Vitamin D and bone metabolism in breast cancer patients in Karachi, Pakistan

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Abstract: Breast cancer is one of the common types of malignancy worldwide and in Pakistan. The heterogeneous disease itself and its complex treatment leads to various bone-affecting complications that make breast cancer patients more vulnerable to bone fractures. Vitamin D deficiency among these women worsens the condition and promotes breast cancer growth. Thus, the purpose of the study was to assess serum levels of 25- hydroxyvitamin D (25OHD) and bone markers in women suffering from breast cancer. Serum levels of 25OHD, alkaline phosphatase (ALP), bone specific ALP, calcium (Ca), phosphorus (P), magnesium (Mg), albumin (Alb) and beta carboxyl terminal collagen crosslink (β-CTx) were analyzed in 201 histological diagnosed patient volunteers from breast cancer clinic. Vitamin D insufficiency was present among the total study population and deficiency was particularly observed among women with metastases. These patients had significantly increased serum levels of β-CTx and bone specific ALP when compared with the non-metastatic group. No significant difference was observed in other biochemical parameters. A weak correlation between serum levels of 25OHD and β-CTx was observed. Therefore, monitoring of serum levels of 25OHD and bone markers at the time of diagnosis and during the course of treatment will endeavor a better overall health status.

Keywords: Breast cancer, beta carboxyl terminal collagen crosslink, bone markers, vitamin D.

INTRODUCTION

The active form (1,25 dihydroxy vitamin D) of vitamin D is known for its important hormonal role in homeostasis of calcium and phosphate by binding to vitamin D receptors (VDR) present in kidney, parathyroid gland, bowel and bone (Bikle, 2014). It is well established now that vitamin D is also produced by other organs including breast, colon, prostrate and it is responsible for the paracrine actions of vitamin D (Ben-Eltriki *et al*., 2016). Due to the paracrine actions of vitamin D, scientists have elucidated its significance in various cancers like prostate, colon and breast cancer (de La Puente- Yague *et al*., 2018).

Studies have elaborated that vitamin D has anticancer activity (Vanoirbeek *et al*., 2011: Yao *et al*., 2017). It has been studied *in-vitro* that active form of vitamin D halted the expansion of cancerous cells resulting in cell death (Diaz *et al*., 2015). In addition to direct inhibition, the active form of vitamin D slows down cancer cell growth via bone environment playing an important role in bone metastasis (Imtiaz and Siddiqui, 2014). Vitamin D deficiency increases bone resorption and accelerates breast cancer cell growth which lead to bone metastasis (Maier *et al*., 2015). Studies have shown that decreased serum levels of 25OHD is associated with poor diagnosis and outcome of therapy (McDonnell *et al*., 2018). Recently bone turnover markers are being used to monitor bone metabolism in breast cancer patients during the course of treatment (Greenblatt *et al*., 2017). The importance of vitamin D in bone metabolism makes it clinically significant to be monitored in breast cancer patients. Evaluation of bone markers is useful to investigate the overall bone health status; the classification of these markers is based on the activity to which they correspond, which is osteoclastic or osteoblastic (Kanis and McCloskey, 1997).

Pakistan has been reported to have about an alarming 23% occurrence rate of breast malignancy (Sarwar and Saqib, 2017). However, rather few studies have focused on the serum levels of 25OHD in breast cancer patients and none encompasses the description of vitamin D and bone markers (Imtiaz *et al*., 2012, 2014; Shaukat *et al*., 2017). The present study elucidated the levels of 25OHD in serum and its relationship with bone markers in women having breast cancer with and without metastasis. This would help to clinically improve the vitamin D levels among patients and their overall bone health status.

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MATERIALS AND METHODS

A cross-sectional study was carried out at Aga Khan University Hospital (AKUH). Approval was obtained from the Ethical Review Committee and diagnosed 201 breast cancer patients were recruited from breast cancer clinic after obtaining their written consent. Inclusion criteria was women ≥ 18 years of age, without chronic disease, secondary hyperparathyroidism, who have not received any vitamin D supplement for last three months and are resident of Karachi for last 5 years. Data related to demography, prognostic markers and metastasis was obtained from medical records of AKUH. Extensive detail related to breast cancer risk including alcohol consumption, smoking, number of abortions, and family history of breast cancer was obtained by interview.

Approximately 7 ml of blood was drawn to separate serum, and aliquots were stored at -80°C until the analysis of biochemical parameters was done. The parameters include phosphorous (P), magnesium (Mg), calcium (Ca), albumin (Alb), and alkaline phosphatase (ALP) were estimated by Advia 2400, Siemens. Serum 25-hydroxy vitamin D (25OHD) was analyzed by electro-chemiluminescence on Liason, Siemens. Bone specific alkaline phosphatase (BALP) and beta carboxyl terminal collagen crosslink (β-CTx) were determined by electro-chemiluminescence on E-170, colorimetric method and ELISA respectively.

Analysis for both descriptive and inferential data was done via SPSS for Windows 15.0; (SPSS Inc., Chicago, IL). The comparison of the mean and standard deviation for the continuous variables between metastatic and non-metastatic groups was done by using an independent sample t-test. Correlation of serum vitamin D (25OHD) with Ca, P, Mg, BALP, albumin, CTx, was performed by Pearson correlation.

RESULTS

In current study, a total of 201 histological diagnosed breast cancer women having average 52 years of age and BMI 26 kg/m^2 were recruited. Among them, 18 (9%) were underweight, 46 (23.1 %) were normal, 48 (24.1%) were obese while 87 (43.7%) of them were overweight. The majority were married, multi para, showed lowest rate of abortions and had irregular menstrual cycle; only 26% of study population had family history of breast cancer and none of them admitted to consuming alcohol at any point in life. Invasive ductal carcinoma was the most prevalent (87.6%) histological type observed. More than 50% study population was found ER/PR+/Her2+ and belonged to tumor grade II while only 7% showed metastasis. Patient characteristics are illustrated in table 1**.**

Most of the study population was observed to have vitamin D insufficiency (< 30ng/mL) fig. 1. Females with metastatic breast cancer showed significantly low levels of 25OHD (almost 18.4 ng/dL) in comparison to the non-metastatic group that had nearly 29.61 ng/dL. Significantly increased bone specific ALP and β-CTx levels were found in serum of females with metastasis when compared to those without metastasis; albumin was significantly lowered in metastasis group (table 2). According to fig. 2 (A and B), there was no significant correlation observed between vitamin D and bone specific ALP via Pearson correlation but β-CTx showed a weak inverse trend with serum 25OHD. Other biochemical parameters were found to be normal and without any significant difference upon stratification (table 2).

Women with tumor grade 1 had significantly increased levels of serum 25OHD, Mg and P as compared to tumor grade II and III as shown in table 3. However, no significant difference was observed in the serum levels of other bone markers.

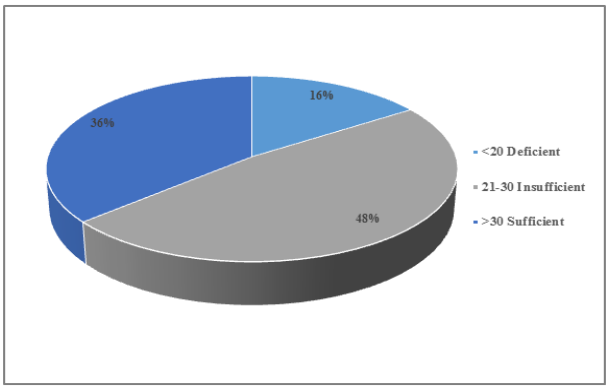

Fig. 1: Frequency of Vitamin D (ng/dL) in Study Population

DISCUSSION

The majority of the population in Pakistan tends to be vitamin D deficient even though they have enough sunlight exposure (Imtiaz *et al*., 2012). Pakistan is also reported to have high heterogeneity of breast cancer (Idrees *et al*., 2018). The main objective of the study was to determine the correlation between serum 25OHD level and bone markers in females with breast cancer in Karachi, Pakistan.

The study population's BMI was calculated to be 26 ± 5.2 which supports the earlier finding of increased weight being a risk factor for breast cancer (Awatef *et al*., 2011). However, weight gain cannot be established as one of the comorbidity of breast cancer. It has been reported in a cross-sectional study that increased plasma 25OHD level is correlated with low risk of breast cancer with an average 22 BMI (Deschasaux *et al*., 2016; Bani-issa *et al*., 2017). Majority (87.6%) of the females in this study were diagnosed with Invasive Ductal Carcinoma (IDC) which is reported to be the most common type of breast cancer

Table 1: Descriptive statistics and characteristics of breast cancer patients

S. No	Descriptive Statistics	Mean ± SD	
1	Age (year)	52 ± 12	
2	Weight (kg)	66 ± 13	
3	Height (ft)	5.3 ± 0.3	
4	BMI (kg/m^2)	26 ± 5.2	
	Characteristics of Patients		
	Variables	Female (n)	Percent Frequency (%)
5	BMI		
	<18.5 kg/m^2 (Underweight)	18	9
	18.5-23 kg/m^2 (Normal)	46	23.1
	23.1-30 kg/m^2 (Overweight)	87	43.7
	>30 kg/m^2 (Obese)	48	24.1
6	Marital Status		
	Single	24	11.9
	Married	177	88.1
7.	Menstrual History		
	Regular	66	32.83
	Irregular	135	67.15
8	Family History		
	Present	52	26
	Not Present	149	74
9	Smoking History	1	0.5
10	Alcohol Intake	0	0
11	Abortions Experienced		
	0 – 2 abortions	187	93.0
	3 – 5 abortions	12	6.0
	6 – 9 abortions	2	1
12	Parity Status		
	Nulli para	41	20.4
	Primi Para	13	6.5
	Multi Para	137	68.2
	Grand Multi Para	10	5
13	Receptor Status		
	ER/PR+, Her2+	103	51.2
	ER/PR+, Her2-	17	8.5
	ER/PR-, Her2+	28	13.9
	ER/PR-, Her2-	53	26.4
14	Tumor Grade		
	Grade I	37	18.4
	Grade II	118	58.7
	Grade III	46	22.9
15	Metastasis Present	14	7

in Pakistan (Kakarala *et al*., 2010). Previous studies have established that increased serum levels of 25OHD have beneficial effect and its association with tumor characteristics (Yao *et al*., 2017). The data show that females with tumor grade I have higher levels of 25OHD as compared to females with high tumor grades. This is in conflict with the study conducted by Hatse and colleagues which reported no correlation between serum levels of 25OHD and tumor characteristics (Hatse *et al*., 2012).

The present data indicate that majority of the breast cancer women were 25OHD insufficient while women with metastasis were vitamin D deficient with 18 ng/mL of 25OHD levels which is consistent with the previous studies (Imtiaz, *et al*., 2012; Imtiaz and Siddiqui, 2014; Younus,*et al*., 2016). The active vitamin D, (1, 25[OH] $_{2D}$), has multiple roles in prevention and development of breast cancer (Riaz, *et al*., 2016). This occurs through active vitamin D forming a complex with the VDR, thus promoting cell cycle regulation, differentiation, increasing cell to cell adhesion, DNA damage protection, and suppression of inflammation. Metabolism of vitamin D mediated by CYP11A1 results in the formation of vitamin D metabolite, 25OHD that has same effects as of active vitamin D (McDonnell *et al*., 2018).

Table 2: Bone markers in breast cancer patients with and without metastasis

S. No.	Groups	Age (year)	BMI (kg/m^2)	Calcium (mg/dL)	Magnesium (mg/dL)	Phosphorus (mg/dL)	Albumin (g/dL)	Vitamin D (ng/dL)	ALP (U/L)	Bone specific ALP (µg/L)	β-CTx (pg/mL)
1	Metastasis	56.3± 10.7	24.9± 4.6	7.9± 1.04	1.7± 0.28	3.2± 0.79	3.1± 0.44*	18.4± 5.51*	77.7± 34.2	20.9± 7.91*	629.2± 391.6*
2	Non-Metastasis	51.6± 12.29	26.03± 5.26	7.8± 0.8	1.8± 0.35	3.2± 0.7	3.5± 0.44	29.61± 9.18	85.6± 50.46	15.6± 6.97	365.6± 245.9

Results are expressed as average ± SD. Statistical significance is indicated by *= $p<0.05$.

Table 3: Bone markers in breast cancer patients with different tumor grades

S. No.	Groups	Age (year)	Calcium (mg/dL)	Magnesium (mg/dL)	Phosphorus (mg/dL)	Vitamin D (ng/dL)	ALP (U/L)	Bone Specific ALP (µg/L)	Albumin (g/dL)	β-CTx (pg/mL)
1	Grade I	47.3± 14.5	7.91±1	3.51±0.76*	1.93±0.29*	30.4± 10.2*	86.9±34.8	16.2±7.78	3.57±0.41	357.7±268.3
2	Grade II	52.2± 11.8	7.85± 0.9	3.11±0.67	1.79±0.33	27.5± 10.7	88.4±58.7	16.8±11.8	3.51±0.47	365.7±262.1
3	Grade III	55.3± 9.79*	7.96± 0.75	3.22±0.66	1.82±0.42	24.1± 12.6	74.8±28.3	15.6±6.34	3.57±0.38	394.1±235.0

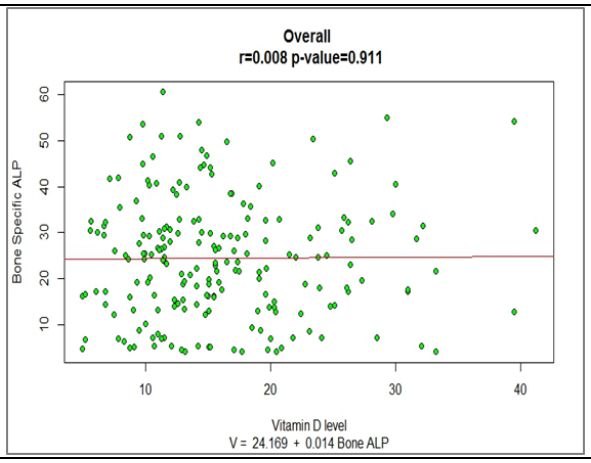

Fig. 2(A): Correlation of Vitamin D (ng/dL) with Bone Specific ALP (µg/L)

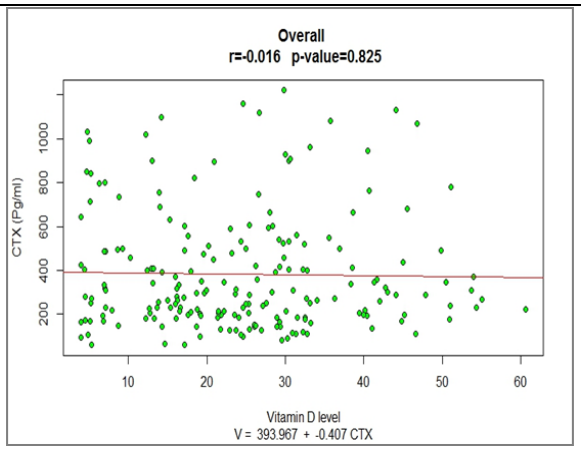

Fig. 2 (B): Correlation of Vitamin D (ng/dL) with β-CTx (pg/mL)

Breast cancer prevalence, its timely diagnosis and treatment is a worldwide challenge (Zhang *et al*., 2017). Many breast cancer patients are not diagnosed sufficiently early, and thus suffer not only from the disease but also from the side effects of the treatment they receive. Chemotherapy and radiation have a detrimental impact on patients' health including bone loss and fractures (Melton *et al*., 2012; Fraenkel *et al*., 2015). Decreased level of vitamin D is associated with breast cancer risk and bone resorption (Yao *et al*., 2011; Imtiaz *et al*., 2012; Yao *et al*., 2017).

In this study, 25OHD levels and bone markers were compared in breast cancer patients with and without metastasis. The serum levels of bone specific ALP and β-CTx in patients having metastasis were almost twice the levels of same markers in group of patients without metastasis, showing a prominent difference ($p<0.05$) between the two (table 2). This indicates that females with metastatic breast cancer having increased bone turnover, could be due to the metastasis or the effect of the therapy received (Salem *et al*., 2007; Zulauf *et al*., 2014). Similarly, a weak inverse trend was observed in between β-CTx and 25OHD though which was not significant (fig. 2B) but highlighting the objective of the study that decrease serum level of 25OHD increase bone resorption**.** However, a study by Lipton and colleagues suggested that there was no difference observed in serum levels of β-CTx in breast cancer patients with pretrial chemotherapy versus those who did not (Lipton *et al*., 2011). Interestingly, there was no correlation observed between serum 25OHD and bone specific ALP (BALP) in the present study (fig. 2A) but the same correlation curve indicates that most of the study population appeared in vitamin D deficient region (>20 ng/dL) with increased levels of BALP which was also confirmed by table 2 that

shows patients with metastasis had decreased vitamin D and increased BALP levels.

CONCLUSION

The study suggests that breast cancer patients with metastasis in Pakistan tend to have high prevalence of vitamin D deficiency. Moreover, increased bone metabolism in women with metastasis is indicated by elevated serum levels of β-CTx and BAP. Thus, present study emphasizes the clinical significance of monitoring the serum levels of 25OHD and bone turnover makers during the course of treatment especially for women with metastasis.

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