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Research Article

Serum bone profile and cathepsin K expression as a prognostic factor in patients with and without breast cancer metastasis

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Abstract

Objectives: Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer-related death among women worldwide. Breast cancer bone metastasis is associated with skeletal events, including acute fractures, compression of the spinal cord, surgery and radiotherapy to the spine, as well as bone pain and hypercalcemia, resulting in decreased mobility and diminished quality of life. Greater understanding of the bone metastasis pathophysiology will highly likely to lead to the discovery of an effective treatment option. This study aims to test whether the serum bone profile and expression level of cathepsin K (CTSK) in breast carcinoma is associated with metastasis.

Methods: In this study, 116 participants, 58 patients who had been diagnosed with breast cancer (n=22 without metastasis and n=36 with metastasis) and 58 healthy controls were included. Serum biochemical profile and immunostaining of CTSK in the breast carcinoma were investigated.

Results: The mean values of calcium, 25-OH Vitamin D, ALP, albumin, phosphorus, magnesium, TSH, cholesterol, PTH and CRP (mg/L) were 11.5±2.03, 28.12±10.5, 93.3±7.9, 3.9±0.3, 3.7±1.64, 1.8±0.24, 2.5±1.5, 165.1±28.02, 63.4±18.9 and 7.7±4.9. Individual data revealed that 70% of patients without metastasis has PTH above normal while 65% has calcium and 62% patients has ALP above normal levels, which were further increased in metastasis. Low Mg levels were detected in 13/58 of the patients with breast cancer and 3/58 of the control group.13/58 of the patients with breast cancer showed low total calcium, and 32/58 of the breast cancer group showed high calcium levels.

Conclusion: The present study results suggest that CTSK expressions are associated with a higher tumour stage and distant metastasis, suggesting serum bone profile and level of CTSK expression are significant parameters in the disease diagnosis and monitoring of breast cancer metastasis.

Keywords: Breast cancer, cathepsin K, immunohistochemistry, serum bone marker

Breast cancer is the most common malignancy among women and the leading cause of death. The majority of the cases are diagnosed at an advanced stage with a higher incidence of skeletal metastases [1-3]. Metastatic disease to the bone has been a crippling devastating complication of breast cancer, leaving patients bedridden or wheelchair-bound and victims of suffering from intolerable pain. The biological mechanisms leading to bone metastasis have been referred to as "vicious cycle" a complex network between cancer cells and the bone microenvironment [4].

Besides clinical and imaging techniques, biochemical tests play a vital role in the assessment and differential diagnosis of bone metabolic disorder in breast cancer [5]. These biochemical indices are non-invasive, comparatively low-cost and when applied and interpreted correctly, it is a great tool among the diagnostic and therapeutic assessment of metabolic bone

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disease. However, the role of serum bone profile as a risk of skeletal metastasis has been under-researched.

CTSK is a papain-like cysteine protease, involved in bone remodeling, produced by cancer cells that metastasize to the bone where it acts in proteolytic pathways that facilitate the invasion of cancer cells and has been widely used as an immunohistochemical marker for osteoclasts *in situ* detection [6, 7]. CTSK expression has shown to be increased considerably in primary cases of breast cancer with skeletal metastasis [8]. CTSK expression has also been very well associated with tumour proliferation and progression in colorectal, gastric, prostate, oral squamous and glioblastoma cancers [9-12].

There are several studies concerning the diagnostic value of breast cancer bone turnover markers for bone metastases. However, their uses in the diagnosis are not yet fully validated. Much of the studies were derived predominantly from retrospective analyses. Bone markers for the diagnosis and management of bone metastasis are significantly hindered by biological and analytical variability multiple confounding factors (tumour burden, malnutrition, chemotherapy, radiotherapy, immobility) causing variations in their concentrations [13]. Therefore, this study aimed to assess the serum bone profile in patients with breast cancer in comparison with healthy controls and to determine the relationship between CTSK expression, including mild, moderate and high levels and breast cancer metastasis. Further, we compared the CTSK expression in different types of breast cancer based on histopathology and receptor status to evaluate the association with specific subtypes.

Materials and Methods

The present study included 58 clinically established breast cancer female patients ranging in age from 34 to 74 years with the mean age of 58.6±12.4. Ethical approvals for this study were taken from Saveetha Medical College & Hospital Chennai and Gleneagles Global Health City Hospital Chennai, India. Patients were prospectively identified and registered. All samples were taken after institutional ethical committee permissions and personal consent of the patients or guardians. All patients had histologically confirmed breast cancer. The histopathological diagnosis of breast cancer, grade, stage of the tumour, and hormone receptor status (estrogen receptor ER, progesterone receptor PR and Her2neu) were recorded from the pathology reports of breast cancer patients.

The blood samples were collected from the patients in heparinized tubes. The collected samples were analyzed for age, weight, and body mass index (BMI) and biochemical profile of blood. The parameters like total cholesterol, triglycerides, HDL-C, LDL-C, C-reactive protein (CRP), calcium, 25-OH Vitamin D and tumour markers CEA, CA 15-3, Vitamin D, parathyroid hormone (PTH), serum alkaline phosphatase (ALP), albumin, phosphorus (Phosphomolybdate), magnesium were included in this study.

Inclusion criteria

The confirmed cases of breast cancer by mammography and histological examination were chosen for this study. Controls were individuals without clinical cases who were seen at the same hospital for an annual physical examination.

Exclusion criteria

Patients suffering from any other cancer as well as diabetes mellitus and dyslipidemia, osteoporosis with drug treatment were excluded from this study.

Immunohistochemistry studies (IHC)

Invasive ductal carcinoma was diagnosed with low to the moderate distinction among donors. The tissue microarray (TMA) slides were made from tissue donors and contained at least two cores per patient (1 mm in diameter). Samples were examined for classification by the vendor's pathologist regarding histopathology, class, the involvement of the lymph node, and tumour grade. Samples were classified based on tumour-node-metastasis (TNM) classification of: the size of the primary tumour (T), degree of regional lymph node involvement (N), and the existence of distal metastasis (M). Endogenous peroxidase was quenched by incubation of tissues in 0.3% hydrogen peroxide in PBS for 10min. Nonspecific binding was blocked for 1h at room temperature with serum (5% goat sera) in phosphate-buffered saline. Endogenous biotin was blocked with an avidin/biotin blocking kit. An affinity pure goat antibody against human CTSK was applied at 40 ng/ml, and the part were incubated in a humidified chamber at 4°C overnight. Sections in Harris hematoxylin and blue in ammonia water were counterstained before mounting. Giant cell tumour tissues were used for CTSK staining as positive regulation.

The criteria used for assessing the immunostaining of the breast tumour were as follows. The degree of staining was taken as a sum of the strength of staining and the percentage of stained cells: negative/mildly stain (-)=0-1; moderately positive (2+)=2-3; strongly positive (3+)=4. Almost all strongly positive had a widely stained area >4.

Statistical analysis

Data were presented as mean±standard deviation (SD). The normality of the data was checked using the Shapiro-wilk test. In case of data not following a normal distribution, the median was presented. For data following normal distribution, differences between groups were assessed by one way ANOVA. Pearson correlation was performed to evaluate correlation analysis between the tumour markers with a bone mineral profile in the breast cancer group. The chi-square test was performed to evaluate the relationship between CTSK expression and clinicopathological features and metastasis. All statistical analyses were conducted with graph pad prism 6.0 software package for windows.

Results

Biochemical profile analysis

The average age of the breast cancer group (n=58) and of the control group (n=58) was 59.1±8.03 median 57.5 and 58.6±12.4 median 56.5. Clinical, demographic, and biochemical characteristics of the study groups are are presented in Table 1. According to baseline parameters, there was no significant difference in the Body Mass Index (BMI) (p<0.0381), fasting plasma glucose (p<0.0844), age (p<0.8409), total cholesterol (p<0.12).

Table 1. Baseline characteristics of this study

Parameter	Control (n=58)	Breast cancer (n=58)	p
Age (years)	59.1±8.03	58.6±12.4	0.8409
Height (cm)	158±5.7	161.07±7.63	0.2054
Weight (kg)	68.2±9.9	66.3±10.33	0.0844
BMI (kg/m^2)	31.4±4.6	27.5±4.3	0.0381
CA 15-3 (U/ml)	7.9±2.3	105.3±41.6	<0.0001
CEA(U/ml)	0.99±0.2	56.3±21.6	<0.0001
Calcium (mg/dL)	8.5±0.5	11.15±2.03	<0.0001
25-OH VitD (ng/ml)	25.39±9.4	27.8±4.8	0.557
ALP (U/L)	76.5±21.3	93.3±7.9	<0.0001
Albumin (g/dL)	4.2±0.2	3.9±0.3	<0.05
Phosphorous (mg/dL)	4.3±1.62	3.7±1.64	<0.0001
Magnesium (mg/dL)	2.3±0.23	1.8±0.24	<0.05
TSH (IU/ml)	2.07±1.3	2.5±1.5	0.478
Cholesterol (mg/dL)	175.1±29.0	165.1±28.02	0.12
PTH (pg/ml)	37.62±22.1	63.4±18.9	<0.0001
HDL (mg/dL)	40.09±6.5	53.9±7.08	0.0002
LDL (mg/dL)	91.5±31	83.2±27.5	0.4954
VLDL (mg/dL)	35.5±5.01	28±3.2	<0.0001
TGL (mg/dL)	177.5±27.3	180.4±16.4	0.034
Chol/HDL ratio	4.1±1.01	3.09±0.5	0.0061
CRP (mg/L)	2.7±1.01	7.7±4.9	<0.0001

Bone profile analysis

Individual data revealed that 70% of patients without metastasis had PTH above normal while 65% had calcium and 62% of the patients had ALP above normal levels which further increased in metastasis.

The patients with breast cancer and control subjects showed significant differences in calcium, PTH and C-reactive protein level, demonstrating an appropriate match in the risk factors for breast cancer. 25-OH Vitamin D deficiency was considered at serum level less than 20 ng/ml, suboptimal 25-OH Vitamin D levels were considered between 21 and 39 ng/ml and optimal levels were more than 40 ng/ml (27). 25-OH Vitamin D deficiency was seen in 36.2% (21/58) patients with breast cancer, while 45.4% (26/58) of the control group was deficient. Phosphorus was deficient in 5/58 and was high in 1/58 of the breast cancer group and 6/58 of the normal control group.

Deficient Mg levels were detected in 13/56 of the patients with breast cancer and 3/22 of the control group. Regarding calcium level, 13/58 of the patients with breast cancer showed low total calcium and 32/58 of the breast cancer group showed high calcium levels (Table 1). Correlation analysis between the breast cancer tumour markers and bone profile in the breast cancer group are presented in Table 2. Serum calcium was significantly high in the patients with breast cancer reflecting the tight control of serum calcium by calcium-regulating hormones such as PTH and 25-OH Vitamin D. Concerning serum PTH, the concentration of PTH in serum was significantly higher in patients with breast cancer than in control subjects (Table 2). The results showed that patients with breast cancer and control subjects were both 25-OH Vitamin D deficient. In this study, the protein albumin level significantly reduced in patients with breast cancer as compared with normal (p<0.05).

Bone profile with some prognostic factors profile between pre and post-menopausal women in breast cancer metastasis and non-metastasis group

Comparing the studied bone profile with some prognostic factors in the breast cancer group is shown in Table 3. The se-

Table 2. Serum tumour marker and bone profile levels in patients with breast cancer (value are mean±SD)

Group	PTH pg/ml	Calcium mg/dl	Vitamin D ng/ml	ALP U/L	CA-153 U/ml	CEA U/ml
Normal control (n=58)	37.62±12.1	8.505±0.57	45.39±9.4	76.5±21.3	76.5±21.3	1.035±02
Breast cancer without metastasis (n=22)						
Pre-menopausal level(n=12)	52.08±17*	9.4±0.5$^{\#}$	47.8±4.8^{ns}	78.2±18.8	64.9±12.3*	37.7±4.4$^{\$}$
Post–menopausal level (n=10)	83.6±8.1$^{\#}$	11.5±1.03*	53.05±5.6*	103.25±28*	99.2±9.4$^{\#}$	60.7±9.7$^{\$}$
Breast cancer with metastasis (n=36)						
Pre-menopausal level (n=9)	62.1±20.4$^{\#}$	12.2±1.2$^{\#}$	47.9±3.8^{ns}	83.75±28$^{\#}$	158.7±8.4$^{\$}$	80.4±12$^{\$}$
Post–menopausal level (n=26)	58.22±8.3$^{\#}$	13.02±1.2$^{\$}$	46.55±3.7^{ns}	119.3±36.2$^{\$}$	156.4±16.8$^{\$}$	79.1±6.1$^{\$}$

*p<0.05 and $^{\#}$p<0.01, $^{\$}$p<0.001 and ^{ns}=non-significant

rum levels of PTH, total calcium and tumour markers CA 15-3, CEA showed a significant difference between the pre and post-menopausal women within the non-metastatic group; however 25-OH vitamin D and ALP did not show any significant difference between both the groups. The serum levels of total calcium, ALP did show a significant difference between the pre and post-menopausal women within the metastasis group; however, PTH and tumour markers CA 15-3, CEA and 25-OH vitamin D did not show any significant difference between both the groups.

Correlations of serum tumour markers with the bone profile in patients with breast cancer

Correlations of serum tumour markers with biochemical markers of bone in patients with breast cancer were made. The differences of the results in the groups were (i) age significantly correlated with both breast cancer markers (r=0.1494, p=0.0030) (ii), whereas ALP, TSH, Mg and phosphorous did not significantly correlate with any markers in the breast cancer group, (iii) whereas calcium level was significantly correlated with both breast cancer markers (p<0.001) (Table 3).

Table 3. Correlation analysis between the tumour markers with biochemical marker of bone in the breast cancer group

Bone mineral profile	CA 15-3		CEA	
	R square	p	R square	p
Age	0.149	0.003#	0.166	0.0016#
Calcium mg/dl	0.13	0.005#	0.429	0.001$
Vitamin D (ng/ml)	0.08	0.0314	0.07	0.034*
ALP (U/L)	0.08	0.0320	0.05	0.0799
Albumin (mg/dl)	0.007	0.5	0.08	0.03
Phosphorus mg/dl	0.004	0.6	0.026	0.2
Magnesium (mg/dl)	0.008	0.8	0.003	0.6
TSH (IU/ml)	0.02	0.2	0.017	0.3
PTH (pg/mL)	0.13	0.004	0.07	0.04*

*p<0.05 and #p<0.01, $p<0.001

Bone profile and tumour marker comparison between metastasis and non-metastasis breast cancer patients

As shown in Table 4, ALP and PTH showed a significant rise in metastatic cases as compared to non-metastatic group (p<0.01), whereas there is no significant difference in the level of vitamin D (p>0.13), albumin (p>0.5), phosphorous (p>0.3), Mg (p>0.4). However, there was a significant difference in the level of serum calcium between the two groups (p<0.001).

Relationship between ctsk expression and clinicopathological factors in breast carcinomas

In this study, 12 (33%) of 36 cases were negative (-) for CTSK, 11 (30.5%) were mildly positive, 9 (25%) were moderately positive (2+) and 4 (11.1%) were strongly positive (3+) for CTSK expression in malignant cells (Fig. 1). No relationship was demonstrated between CTSK expression and patient age, tumour size (major axis) and estrogen receptor status (Table 5). However, a significant positive relationship was found between CTSK expression and histological grade (p<0.001), CTSK expression and presence or absence of distant metastasis (p<0.05).

Table 4. Bone profile and tumour markers comparison between metastasis and non-metastasis patients with breast cancer using T-test

Parameters	Non-Metastasis group(n=22) Mean±SD	Metastasis group (n=36) Mean±SD	p
CA 15-3 (U/ml)	85.2±22.3	139.9±34.7	p<0.01
CEA (U/ml)	48.05±9.07	76±15	p<0.05
Calcium (mg/dl)	9.4±0.6	13.14±1.1	p<0.001
Vitamin D (ng/ml)	25.3±11.3	26.8±10.8	p>0.13
ALP (U/L)	110.1±54.2	82.9±51.3	p<0.05
Albumin (g/dl)	3.9±2.3	4.0±1.3	p>0.5
Phosphorous (mg/dl)	3.8±0.8	3.5±0.5	p>0.3
Magnesium (mg/dl)	1.8±0.29	1.9±0.2	p>0.4
TSH (IU/ml)	2.8±1.6	2.4±1.9	p>0.31
PTH (pg/mL)	56.3±18.7	68.4±19.1	p<0.05

TSH: Thyroid stimulating hormone, PTH: Parathyroid hormone

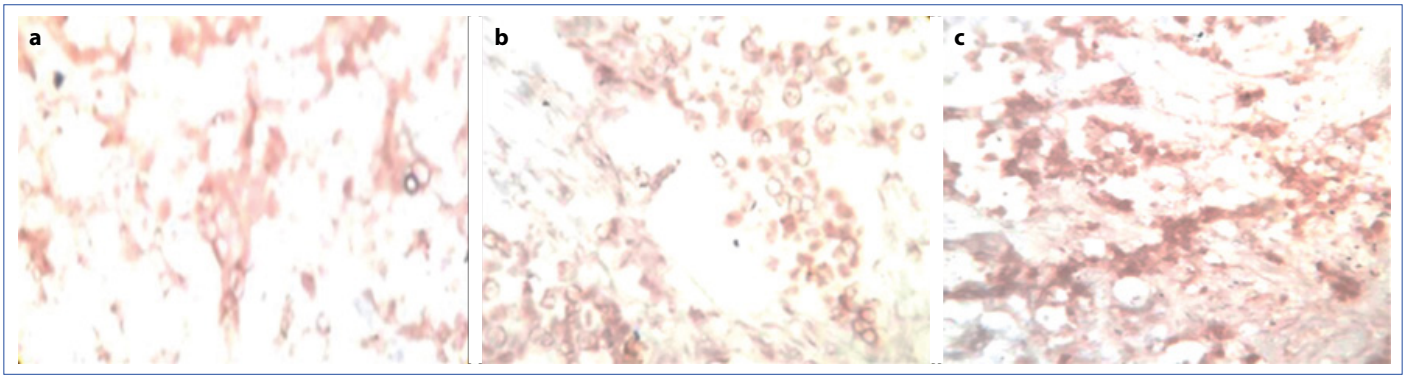

Figure 1. (a-c) Depicted is the representative immunostaining pattern of CTSK (a) mildly positive stain benign breast tissues, (b) moderately positive stain grade II breast cancer tissues, (c) strongly positive stain grade III breast cancer tissues (Magnification 40x).

Table 5. Relationship between CTSK expression and clinicopathological factors in breast carcinomas by Chi-square test

Factors	n	- (%)	+ (%)	++ (%)	χ^2-test
Age					
>50	14	5 (35.7)	8 (54.1)	1 (7.1)	ns
<50	24	7 (29.1)	12 (50)	3 (12.5)	
Tumour size					
<2.5	13	8 (61.5)	5 (38.4)	0	ns
>2.5	23	4 (27.4)	15 (65.2)	4 (17.3)	
Distance metastasis					
M0	27	12 (44.7)	15 (55.2)	0	p<0.05
M1	9	0	0	4 (44)	
Histological					
Grade (I)	9	8 (88.8)	1 (11.1)	0	p<0.001
Grade (II)	18	4 (22.2)	14 (77.7)	0	
Grade (III)	9	0	5 (55.5)	4 (44.4)	
Estrogen receptor					
(+)	30	12 (40)	15 (50)	3 (10)	ns
(-)	6	1 (16.6)	5 (83.3)	0	

Discussion

The present study has shown that women with breast cancer have higher levels of total serum calcium and higher levels of ALP and PTH than control subjects. Other malignancies also reported hypercalcemia and high ALP and PTH activity [14-16]. Hypercalcemia has been linked to osteolytic bone metastases, responsible for 20–30% of breast cancer metastasis. According to the previous studies, increased skeletal invasion and tumour destruction triggered by tumour development of various cytokines, such as growth factors (TGF-β), tumour necrosis factor-α (TNF-α), interleukin-1 and interleukin-2 leads to increasing bone osteolysis and modification of the reabsorption, excretion and resorption of calcium and phosphate ion, causes a high level of calcium [17].

ALP has many isoenzymes located in the liver, bones, and smaller amounts in intestines, placenta, kidneys, and leucocytes [18]. Another ALP isoenzyme called Regan isoenzyme has also been identified in various malignancies [19], which may contribute to increased ALP activity in breast cancer patients. This enzyme elevated activity seen in study participants can also be linked to osteolytic bone metastases in breast cancer, leading to increased osteoclastic activity and bone resorption. However, the rise in serum ALP levels is non-specific, as it is also frequently associated with many other diseases. Also, the elevation of ALP activity to less than three times the normal level is usually not considered significant [14]. In the present study, ALP and calcium showed a non-significant rise in non-metastatic cases and registered a significant rise (p<0.001) in metastatic patients, respectively, which is consistent with the findings of Uemura et al. [20] and Mulholland et al. [21] which indicate no significant difference in ALP levels in non-metastatic breast cancer. Atoe et al. [22] revealed a significant rise in ALP and calcium in metastasis and no change in non-metastatic groups, which suggested the involvement of bone in cancer metastasis.

Multiple epidemiological studies have shown the link between C-reactive protein (CRP) and the risk of breast cancer [23]. Nonetheless, the findings of multiple studies testing the interaction of CRP with breast cancer in different ethnic groups have shown inconsistencies [24]. Some studies showed an association between elevated CRP and poor prognosis, while other studies found no association [25]. Guo et al. [26] conducted the largest study, which involved 5.286 patients with breast cancer. This meta-analysis found that elevated levels of CRP are correlated with increased risk of breast cancer [26]. Another study reported a high level of CRP at the time of breast cancer diagnosis which was associated with decreased overall survival and disease-free survival and increased breast cancer death [23]. In the present study, we found that CRP levels in patients with breast cancer significantly elevated at the time of diagnosis (p<0.001) compared with healthy controls.

Carcinogenesis causes magnesium mobilization through blood cells and magnesium depletion in non-neoplastic tissue. At the same time, Mg deficiency seems to be carcinogenic. It has been found that supplementation of a high level of magnesium inhibits carcinogenesis in case of solid tumours [27]. Serum magnesium lower than 1.8 mg/dL is considered low. In the present study, magnesium was deficient in 42% of the patients with breast cancer and in 5% of the control group. Magnesium levels were significantly lower in the breast cancer group (p<0.001), which is in agreement with Sartori et al. [23] and Atoe et al. [22], which suggests that serum Mg was significantly lower in the patients with breast cancer compared to the control group and contradictory to the findings by Arinola et al. [28] who reported

slight hypomagnesaemia in patients with breast cancer. Abdelgawad et al. [29] found no significant difference when comparing Mg levels between the breast cancer and the control group. According to previous study, magnesium deficiency has been found to be involved in both cancer risk and prognosis, including breast cancer [26, 30-32]. Several studies also indicate the impact of dietary magnesium on breast cancer prognosis [24, 33]. Their results suggest that a higher dietary intake of magnesium among patients with breast cancer is inversely linked to mortality [34]. To date, the link between dietary magnesium consumption and the risk of breast cancer has been investigated by few epidemiological trials. A case-control study from Italy found that the serum magnesium level among patients with breast cancer was significantly lower than among control subjects [26], in line with our result.

25-OH Vitamin D status is known to be inversely related to serum PTH. The higher level of PTH in patients with breast cancer in the present study does not appear to be as a result of lower circulating 25-OH Vitamin D because there was no difference in serum 25-OH Vitamin D between patients with breast cancer and control subjects. The mean serum 25-OH Vitamin D in both groups was below 28.3 ng/mL, which is consistent with previous reports suggesting that 25-OH Vitamin D deficiency or insufficiency is prevalent across the globe in almost all age groups and geographic areas [35].

The total cholesterol observed among the patients with breast cancer in the present investigation was within the normal range, but there is a significant change in the level of HDL and VLDL and no change in LDL level. This finding is consistent with the studies of Ramaswamy et al. [36] and Damodar et al. [37] which reported a non-significant change in total serum cholesterol of breast cancer cases. However, this is in contrast to the studies of Qi and Owiredu et al. [38], which reported that elevated total serum cholesterol with increased breast cancer risk.

Several studies have consistently reported the prognostic value of serum albumin in patients with breast cancer. Low levels of albumin have been associated with increased cancer risk, and elevated levels of albumin (>3.5 g/dl) are significantly associated with improved overall survival among patients with breast cancer [39]. The present results are consistent with previous findings of Boonpipattanapong et al. [40], Win et al. [41] and Neal et al. [42]. The result provides strong evidence that lower serum albumin level is a prognostic factor for poor survival in early-stage patients with breast cancer regardless of stages. We observed an independent association between low baseline levels of serum albumin and survival. It is likely that serum albumin is a marker for patients with severe disease. Interestingly, our analysis suggests that low levels of serum albumin identify patients with the most severe disease within each tumour stage.

In the present study, serum TSH and phosphorus level in breast carcinoma women were within a normal range. This finding is in agreement with the report of non-significant change in total serum level of TSH and phosphorous [43]. Elevated levels of serum TSH and phosphorus are associated with advanced breast cancer [44].

The predominant expression of CTSK resulted in acute increase in serum calcium level and CTSK inhibition by SI-591 decreased serum calcium level in a rodent in-vivo study [45]. The current study reported that positive CTSK staining was detected in 55% of the breast tumours. There have been many studies concerning CTSK expression in breast carcinoma by immunohistochemistry [46, 7].

Evaluating the association of elevated CTSK with various histological grade ($p<0.001$), presence or absence of distant metastasis ($p<0.05$), we found that elevated CTSK levels associated significantly with histological grade I, II and III. This is in contradiction with the recent study that reported a significant association of negative ER status with elevated CTSK levels [46]. However, this study was carried out involving only 58 patients with breast cancer and, therefore, needs to be confirmed involving a larger cohort of patients. In addition, there was no significant difference in CTSK levels among premenopausal and postmenopausal patients with breast cancer ($p<0.5$). Higher and moderate CTSK levels were associated with a significantly presence or absence of distant metastasis ($p<0.05$). Therefore, moderate and high CTSK levels were possibly associated with significantly with poor outcomes, including death, recurrence and metastasis. To our knowledge, there is no published literature on CTSK in association with histological grade and distance metastasis and this will be the first study. The levels of CTSK were assessed at the time of disease diagnosis and the outcome measures were robust. However, limitations of this study are that we could not collect detailed data on receptor measurements, organ specific metastasis and lymph node status.

Conclusion

The estimation of serum bone profiles has a potential role in the early detection and monitoring of patients with breast cancer. The present study suggests that CTSK expression is not only correlated with metastasis but also related to the progression of breast carcinoma, and its overexpression could be potential prognostic factor for human breast carcinoma. The present study indicates CTSK as a potential molecule for diagnosis and therapeutic target for the treatment of breast cancer metastasis. However, none of the previous studies, as well as this study, determine whether this association has diagnostic value. If CTSK plays a critical role in breast cancer outcomes, then the future researchers need to focus on understanding how interventions can reduce the concentration of this bone resorption marker. In accordance with our study, if CTSK is a novel prognostic marker, then future studies are required to understand if it is responsive to drug and lifestyle interventions need to be designed to reduce the risk of skeletal metastasis in breast cancer women.

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Conflict of Interest: The authors do not have any conflict of interest in the manuscript.

Ethics Committee Approval: This study was approved by the ethics board of Saveetha Medical College & Hospital Chennai and Gleneagles Global Health City Hospital Chennai, India. (Date: 11.04.2015)

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