ORIGINAL ARTICLE

Estimation of Serum Level of Vitamin E in Breast Cancer Patients and Apparently Healthy Women of Lahore, Pakistan

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ABSTRACT

Background: Breast cancer is one of the most frequently diagnosed cancers and is also the major cause of death in females worldwide. Pakistan has the highest number of patients of breast cancer among other countries of Asia. Multiple factors, in addition to genetic predisposition, contribute to its development e.g., chronic inflammation and hormonal imbalances. These cause redox disruption thus increasing ROS that alters gene expression leading to carcinogenesis. Antioxidants like Vitamin E may help in reducing tissue-level inflammation through different mechanisms by maintaining redox balance within the tissue. In this cross sectional comparative study was we measured the serum level of vitamin E in breast cancer patients and in apparently healthy women of same age group to determine the involvement, if any, of this vitamin in breast cancer etiology, we recruited 90 women Lahore. Out of those 60 were breast cancer patients and 30 were apparently healthy women. Vitamin E level in serum was measured by Enzyme Linked Immunosorbant Assay (ELISA). The vitamin E was within the normal reference range of WHO in both, normal women and breast cancer patients. Though levels were within normal range, patients had significantly low levels as compared to controls. Age did not affect the vitamin E levels in both groups of women.
Key words: breast cancer, ROS, vitamin E, anti oxidants

INTRODUCTION

Breast cancer is one the most commonly occurring types of cancer in women. The incidence and mortality rate due to CA breast is very high among females worldwide[1]. Around 23% of the total newly diagnosed cases of cancer and 14% of the total deaths due to cancer, reported in 2008, were caused by breast cancer[2]. Younger women are getting more affected in Pakistan as compared to west. In addition to other causes, ROS induced alteration in transcription factors contributes to carcinogenesis[3,4]. An imbalance occurs between the production of free radicals and their scavengers, increasing the oxidative stress during different stages of carcinogenesis. Causative factors of imbalance can be exogenous factors e.g., diet, smoking or endogenous factors e.g., chronic inflammation and hormonal disturbances[4,5]. Vitamin E is considered the most effective natural antioxidant and scavenger of ROS that protects the cell membranes from damage caused by lipid per-oxidation[6]. It has been suggested that one of the possible ways in which vitamin E retards the growth of breast cancer cells is that it modifies the response of cells to estrogen and inhibits ER-positive cells more effectively as compared to ER-negative cells. Studies on animal models with breast cancer and in vitro have suggested that vitamin E causes activation of the peroxisome proliferator-activated receptor (PPAR) pathway which in turn, interferes with NF-κB pathway and leads to the inhibition of certain inflammatory markers such as cyclooxygenase-2(COX-2). These are the likely mechanisms that show the relationship between vitamin E and inflammation and can be used in treatment of breast cancer[7]**.** In different studies lower serum levels of vitamin E have been found in patients of

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CA breast (0.44mg/100ml) when compared to their matched healthy controls (1.108 mg/100 ml)[8].

PATIENTS AND METHODS

The study was approved by the advanced studies and research board of UHS. The purpose of the study was explained to all participants and investigation was carried out with their written consent. A total of 60 newly diagnosed patients of breast cancer, after confirmation of diagnosis on histopathology, were recruited from Inmol Hospital, Lahore. Selected patients were of stages (TNM) 1 and 2. Blood samples of patients were collected before initiating chemotherapy or radiotherapy. The subjects were ranging in age 20–60 years. They had all a body mass index of 23.1±3.85. None of them had concomitant diseases such as diabetes mellitus, liver disease, hypertension and previous history of any other cancer. None of them was using vitamin supplements. Thirty healthy age matched (between 20 and 60 years) women were selected as controls. They had all a BMI of 21.0±2.6 and were considered normal weight. None of the controls had a previous history of breast cancer and other diseases. A questionnaire with epidemiologic information on demographic and lifestyle factors, personal and medical history, and family history of breast cancer was completed for each participant.

Sample collection: Five milliliters of blood were taken from the ante-cubital vein of each subject, under aseptic conditions, in gel coated vacutainer tube. After centrifugation, serum was aliquoted and stored at -80 degree Celsius until analyzed. Serum vitamin A levels were estimated by sandwich enzyme linked immunosorbant assay (ELISA) using automated EIA analyzer (Bio-Rad Laboratories, Hercules, CA, USA) with commercially available human vitamin A ELISA kit.

Statistical analysis: For the purpose of comparison and analysis we divided them into subgroups according to their ages. Group A had female patients of CA breast and was subdivided into two groups A1 and A2. Group A1 comprised of 30 breast cancer patients of ages 20 - 40 yrs and group A2 had 30 patients of ages 41 - 60 yrs. Group B was the control group (healthy females) and was subdivided into B1 and B2. In group B1, women were of ages 20 - 40 yrs (n=15) and in group B2 women were of 41 – 60 yrs (n=15). The data were entered and analyzed using IBM SPSS (Statistical Package for Social Sciences) version 20.0. A *p*-value of < 0.05 was considered statistically significant for all purposes. Two way ANOVA was used to check relationship between vitamin E levels and age of subgroups of patients and controls.

The plot of mean score of vitamin E for each combination of groups (patients and controls) and subgroups on the basis of Age (20-40 and 41-60 years) is plotted in a line graph as shown above. It is providing a good graphical presentation of our results obtained through the calculation of two-way ANOVA. No interaction effect can be seen as graph is showing parallel lines of mean levels of dependant variable (Vitamin E) in both groups on the basis of age. If the lines appeared to be non-parallel and crossed each other then we could interpret this line graph as some significant interaction on the basis of two-way ANOVA. It also shows that the vitamin E levels were not affected much by age as in case of vitamin A but the patients had significantly low serum vitamin E compared with normal subjects.

Table 1: Serum Level Of Vitamin E (µg/ml) In Patients And Controls According To Age Groups

Age	Groups	Mean±SD	p value
Group A (Patients)	20-60 yrs	8.54±3.16	0.044 [a]
Group B (Controls)		9.98±3.10	
Group A1 and A2 (Patients)	20-40yrs	7.56±4.34	0.354 [a]
	41-60yrs	7.58±6.67	
Groups B1 and B2 (Controls)	20-40yrs	14.9±7.32	0.59 [b]
	41-60yrs	13.2±7.8	

[a] p-value generated by Independent Sample "t"-Test
[b] p-value generated by Mann-Whitney U Test
p-value ≤ 0.05 is considered statistically significant

Table 2: Result of Two-way ANOVA Tests of Between-Subjects of different age Groups and their Vitamin E levels in apparently healthy and CA breast patients

Tests of Between-Subjects Effects (Vitamin E)					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	877.343[a]	3	292.448	7.274	.000
Intercept	9414.184	1	9414.184	234.149	.000
Age_groups	13.388	1	13.388	.333	.565
Group	856.871	1	856.871	21.312	**.000**
Age_groups * Group	13.906	1	13.906	.346	.558
Error	3457.719	86	40.206		
Total	12902.977	90			
Corrected Total	4335.062	89			
a. R Squared = .202 (Adjusted R Squared = .175)					

Fig 1: Plot of mean score of vitamin E for each combination of groups (patients and controls) and subgroups on the basis of Age (based on two-way ANOVA)
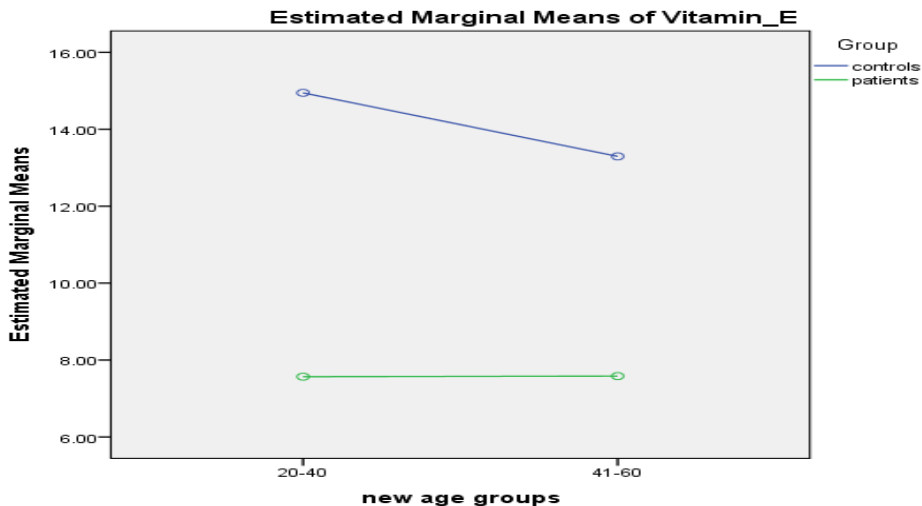
DISCUSSION

In the present study, we recruited 60 patients of breast cancer and 30 healthy women. Vitamin E level was moderately low in 7 (11.6%) patients while 10% controls also had less than the normal reference values. However, on the overall basis, significant low levels of vitamin E were seen in patients as compared to normal females. According to the international standard reference values, the normal range of vitamin E in serum is 11.5 - 46 µmol/L (4.9 - 19.8µg/ml) and less than 11.5 µmol/L (4.95 µg/ml) is considered as vitamin E deficiency[9]. In the present study, the mean levels of vitamin E of apparently healthy women was 9.98 ± 3.10 µg/ml and in the breast cancer patients, it was 8.54 ± 3.16 µg/ml. These values are towards the lower side of the normal range. While vitamin E levels of two subgroups according to age (20-40 and 41-60 years) of apparently healthy females was 14.9±7.32µg/ml and 13.2±7.8µg/ml while in patients it was 7.5±4.3 and 7.5±6.6 respectively.

Similar findings were reported in which the mean vitamin E levels among cases was 4.7µg/ ml and controls was 6.0µg/ml. The women with serum vitamin E levels in the lowest quintile had about 5-times higher risk of developing CA breast than women with serum levels in the highest quintile[10,11]. Chamras et al. (2005) also observed lower serum levels of vitamin E in patients of CA breast when compared to healthy controls (0.44 mg/100 ml vs. 1.108 mg/100 ml(8). The level of vitamin E was found lower in breast cancer patients than healthy women in another study conducted in Algeria. In that study, the authors suggested that low plasma level of vitamin E may reflect its high utilization rate in breast cancer patients, showing that this vitamin may be used to reduce oxidative stress in breast cancer patients[12].

Several investigations have been done on this vitamin and most of them have showed low serum levels of vitamin E in breast cancer patients. A review indicates that vitamin E is capable of inhibiting and restricting tumor growth and its incidence while others do not support this observation[8]. Another work done in Korea demonstrated that consumption of antioxidants like vitamin E was associated with significant reduction in the oxidative stress[5]. A recent meta-analysis reported a significant association between plasma CA breast and α-tocopherol[6]. A study conducted in Brazil showed that the post-menopausal women had a more favorable antioxidant profile, causing less oxidative damage and higher antioxidant capacity. That also showed higher mean values of α-tocopherol levels among the postmenopausal women[1].

From our and different earlier studies, it can be said that low levels of vitamin E is an important feature in breast cancer patients. From these results we can say that the nutritional status for vitamin E of women of this study was compromised and that age is not a factor in this regard. We suggest a comprehensive study on the status of vitamin E in our population taking into consideration the population income group, nutritional habits, life styles and disease status.

CONCLUSION

Low levels of vitamin E were found in patients than apparently healthy women. Age did not affect the vitamin E levels. But due to small sample size of our study and discrepancies in the available earlier experimental results, there is currently a need for a proper population based study on the role of vitamin E in diseases and their supplementation for the prevention of chronic diseases including breast cancer.

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