



Measurement of Alkaline Phosphatase Activity and Calcium Levels in Sudanese Patients with Breast Cancer

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Abstract

Breast cancer is the most common cancer affecting women in Sudan, it is characterized by metastatic pattern in liver and bone. Changes in serum levels of biochemical parameters especially alkaline phosphatase (ALP) may be valuable in diagnosis of breast cancer. This study was conducted to detect alkaline phosphatase activity and calcium level in Sudanese patients with breast cancer, who attended the Radiation and Isotope Center of Khartoum hospital during the period from March -May 2015.

In this retrospective, case-controlled, hospital-based study; 40 female patients with breast cancer (aged 25-85 years), and 40 healthy female control (aged 25-85 years) were enrolled in the study. A questionnaire containing demographic and clinical data was completed for every patient. Venous blood (2.5ml) was taken in a plane container from every volunteer. Samples for alkaline phosphatase and calcium were processed and analyzed using spectrophotometric method.

The present study showed that there was a highly significant increase in alkaline phosphatase activity in patients compared to control ($P < 0.01$), whereas insignificant increase in calcium level in patients compared to healthy control ($P > 0.05$). Thus, it was concluded that analysis of serum alkaline phosphatase and calcium in patients with breast cancer may help in monitoring metastasis, and to follow up of treatment response.

Key words: Breast cancer, alkaline phosphatase, calcium

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Introduction

Among females, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death (Freddie *et al.*, 2018; Jemal. *et al.* 2011). There are several types of breast cancer, include noninvasive breast cancer which may be ductal carcinoma *in situ* (DCIS), in which a malignancy involving the ductal system of the

breast that is wholly confined within basement membrane (Malone *et al.*, 1993). Another type is invasive breast cancer (secondary or metastatic breast cancer), in which the cancer cell spreads from the ducts or lobules into surrounding breast tissue, lymph node in the armpit or to other areas of the body such as bones, liver or lung. No single agent has been found to cause the disease, but a

number of predisposing factors have been identified, such as geriatric factors (Allen *et al.*, 2009), genetic and family history (Bishop *et al.*, 2010), which includes menstrual cycle, pregnancy history, breastfeeding, overweight, history of breast cancer, oral contraceptive use and ionized radiation, alcohol, smoking, lack of exercise, race, chemicals used in cosmetic, food, water and plastic (Singh *et al.*, 2013).

Breast cancer is characterized by a distinct metastatic pattern mostly in liver and bones and leads to elevation of some biochemical metabolites such as alkaline phosphatase (ALP) and calcium. Alkaline phosphatase belongs to a group of enzymes that catalyzes the hydrolysis of various phosphor-monoester at an alkaline pH to liberate inorganic phosphate from the organic phosphate ester with the concomitant production of an alcohol. It is present in high concentration in intestine, liver, bones, spleen, placenta and kidneys (Singh *et al.*, 2013).

Elevation of alkaline phosphatase level is frequently associated with a variety of diseases such as extra hepatic bile obstruction, intrahepatic cholestasis, hepatitis, cirrhosis, cancer, and bone disorder. Some studies found that women with breast cancer have alkaline phosphatase activity higher than normal healthy women and the progressive increase in serum alkaline phosphatase activity with breast cancer is an indication of metastasis (Kamal Eldin, 2007).

Calcium is one of abundant mineral in the human body. Ninety nine percent of the total calcium in the body is found in bones and 1% is found extracellular in soft tissue and plasma (Ferro and Roberto, 2012). The calcium ion is an essential structure component of skeleton and play a key role in muscle contraction, blood coagulation, enzyme activity, neural excitability, secondary messenger hormone release and membrane permeability (Ferro and Roberto, 2012).

Methods

This study was conducted to detect alkaline phosphatase activity and calcium level in Sudanese patients with breast cancer during the period from March 2015 to May 2016 who attended the Radiation and Isotope Center of Khartoum hospital. Forty female patients with breast cancer (aged 25-85 years), and 40 healthy female control (aged 25-85 years) were enrolled in this study. Patients with myocardial infarction, liver disease, pancreatic disease, diabetes mellitus were excluded from the study.

Informed consent was obtained from each participant and protocol was approved by Omdurman Islamic University, Radiation and Isotopes Center Khartoum and Ministry of health, and conducted in accordance with international ethical guidelines.

Venous blood samples, (2.5 ml) each, were obtained from participants using standard vein puncture

technique, blood specimens were collected in plain blood containers, allowed to clot at room temperature for 30 minute and centrifuged at 500 x g for 5minutes to obtain the serum. Then serum was separated and preserved at- 20 °C until analysis of sample.

To monitor the reliability of result, control sera with known alkaline phosphatase and calcium values were run with samples. Alkaline phosphatase activity was measured by enzymatic method using

photometer 4040. The kit supplied by Biosystems (Spanish, Cat. NO 11805).Calcium was measured by chemical method using Med Lab Plus; the kit supplied by Biosystem (Spanish, Cat. NO 11583). Data were analyzed using statistical package for social sciences (SPSS) software (version 11.5).A p value <0.05 was considered statistically significant.

Results:

General characteristics of the participants were shown in table (1)

Table (1): Characteristic of study subjects

Variables		Control (n= 40)	Patients (n= 40)	Percentage (%)
Age	(years)	34. 3± 11. 4	46. 1± 13.1	-
Stages	Stage1 Stage 2 Stage 3	- - -	5 25 10	12.5% 62.5% 25%
Menopausal	Premenopausal Postmenopausal	- -	19 21	47.5% 52.5%
Cancer site	Left Right	- -	18 22	45% 55%
Metastasis	With Without	- -	3 37	7.5% 92.5%
Treatment	Untreated Hormonal Surgical Chemotherapy	- - - -	4 30 5 1	10% 75% 12.5% 2.5%

As shown in table (2), ALP was found to be significantly higher in patients compared to control (P=0.01), whereas calcium showed insignificant difference between patients and control (P=0.05), although women with breast.

Table (2) Comparison between controls and patients regarding ALP and calcium level

Variables	Control	Patient	P. value
ALP(U/L)	48.15	99.39	0.01**
Calcium (mmo/l)	2.12	2.26	0.05 ^{ns}

** : significant at 0.01 level of probability, ns: no significant difference

Regarding age factor, it is obvious that ALP activity decreases significantly in patients with the increase of age ($P=0.03$); whereas calcium level is not ($P=0.246$)

Table (3): Age factor regarding ALP activity and calcium levels (using “Duncan’s New Multiple Range Test”

Age (years)	ALP (U/L)		Calcium (mmol/l)	
	Patients	Control	Patients	Control
25- 40	107 ^a	46.92 ^c	2.18 ^a	2.13 ^a
41-55	98 ^{ab}	52.02 ^c	2.30 ^a	2.14 ^a
>55	88.0 ^b	53.07 ^c	2.30 ^a	2.01 ^a
	0.03 ^{**}		0.246 ^{ns}	

Means within the Column which having similar letters are not significantly difference between level of probability according to DNMRT,

** : significant at 0.01 level of probability,

Table (4): Showing ALP activity and calcium levels regarding the stage of breast cancer

Stage of disease	ALP(U/L)	Calcium (mmol/l)
Stage1 (n=5)	48.00	2.30
Stage 2 (n=25)	104.75	2.23
Stage 3 (n=10)	111.69	2.31
P-value	0.22 ^{ns}	0.81 ^{ns}

Comparing premenopausal and postmenopausal phases regarding the activity of ALP and calcium levels, showed no significant difference concerning both ALP ($P= 0.25$) and calcium level ($P = 0.44$), (table 5).

Table (5): Level of ALP and calcium as affected by menopausal of breast cancer patients

Variables	Premenopausal	Postmenopausal	P. value
ALP(U/L)	113.1	87.0	0.25 ^{ns}
Calcium(mmo/l)	2.21	2.30	0.44 ^{ns}

As shown in (table 6), metastasis has no role concerning ALP activity or calcium levels ($P=0.07$) and ($P=0.88$) respectively.

Table (6): Showing ALP activity and calcium levels in patients with metastasis

Variables	With metastasis	Without metastasis	P. value
ALP(U/L)	77.4	117.4	0.07
Calcium(mmo/l)	2.27	2.25	0.88

As shown in (table7), both ALP activity and calcium level insignificantly affected by treatment of breast cancer, but untreated patients reported higher mean of ALP activity, followed by hormonal treatment, chemotherapy and finally surgical, while the highest level of calcium was obtained by surgical, followed by chemotherapy, untreated women and hormonal treatment.

Table (7): Effect of treatment on ALP activity and the calcium levels using “Duncan’s New Multiple Range Test”.

Parameters	ALP (U/L)	Calcium (mmol/l)
No treatment	108.8a	2.25a
Chemotherapy	77.2a	2.27a
Hormonal	98.0a	2.20a
Surgical	57.3a	2.36a
P. value	0.503ns	0.954ns

As shown in (table 8), no correlation was found when comparing the three parameters (age, ALP activity, and calcium levels) with each other.

Table (8): Showing no correlation when comparing the three parameters (age, ALP activity, and calcium levels)

Variables	Coefficient	Age	ALP	Calcium
Age	r	1.000	- 0.096 ^{ns}	0.215 ^{ns}
	P	-	0.555	0.183
ALP (U/L)	r	-	1.000	0.187 ^{ns}
	P	-	-	0.248
Calcium (mmol/l)	r	-	-	1.000
	P	-	-	-

ns: no significant correlation

Discussion

Breast cancer mostly cancer affects women in Sudan. Generally, in order to improve breast cancer consequences and ensure survival, early detection is crucial. Early detection approaches are screening and early diagnosis. When breast cancer cells invade bones, they cause an osteolysis lesion which

increases alkaline phosphatase and calcium in blood, hence alkaline phosphatase and calcium may help in monitoring the stage of breast cancer.

The present study was primarily designed to measure the calcium level and ALP activity in Sudanese patients suffering breast cancer compared to healthy individuals. In this study, 40 females with

breast cancer (aged 25-85 years), in addition to 40 healthy females(aged 25-85 years) were enrolled as control. This study revealed that, mean alkaline phosphatase activity was markedly elevated in patients with breast cancer compared to the control ($P < 0.01$). These findings agreed with the results obtained by Nathaniel *et al.* 2010 and Singh *et al.* 2013. Although some studies have reported high sensitivity of ALP for bone and overall metastases detection, but these studies use specific isoenzymes in addition to total ALP, which reported by Keshaviah *et al.*, 2007. Also, our study revealed that alkaline phosphatase activity was found to increase significantly with the decrease of patients ages, while there was no significant difference in control, these findings agreed with the results obtained by Nathaniel *et al.* 2010. The increased activity of this enzyme observed in patients enrolled in our study may be due to osteolytic bone metastases in breast cancer leading to increased osteoclastic activity and bone resorption.

In addition to that the present study found increased ALP activity in the advanced stage of breast cancer patients. These findings agreed with the results obtained by Singh *et al.* 2013, Amritpal Kaur in 2015 who found that serum ALP activity was significantly increased with the progress of stage of cancer and the maximum increase being observed at stage IV, and Chan dracket *et al.* 2016 serum ALP

levels increased significantly as the stage of the cancer progressed.

Regarding treatment of patients on ALP activity: This study revealed that alkaline phosphatase showed insignificant decrease for all treatments type but after surgery showed low one. These findings agreed with the results obtained by Chandrakanth *et al.* 2016. This may be due to removal of cancer tissue. But disagree with Keshaviah *et al.* 2007 and Prabasheela *et al.* 2012; they reported an increased ALP activity in some of the cases after surgery, which might be due to recurrence.

The present study showed that calcium levels are insignificantly increased in patients compared with control ($P = 0.05$). Some studies such as that carried out by Nathaniel and his colleagues (2010), reported a significant increase in serum levels of calcium. The hypercalcemia in breast cancer has been attributed in part to osteolytic bone metastases and this account for 20-30% of the hypercalcemia cases in oncology patients. The skeletal invasion and destruction by tumor induced by tumor production of various cytokines such as transforming growth factor- α (TGF- α), tumor necrosis factor α (TNF- α), TNF- β , interleukin-1 and interleukin-2, leads to increasing bone osteolysis and modification of their absorption, excretion and resorption of calcium and phosphate ion (Francini *et al.*, 1993).

Conclusion

From this study it could be concluded that alkaline phosphatase activity increases significantly in patients with breast cancer, but unlike calcium level which showed no significant increase. The increase in the serum ALP activities with breast cancer is an indication of metastasis. Therefore, measurement of ALP activities could be valuable in monitoring of metastasis and follow up of treatment response. However, more studies (better if cohort study), are required including more sample size to determine the role of ALP and calcium in monitoring breast cancer.

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