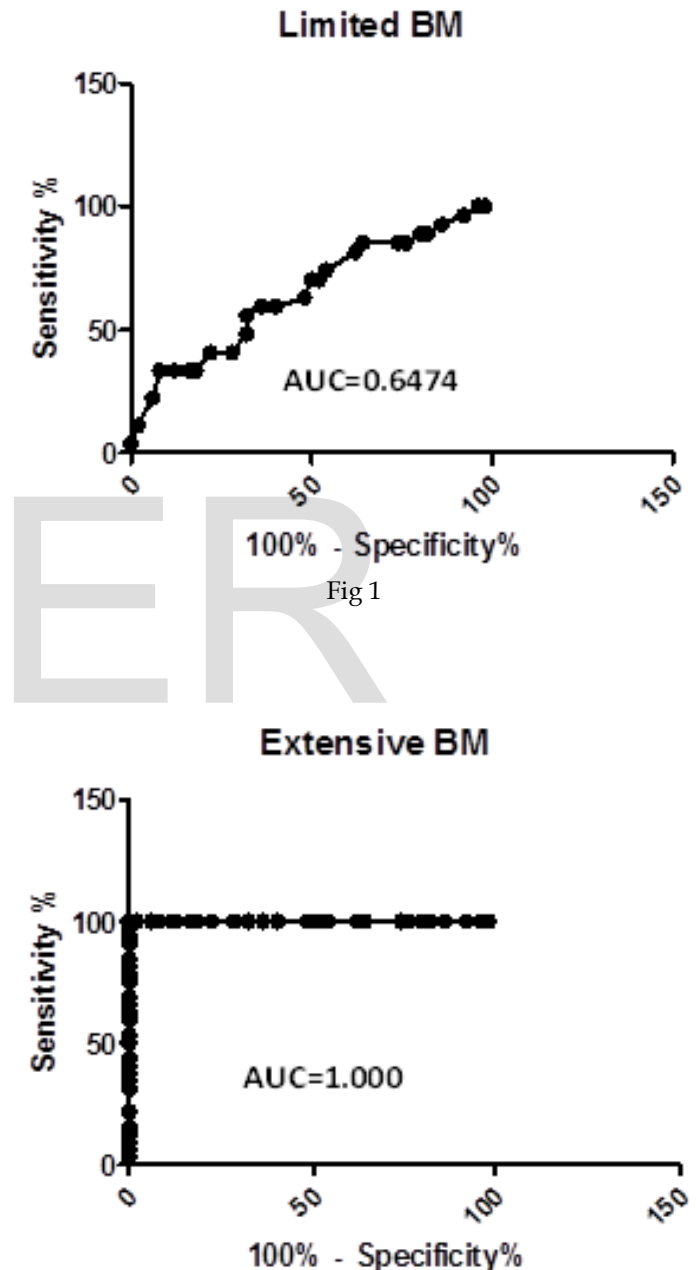


Fig 1

3.2 Sensitivity and Specificity of Serum Alkaline Phosphatase:

The Receiver Operating Characteristic (ROC) Curves plotted for serum alkaline phosphatase in 38 PC patients without BM vs. 50 control subjects. The area under the curve (AUC) generated from the serum calcium was 0.5713 (95%CI, 0.4516 to 0.6911) and the p value is 0.2537. The ROC curves plotted for serum ALP in 27 PC patients with Lim BM vs. 50 control subjects. The area under the curve (AUC) generated was 0.8070 (95%CI, 0.7112 to 0.9029) and the p value is < 0.0001 (Fig.3). The area under the curve increased to 1.000 (95% CI 1.000 to 1.000) for the 32 patients with extensive metastases vs. 50 control subjects, and the p value is < 0.0001 (Fig. 4).

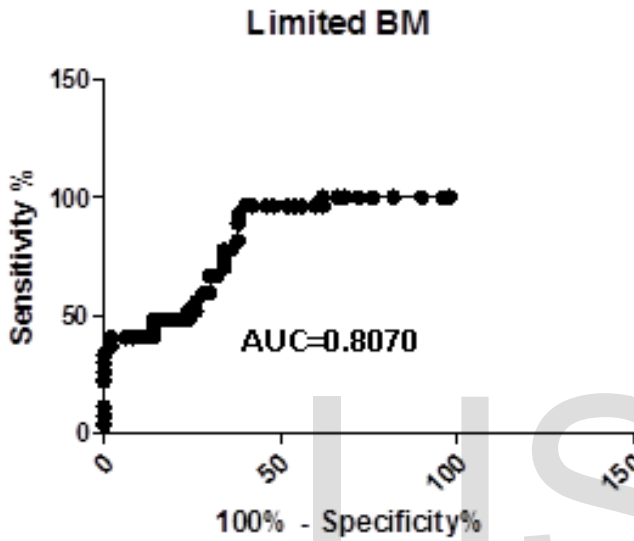


Fig 3

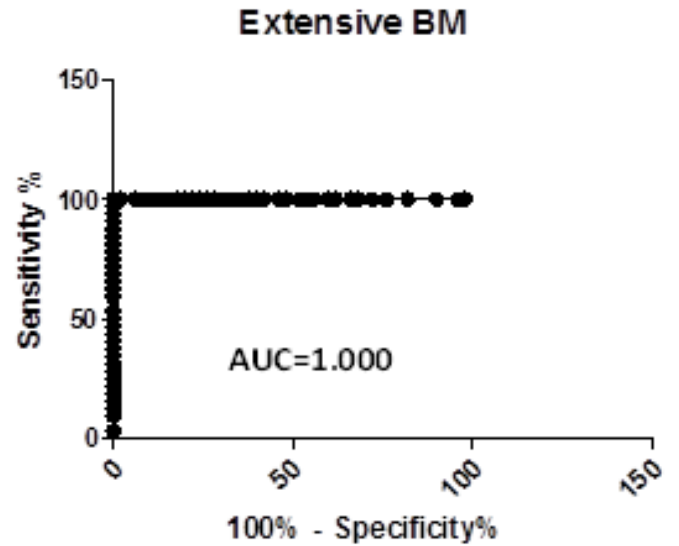


Fig 4

3.3 Sensitivity and Specificity of Serum calcium:

The Receiver Operating Characteristic (ROC) Curves plotted for serum calcium in 38 PC patients without BM vs. 50 control subjects. The area under the curve (AUC) generated from the serum calcium was 0.6047 (95%CI, 0.4840 to 0.7255) and the p value is <0.09. The ROC curves plotted for serum calcium in 27 PC patients with Lim BM vs. 50 control subjects. The area under the curve (AUC) generated from the serum calcium was 0.7330 (95%CI, 0.5997 to 0.8663) and the p value is <0.0007(Fig.5). The area under the curve increased to 0.8666 (95% CI, 0.7530 to 0.9802) for the 32 patients with extensive metastases vs. 50 control subjects, and the p value is < 0.0001 (Fig. 6).

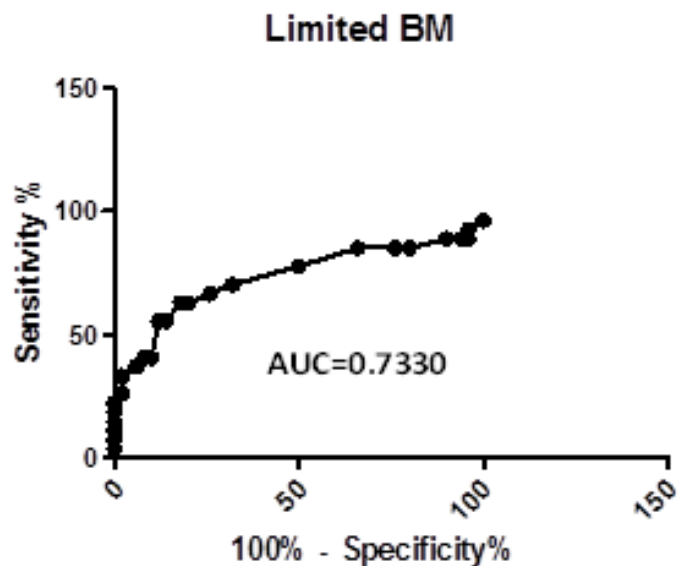


Fig. 5

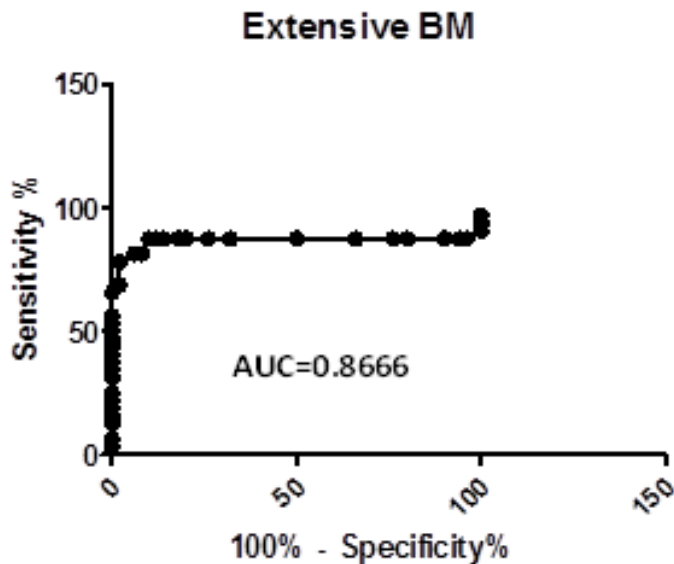


Fig. 6

4. DISCUSSION:

Prostate cancer is the most frequently diagnosed non-cutaneous cancer and the second leading cause of cancer deaths among men in the United States [19]. In prostate cancer patients the incidence of bone metastases is observed to be very high at about 70-80% [20], [21], [22]. When treating patients with prostate cancer, identification of bone metastases is an important issue. Image studies such as plain radiography, bone scintigraphy, computerized tomography and magnetic resonance play a major role in detection and follow-up of bone metastases of prostate cancer patients. But each image measure has its own limitation. The advantage of bone biochemical markers over image studies are, non-invasive, cost-effective, no fear of radiation, fast and easy to perform repeatedly and also show rapid response to treatment, differentiate healing lesions from progressive lesions and provides more information on the mechanisms and cellular dynamics of bone destruction [23], [24], [25], [26], [27], [28]. Metastases in prostate cancer are characterized by excess of abnormally dense bone showing increased bone turnover. This is due to increased activity of both osteoblasts and osteoclasts. The relative amount of osteoblastic activity exceeds that of the osteoclasts, resulting in excess bone formation.

Recently a novel bone resorption marker TRACP 5b activity is used as specific and sensitive marker in the detection and follow-up in bone metastases. In this study, we have compared serum TRACP 5b activity, along with total alkaline

phosphatase and serum calcium with whole body skeletal scintigraphy with Technetium^{99m} MDP. Serum TRACP 5b activity and serum ALP are higher in prostate cancer patients with bone metastases than normal subjects and are correlated with each other. Serum TRACP 5b activity is not significantly increased in BC patients with limited bone metastases (3 or less than 3 skeletal lesions) (Fig.7) and increased activity is shown only in patients with extensive BM (4 or more than 4 skeletal lesions) (Fig.7). Serum ALP is increased significantly in limited bone metastases (Fig.8) may be due to liver metastases as lung and liver are the most frequent sites of distant prostate cancer metastases. Bone specific ALP is more specific osteoblastic marker for bone metastases along with TRACP 5b.

Among patients with limited BM, 11% of cases showed hypercalcemia and 14.8% cases showed hypocalcemia and in patients with Ext. BM 13% cases showed hypercalcemia and 53% showed hypocalcemia. Prostate cancer patients tend to have more hypocalcemia than hypercalcemia and this could be due to the metastases that is predominantly osteoblastic [29]. Intense osteoblastic response seen in prostate cancer is preceded at a cellular level by osteoclast activation [30]. Avid uptake of calcium by osteoblastic metastasis of prostate is the main cause of hypocalcemia Fig. 9[30].

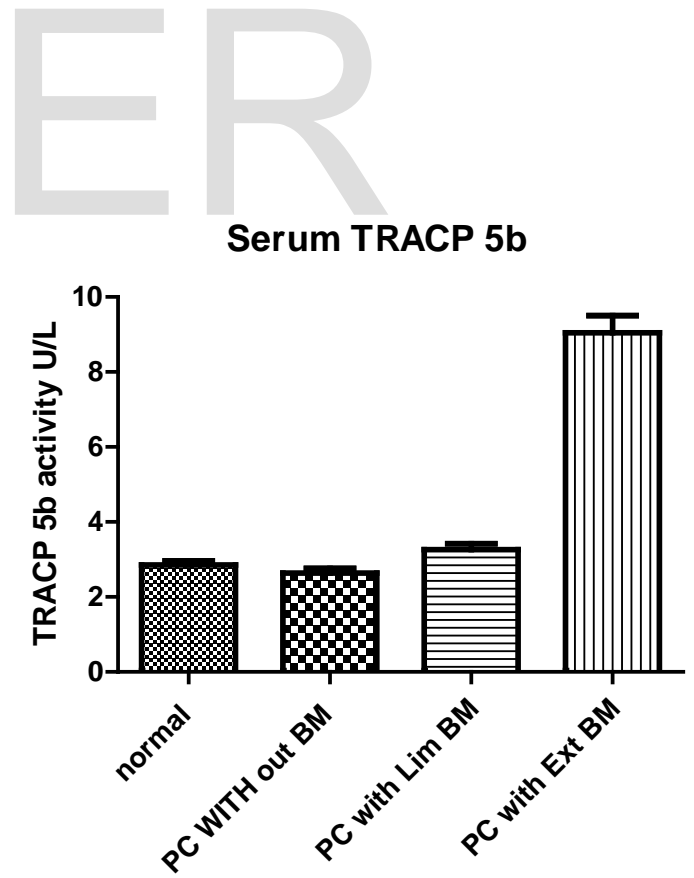


Fig 7

Serum ALP

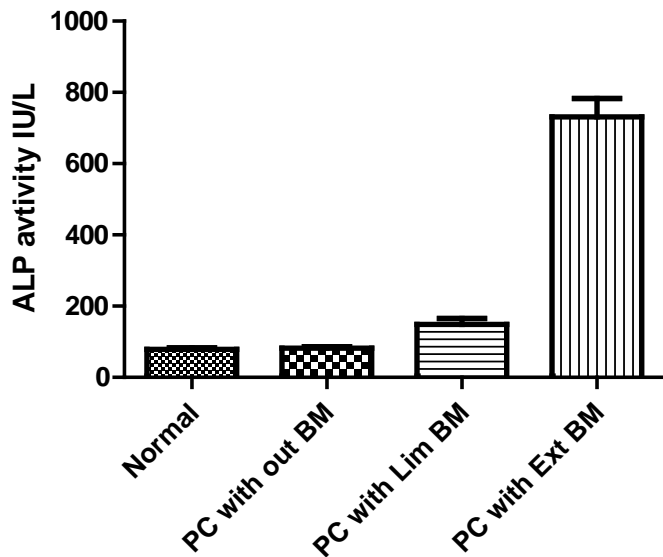


Fig 8

Serum calcium

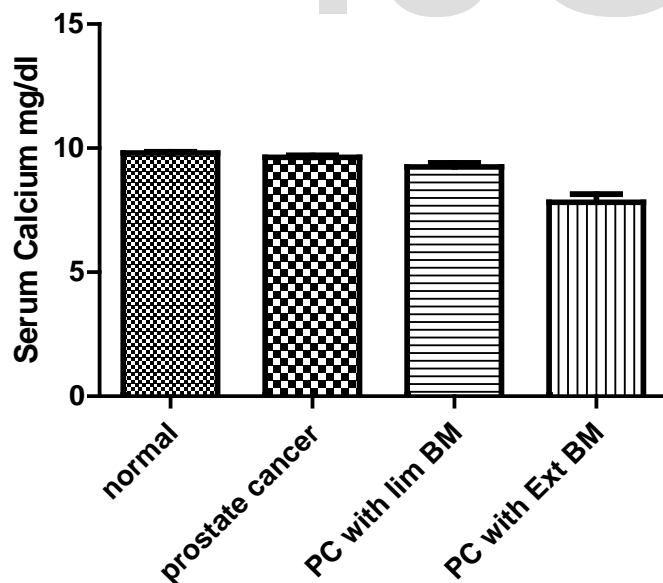


Fig 9

5. CONCLUSION:

In conclusion, our results suggest that measurement of serum TRACP 5b concentration is a powerful test alone as a resorption marker or in combination with routinely used bone formation marker ALP to detect bone metastatic spread. Serum calcium will give additional background values for clinical guidance. This study shows serum TRACP 5b increasing consistently, with the presence of bone metastases and the extent of skeletal involvement.

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