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Review

# Nutritional risk factors and status of serum 25(OH)D levels in patients with breast cancer: A case control study in India

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ABSTRACT

To study the nutritional risk factors and status of serum 25(OH)D levels in patients with breast cancer. A total of 100 women (cases) with confirmed breast cancer (BC) matched with equal number of healthy females (controls) of similar age and socioeconomic status (SES) were included in study. Controls included were nonbreast cancer patients who accompanied the patients to a tertiary care hospital. All the subjects (cases and controls) were administered a questionnaires to collect data on socioeconomic status, dietary pattern and the frequency of food consumption using a validated food frequency questionnaire. Anthropometric assessment was done for waist and hip circumference to calculate waist to hip ratio (WHR). Non fasting blood samples were collected for serum 25-hydroxyvitamin D [25(OH)D] levels estimation using chemiluminescent immunoassay technique and total serum calcium levels by colorimetric assay technique. Serum 25(OH)D and total calcium levels were expressed in ng/ml and mg/dl. Vitamin D deficiency was defined as per the guidelines set by United States Endocrine Society. The mean age of cases and controls was $45 \pm 9$ and $46 \pm 10$ years respectively. On multivariate analysis, an inverse association with BC was found for less frequency of fruits consumption with an adjusted (ORs, 95% CI) (2.7, 0.5–15.7) respectively. Mushroom intake was inversely associated with risk of BC (ORs, 95% CI) (5.6, 1.9–16.6). Saturated fat intake and high WHR were significantly associated with high risk of BC with adjusted ORs, 95% CI of (3.4, 1.4–8.1) and (5, 1.4–17). A significant association ($p < 0.05$) was found between low serum 25(OH)D levels and the risk of BC with adjusted ORs, 95% CI of (2.5, 0.9–7.4). Majority of the patients with BC were suffering from vitamin D deficiency. Dietary intake of mushrooms containing vitamin D naturally was found to be associated with decreased risk of breast cancer. A significant association was found between low serum 25(OH)D levels (<20 ng/ml) with the risk of BC. Obesity as a consequence of nutritional risk factors determined by higher WHR was found to be significantly associated with the risk of BC.



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## 1. Introduction

Cancer is a major public health problem and cause of death worldwide. According to WHO, cancer accounted for 7.6 million deaths in 2008, which is projected to rise with an estimated 13.1 million deaths in 2030. Breast cancer (BC) is the most common cancer among women, accounting for 25% of all new cancer cases [1]. In India, BC is the leading cancer diagnosed in women overtaking cervical cancer [2]. A number of risk factors are associated with BC like age, family history, genetic mutation, breast density, reproductive factors, nutritional status, obesity, alcohol use and socioeconomic status (SES). Epidemiological and clinical evidence supports that nutrition in its broadest sense, plays a role in BC [3,4]. Evidence suggests that vitamin D intake (ergocalciferol and cholecalciferol) in association with calcium may be protective against BC [5–7]. Women with 25(OH)D concentrations $\geq$40 ng/ml have a significantly lower risk of cancer ($\sim$70%) compared with concentrations $<$20 ng/ml [8].

Vitamin D has been shown to have anti-carcinogenic properties like effects on cell proliferation and differentiation [9]. Synthesis of 1,25-dihydroxyvitamin D in breast tissue may contribute to maintenance of normal cell function, which could be impaired in vitamin D deficiency. A large number of Indian women are affected with BC. The literature available on the nutritional risk factors in BC has provided mixed results [10]. Hence, the present case control study aimed to investigate the nutritional factors and status of serum 25(OH)D levels and the risk of BC was conducted.

## 2. Materials and methods

A hospital based case control study was conducted. Women (cases) with confirmed diagnosis of BC attending a tertiary health care hospital located at latitude of 28.56N and longitude of 77.21E in the national capital territory of India were enrolled. Patients were referred to the current hospital from peripheral hospitals, clinics or physicians in private practices.

Considering average serum 25(OH) levels in cases as $9.5 \pm 6.5$ ng/ml and controls as $15 \pm 13$ ng/ml [11] Power of 90 with alpha equal to 5%, the required estimated sample size was seventy seven (77), we recruited a total of 100 cases and 100 controls of similar age.

Controls were healthy females of similar age and socioeconomic status (SES) who accompanied the nonbreast cancer patients to hospital. The case to control ratio was maintained as 1:1. The inclusion criteria adopted was i) confirmed BC by histopathological and fine needle aspiration cytology (FNAC) reports ii) No history of specific treatment for BC, iii) absence of any chronic disease like diabetes or crohn's disease that may affect the dietary intake iv) written consent to participate in the study. Exclusion criteria adopted was i) any vitamin, protein or mineral supplement intake during last one year, ii)corticosteroid therapy, iii) use of antiepileptic drugs, iv) suffering from hepatic disorders v) severe malnutrition vi) non ambulatory patient. The study was conducted from November 2014 till December 2015.

We had a total of (n = 100) cases who fulfilled the inclusion criteria. A semi structured questionnaire was used to record data on parameters like age and socio economic status. Socio economic status was determined by using modified Kuppuswamy's SES scale [12]. Dietary consumption pattern for different food items was done by using a validated food frequency questionnaire method for selected food groups. Waist and Hip circumference to calculate Waist to Hip Ratio (WHR) was done by using standard tools and the cut offs used were adopted from the guidelines set by World Health Organization [13]. The approval for the study was obtained from the Institutional ethics board.

Non fasting blood sample collection was done for the biochemical estimation of serum 25(OH)D and total calcium (Ca) levels. The season for blood with draw was similar for cases and controls. Three milliliters of blood was withdrawn from the median cubital vein of cases. The serum separation was carried out within 2 h after collection by centrifugation at 2100 revolutions per minute (rpm) for seven minutes. Serum samples were stored at minus 80 °C till biochemical analysis was done. Serum 25(OH)D and total calcium levels were estimated by chemiluminescent immunoassay (chemiluminescence) and colorimetric assay (Roche Cobas) technique. The values were documented in ng/ml for 25 (OH)D and mg/dl for serum calcium levels. Vitamin D deficiency was defined as per the criteria of United States Endocrine Society [14]. Cases and controls were subjected to similar investigations. Internal and external quality control was maintained throughout the assay.

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

25(OH)D levels in serum was measured as a standard procedure at the department of biochemistry at an apex healthcare institute. The LIAISON® 25-hydroxyvitamin D Assay (DiaSorin) uses chemiluminescent immunoassay technology. The lower limit of Quantitation of the assay was 4.0 ng/ml [15]. Specific antibody to vitamin D is used for coating magnetic particles (solid phase) and vitamin D is linked to an isoluminol derivative. During the incubation, 25(OH)D was dissociated from its binding protein and competes with labelled vitamin D for binding sites on the antibody. 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 was maintained by running a sample of known concentration of 25(OH)D along with the samples for analysis. The coefficient of variance calculated for low QC was 8% and high QC was 13% which was found to be similar to recent research study for measurement of 25(OH)D assays [16].

### 2.2. Measurement of serum calcium levels by colorimetric assay (Roche cobas) technique

Serum total calcium estimation was done on an automated analyzer, COBAS INTEGRA 400 Plus. Calcium ions react with O-cresolphthelein under alkaline conditions to form a violet 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 [17]. It is determined by measuring the increase in absorbance at 552 nm. The value is expressed in mg/dl.

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