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Selenium in Breast Cancer

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Key Words
Selenium · Trace elements · Neoplasm · Breast cancer

Abstract
Aim: Controversy surrounds the hypothetical relationship between low serum levels of selenium and reduced activity of selenium-dependent enzymes, such as glutathione peroxidase, and an increased risk of cancer in humans. This study investigated serum concentrations of selenium in women with and without breast cancer. ***Methods:*** In this case-control study, we compared serum concentrations of selenium in women with breast cancer (n = 200), healthy women (n = 100), and women with chronic diseases (n = 100). Patients with breast cancer were divided into premenopausal (n = 99) and postmenopausal subjects (n = 101). ***Results:*** Mean serum concentrations of selenium were 81.1 μg/l in women with breast cancer and 98.5 μg/l in women with non-tumoral disease ($p < 0.001$). ***Conclusion:*** Alterations in serum concentrations of selenium in women with breast cancer appear to be a consequence, rather than a cause of cancer. In accordance with the hypothesis, the findings suggest that very low selenium status could be due to the nature of cancer.

Introduction

Selenium is not uniformly distributed in the earth's crust [1]. It is present in the human diet as selenomethionine in plants [2] and as selenocysteine in meat [3]. The geographical variations in selenium distribution, and the fact that this trace element is present in food, led some researchers to hypothesize, on the basis of differences in the incidence of cancer, that selenium deficiency might be a risk factor for cancer in some areas. Animal organs and fish contain between 0.5 and 1.5 μg selenium per gram of tissue; these sources represent 45–50% of the dietary intake of this element. Selenium levels in grains vary widely from 0.1 to 0.8 μg/g, and account for 25–35% of the dietary supply. Dairy products contain 0.1–0.3 μg/g, this source making up 10–20% of the dietary intake of selenium [4]. Diet varies across different cultures, and this variability, together with geographical differences in the presence of selenium in the environment, may contribute to differences in the risk of cancer [5].

The first suggestion that selenium was related with cancer appeared in a 1969 study by Shamberger and Frost [6], who reported that mortality from cancer in the USA correlated inversely with the geographic distribution of selenium. Salonen et al. [7] and Shamberger and Willis [8] also found that mortality from cancer correlated inversely with plasma levels of selenium. Clark et al. [9] measured plasma selenium levels in patients with non-melanoma skin tumors and concluded that decreased plasma selenium was associated with an increased frequency of skin cancer.

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Salonen et al. [7] studied 8,113 Finnish subjects without cancer at the time of study entry for 6 years. Subsequently, in 128 cases cancer was diagnosed; these patients had lower plasma levels of selenium in comparison with the remaining control subjects [7]. In a retrospective study, Willet et al. [10] followed up 11,000 patients with hypertension for 5 years. A comparison of plasma selenium concentrations disclosed that concentrations were lower in 111 patients who developed cancer than in 210 controls [10]. Kok et al. [11] found similar results in their comparison of 69 patients with cancer and 164 controls.

In contrast, several case-control studies found no differences between groups in plasma selenium levels [12–14]. Selenium intake cannot be measured accurately by means of dietary intake assessment because of the high variability in the selenium content of individual foods, depending on the geographical area in which the foods were grown. However, selenium levels in tissues, e.g. blood and toenails, do reflect selenium intake and thus provide an informative measure of diet [15]. Van den Brandt et al. [16] designed a cohort study in the Netherlands to investigate the relationship between toenail selenium levels and the risk of stomach cancer and colorectal cancer, as other studies found no association between the selenium status and colorectal cancer.

In a review of five case-control studies by Garland et al. [17], four studies reported lower plasma levels of selenium in patients than in control subjects. However, Hunter et al. [18] reviewed another five studies in search of a correlation between selenium levels in the blood and nails and the risk of breast cancer, but no statistically significant correlations were noted.

The possible mechanism by which selenium is involved in carcinogenesis in humans is related with its antioxidant effects via glutathione peroxidase, which reduces hydrogen peroxide and organic hydroperoxides, using reduced glutathione as an electron donor [19, 20].

In the present study we compared serum concentrations of selenium in patients with breast cancer of different clinical status with those in patients with non-tumoral disease and in healthy women.

Subjects and Methods

Study Design. In this case-control study comparing serum concentrations of selenium, healthy women, women with non-tumoral diseases, and women with active breast cancer or with breast cancer in complete remission were included. A double-blind study design was used.

Null Hypothesis. The null hypothesis was that there was no difference in serum selenium concentrations between healthy controls and women with breast cancer (active disease or cancer in remission). The alternative hypothesis was considered valid when $p < 0.05$.

Study Cohort. The study group consisted of women with breast cancer in whom the diagnosis was confirmed histologically before the study began. Clinical status was determined in accordance with WHO criteria as active disease or disease in complete remission [21]. The normal control group consisted of asymptomatic women matched to the patients for age and socioeconomic status, and a second control group consisted of women with another chronic disease (type 2 diabetes mellitus and chronic obstructive pulmonary disease), also matched to patients for age and socioeconomic status.

Sampling. Consecutive and random sampling was performed and the population included both outpatients and inpatients of the University Hospital of Puerta del Mar and Puerto Real in the city of Cadiz in southern Spain, during the period from October 1999 to September 2001. During this period, a total of 237 cases of breast cancer were diagnosed at the two hospitals. Since 37 patients died during the study, they were excluded and 200 patients remained for the study. Their mean age was 54 years (range: 32–82). Control subjects were recruited from women who accompanied the patients during their hospital stay or from visitors of the outpatient clinic. Informed consent to participate in the study was obtained from each patient and control.

Sample Size. The sample consisted of 200 women with breast cancer and two control groups: 100 healthy women and 100 women with chronic non-tumoral disease. Of the 200 women with breast cancer, 99 were premenopausal and 101 postmenopausal. Of the 200 control subjects, 100 were premenopausal and 100 postmenopausal. In the breast cancer group, 90 women had been in complete clinical remission for at least 5 years, and 110 had active disease but received no treatment at the time of the study.

Variables. As quantitative variables we recorded age (<40, 41–60, or ≥61 years) and serum selenium concentration. A 10-ml blood sample was obtained by venipuncture, and the serum was separated and stored at −80°C until analysis. Dichotomous variables were breast cancer/non-tumoral disease (case/control), pre-/postmenopausal status, and complete remission or active, clinically progressing disease (qualitative variable).

Techniques and Apparatus. Serum selenium concentrations were determined by a graphite furnace atomic absorption spectrophotometry method [22–24] with a Varian Spectra 800-Zeemat GTA 100 apparatus. Accuracy of the technique was checked by constructing calibration curves with a certified reference serum (Seronorm) containing a known concentration of selenium. The determinations were found to b e accurate to 97.5%.

Statistical Analysis. The Systat Program was used for all statistical analyses [25]. Confidence limits were determined as recommended by Gardner and Altman [26].

Results

General Findings

The results are summarized in table 1. In the 200 patients with breast cancer, mean serum concentration of selenium was 81.1 μg/l, with a 95% confidence interval of

77.0–85.1 μg/l. In the 200 control subjects without tumoral disease, the mean concentration was 98.5 μg/l (95.7–101.4 μg/l). The results of Student's t test and the Mann-Whitney test showed that this difference was significant at $p < 0.001$. When we compared the serum concentration of selenium in the 100 healthy women (96.1 μg/l, 92–99.1 μg/l) and the 100 patients with non-tumoral disease (100.2 μg/l, 96.7–104.3 μg/l), the difference was not statistically significant ($p > 0.05$).

Differences with Age

The correlations between age and serum selenium concentrations in healthy women ($r = -0.14$) and in women with chronic non-tumoral disease ($r = -0.15$) were not statistically significant ($p > 0.05$). However, in women with breast cancer this correlation was significant ($r = 0.35$, $p < 0.05$). In women with non-tumoral disease, mean serum selenium concentrations in the three age subgroups were 100.8 (<40 years), 97.9 (41–60 years) 98.5 μg/l (>60 years). None of the differences between any of the three subgroups was significant ($p > 0.05$). In women with breast cancer, these values were 95.6 (<40 years), 77.1 (41–60 years) and 82.0 μg/l (>60 years). The difference between the first two subgroups was significant ($p < 0.001$) as well as the difference between the last two subgroups ($p < 0.05$). The difference between women <40 years and those >61 years was not significant ($p > 0.05$).

Menopausal Status

In the 100 premenopausal women with non-tumoral disease, mean serum selenium concentration was 98.6 μg/l (96.7–100.5 μg/l); in the 100 postmenopausal women in this group, this figure was 98.4 μg/l (96.2–100.6 μg/l). The difference was not statistically significant ($p > 0.05$).

In the 99 premenopausal women with breast cancer, the mean concentration was 86.1 μg/l (82.2–89.8 μg/l); in the 101 postmenopausal women in this group, the mean concentration was 78.8 μg/l (76.3–81.2 μg/l). This difference was statistically significant ($p < 0.05$).

Clinical Status

Of the 90 patients with breast cancer who were in complete remission at the time of the study, the mean serum concentration of selenium was 90.0 μg/l (83.1–96.9 μg/l); this value did not differ significantly from that found in healthy women ($p > 0.05$), but did show a significant difference in comparison to patients with non-tumoral disease and patients with breast cancer as a whole ($p < 0.05$). Of the 110 patients with clinically progressing disease, the mean concentration was 66.5 μg/l (60.2–72.9 μg/l); this value was significantly lower than the concentration found in healthy subjects ($p < 0.001$) and the group of patients with breast cancer as a whole ($p < 0.001$).

Table 1. Mean serum concentrations of selenium in Spanish women with breast cancer, healthy women and women with chronic diseases

Study groups	n	Mean serum selenium, μg/l	95% CI
Breast cancer	200	81.1	77.0–85.1
Clinical remission	90	90.0	83.1–96.9
Active disease	110	66.5	60.2–72.9
Stage I–II	50	81.5	74.5–88.6
Stage III–IV	60	75.4	67.6–83.3
Control subjects	200	98.5	95.7–101.4
Healthy women	100	96.1	92.0–99.1
Chronic disease	100	100.2	96.2–104.3

Clinical Stage

In 50 patients, the disease was classified as locoregional stage I or II; mean serum concentration of selenium in this group was 81.5 μg/l (range 74.5–88.6 μl). In 60 women with stage III or IV disease, mean concentration was 75.4 μg/l (67.6–83.3 μg/l). The difference between these two subgroups was not significant.

Discussion

The results of epidemiologic investigations regarding the possible role of selenium in the etiology of breast cancer are controversial. In our sample of patients with breast cancer, the mean serum concentration of selenium was lower than in healthy women and women with chronic non-cancerous disease. The differences between groups were unquestionable, and the technique used to measure selenium concentration was highly accurate. In agreement with other authors, we found a clear reduction in serum selenium levels in relation with breast cancer [6–11, 17].

However, the lower selenium concentrations in patients with breast cancer are not in themselves proof that these women consumed a diet poor in selenium. Homeostasis can prevent or lessen large variations that might be caused by the diet [5]. Biomarkers of dietary exposures are also being developed and used in observational epidemiologic studies [27]. In recent years, toenail selenium has gained increased interest as a biomarker of the seleni-

um status, following observations that this marker is an indicator of the long-term selenium status [16]. No biomarkers have yet been validated as surrogate endpoints for cancer. The notion that dietary selenium deficiency led to lower serum levels of this trace element has been so strongly defended that cancer prevention programs have been based on dietary supplementation with this element [28]. In a prospective study by Hunter et al. [18] no association between toenail selenium and the risk of breast cancer was observed during a 4-year follow-up. Several prospective investigations have also failed to detect any association between serum or toenail selenium and breast cancer [29].

However, a low dietary intake cannot be blamed for low serum concentrations in all patients with breast cancer, nor can the negative findings in some studies be assumed to be spurious [12–14, 18]. Serum selenium levels did not vary with age in healthy women or women with chronic non-tumoral disease; however, in women with breast cancer we noted that the concentration was lowest in women aged 41–60 years. In patients <40 years, selenium levels were not lower than in healthy women.

There were no differences in serum selenium concentrations between pre- and postmenopausal women with non-tumoral diseases. However, in the group with breast cancer, serum levels of this element were lower in postmenopausal than in premenopausal women.

Patients whose breast cancer was in complete remission had normal or near-normal serum levels of selenium. In contrast, the subgroup of women with clinically active disease had the lowest mean level of all subgroups studied. The decrease in serum selenium in patients with stage I disease was minimal; however, the difference was greater in women with larger tumors and extended disease.

The apparently contradictory results reported in different studies may be explained by methodological differences, as some authors did not take age, menopausal status, clinical stage of the tumor or clinical status (active disease or remission) into account. Statistical significances in the decrease in serum selenium may be influenced by age, menopausal status, or clinical status in the sample of patients studied.

Selenium is an important inorganic antioxidant, and diminished circulating levels of this element may largely result from uptake by tumoral tissue, which uses reduced glutathione as an electron donor [19, 20]. The decrease in serum selenium may be the result of increased activity or increased tumoral mass, which in turn may increase the amount of free radicals in the tumoral tissue. These free radicals may attract greater amounts of selenium through electrophilic mechanisms, as found in a study of blood selenium levels in patients with laryngeal cancer [30].

Our results suggest that the decrease in serum concentrations of selenium in women with breast cancer was a consequence rather than a cause of cancer. This hypothesis would explain why the levels of selenium were lower in women with more advanced cancer than in patients whose disease was in an earlier stage, when the tumoral mass was smaller. If selenium deficiency were a cause of cancer, the greatest decreases in serum concentrations would be expected in the initial phases of the disease. If exogenous causes were responsible, the alterations in selenium levels observed in the absence of active disease (as in complete clinical remission) would be expected to be similar to those found in patients with active disease; our findings, however, suggest that this was not the case. The differences we found between premenopausal and postmenopausal women are further evidence that endogenous rather than exogenous factors are responsible for alterations in serum concentrations of selenium.

Prospective studies are needed to document whether the clinical course of the tumor is associated with decreases in serum selenium concentrations and increases in tumoral tissue. We believe that this hypothesis accounts for the findings of the present study. The issue can be further elucidated by studies designed to determine the daily serum selenium balance and its relationship with the changes in selenium concentrations in tumoral tissue.

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