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Plasma Level of Antioxidant Vitamins and Lipid Peroxidation in Breast Cancer Patients

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Abstract

Oxidative stress arises when there is an imbalance between reactive oxygen species (ROS_s) and scavenging capacity of antioxidants, and it can induce and progress many diseases such as breast cancer. The present study was conducted to investigate the status of plasma antioxidative vitamins (E and C) and lipid peroxidation on 50 untreated breast cancer patients and 50 healthy age-matched women. The results revealed that plasma vitamin E and vitamin E adjusted for the sum of cholesterol and triglycerides decreased significantly in patients group ($P< 0.05$). We could also observe that vitamin E adjusted for lipid was significantly different in various stages of breast cancer. On the other hand, the level of malondialdehyde increased significantly in patients as compared to the controls ($P <0.05$). There were no significant changes in plasma vitamin C between two groups. According to the findings, attention to the level of plasma antioxidant vitamins and lipid peroxidation is of great importance to promote the level of health in women suffering from breast cancer.

Keywords: *Breast cancer, Oxidative stress, Vitamin E, Vitamin C, Malondialdehyde*

Introduction

Breast cancer is the second most common malignant neoplasms after lung cancer in the world (1), with approximately 410 000 cases of death of infected with this disease annually (1, 2). According to the GlOBOCAN database of the International Agency for Research on Cancer (IARC), there were over one million new cases in the world in 2002; including 636 128 cases in more developed countries and 514 072 cases in less developed countries (2). In Iran, breast cancer ranks the first among cancers of women, comprising 21.4 percent of all malignancies in females (3). Breast cancer is again the most common cancer among women in Tehran, capital of Iran (25.5 %) (3).

The etiology of breast cancer is multifactoral. Hormonal, genetic and environmental factors appear to interplay in the pathogenesis of breast cancer (4). The risk factors associated with breast cancer, may exert their effects via generation of reactive oxygen species (ROS_s) such as super oxide(o_2^-), hydroxyl radical($^{\cdot}OH$), peroxyl($RO2^{\cdot}$), hydrogen peroxide(H2O2) and hydroperoxid(LOOH), which induce oxidative damage of DNA, lipid peroxidation (LPO) and neoplastic transformation (5-8). The lipid peroxide formation is normally prevented or scavenged by a host of antioxidants (8). Experimental evidences reveal that reactive oxygen metabolites (ROM_s) are involved in initiation, promotion and progression of carcinogenesis, where inactivation or loss of certain tumor suppressor genes is occurred (9). The extent of ROS- induced oxidative damage can be exacerbated by a decreased efficiency of antioxidant

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defense mechanisms (6, 8). Therefore, it is important to pay more attention to antioxidants defense. Vitamin C is important water–soluble, chain– breaking antioxidant which reacts directly with superoxide singlet oxygen and regenerate tocopherol from the tocopheroxy radical produced during scavenging of free radicals (8, 10). Vitamin E is a major lipid soluble antioxidant present in plasma and erythrocyte membrane that can prevent cellular damages by inhibiting DNA breakage induced by the ROM (8, 11).

In recent years, there has been a growing interest in studying the role of lipid peroxidation and antioxidant status in cancer patients, that demonstrated enhanced lipid peroxidation and impairment of antioxidant defense mechanism as mentioned in the studies by Kumaragunparan (12,13), Ray (14,15), Khanzode (16), and their colleagues.

To our knowledge, the relationship between the changes of vitamins and oxidative stress in plasma of breast cancer patients has never been determined in Iran. Therefore, we undertook the present study to assess the extent of lipid peroxidation by measuring malondialdehyde (MDA), the end product of LPO, and the status of plasma vitamin E and C in breast cancer patients.

Materials and Methods

Fifty newly diagnosed breast cancer patient, mean age of 48± 9.75 yr, with a range of 30-68 yr, from Imam Khomeini Hospital in Tehran, Iran, who had not undergone any previous treatment for malignancy were selected for the study. The patients were clinically categorized as stage I (10 Patients), Stage II (28 patients), and Stage III (12 patients). Fifty healthy women ranging in mean age of 45.88 ±9.07 yr, with a range of 30-68 yr were served as control group. None of the patients or controls had concomitant diseases such as diabetes mellitus, liver disease and rheumatoid arthritis and none of them was using vitamin supplements. All of them belonged to the same socioeconomic strata. At the same time, confounding variables such as smoking and menopausal status were considered.

Fasting blood samples were collected from patients and controls in the presence of EDTA anticoagulant. The plasma was separated by centrifugation at 3000 rpm for 10 min. Both patients and controls' plasma were freezed and stored at -70 ^{o}C until analysis.

Lipid peroxidation was estimated by measurement of thiobarbituric acid reactive substances (TBARS) in plasma according to Satoh method (17). The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde was measured at 530 nm.

Plasma vitamin E was measured by high performance liquid chromatography (HPLC), according to Cuesta–Sanz method (18). Plasma vitamin E was standardized with cholesterol and triglycerides to correct any effect of plasma lipid on vitamin E concentrations. Cholesterol and triglyceride were measured by enzymatic procedure with diagnostic kits. Plasma vitamin C was estimated by Lowry method (19) in which dehydro ascorbic acid reacted with 2, 4 dinitrophenyl hydrazin (DNPH) to form an orange red color compound , which was measured at 520 nm .

The biochemical data are expressed as mean± SD. Statistical analysis was performed using Student's *t*–test, Mann-Whitney, Chi square and one way Anova. *P*-value less than 0.05 was considered significant. All data were analyzed using SPSS software.

Results

Table 1 shows the levels of plasma vitamin E, C, and MDA in BC patients. The level of vitamin E and vitamin E/Chol+TG in BC patients was significantly lower as compared to controls. The extent of lipid peroxidation in patients was significantly increased compared to normal subjects.

In addition, in the present study, vitamin E, vitamin E/Chol+TG, vitaminC and MDA were allocated in relation to different menopausal

and smoking status among BC patients. According to Table 2, plasma vitamin E was found significantly decreased among postmenopausal BC patients as compared to their corresponding controls.

Moreover, in patients and control groups the level of vitamin E in postmenopausal subjects was higher than premenopausal ones. Table 3 shows that the level of vitamin E in non-smoker patients was lower than non-smoker controls, while plasma MDA in non-smoker BC patients was higher than corresponding controls.

Considering clinical stage, changes in biochemical parameters were observed in different stages of breast cancer in patient group (Table 4). Significant changes in plasma vitamin E/Chol+TG were found especially between stages I and III.

Table 1: Plasma concentration of vitamin E, C and MDA in controls and patients with breast cancer

Measured parameters	Patiesnts	Controls
Vitamin E(µg/ml)	12.28±3.06[a]	13.56±3.23
Vitamin E/ Chol+ TG(µg/ mg)	3.72±1.05[a]	4.17±1.2
Vitamin C(mg/ dl)	0.83±0.4	0.89±0.3
MDA(nmol/ ml)	1.76±0.47[a]	1.57±0.45

Significant differences between groups: [a]) $P<0.05$

Table 2: Comparison of plasma vitamin E, C and MDA between menopausal status of breast cancer patients and controls

Menopausal status	Vitamin E (µg/ml)	Vitamin /Chol+TG (µg/mg)	Vitamin C (mg/dl)	MDA (nmol/ml)
Premenopausal Patients (n=27)	11.47±2.76	3.6±1.04	0.89±0.36	1.69±0.39
Premenopausal Controls (n=32)	12.19±2.05	3.6±1.09	0.83±0.26	1.56±0.47
postmenopausal Patients (n=23)	13.24±3.17 [a,b]	3.87±1.06	0.78±0.41	1.85±0.55
postmenopausal Controls (n=18)	15.98±3.53 [c]	4.56±1.27	0.99±0.34	1.58±0.4

Significant differences between:
Patients versus controls: [a]) $P<0.05$
Premenopausal versus postmenopausal in each group: [b]) $P<0.05$; [c]) $P<0.0001$

Table 3: Comparison of plasma vitamin E, C and MDA between smoking status of breast cancer patients and controls

Smoking status	Vitamin E (µg/ml)	Vitamin E/Chol+TG (µg/mg)	Vitamin C (mg/dl)	MDA (nmol/ml)
Non-smoker Patients (n=48)	12.26±3.12 [a]	3.75±1.06	0.84±0.38	1.78±0.47 [a]
Non-smoker Controls (n=49)	13.64±3.2	4.2±1.88	0.89±0.3	1.57±0.45
Smoker Patients (n=2)	12.84±1.06	3.08±0.23	0.35±0.06	1.4±0.51
Smoker Controls (n=1)	9.4±0	2.97±0	0.52±0	1.39±0

Significant differences between:
Patients versus controls: [a]) $P<0.05$

Table 4: Plasma concentration of vitamin E, C and MDA in different stage of breast cancer patients

Measured parameters	Stage I (n=10)	Stage II (n=28)	Stage III (n=12)
Vitamin E(µg/ml)	12.46± 2.9	12.6±3.4	11.24±2.22
Vitamin E/Chol+TG(µg/mg)	4.54 ±1.21	3.6±0.99	3.33±0.66
Vitamin C(mg/dl)	0.7± 0.36	0.93±0.42	0.67±0.23
MDA(nmol/ml)	1.74± 0.45	1.71±0.5	1.9±0.44

Significant differences between:
Stages for vitamin E/Chol+TG: $P<0.05$

Discussion

Lipid peroxidatin plays an important role in the control of cell division (5). Low concentration of oxygen free radicals have been reported to stimulate cell proliferation; whereas high levels induce mutagenecity, cytotoxicity and cell death (5-7). The improper balance between ROS production and antioxidant defenses results in oxidative stress (8).

In the present study, we observed increased levels of plasma MDA in BC patients, which may be attributed to over production of ROS or a deficiency of antioxidant defense.

ROS have been found to play a functional role in the pathogenesis of malignancy, including breast cancer (5-8). The mammary epithelium damage by ROS can lead to fibroblast proliferation, epithelial hyperplasia, cellular atypia, and ultimately breast cancer (6, 7). Studies have shown lipid peroxidation increases in plasma and solid tumors (13-16, 20). The results of the present study are consistent with the findings of Kumar et al. (21), Hristozov et al. (22), and Gonenc et al. (23). In addition, Huang (24) has found significant difference between plasma levels of MDA in BC patients and controls (1.5±0.4nmol/mL compare to1.27±0.4nmol/Ml).

On the other hand, antioxidant depletion in plasma maybe due to increased scavenging of lipid peroxides by antioxidants as well as sequestration by tumor cells (25). Tumor cell have been reported to sequester essential antioxidants to meet the demands of growing tumor (7, 25). Antioxidant vitamins have a number of biological activities such as immune stimulation, and an alteration of metabolic activations of carcinogens (8, 25). They can prevent genetic changes by inhibiting DNA damage induced by the ROS (26). The mechanism through which vitamin E contributes to anticarcinogenic effects is that it can directly act with a variety of radicals, including the peroxylipid and ·OH (27). There is also mechanism by which vitamin C plays important roles in preventing carcinogenesis. It can readily scavenge ROMs and reactive nitrogen species, including peroxynitrite, nitrogen dioxide, nitric oxide radicals, thereby effectively protecting cellular biopolymers, such as genetic material from oxidative damage (10). Besides, vitamin C may reduce carcinogenesis through the stimulation of immune systems, where cytotoxic T lymphocyte, macrophages, and natural killer cells stimulated to lyse tumor cells (10, 25).

In the present study, plasma vitamin E and vitamin E/Chol+TG were found significantly lower in BC patients. There are several reports (12, 14, 28), which reveal decrease vitamin E concentration in BC patients, while Gerber et al.(29) have observed increase plasma vitamin E/chol especially in young BC patients and Hacisevki et al. (30) have seen no changes in vitamin E concentration.

None of the studies adjusted vitamin E for lipid, except Gerber et al. (29). In this study, we also adjusted vitamin E for lipid (Cholesterol and triglyceride) and observed vitamin E/Chol+TG changes among the BC patients in different stages of disease.

However, there were no significant differences in plasma vitamin C concentration between two groups. Though in the studies conducted earlier

(12, 14, 31), an increase in plasma vitamin C have been seen, which is different with our results; the difference may be due to our method for measuring vitamin C, and while plasma vitamin C is dependent on recent vitamin C intake, it cannot be considered as a sensitive indicator for vitamin C status. So other methods such as measuring white blood cell vitamin C is more reliable and recommended for future research.

Since variables like menopausal and smoking status can influence plasma antioxidant and MDA concentration, we analyzed the effects of them on biochemical parameters that we measured. According to our data, only menopausal status affected plasma vitamin E in patients and controls, while it had no effect on plasma vitamin E/Chol+TG. (Probably aging and following changes of the plasma hormones and lipids level are the main causes of it).

In conclusion, plasma vitamin E and vitamin E/Chol+TG are lower and lipid peroxidation (MDA) is higher in BC patients than healthy women are. Further studies are required to investigate the antioxidant and lipid peroxidation status in BC patients in different stages of disease.

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