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Review

Reproductive factors, nutritional status and serum 25(OH)D levels in women with breast cancer: A case control study

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ABSTRACT

The study was conducted with an objective to investigate the association between reproductive factors, nutritional status and serum 25(OH)D levels among women diagnosed with breast cancer (BC). A total of 200 women with BC attending a tertiary healthcare institute of Delhi, India matched with 200 healthy women for age (± 2 years) and socio economic status were included in the study. Data was collected on socio-demographic profile, reproductive factors, physical activity and dietary intake (24 h dietary recall and food frequency questionnaire) using interviewer administered structured questionnaires and standard tools. Non fasting blood samples (5 ml) were collected for the biochemical estimation of serum 25(OH)D and calcium levels by chemiluminescent immunoassay and colorimetric assay technique. Data was analyzed by univariable conditional logistic regression and significant variables with ($p < 0.05$), were analyzed in final model by conditional multivariable logistic regression analysis. The mean age of patients at diagnosis of BC was 45 ± 10 years. Results of multivariable conditional logistic regression analysis revealed significantly higher odds of BC for reproductive factors like age at marriage (more than 23 years), number of abortions, history or current use of oral contraceptive pills (OCP), with [OR (95% CI)] of [2.4 (1.2–4.9)], [4.0 (1.6–12.6)], [2.4 (1.2–5.0)]. Women with physically light activities and occasional consumption of eggs were found to have higher odds of BC [4.6 (1.6–13.0)] and [3.2 (1.6–6.3)]. Women with serum 25(OH)D levels less than 20 ng/ml and calcium levels less than 10.5 mg/dl had higher odds of having BC [2.4 (1.2–5.1)] and [3.7 (1.5–8.8)]. A protective effect of urban areas as place of residence and energy intake greater than 50% of Recommended Dietary Allowance (RDA) per day against BC was observed ($p < 0.05$). The findings of the present study revealed a significant association of reproductive and dietary factors in addition to sedentary physical activity and low serum 25(OH)D levels in women diagnosed with BC.

1. Introduction

Breast cancer (BC) is one of the major public health problems. Globally, more than one million cases are diagnosed annually with one woman being diagnosed with BC in every three minutes [1]. The incident cases of BC diagnosed in 2012 accounted for 25% of all cancers, with 8,83,000 cases reported in developed countries and 7,94,000 in developing countries [2,3]. In India, BC is biologically more aggressive disease than in developed countries with incidence at a younger age and delayed presentation for diagnosis thereby, making it the most common cause of cancer related deaths in Indian women [4]. It is hormonally mediated disease predisposed by the effect of risk factors on hormonal status or change in markers of hormonal status. However, many of these risk factors are non modifiable such as sex, age, race, genetic (BRCA1 and BRCA2) mutation and family history of BC [5]. Reproductive factors like age at marriage, use of oral contraceptives, number of pregnancies, number of abortions and breastfeeding, dietary factors and status of physical activity are the modifiable factors [6–8]. Vitamin D deficiency is yet another most common nutritional deficiency worldwide. Scientific evidence has documented a positive

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association of vitamin D deficiency with risk of BC [9,10]. Majority of the previous studies in India have investigated reproductive risk factors or nutritional factors separately in BC patients. BC is a multifactorial disease and study of the possible modifiable risk factors may assist in better understanding for their association in addition to the established non modifiable risk factors. Therefore, the present study was undertaken to study the risk factors together in terms of reproductive, nutritional factors, vitamin D and calcium status of women recently diagnosed with BC.

2. Materials and methods

A hospital based case control study was conducted on women (cases) with confirmed BC attending a tertiary healthcare institute in the national capital territory of India. The study was conducted from January 2015 to February 2017. Women with pathologically confirmed incident cases of BC (International Classification of Diseases code 174 and code C50 from the Ninth Revision and Tenth Revision, respectively) diagnosed within past 6 months from the date of interview were eligible as cases. Healthy women as attendants of non cancerous patients were eligible as controls. With the presumption for the prevalence of the proportion of risk factor of early menarche amongst control group for 30% [5], 80% power with an odds ratio (OR) of 2.0 (where $Z\beta = 0.84$, $Z\alpha/2 = 1.96$) $p < 0.05$, the required sample size calculated for the proposed study was 144 cases and 144 controls. Based on the availability of the cases and controls, a total of 200 BC cases and 200 healthy controls were included in the study. The criteria adopted for inclusion of BC patients (cases) were i) women with pathologically confirmed BC, diagnosed during last six months from the date of interview ii) no history of any treatment specific for BC prior to inclusion, iii) ambulatory patients. The criteria for selection of the controls was i) healthy attendants of nonbreast cancer patients attending the healthcare institute for treatment. The exclusion criteria for cases and controls were i) past history of benign tumor, breast or any other cancer, ii) history of any chronic disease like diabetes, hepatic disorders/severe malnutrition and corticosteroid therapy, iii) any changes in dietary pattern, iv) history of vitamin or mineral supplements during the last one year and v) subjects not willing to participate.

A total of 500 cases with BC and 250 apparently healthy controls were screened for inclusion in the study out of which 200 cases (age 25 years and above) with newly diagnosed BC and 200 healthy women fulfilling the inclusion criteria were included in the study. The healthy controls were the attendants accompanying the nonbreast cancer patients to general medicine and surgical departments of the same tertiary healthcare institute. For each case, a control of similar age (± 2 years) and socio economic status (SES) was selected. A semi structured questionnaire was used to record data on background information, sociodemographic profile, reproductive variables like age at menarche, age at marriage, age at first child birth, breast feeding and duration of breast feeding, number of abortions, OCP use and menopausal status. Information was also collected on daily physical activities performed prior to diagnosis of BC. Nutritional and dietary intake with adequacy of diet consumed was assessed by 24 h diet recall method and validated food frequency questionnaire (FFQ) method for selected food groups using standard tools and techniques. Information for the dietary intake of vitamin D containing foods (fish, eggs, meat, milk and mushroom) was also recorded. For the calculation of nutrient intake for “energy, protein, fat, carbohydrate and calcium”, Indian Nutrition Software- “DietCal- A Tool for Dietary Assessment and Planning” (software version-3.0) developed from Nutritive Value of Indian Foods, by Indian Council of Medical Research (ICMR) was used. Serum 25(OH)D and total calcium levels were estimated by chemiluminescent immunoassay and colorimetric assay (arsenazo) technique. Serum samples collected after centrifugation of non fasting blood samples (5 ml) were stored at −80 °C till biochemical analysis. The values for serum 25(OH)D and calcium were documented in ng/ml and mg/dl. Vitamin D deficiency was defined as per the criteria of United States Endocrine Society [11]. Internal and external quality control was maintained throughout the assay. Subjects in case and control group were subjected to similar investigations.

2.1. Measurement of serum 25(OH)D levels by chemiluminescence technique

Serum 25(OH)D levels were estimated by LIAISON 25-hydroxyvitamin D total assay (DiaSorin, Stillwater, MN, USA) by chemiluminescent immunoassay technique (CLIA). Analytical measurement range for the assay was 4 ng/ml–150 ng/ml. The lowest reportable value was 4 ng/ml based on an inter-assay precision that approximates 20% coefficient of variance (CV) for functional sensitivity. Specific antibody to vitamin D was used for coating magnetic particles (solid phase) and vitamin D was linked to an isoluminol derivative. After the incubation, the unbound material was removed with a wash cycle. Subsequently, the starter reagents were added and a flash chemiluminescent reaction was initiated. The light signal was measured by a photomultiplier as relative light units and was inversely proportional to the concentration of 25(OH)D present in samples. Internal and external quality control (QC) was maintained by running a sample of known concentration of 25(OH)D along with the samples for analysis. The CV calculated for low QC was 8.5% and high QC was 6.5% which was found to be similar to recent research study for measurement of 25(OH)D assays [12]. The intra and interassay precision was acceptable, with CV less than 10%.

2.2. Measurement of serum calcium levels by colorimetric technique

Serum total calcium estimation was done on an automated analyzer, COBAS INTEGRA 400 Plus (Roche Diagnostics India Pvt. Ltd, Mumbai, India). Calcium ions react with *O*-cresolphthelein under alkaline conditions to form a purple colored complex. The addition of 8- hydroxyl quinoline prevents interference by magnesium and ferric ions. The color intensity of the complex formed was directly proportional to the calcium concentration. It was determined by measuring the increase in absorbance at 650 nm. The value was expressed as mg/dl.

2.3. Statistical analysis

The present study was a matched case control where matching was done based on age and socioeconomic status. Matching was assessed using paired *t*-test and Stuart-Maxwell chi-square ($\chi 2$) test. All categorical variables were presented as frequency (%) and continuous variables were presented as Mean ± SD. First of all bi-variable analysis was carried out where unadjusted odds ratio and their 95% confidence interval were calculated using conditional logistic regression for all explanatory variables. Explanatory variables which showed association with BC in bi-variable analysis at 10% level of significance and all clinical important variables were considered as candidate predictors for multi-variables analysis. Conditional logistic regression with step-wise procedure was adopted for multi-variables analysis. P-value of 0.05 was considered as significant in multivariate analysis. The data was analyzed using Stata 12.0 (Stata Corp College Statition) software and electronically entered in Epi-info-7 software.

3. Results

3.1. Socio-demographic factors and risk of BC

The age at diagnosis for the cases ranged from 25 to 85 years with a mean of 45 ± 10 years. The mean age for the controls was 47 ± 10 years. Majority of the cases 68.5% (n = 137) were in the age group of 36 to 55 years (Table 1). Nearly 30% (n = 60) of cases and 26% (n = 52) of controls had attained higher education (graduation

Table 1
Distribution of subjects according to demographic profile, reproductive factors, dietary intake and their association with the risk of breast cancer: Univariable analysis.

Variable	Cases N (%)	Controls N (%)	Unadjusted OR (95%CI)	p-value
Age at enrollment (years)				
25–35 (Ref)	35 (17.5)	42 (21.0)	1.0	
36–45	82 (41.0)	75 (37.5)	9.3 (1.1–8.9)	0.042
46–55	55 (27.5)	54 (27.0)	7.5 (0.6–9.5)	0.120
≥56	28 (14.0)	29 (14.5)	3.3 (0.2–5.6)	0.407
Type of family				
Nuclear (Ref)	135 (67.5)	128 (64.0)	1.0	
Joint	65 (32.5)	72 (36.0)	0.8 (0.5–1.3)	0.426
Educational status				
High (Grad and ab) (Ref)	60 (30.0)	52 (26.0)	1.0	
Middle (middle to grad)	53 (26.5)	51 (25.5)	0.8 (0.4–1.4)	0.504
Low (upto middle)	87 (43.5)	97 (48.5)	0.7 (0.3–1.2)	0.198
Religion				
Hinduism (Ref)	164 (82.0)	176 (88)	1.0	
Islam	22 (11.0)	17 (8.5)	1.3 (0.7–2.4)	0.425
Sikhism	14 (7.0)	7 (3.5)	2 (0.8–4.9)	0.134
Family history of breast cancer				
No (Ref)	170 (85.0)	168 (84.0)	1.0	
Yes	30 (15.0)	32 (16.0)	1.16 (0.6–2.2)	0.631
Age at menarche (years)				
≥14 (Ref)	101 (50.5)	127 (63.5)	1.0	
< 14	99 (49.5)	73 (36.5)	1.74 (1.1–2.6)	0.009
Parous				
Yes (Ref)	186 (93)	188 (94)	1.0	
No	14 (7)	12 (6)	0.37 (0.2–1.4)	0.147
Age at first child birth (years)				
Total	**186 (100)**	**188 (100)**		
≤25 (Ref)	142 (76.3)	160 (85.1)	1.0	
> 25	44 (23.7)	28 (14.9)	1.8 (1.0–3.4)	0.042
Duration of breastfeeding (Mo)				
Total	**186 (100)**	**188 (100)**		
< 12 (Ref)	168 (90)	154 (82)	1.0	
≥12	18 (10)	34 (18)	0.5 (0.2–0.9)	0.041
Menopause status				
Pre (Ref)	124 (62.0)	139 (69.5)	1.0	
Post	76 (38.0)	61 (30.5)	2.3 (1.1–4.4)	0.019
Mushroom				
Daily – 1/wk (Ref)	3 (1.5)	10 (5.0)	1.0	
Twice-once/mo	8 (4.0)	19 (9.5)	1.3 (0.3–6.2)	0.665
Occ/Never	189 (94.5)	171 (85.5)	4.1 (1.1–15.4)	0.038
Roots and tubers				
Daily – 4/wk (Ref)	30 (15.0)	47 (23.5)	1.0	
3/wk – 1/wk	104 (52.0)	100 (50.0)	1.6 (0.9–2.8)	0.059
Twice – once/ month	29(14.0)	27 (13.5)	1.7 (0.8–3.4)	0.142
Occasional/never	37 (0.5)	26 (13)	2.3 (1.1–4.7)	0.018
Type of fat consumed				
Saturated (Ref)	40 (20.0)	16 (8.0)	1.0	
Unsaturated	160 (80.0)	184 (92.0)	0.3 (0.2–0.6)	0.001

and above), with 67.5% (n = 135) cases and 64% (n = 128) controls from nuclear families. A total of 82% (n = 164) cases and 88% (n = 176) controls were Hindus whereas 11% (n = 22) cases and 8.5% (n = 17) controls were Muslims. Sixty five percent (n = 130) cases and eighty two percent (n = 164) controls were from urban areas. History of BC was positive in 15% (n = 30) cases and 16% (n = 32) controls. A non significant association was found for type of family, religion, educational status and family history of BC.

3.2. Reproductive factors and risk of BC

Table 1 shows the results of univariable regression analysis, [OR (95% CI)], for the association of reproductive factors with BC. A significant association for higher risk was found between age at menarche (less than 14 years), age at marriage (more than 23 years), number of abortions (more than three abortions), OCP use, age at first child birth (more than 25 years) and post menopausal women ($p < 0.05$). History of breastfeeding the youngest child for more than 12 months was found to be significantly associated with lesser odds of BC. More than 90% of cases and controls were married and parous.

3.3. Dietary factors and risk of BC

The results for frequency of consumption for cereals and pulses were daily to once per week for all subjects in both the groups and were thus excluded from analysis. The nutritional intake for energy (Kcal/d), fat (g/d) and calcium (mg/d) was greater than 50% of RDA for more than 50% of cases and controls. The dietary intake for protein was greater than 50% of RDA for only 9% of cases and 6% of controls. Carbohydrate intake was greater than 50% of RDA for more than 96% of subjects in both case and control group. There was no significant association of dietary factors like consumption of green leafy vegetables, fruits, milk and vitamin D containing foods, viz., fish, poultry, and meat with the risk of BC. A positive association was found for occasional consumption of eggs, roots and tubers and mushroom with the risk of BC as compared to regular consumption ($p < 0.05$). Nutritional intake for dietary intake of fat, protein and calcium per day revealed a non significant association with BC. However, energy intake (Kcal/d) greater than 50% of RDA and unsaturated fat intake were associated with lesser odds of BC ($p < 0.05$).

In the final model (Table 2), urban areas as place of residence and daily dietary energy intake (Kcal/d) greater than 50% of RDA were associated with a protective effect against BC. On the contrary, women with age at marriage (more than 23 years), history of more than three abortions, OCP use, occasional consumption of eggs, light physical activities, low serum 25(OH)D (less than 20 ng/ml) and calcium levels (less than 10.5 mg/dl) had significantly higher odds of having BC.

4. Discussion

The present case control study revealed the information about the reproductive and nutritional risk factors in BC with an emphasis on serum 25(OH)D and calcium levels. The mean age of the cases was 45 ± 10 years. Women from urban areas were found to have lesser odds of having BC compared to women from rural areas which is similar to findings by earlier studies where women from urban areas were better protected against BC as compared to women from rural areas with higher mortality to incidence ratio in rural areas as compared to urban areas [13–15]. The possible reason for the lesser odds of BC in urban areas may be linked to increased awareness and easy access to healthcare for preventive diagnosis which is not the available in rural areas. Religion may play a role in screening process due to difference in cultural beliefs and faith, however the present study did not find any association of religion with BC. Whereas higher education status and SES has been conjectured to have an association with higher risk of BC due to late preference for marriage and child bearing, our study could not find any association of educational status with BC. The possible reason could primarily be similar number of subjects under each category of educational status. Positive family history of BC is one of the strong risk factors for the disease, however our study failed to show any association between family history and BC possibly due to similar number of subjects in both the groups. Results for univariable regression analysis revealed a significantly positive association between reproductive factors like age at menarche (less than 14 years), post menopausal status, age at marriage (more than 23 years), age at first child

Table 2
Association of risk factors with breast cancer: Results of Univariable and Multivariable regression analysis.

Variable	Cases N (%)	Controls N (%)	Unadjusted		Adjusted	
			OR (95%CI)	p-value	OR (95% CI)	p-value
Residence						
Rural (Ref)	70 (35.0)	36 (18.0)	1.0		1.0	
Urban	130 (65.0)	164 (82.0)	0.4 (0.3–0.7)	< 0.001	0.4 (0.2–0.7)	0.004
Energy (Kcal/day)						
≤50% RDA (Ref)	116 (58)	63 (31.5)	1.0		1.0	
> 50 to 100% RDA	84 (42)	137 (68.5)	0.6 (0.4–0.9)	0.027	0.5 (0.3–0.9)	0.028
Age at marriage (years)						
Total	**195 (100)**	**191 (100)**				
≤23 (Ref)	147 (75.4)	163 (85.3)	1.0		1.0	
> 23	48(24.6)	28 (14.7)	1.8 (1.0–2.3)	0.039	2.4 (1.2–4.9)	0.017
Number of abortions						
Total	**186 (100)**	**188 (100)**				
≤3 (Ref)	168 (90)	179 (95)	1.0		1.0	
> 3	18 (10)	9 (5)	2.3 (0.9–5.5)	0.068	4.0 (1.6–12.6)	0.017
Oral contraceptive use						
Total	**195 (100)**	**191 (100)**				
No (Ref)	171 (87.6)	143 (75)	1.0		1.0	
Yes	24 (12.4)	48 (25)	2.8 (1.5–5.0)	0.001	2.4 (1.2–5.0)	0.015
Physical activity						
Active (Ref)	11 (5.5)	28 (14.0)	1.0		1.0	
Light	189 (94.5)	172 (86.0)	2.8 (1.3–6.2)	0.006	4.6 (1.6–13.0)	0.004
Frequency of egg consumption						
Daily – 1/wk (Ref)	31 (15.5)	61 (30.5)	1.0		1.0	
Twice-once/mo	22 (11.0)	11 (5.5)	3.7 (1.6–8.6)	0.002	6.2 (2.0–18.7)	0.001
Occ/never	147 (73.5)	128 (64.0)	2.3 (1.3–3.9)	0.001	3.2 (1.6–6.3)	0.001
Serum 25(OH)D levels (ng/ml)						
≥20 (Ref)	19 (9.5)	35 (17.5)	1.0		1.0	
< 20	181 (90.5)	165 (82.5)	1.9 (1.1–3.5)	0.026	2.4 (1.2–5.1)	0.025
Serum calcium levels (mg/dl)						
≥10.5 (Ref)	18 (9.0)	38 (19.0)	1.0		1.0	
< 10.5	182 (91.0)	162 (81.0)	2.5 (1.3–4.8)	0.004	3.7 (1.5–8.8)	0.003

birth (more than 25 years), number of abortions and OCP use with BC. However, a protective effect of duration of breastfeeding against BC was observed. Similar results were documented by a recent study where compared to less than six months, breast feeding for more than 6 months was found to be significantly associated with a reduced risk of BC [16]. Our study concluded a higher risk of BC for women with age at marriage of more than 23 years, number of abortions and OCP use. Similar results were documented in earlier other studies where advanced age, history of abortions and OCP use were significantly associated with higher odds of BC [13,17,18]. The complete differentiation of epithelial cells of the breast as a result of full-term pregnancy is responsible for a reduced risk future BC in women of reproductive age. In contrast, a disruption by abortion may result in seizure of differentiation process which in turn elevates the risk of BC. In India, there is no law limiting the number of children per family. The recommendation for the use of OCP as part of family planning policy should be limited to fertile women with a healthy reproductive outcome. More research is warranted to understand the role of OCP undertaking the type, combination of estrogen and progestrin and duration of usage in view of the association of OCPs with higher risk of BC. Women with family history of BC in their first degree family, have higher risk of developing BC [19]. Our study found a positive but non significant association of family history of BC with higher risk of BC. This is contradictory to the findings of earlier studies where family history of BC was found to have a significant association with higher risk of BC [4,18]. The possible reason for non significant association could be due to the similar number of subjects with and without family history of BC for both case and control group.

The potentially modifiable role of dietary factors against the risk of BC has been extensively investigated; however, the results have been inconsistent [20]. The present study revealed lesser odds of BC associated with dietary intake of unsaturated fat. Similar results were documented by earlier studies [21,22]. The mechanism proposed for the effect of dietary fat on BC involves increased estrogen production in adipose tissue which causes inflammation and modifications in some physiologic processes leading to higher risk of BC [22]. A non significant association was found for the daily intake of dietary protein, fat and calcium with BC. Owing to the anticancerous effects of fruits and vegetables, an inverse yet non significant association was found between daily consumption of fruits and vegetables with risk of BC. Similar results have been documented by an earlier research study [16] whereas in another nested case control study, a null association was found between dietary intakes of vegetable, fruit, dietary fiber and BC [23]. Among vitamin D containing foods, occasional consumption of eggs was associated with lower risk when compared with twice to once per month but higher when compared with daily to once per week. Occasional consumption of mushrooms was found to be significantly associated with higher risk of BC compared to daily consumption. Similar results were documented earlier for dietary intake of vitamin D containing foods and risk of BC [24–26]. The variation in results could be possibly due to major difference in the number of subjects in three categories for egg and mushroom consumption. Women with active lifestyle had less odds of having BC compared to women with physically light lifestyle ($p < 0.05$). Similar results have been documented earlier [27]. The protective effect of physical activity is associated with secretion of cytokines from the muscles which is related to inhibition of mammary cancer cell growth [28].

There are different methods of analyzing vitamin D status [29].

However, the samples in present study were analyzed by chemiluminiscence technique for serum 25(OH)D levels. It was found that women with serum 25(OH)D (less than 20 ng/ml) and calcium levels (less than 10.5 mg/dl) had higher odds of having BC. Similar results have been documented earlier [26].Vitamin D in association with calcium leads to suppression of proliferation induced by 17-beta estradiol and down-regulation of estrogen receptors limiting the availability of free estrogen and thereby decreasing risk of BC.

BC in Indian women is the most common form of cancer and continues to affect our country adversely. The present study revealed that modifiable risk factors related to reproductive, dietary and physical activity were associated with BC in Indian women. A review conducted earlier concluded that vitamin D intake was essential for overall cancer prevention including BC [30]. On the other hand, vitamin D deficiency continues to be highly prevalent in our country. Therefore, given the fact that vitamin D deficiency is related to cancer cell proliferation and is possibly one of the risk factors for BC, vitamin D supplementation or fortification of foods with vitamin D in addition to changes in certain reproductive factors, diet and lifestyle may together promote prevention and healthy outcome in patients with BC. More research is warranted to identify changing aspects in reproductive and lifestyle patterns and determining vitamin D status to correct deficiencies and thus making women aware about decreasing their risk of BC.

5. Limitations to the study

The present study has some limitations. Firstly, we couldn't access the dietary vitamin D content of the foods containing vitamin D naturally as the country has no standard system in place for estimation of vitamin D content in foods. Secondly, similar to other case–control studies, this study is prone to recall, selection, and measurement biases. Other biochemical parameters could not be estimated due to operational constraints.

Conflict of interest

The authors declare no conflict of interest.

Ethical clearance

Ethical clearance was obtained from the ethics committee of All India Institute of Medical Sciences, New Delhi. All procedures performed in the study involving human participants were in accordance with the ethical standards and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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