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Impact Of Vitamin D Deficiency On Breast Cancer

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Abstract

Breast cancer was found to be the most recurrent cancer among women. The study was taken to find out the effect of body composition, vitamin D and other associated biochemical factors for breast cancer among women in their reproductive age groups in connection with the mammographic density. Newly diagnosed subjects with breast cancer and subjects without breast cancer were selected for the study. Mammographic densities of subjects were taken from Hospital Information System through picture archiving and communication system. Human body composition and biochemical analysis were found out using universally acceptable techniques and formulae. The research revealed that certain factors like age, body composition, and biochemical factors like calcium, phosphorus along with vitamin D had a great influence on breast cancer.

Introduction

A rise in cases of breast cancer is undeniably a matter of anxiety in Kerala. Earlier, cervical cancer was on the top of the registered cases of cancers among women. Now breast cancer has taken up the position. It was reporting in generally that every third women who approaches for oncology consultation has breast cancer. In Kerala, breast cancer is more prevalent among women in their thirties. While breast cancer frequency is high among women in more urbanized regions, frequency is growing in approximately every region worldwide (World Health Organisation, 2018). About 70% of the world's cancer cases is predicted to be in developing countries, with India in the fifth position. Breast cancer is fixed to go beyond cervical cancer as the usual form of cancer among all females, by 2020 (Shetty, 2012).

As per the World Health Organisation (2018), breast cancer is the top cancer in women both in the developed and the developing world. The prevalence of breast cancer is growing in the developing world as life expectancy increased, urbanization increased and the adoption of western standard of living (THE TIMES OF INDIA, 2018). Mostly breast cancers are identified in women above 50 years of age, but its' not vivid that some women were only getting breast cancer (women with no risk factors) but others were not (women with risk factors).

Usually, breast cancer develops in either the ducts or in the lobules. Cancer can also be found, less often, in fatty tissue or the fibrous connective tissue in the breast. Breast cancer is an ailment that happens when cells in breast tissue changes (or mutate) and starts replicating. These unusual cells typically gather together to form a tumour. When these atypical cells conquer other parts of the breast or when they spread (or metastasize) to other parts of the body through the bloodstream or lymphatic system, the tumour converts into cancerous (or malignant).

In recent years, the dietary risk factors with special reflection to vitamin D have increased much prominence. Many researches had recommended about the protective procedure of vitamin D against breast cancer by autocrine/paracrine manner. From i*nvitro* studies on animals, it was observed that calcitriol, the hormone form of vitamin D employs pro-apoptotic, multiple anti-proliferative and pro-differentiating activities on various cancerous cells and prevents tumor growth . Obstruction in cell proliferation and increase apoptosis in cancer cells culture by vitamin D3 and its analogues were proved through laboratory experiments. Many clinical and preclinical studies support the hypothesis that decreased levels of vitamin D are associated with a high risk of breast cancer. Facts from both experimental and epidemiological studies advocate a relationship of vitamin D deficiency with breast cancer prevention and survival.

Vitamin D deficiency is found to be very common in India. Winter season could be seen in many parts of India. But many parts of South India had abundance sunshine throughout the year. Still South Indians were suffering from Vitamin D deficiency. But vitamin D is termed as 'Sunshine Vitamin'. Many people from the south were suffering from this deficiency due to various reasons. People are avoiding sun for many reasons. Among city dwellers, people are spending most of their time in air conditioned rooms and whenever they step out, move to the air conditioned car in the basement park and get into other air conditioned space. Also kids are abandoned from playing outside, in fear of pollution and other factors. The fairness angle also plays a pivotal role in avoiding sunlight as everyone wants to be fairer or prevention from tanning (Shravan, 2017).

Besides vitamin D, mammographic density is also a risk factor for breast cancer. In a mammogram, fat looks radiologically lucent and seems dark. Epithelial and connective tissues remain radiologically dense which appears light in colour. Mammographic density is nowadays considered to be one of the major independent predictor of breast cancer and contributory factor in decreasing the mammographic sensitivity. Today radiologists compare the previous mammograms with the present mammogram and detect whether any changes are present. This would help them to indicate the onset of cancer. If mammographic density is included as a criterion in screening programme for choosing women who want extra examination it would be more effective rather than it could increase the detection rates for cancer (Kavanagh *et al.,* 2008). Many times dense breast tissue renders breast cancers low sensitive to screen detection, leading to greater chances of breast cancer in the negatively screened mammograms earlier.

The present study aims to find out the relation between the impact of vitamin D deficiency on breast cancer in relation with the associated biochemical parameters and body composition.

Objectives

1. The present study was aimed to find out the impact of vitamin D levels and the associated biochemical parameters on breast cancer.
2. The study was analysing the effect of body composition on breast cancer in relation with vitamin D.

METHODOLOGY

Selection of area and sampling

The study was conducted in Kerala, one of the Southern states of India, in association with Amala Institute of Medical Sciences (AIMS), Thrissur, Kerala, India. Newly diagnosed breast cancer patients in reproductive age groups registered in AIMS for different treatment modalities and who were willing to provide informed consent formed the subjects for the study. Green *et al.,* (2010) indicated that the association between mammographic density and breast cancer is independent of plasma vitamin D metabolites among post-menopausal women. Hence post-menopausal women were excluded from the study.

Study Design

The design of the study was prospective case control design.

Sample Size

About 178 females were diagnosed annually with BCA in Cancer registry. Considering this as the baseline data and after providing 95 per cent of confidence interval at 5 per cent level of significance and ±10 per cent margin of error, the sample size for the study was calculated by using Rao Soft online sample size calculator and was

fixed as 73 newly diagnosed BCA subjects rounded off to 75 newly diagnosed BCA subjects. Fox *et al.*, (2009) indicated that it is costly and unethical to have a large sample in biomedical research. Forty percent of the total sample size i.e., thirty female subjects aged ≥30 years without any chronic communicable or diet related non-communicable diseases and metabolic disorders formed the control subjects for the study.

Tools and techniques

Data collection chart approved by the Institutional Review Board (IRB) of Hospital was used to collect the data related to mammographic density and histopathology of the disease. International Union of Pure and Applied chemistry (IUPAC) approved biochemical methods were used to analyse biochemical variables such as serum vitamin D, serum calcium, serum phosphorus, serum iron, serum ferritin, serum Parathyroid Hormone (PTH) and serum estradiol.

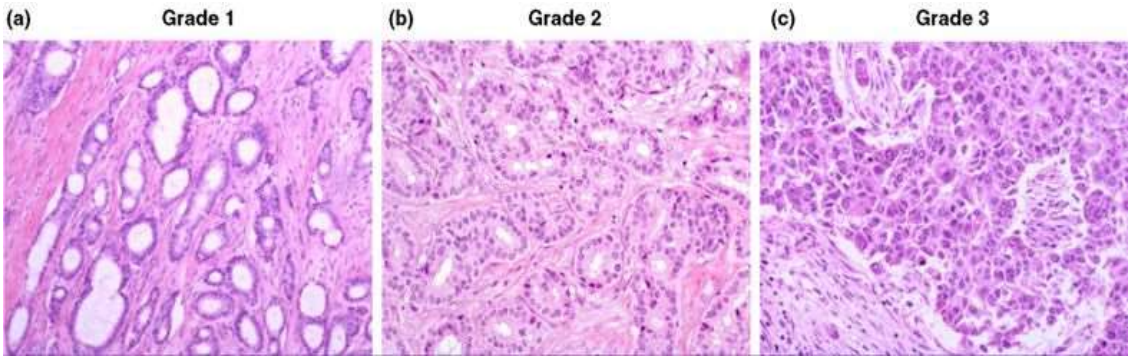
Assessment of mammographic density of the subjects

Mammographic densities of the selected subjects were taken from Hospital Information System of Amala Institute of Medical Sciences through picture archiving and communication system (PACS). In order to assess the mammographic densities of the subjects who formed the control, three breast cancer screening camps were conducted and mammograms were taken.

A, B, C, D categorization of mammographic density by Breast Imaging Reporting and Data Systems (BI-RADS) of the American College of Radiology (ACR) otherwise classified as Type I, Type II, Type III and Type IV (D'Orsi *et al.*, 2013) was used.

Assessment of histopathological profile of the subjects

Histopathology of the tumor cells were done by using hematoxylin-eosin-stain and the tumor cells were graded following Nottingham grading system (Elston and Ellis,1991) as given in Figure 1 and Table 1 below:



(Ref: Rakha *et al*., 2010**)**

Fig. 1 Histological grade of breast cancer as assessed by the Nottingham Grading System

Table 1 Grading of histopathological profile of the subjects

Grade	Details of histopathological profile
I	**Low grade or well differentiated,** have relatively normal-looking cells that do not appear to be growing rapidly and are arranged in small tubules for ductal cancer and cords for lobular cancer. These cancers tend to grow and spread slowly and have a better prognosis (outlook).
II	**Intermediate/moderate grade or moderately differentiated,** lacking normal features, tend to grow and spread faster, and have a worse prognosis.
III	**High grade or poorly differentiated** in which cancer cells look very different from normal cells and are fast-growing.

Assessment of Body Composition

Body composition factors like BMR, body fat and total body water content were computed with verified and known equations. BMR is the quantity of the number of calories needed to accomplish the body's most basic functions, like breathing, circulation and cell production. Mifflin St. Jeor Equation (Mifflin *et al*., 1990) given below was used in the study to assess the BMR of the subjects.

BMR = 10 x weight (kg) + 6.25 x height (cm) – 5 x age (years) – 161)

Body Fat percentage of the subjects was calculated by using formula developed by Deurenberg *et.al*.(1991) which is based on the criteria of body mass index (BMI), age in years, and gender (S), (0 for female and 1 for male).

Body fat % = (1.2 x BMI) + (0.23 x age) - (10.8 x S) - 5.4

For women between ages 18-39, the overweight body fat percentage is between 34-39% and anywhere above 39% is considered obese. Women who ages 40-59, the overweight body percentage is 35-40% and anywhere over 40% is considered obese (Gallagher *et al*., 2000).

Total Body water

The percentages of body water stored in various fluid compartments, altogether known as total body water (TBW). Watson equation (Watson *et al.,* 1980) for calculating TBW, given below, was used for the present study.

TBW = -2.097 + 0.1069 * height (cm) + 0.2466 * weight (kg).

Data management and analysis

The data was entered in MS Excel (2016) edited and exported to SPSS (version 21). Descriptive statistics for the continuous variables were reported as mean ± standard deviation and categorical variables were summarized as frequencies and percentages.

The continuous variables were compared by Student's independent t-test or one-way ANOVA as applicable; while the categorical variables were compared by Chi-square test (Fisher's exact test will be used when expected counts are less than 5).

Ethical considerations

The Institutional Review Board and Ethical committee of Amala Institute of Medical Sciences, Thrissur approved the study. After giving personnel counselling, informed written consent in the subject's mother tongue was obtained from the subjects prior to the start of the study. As per Helsinki Declaration at most precaution was taken to protect the privacy of research subjects and the confidentiality of their personal information.

RESULTS AND DISCUSSION

Agewise distribution of Vitamin D status of Subjects

Distribution of subjects according to age based on Vitamin D status is displayed in Table 2 and Figure 2.

Table 2 Age wise distribution of Vitamin D status of Subjects

Category	Age * (years)	Normal D_3	Insufficient D_3	Deficient D_3	Total	$\chi 2$	*p*-value
Subjects with BCa (n_1=75)	<30	0	0	$1_{(1.9)}$	$1_{(1.3)}$	14.626	0.023**
	30-39	0	$3_{(16.7)}$	$15_{(28.3)}$	$18_{(24)}$		
	40-49	$2_{(50)}$	$14_{(77.7)}$	$35_{(66)}$	$51_{(68)}$		
	>50	$2_{(50)}$	$1_{(5.6)}$	$2_{(3.8)}$	$5_{(6.7)}$		
	Total	$4_{(100)}$	$18_{(100)}$	$53_{(100)}$	$75_{(100)}$	5.545	0.236
Subjects without BCa (n_2=30)	30-39	0	$6_{(35.3)}$	$1_{(25)}$	$7_{(23.3)}$		
	40-49	$7_{(77.8)}$	$10_{(58.8)}$	$3_{(75)}$	$20_{(66.7)}$		
	>50	$2_{(22.2)}$	$1_{(5.9)}$	0	$3_{(10)}$		
	Total	$9_{(100)}$	$17_{(100)}$	$4_{(100)}$	$30_{(100)}$		

**Numbers in parentheses indicate percentage* ***Significant at 5% level*

Table 2 pointed out that among subjects with BCa, majority of the subjects with deficient (60%), insufficient (77.7%) and normal (50%) serum vitamin D level belonged to the age group 40-49 years. Also about 50% subjects with normal vitamin D were above 50 years of age. Statistical analysis at 5% level significance (χ^2 = 14.626, p = 0.023) indicated that age had a significant impact on the vitamin D status. The prevalence of hypovitaminosis D among women about 25-35 years of age (Shivane *et al.,* 2011) and 20-50 years (Le Goaziou *et al.,* 2011) of age were reported.

Among subjects without BCa many of them were found to have insufficient serum vitamin D. Majority of the subjects with normal (77.8%) insufficient (58.8%) and deficient (75%) serum vitamin D belonged to 40-49 years of age. About 35.3% of subjects with insufficient serum vitamin D and 25% of subjects with deficient serum vitamin D belonged to age group, 30-39 years. Statistical analysis (χ^2 = 5.545, p = 0.236) did not indicate any influence between age and vitamin D among subjects without BCa.

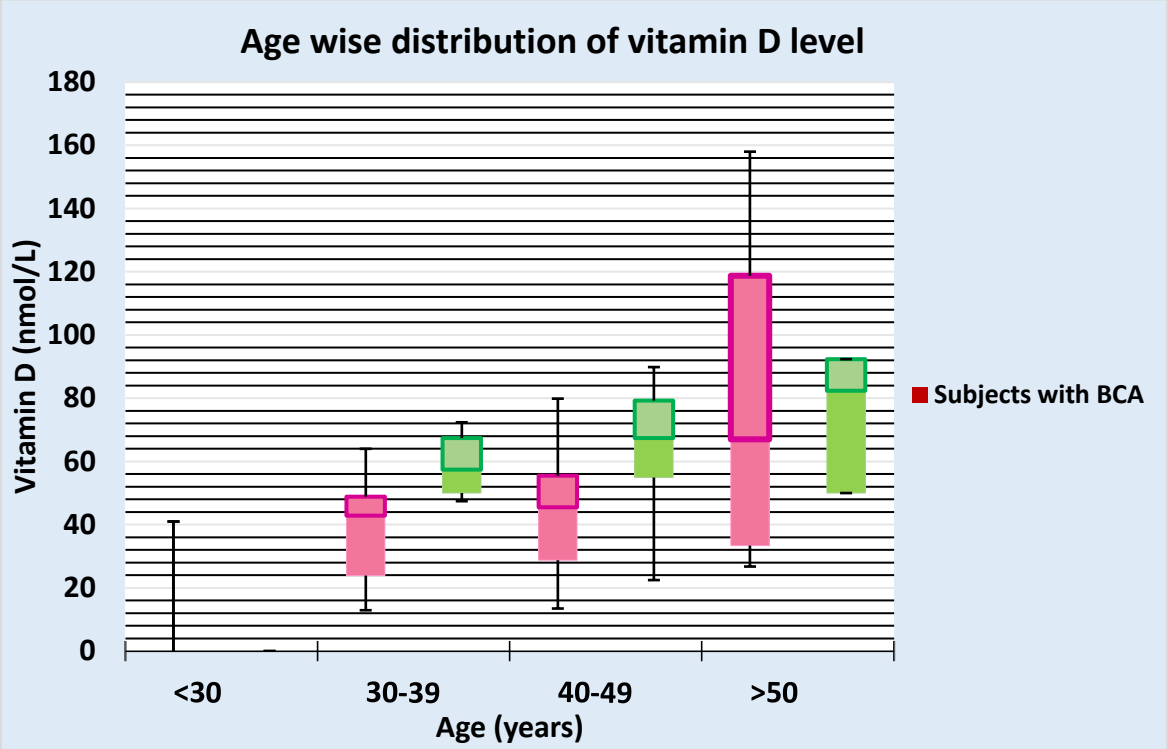


Figure 2. Age wise distribution of vitamin D level

The Figure 2 explains more about the age wise distribution of vitamin D level. Among the subjects with BCa the median value of Vitamin D for 30-39 years was found to be 42.85nmol/L with a minimum of 12.925 nmol/L and a maximum of 64 nmol/L. It was observed 45.5 nmol/L as median for 40-49years subjects with a minimum of 13.47nmol/L and a maximum of 79.87nmol/L. But among subjects without BCa, 57.41nmol/L was median among 30-39 years with a minimum of 47.42nmol/L and a maximum of 72.38nmol/L. Also, among 40-49 years, 67.39nmol/L formed to be the median with 22.46nmol/L as minimum and 89.85nmol/L as maximum.
The study showed that the vitamin D level of 30-49 years' age group among subjects with BCa was found to be low compared with others. The level of 25(OH)D3 was considerably lesser in breast cancer subjects than in controls was indicated in a study conducted among African-American and Hispanic units. The lowermost level of vitamin D was noted between 31 to 50 years of age group in African-American women (Wu *et al.*, 2017). A study was conducted by Goodwin *et al.*, (2009) in a potential initiation unit of 512 women with initial breast cancer diagnosed. Average age was found to be 50.4 years, with mean 25(OH)D as 58.1 ± 23.4 nmol/L. Vitamin D levels were lacking (< 50 nmol/L) in 37.5%, insufficient (50 to 72 nmol/L) in 38.5%, and sufficient (> 72 nmol/L) in 24.0% of subjects with breast cancer.

Histopathological profile of the subjects

Histology explains about the form of tissue where the breast cancer starts which can define the nature of cancer. Based on histology the cancers can be of ductal and lobular. Ductal carcinoma is a kind of cancer which forms in the lining of a milk duct. Lobular carcinoma occurs when cells in lobules of the breast, where breast milk is produced, are affected. If the cancer cells remain within the ducts or lobules it is non-invasive cancer or in situ, or if it spreads out of the ducts or lobules it is termed as invasive carcinoma.
A complete histology report comprises of tumour size, mitotic activity, lymph node status, nuclear grade lymphatic and vascular invasion. Histopathological report indicates the degree of aggressiveness of the disease and hence has a vital role in defining the treatment strategy.
Histopathological profile of subjects with breast cancer is presented in Table 3.

Table 3 Histopathological profile of the subjects with breast cancer

Histopathological variable	Classification	Details of subjects*	Total
Histology type (n=75)	Ductal	64(85.3)	75(100)
	Lobular/ Special type	11(14.7)	
Tumour stage at diagnosis (n=75)	I	6(8)	75(100)
	II	18(24)	
	III	51(68)	
Histologic grade (n=47)	1	8(17)	47(62.67)
	II	32(68.1)	
	III	7(14.9)	
Estrogen receptors ER (n=47)	Positive	27(57.4)	47(62.67)
	Negative	20(42.6)	
Progesterone receptors PR (n=47)	Positive	28(59.6)	47(62.67)
	Negative	19(40.4)	
HER2/neu (n=47)	Positive	22(46.8)	47(62.67)
	Negative	25(53.2)	
Depth of invasion (n=62)	pT1	10(16.1)	62(82.67)
	pT2	39(62.9)	
	pT3	9(14.5)	
	pT4	4(6.5)	
Lymph node metastasis (n=62)	pN0	17(27.4)	62(82.67)
	pN1	30(48.4)	
	pN2	9(14.5)	
	pN3	6(9.7)	

**Numbers in parentheses indicate percentage*

The present study showed that majority of the cases (85.3%) had ductal carcinoma. Li *et al*., (2003) stated that lobular carcinoma is found to be less common than ductal carcinoma. Also, Li *et al*., (2003) opined that, it was more difficult to identify ductal carcinoma by both physical examination and mammography. Virnig *et al.,* (2010) reported that there was 5.75per cent increase in the incidence of ductal carcinoma in situ during the period from 1975-2004 which can be attributed to increased rate of mammography screening (Claus *et al.,* 2001).

The prediction of breast cancer is strongly influenced by the stage of the disease ie, tumor size and the degree to which cancer has spread when it is initially diagnosed. Stage 0 being in situ (abnormal cells have not penetrated the ducts or glands from which they originated), stage I being early-stage invasive cancer, and stage IV being the most progressive disease. It was observed that majority (68%) of the subjects with BCa had tumour in Stage III at diagnosis, followed by 24% in stage II and 8% in stage I. The study results were on par with the findings of Agarwal and Ramakant (2008), that many patients with breast cancer were diagnosed at a relatively late stage in developing countries.

Histological grade represents the morphological assessment of tumour, biological characteristics and has been shown to be able to generate important information related to the clinical behaviour of breast cancers (Rakha *et al.,* 2010). The assessment of histologic grade, a combination of mitotic activity, tubular differentiation and nuclear features, is a significant component in the assessment of breast cancers and is a required pathologic parameter in diagnosing breast cancers. In addition to the details about subtypes of breast cancer, histologic tumor grade gives significant prognostic information about the disease (Ehinger *et al*., 2017). Majority of the subjects (68.1%) had their histologic grade II, followed by 17% in grade I and 14.9% in grade III.

Hormone receptor tests are very important as the results help to decide whether the cancer is probable to respond to treatments like hormonal therapy. Hormone receptor status revealed majority of them had positive progesterone receptors (59.6%) and estrogen receptors (57.4%). It was reported that for some subjects with breast tumours with greater intensities of a protein known as HER2/neu (human epidermal growth factor receptor 2). In the study, HER2 negative status was indicated by 53.2 per cent of the subjects.

Prognosis is better when cancer has not spread to the lymph nodes (lymph node-negative). The more lymph nodes that contain cancer, the poorer prognosis tend to be. Tumour size and regional lymph node status determines the prognosis of BCa among women (Wang *et al*., 2014) or degree of cancer is more described by tumour size (T) and lymph node status (N). 48.4% had pN1 lymph node metastasis (cancer cells lies in the lymph nodes in the armpit but the nodes are not held to adjacent tissues) and 62.9% had pT2 depth of invasion

(Tumor > 2cm but ≤ 5cm in greatest dimension). The incorporation of histologic grade in TNM (Tumor Lymph Node Metastasis - to study the clinical pattern of malignancies and to define the prognosis,) staging for breast cancer provides important prognostic information (Schwartz *et al.*, 2014).

Body composition of the subjects

Research (Shachar *et al.*, 2017) showed that body composition parameters like body surface area were found to be similar in early breast cancer and metastatic breast cancer patients whereas skeletal mass measures exhibited significant differences. Metastatic breast cancer had lower lean body mass, skeletal muscle index and skeletal muscle density. More advanced breast cancer was related with higher proportions of skeletal muscle loss or sarcopenia.

Basal metabolic rate which is defined as the energy essential for keeping the body's vital functions under resting situations, and characterizes 45 to 70 percent of daily overall energy expenditure. BMR is determined by the individual's age, body size, gender and body composition (FAO/WHO/UNU, 2001).

Body composition parameters such as Total body fat and water content were assessed by using universally approved equations (Body fat percentage - Deurenberg *et.al*., 1991 and Total Body water - Watson *et al.*, 1980). Table 4 & 5 details about the body composition of the subjects.

Table 4 Body composition (BC) of the subjects with BCa

Parameters with respect to BC	**Mammographic density of the subjects (N=75)***						
	Type I (n=20)	Type II (n=17)	Type III (n=19)	Type IV (n=19)	Total	χ^2	*p* -value
Total Body Water							
<45%	3 (15)	4 (23.5)	5(26.32)	5(26.32)	17(22.7)	3.567	0.735
45-60%	16 (80)	13 (76.5)	14 (73.68)	14(73.68)	57(76)		
>60%	1 (5)	0	0	0	1(1.3)		
Total	20(100)	17(100)	19(100)	19(100)	75(100)		
Total Body Fat percentage							
Healthy	10(50)	5(29.4)	7(36.84)	7 (36.84)	29(38.7)	3.712	0.716
Over fat	7(35)	7(41.2)	9(47.37)	6(31.58)	29(38.7)		
Obese	3 (15)	5(29.4)	3(15.79)	6(31.58)	17(22.7)		
Total	20(100)	17(100)	19(100)	19(100)	75(100)		

**Number in parentheses indicates percentage*

Table 5 Body composition (BC) of the Subjects without BCa

Parameters with respect to BC	**Mammographic density of the subjects (N=30)***						
	Type I (n=9)	Type II (n=3)	Type III (n=11)	Type IV (n=7)	Total	χ^2	*p* -value
Total Body Water							
<45%	2(22.2)	0	0	0	2(6.7)	8.272	0.219
45-60%	7(77.8)	3(100)	11(100)	6(85.71)	27(90)		
>60%	0	0	0	1(14.29)	1(3.3)		
Total	9(100)	3(100)	11(100)	7(100)	30(100)		
Total Body Fat percentage							
Healthy	1 (11.1)	0	3(27.27)	6(85.71)	10(33.3)	14.428	0.025**
Over fat	7(77.8)	3 100)	7(63.64)	0	17(56.7)		
Obese	1(11.1)	0	1 (9.09)	1(14.29)	3(10)		
Total	9(100)	3(100)	11(100)	7(100)	30(100)		

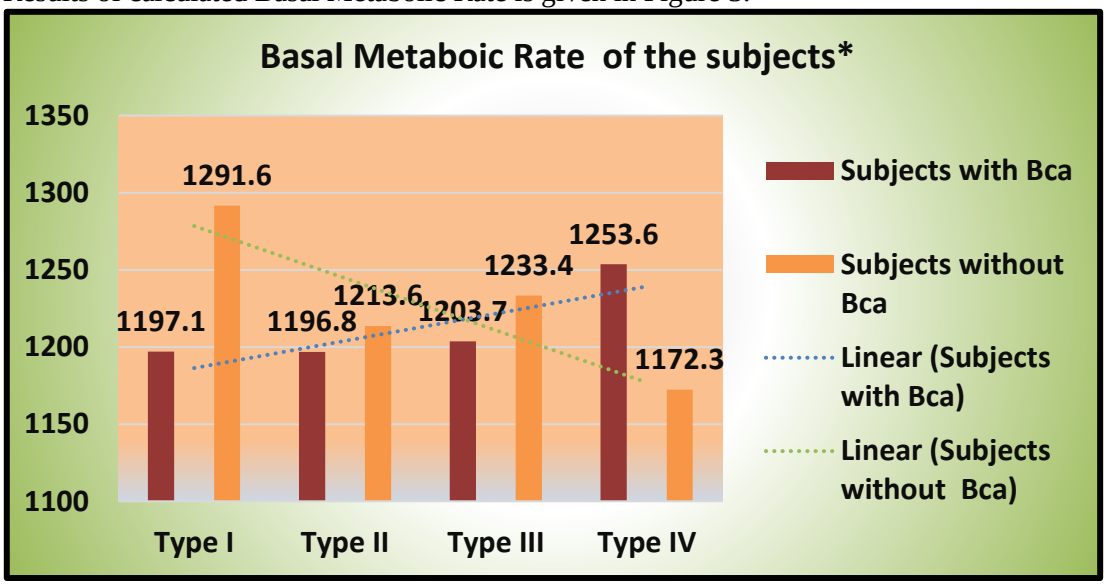
Number in parentheses indicates percentage* * Significant at 5% level*

Total body water assessment showed majority (76%) of the subjects with BCa and subjects without BCa (90%) belonged to the 45-60% category. Statistical analysis showed no significant influence between total body water and mammographic density (Cases: $\chi^2 = 3.567$, $p = 0.735$, Control: $\chi^2 = 8.272$, $p = 0.219$). Assessment of total body fat percentage showed 38.7% were over fat and 38.7% were found to be healthy among subjects with breast cancer. Similarly, 56.7% were found to be over fat and 33.3% were healthy among subjects without BCa. Statistical analysis observed a significant influence between total body fat percentage and mammographic density among the subjects without BCa ($\chi^2 = 14.428$, $p = 0.025$).

A lower percentage of total body lean mass and a higher percentage of total body fat mass were correlated with higher breast dense areas in premenopausal women but with lower breast dense areas in postmenopausal

women among Hispanic and non-Hispanic white women (Caire-Juvera *et al.*, 2008). Body fat distribution which increased from central to peripheral forecasts breast cancer risk independently of the degree of adiposity and become a definite indicator of a premalignant hormonal pattern than degree of adiposity (Ballard-Barbash *et al*., 1990). A high caloric intake above expected levels could increase mammographic density (del Pozo *et al.*, 2018).

Results of calculated Basal Metabolic Rate is given in Figure 3.



**Calculated by using St. Jeor's equation*

Figure 3. Basal Metabolic Rate of the subjects

As indicated in Figure 3 linear trend line indicated that Basal Metabolic Rate increased with the increase in mammographic density whereas subjects without BCa indicated a reverse trend with increase in mammographic density. Statistical analysis pointed out that there existed no significant differences between the means of Basal Metabolic Rate (Cases: $F = 0.852$, $p = 0.470$, Control: $F = 1.109$, $p = 0.363$) for subjects with different mammographic densities.

Biochemical profile and its influence on mammographic density

In order to assess the effect of serum nutrients and on mammographic density, vitamin D, calcium, phosphorus, iron and ferritin were assessed biochemically. Vitamin D was found to be associated with a reduction in breast density among premenopausal women (Yaghjyan *et al*., 2012). Also vitamin D and calcium were inversely associated to breast density, an intermediary end point for breast cancer; calcium had been linked with a reduced risk of benign proliferative epithelial disorders of the breast, accepted precursors of breast cancer (Cui & Rohan 2006). Breast cancer incidence by the effect of over production parathyroid hormone was reported by Michels *et al.*, (2004). Iron deficiency was observed as a risk factor in breast cancer aggressiveness of young women was pointed out by Jian *et al.*, (2013). Ferritin concentrations were found to be higher in cancer subjects compared with normal women. Patients with an initial circulating ferritin level above 200 µg/l were reported with a higher tumour recurrence rate during the following years (Jacobs *et al.*, 1976). Similarly, a significant rise in serum ferritin level was observed among breast cancer subjects (Narkhede *et al.*, 2017).

Table 6 & 7 shows the effect of serum nutrients on mammographic density of the subjects.

Table 6 Biochemical profile of the subjects with BCa (N=75)

Nutrients	Mean ± SD	Mammographic density				*F* test	*p* value
		Type I (n=20)	Type II (n=17)	Type III (19)	Type IV (19)		
Serum Vitamin D (nmol/L)	44.25± 21.02	49.45± 30.9	48.58± 10.2	36.18±16.14	42.97±18.3	1.645	0.187
Serum Calcium (mg/dl)	8.99 ± 0.63	9.3± 0.6	8.88±0.63	9.02± 0.6	8.72± 0.6	3.139	0.031*
Serum Iron (µg/dl)	81.21± 43.26	81.1± 51.3	88.24± 47.86	79.48±31.3	76.79±42.8	0.219	0.883
Serum Ferritin (ng/ml)	147.35± 256.30	168.85 ± 336.1	126.69± 147.70	155.35± 332.1	135.19± 141.5	0.100	0.960
Serum Phosphorus(mg/dl)	5.61± 1.68	6.92± 4.7	4.91± 0.91	5.82± 2.0	4.77±0.2	2.998	0.036*

**Significant at 5% level*

Table 7 Biochemical profile of the subjects without BCa (N=30)

Nutrients	Mean ± SD	Mammographic density					
		Type I (n=9)	Type II (n=3)	Type III (n=11)	Type IV (n=7)	*F* test	*p* value
Serum Vitamin D (nmol/L)	63.99±17.24	61.59± 16.8	60.77± 14.6	62.4± 22.6	70.96± 8.4	0.478	0.700
Serum Calcium (mg/dl)	9.86± 0.41	9.78± 0.6	10.07± 0.2	9.87± 0.3	9.8± 0.4	0.361	0.782
Serum Iron (µg/dl)	69.97±28.25	78.25± 33.6	65.84± 20.8	67.23± 31.4	65.41± 20.3	0.349	0.790
Serum Ferritin (ng/ml)	44.74±32.73	78.25± 33.6	42.95± 36.1	46.11± 22.3	46.01± 39.9	2.230	0.109
Serum Phosphorus(mg/dl)	3.91± 0.55	3.9± 0.4	3.93±0.4	3.9± 0.7	3.93± 0.7	0.006	0.999

Serum vitamin D analysis explained that low level of vitamin D was observed among subjects with BCa (44.25±21.02 nmol/L) compared with subjects without BCa (63.99±17.24 nmol/L). Statistical analysis did not show any significant difference between the means of vitamin D (Cases: $F = 1.645$, $p = 0.187$, Control: $F = 0.478$, $p = 0.700$) for subjects with different mammographic densities.

Vitamin D deficiency is related with several variations in mineral metabolism (Rao *et al.*, 1985). Mean serum calcium level was found to be less among cases (8.99± 0.63 mg/dl) compared with control (9.86± 0.41 mg/dl) group. Statistical analysis indicated a significant difference between the means of calcium on mammographic density at 5% level ($F = 3.139$, $p = 0.031$) for subjects with different mammographic densities among cases and no significant difference ($F = 0.361$, $p = 0.782$) was observed in the other group. In primary breast cancer patients, an inverse association was perceived between serum calcium levels and percentage mammographic density adjusted to serum albumin levels (Hack *et al*., 2017). Fair *et al*., (2015) found a trend of decreasing breast density with increasing calcium and vitamin D intake among premenopausal after statistical adjustment for age, race, and body mass index, but not among postmenopausal women.

Serum iron status was found to be relatively high among the subjects with breast cancer. Average level showed 81.21± 43.26 µg/dl for cases and 69.97 ± 28.25 µg/dl for the control group. Statistically significant difference was not observed (Cases: $F = 0.219$, $p = 0.883$ Control: $F = 0.349$, $p = 0.790$) between the means of serum iron level for subjects with different mammographic densities. An association of high serum iron with high risk for all cancers collectively and for breast cancer and liver cancer specifically was reported by Wen *et al*., (2014). Bae *et al*., (2009) had opined that high serum iron to be a risk element for carcinogenesis in breast tissues.

High level of serum ferritin was reported among the subjects with BCa (147.35± 256.3 ng/ml) compared with subjects without BCa (44.74 ± 256.3 ng/ml). Statistical analysis did not indicate a significant difference between the means of serum ferritin levels for subjects with different mammographic densities in both the groups (Cases: F=0.100, p= 0.960, Control: F=2.230, p= 0.109). Increased serum ferritin caused carcinogenesis in breast owing to the discharge of free iron as the activating factor for free radical induced carcinogenesis (Goswami *et al*., 2008b). Research showed that elevated serum ferritin level among subjects with breast cancer and increased ferritin caused tumourigenesis as ferritin act as source of free iron (Moore *et al*., 2009). Amrita *et al*., (2018) observed that breast tumours caused increased level of ferritin and iron.

Subjects with BCa were having high serum phosphorus level (5.61±1.68 mg/dl) compared with the other group (3.91± 0.55 mg/dl). Statistically significant difference (5%) was observed between the mean levels of serum phosphorus among the cases (Cases: F=2.998, p= 0.036, Control: F=0.006, p= 0.999) for subjects with different mammographic densities. Studies indicated that raised phosphate levels boost up cancer growth by elevating cell multiplication (Jin *et al*., 2009). Also it was analysed (Lee *et al.*, 2011) that inadequate fibroblast growth factor 23-a bone derived hormone, response causes hyperphosphatemia in vitamin D-deficient patients with either hypocalcemia and/or low 1,25-vitamin D values.

Vitamin D is one of the vital factor for female reproductive health (Shao *et al.*, 2012) which had protective functions against many cancer types, including breast cancer. Severe vitamin D deficiency caused a three-fold rise in the risk of breast cancer while it was not the case for mild and moderate deficiency (Alipour *et al.*, 2014a). In Iran, breast cancer was ranked as the most widespread malignancy among women and its possibility increased with the age commonly (Harirchi *et al.*, 2011). Vitamin D has varied biological effects applicable to carcinogenesis, including known cross-talk between the vitamin D receptor (VDR) and insulin-like growth factor (IGF) signaling ways. Based upon data, women with serum 25(OH) D status higher than 40-50 ng/ml had a 50% poorer risk of breast cancer associated to women with vitamin D deficiency.

Table 8 & 9 describes about the classification of vitamin D levels among the subjects.

Table 8 Classification of Vitamin D levels among the subjects with BCa

(N=75)

Vitamin D (nmol/L)	**Distribution of mammographic density***						
	Type I (n=20)	Type II (n=17)	Type III (n=19)	Type IV (n=19)	Total	χ^2	*P* value
75-250 (Normal)	$3_{(15)}$	0	0	$1_{(5.3)}$	$4_{(5.3)}$	14.627	0.023**
50-75 (Insufficiency)	$2_{(10)}$	$8_{(47.1)}$	$2_{(10.5)}$	$6_{(31.6)}$	$18_{(24)}$		
<50 (Deficiency)	$15_{(75)}$	$9_{(52.9)}$	$17_{(89.5)}$	$12_{(63.2)}$	$53_{(70.7)}$		
Total	$20_{(100)}$	$17_{(100)}$	$19_{(100)}$	$19_{(100)}$	$75_{(100)}$		

**Numbers in parentheses indicate percentage* ***5% level significance*

Table 9 Classification of Vitamin D levels among the Subjects without BCa

(N=30)

Vitamin D (nmol/L)	**Distribution of mammographic density***						
	Type I (n=9)	Type II (n=3)	Type III (n=11)	Type IV (n=7)	Total	χ^2	*p* value
75-250 (Normal)	$2_{(22.2)}$	$1_{(33.3)}$	$4_{(36.4)}$	$2_{(28.6)}$	$9_{(30)}$	2.975	0.812
50-75 (Insufficiency)	$5_{(55.6)}$	$2_{(66.7)}$	$5_{(45.5)}$	$5_{(71.4)}$	$17_{(56.7)}$		
<50 (Deficiency)	$2_{(22.2)}$	0	$2_{(18.2)}$	0	$4_{(13.3)}$		
Total	$9_{(100)}$	$3_{(100)}$	$11_{(100)}$	$7_{(100)}$	$30_{(100)}$		

**Numbers in parentheses indicate percentage*

Majority (70.7%) of the subjects with BCa were found to be deficient with serum vitamin D followed by 24% with insufficient vitamin D. Only 5.3% were found to be normal. Regarding the subjects without BCa, 56.7% were insufficient in Vitamin D, followed by 13.3% deficient and 30% being with normal vitamin D status. Vitamin D status showed a significant influence on the mammographic density among subjects with BCa (χ^2 = 14.627, p = 0.023). Higher levels of 25(OH)D status in pre-menopause and vitamin D substitution were related with lower breast density and could decrease the risk of breast cancer was reported by Straub *et al.*, (2017). But, study conducted by Bertrand *et al.*, (2015) among 835 pre-menopausal women showed considerably increased percent breast tissue densities for women with vitamin D levels in the highest 25(OH)D quartile than levels in the lowest 25(OH)D quartile.

Conclusion

Regarding the various factors influencing the mammographic density, serum calcium, serum phosphorus and vitamin D status were found to be the factors influencing mammographic density among subjects with BCa. Similarly, total body fat percentage was a factor affecting mammographic density among the subjects without BCa. Total body water percentage also had shown significant effect on serum vitamin D status. total body water percentage was found to be a predisposing factor for vitamin D deficiency. Vitamin D deficiency is likely to play a significant role in the very high prevalence of breast cancer in Kerala. Mammographic density is a strong risk element for breast cancer.

The research revealed that certain factors like age, body composition, and biochemical factors influenced mammographic density. Based on the findings of the study, it is recommended to have good exposure to sunlight for about 20-30 minutes. Supplementation of Vitamin D at the government level irrespective of age to reduce the prevalence of Vitamin D deficiency should be done. Vitamin D deficiency should be recognized as a public health problem. Population based educational schemes like nutrition education should be implemented to combat this menace by the government bodies.

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