RESEARCH COMMUNICATION

Association between Breast Cancer and Vitamin C, Vitamin E and Selenium Levels : Results of a Case- control Study in India

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Abstract

In women, cancer of the breast is generally the most prevalent neoplasm and cause of cancer death. Though a large number of women are affected with breast cancer, very few studies have been undertaken in India on the association between micronutrients and the risk of breast cancer. We conducted a hospital based case- control study to examine the associations of vitamin C, vitamin E and selenium with breast cancer. One hundred and sixty breast cancer patients and an equal number of normal healthy individuals constituted the study population. Venous blood was collected from the cases and controls for estimation of vitamin C, vitamin E and selenium utilizing standard procedures. Univariate logistic regression analysis was carried out to calculate odds ratios and confidence intervals. The mean vitamin C, vitamin E and selenium levels were lower in patients as compared to the controls. There was a 84% and 77% lower risk of breast cancer if the levels of vitamin C and vitamin E were increased by 1 unit, respectively. Similarly, there was a 7% lower risk of breast cancer if the level of selenium was increased by 1 unit. The results of the present study thus indicated a strong association of vitamin C, vitamin E and selenium with breast cancer in the Indian population.

Key Words: Breast Cancer - Nutrition - Micronutrients - Risk Factors

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Introduction

Carcinogenesis, may arise as a result of chemical, physical, biological and genetic insults to cells (Rozenberg et al., 1996) In women, cancer of the breast is one of the most common incident cancer and cause of death from cancer (Parkin 1989). The etiology of breast cancer is multifactorial and various risk factors have been postulated (Kelsey et al., 1993). The American Cancer Society has estimated that about one-quarter of breast cancer cases can be accounted for by known risk factors (Seidman et al., 1983). Epidemiological studies tentatively and inconsistently suggest that among individual women, high intake of vitamin A, carotenoids, vitamin E, selenium and vitamin C may be protective (Brinton 1994). These are potent antioxidants, and thus may provide a cellular defense against reactive oxygen species which damage DNA and initiate actions such as lipid peroxidation and may have implications not only in the initiation and promotion of breast cancer but also in its spread (Hunter and Willett, 1993; Frei 1994). An improper balance between reactive oxygen molecules (ROMs) production and antioxidant defenses results in oxidative stress, which deregulates the cellular functions leading to cancer (Ray and Husain, 2001). Though a large number of women are affected by breast cancer, very few studies have been undertaken in India on the association between these micronutrients and the risk of breast cancer. We therefore conducted a hospital based case- control study to examine the association of vitamin C, vitamin E and selenium with breast cancer.

Methodology

The present study was a hospital based case – control study. One hundred and sixty breast cancer patients (all consecutive cases) from the out-patient and hospital admissions of the departments of Surgery/ Surgical oncology at the All India Institute of Medical Sciences, New Delhi constituted the study population. The criteria for selection of the patients was i) they should be proven cases of breast cancer by histopathology / cytopathology ii) they should have not undergone any treatment specific for breast cancer iii) they should not have suffered from any major chronic illness in the past, before the diagnosis of breast cancer so as to change their dietary pattern iv) They should not have taken long course of any vitamin or mineral

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supplements during the last one year. v)they should not be on corticosteroid therapy or suffering from hepatic disorders/ severe malnutrition. The participation rate amongst the cases was 100%.

One hundred and sixty normal healthy individuals accompanying the patients in the department of Gastroenterology, Medicine and Surgery at All India Institute of Medical Sciences, New Delhi constituted the control group. The criteria for selection were i) the attendants of patients who did not suffer from any major illness in the past ii) they should not have taken long course of any vitamin or mineral supplements during the last one year iii) they should not be on corticosteroid therapy or suffering from hepatic disorders or severe malnutrition. The participation rate amongst controls was 80%. The study was ethically approved by the Ethics Committee of All India Institute of Medical Sciences, New Delhi. All the investigations to be performed were explained to the subjects and only those who consented for participation were included in the study.

Venous blood (8-10ml) from antecubital vein was drawn from the cases and controls and collected in i) heparin coated and ii) uncoated amber coloured polypropylene tubes for separation of plasma and serum, respectively. The tubes were flushed with nitrogen for 30-60 seconds and tightly capped prior to blood collection. The tubes were then placed in an ice box and transported for centrifugation. The samples were centrifuged at 3500 rpm at 4°C for 30 minutes for the collection of serum and plasma. Plasma was separated and 0.5 ml was mixed with 2 ml of freshly prepared metaphosphoric acid and stored in previously labeled eppendorf vials for estimation of vitamin C. Serum was collected in 2.5ml Eppendorf vials for estimation of vitamin E and selenium and stored at -70° C until analysis. Estimation of vitamin C, vitamin E and selenium was done utilizing the standard procedures (Oramura 1980; Hashim and Schuttringer, 1966; Elmer and Conn, 1975).

The independent sample 't' test was utilised to compare the mean serum levels of vitamin C, vitamin E and selenium between breast cancer patients and controls. The result was considered significant at the 5% level of significance. Univariate logistic regression analysis was also carried out to calculate odds ratios and confidence intervals.

Results

A total of 160 breast cancer patients and 160 controls were included in the present study. The mean age of the patients was 45.29 ± 10.64 years and of the controls was 40.98 ± 10.32 years. Nearly 62.5% of the patients and 67.5% of the controls were in the age group of 30-49 years. Majority of the patients (56.9%) belonged to urban area of residence. All the patients and controls were married and about 95.6% of the patients and 95.6% of the controls were housewives. The patients and the controls were similar with respect to their occupational status. Thirty eight percent of the patients and 61.3% of the controls were illiterate. Forty seven percent of the patients and 65.0% of the controls belonged to upper lower socio-economic status.

The distribution of breast cancer patients and controls according to their plasma vitamin C, serum vitamin E, selenium, total cholesterol and triglyceride levels is depicted in Table 1. There was a statistically significant difference in the mean vitamin C level in patients (0.68 ± 0.45 mg/dl) and controls (1.09 ± 0.50 mg/dl). Similarly, vitamin E levels were also found to be significantly lower in patients (0.92 ± 0.68 mg/dl) as compared to the controls (1.73 ± 0.78 mg/dl). There was no significant difference in the selenium levels of the patients and controls. There was a 84% and 77% lower risk of breast cancer if the levels of vitamin C and vitamin E were increased by 1 unit, respectively. The odds ratio for selenium was 0.93 resulting in a 7% lower risk of breast cancer if the level was increased by 1 unit.

Discussion

The findings of the present study indicated a strong association of vitamin C with breast cancer. The cases had significantly lower serum levels of vitamin C as compared to the controls. Similar results have been reported in another case- control study conducted in India, the vitamin C levels were found to be significantly lower in breast cancer patients (1.10 mg/dl) as compared to controls (1.98 mg/dl) (Ramaswamy and Krishnamoorthy, 1996). Results of another case- control study conducted in India revealed that the vitamin C levels were significantly decreased in breast cancer patients (155.0 μmol/l) than in controls (186.3 μ mol/l) (P<0.01) (Ray and Husain, 2001). Vitamin C is as antioxidant and has been shown to inhibit the formation of nitrosamines. It also acts on the immune system, thereby reducing the risk of breast cancer (Ramaswamy and Krishnamoorthy, 1996; Watternberg 1985; Freudenheim et al., 1996). Vitamin C also plays an important role in the hydroxylation of lysine and proline, in the synthesis of connective tissue proteins such as collagen. A deficiency of vitamin C therefore, may affect the integrity of

Table 1. Distribution of Breast Cancer Patients and Controls According to Their Plasma Vitamin C, Serum Vitamin E, and Selenium Levels

	N	Breast Cancer Patients Mean ± SD	Controls Mean ±SD	OR	CI
Vitamin C (mg/dl)	160	0.68 ± 0.45	1.09 ± 0.50	0.16	0.09-0.27
Vitamin E (mg/dl)	160	0.92 ± 0.68	1.73 ± 0.78	0.23	0.15-0.33
Selenium (μmol/l)	160	3.29 ± 0.76	3.34 ± 0.90	0.93	0.72-1.22

OR = Odds Ratio C.I. = Confidence Interval

intercellular matrices and thus may promote tumour growth or inhibit tumor encapsulation (Steinmetz and Potter, 1991).

There was also a strong association observed between vitamin E levels and breast cancer in the present study. In another case- control study conducted in India the vitamin E levels were observed to be significantly decreased in breast cancer patients (24.87 μmol/l) than in controls (28.3 μmol/l) ($P<0.01$) (Ray and Husain, 2001). Two other case-control studies that have examined levels of vitamin E in blood have reported OR of 0.8 and 4.2 for the highest quintile levels. Results of two other studies have also found marginally to significantly higher levels of vitamin E in plasma, erythrocytes and leucocytes in cases, as compared to controls (Garland et al., 1993). Vitamin E has a role in inhibiting cancer via its action as an antioxidant, as well as its potential effects on selenium. It reduces nitrite, thereby inhibiting the production of carcinogenic nitrosamines and nitrosoamides and expression of certain oncogenes (Freudenheim et al., 1996; Kimmick et al., 1997). Vitamin E can neutralise reactive oxygen species, may reduce oxidative DNA damage, genetic mutations and also enhance host immunological functions. These reactions may help to protect against breast carcinogenesis (Frei 1994). Vitamin E is effective against both tumor initiation and promoters. It also enhances body's immune response and it may regulate the gene expression in mammalian cells (Garland et al., 1993; Dorgan and Schatzkin, 1991; Knekt 1991; Parker 1991).

The results of the present study revealed lower selenium levels in breast cancer patients as compared to the controls, however the difference was not statistically significant. Earlier case- control studies have shown serum selenium to be lower in breast cancer cases as compared to controls (McConnell et al., 1980; Chaitchik et al., 1988; Basu et al., 1989).

A case- control study conducted in Spain revealed that the mean serum concentrations of selenium was 61.1 μg/l in women with breast cancer and 98.5 μg/l in women with non tumoral disease ($p<0.001$) (Lopez-Saez et al., 2003). Results of another case- control study conducted in Netherlands revealed lower mean plasma selenium concentrations in cases (89 μg/l) as compared to the controls (93 μg/l). However, there was no substantial association between selenium and breast cancer (Van't Veer et al., 1990). Selenium is an essential trace element in human nutrition and is a co-factor for enzyme glutathione peroxidase. The metabolic function of this enzyme is vital for cells, as it is a part of the mechanism responsible for the metabolism and detoxification of oxygen. It is assumed that glutathione peroxidase can protect the DNA from oxidative damage and consequently from mutation leading to neoplastic transformation of cells (Watternberg 1985; Biesalski 2002).

Nutrition, in its broadest sense, plays a role in breast cancer, because the nutrient deficiencies in developing countries can be a significant contributing factor in modifying the multistage process of carcinogenesis. The etiology of breast cancer is multifactorial and various risk factors have been postulated. Our data does not allow us to conclude whether vitamin C, vitamin E and selenium deficiency preceded or occurred as a result of the cancer. However, the low mean vitamin C, vitamin E and selenium levels in breast cancer patients as compared to the controls indicated there strong association with breast cancer in the Indian population.

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